



**ASSESSMENT OF HEMATOLOGICAL AND GROWTH RESPONSES OF
HETEROBRANCHUS LONGIFILIS FINGERLINGS TO
PROBIOTIC-SUPPLEMENTED DIETS**

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ABSTRACT

The study assessed the hematological and growth responses of *H. longifilis* fingerlings fed diets supplemented with Aquapro, CHC, and their combination at 0.00%, 0.50%, 1.00%, and 1.50% inclusion levels for seven months. A total of 280 fingerlings (mean weight 5.3 ± 0.1 g) were distributed in a completely randomized design with triplicate treatments. Growth and hematological parameters were analyzed using standard methods. Results revealed that probiotic supplementation significantly ($p < 0.05$) enhanced growth performance and blood profiles compared to the control. Fish fed the combined probiotics at 1.50% inclusion achieved the highest final weight (348.79 ± 4.33 g) and specific growth rate ($1.97 \pm 0.41\%$), while the control recorded the lowest values (166.81 ± 2.37 g; $1.50 \pm 0.00\%$). Feed conversion ratio improved from 1.95 in the control to 1.61 in probiotic-fed fish. Similarly, red blood cell count increased from $2.95 \times 10^6 \text{ mm}^{-3}$ in the control to $4.01 \times 10^6 \text{ mm}^{-3}$ in the combined probiotic group, while hemoglobin concentration rose from 10.17 ± 0.02 g/dL to 13.58 ± 0.20 g/dL. Packed cell volume also improved significantly from 33.00% to 35.80%. It was concluded that probiotic supplementation especially combined Aquapro + CHC diets at 1.0–1.5% significantly improved both hematological indices and growth performance in *H. longifilis* fingerlings. Such inclusion levels are therefore recommended to enhance productivity and physiological health in intensive aquaculture systems.

Keywords: *Heterobranchus longifilis*, Probiotics, Growth, Performance; Hematology,

INTRODUCTION

Aquaculture remains a critical contributor to global food security, providing more than half of the world's fish consumed annually. In sub-Saharan Africa, species such as *Heterobranchus longifilis* have gained prominence due to their fast growth rate, adaptability, and high market value (Akanmu and Omitoyin, 2022). However, intensification of aquaculture practices often leads to stress, poor water quality, disease outbreaks, and reduced growth performance. The reliance on antibiotics for disease control has raised environmental and public health concerns, prompting a shift toward natural growth promoters such as probiotics (Sohel *et al.*, 2023; Hang *et al.*, 2022). Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts have demonstrated potential to enhance fish immunity, improve feed utilization, and stabilize gut microbiota (Tanveer *et al.*, 2024). In fish, probiotics such as *Lactobacillus plantarum* and *Saccharomyces cerevisiae* have been shown to improve hematological and biochemical parameters, leading to better resilience against pathogenic stressors and improved overall physiological performance (Akanmu and Omitoyin, 2022; Sohler *et al.*, 2023). Hematological indices including red and white blood cell counts, hemoglobin concentration, and hematocrit levels are reliable indicators of the physiological status and stress response of fish (Witeska *et al.*, 2023). These parameters, alongside growth performance indicators such as specific growth rate and feed conversion efficiency, provide an integrated picture of the nutritional and health status of cultured species.

Despite numerous studies on probiotics in aquaculture, data on their influence on the hematological profile and growth response of *Heterobranchus longifilis* remain limited and inconsistent. While some studies have documented significant improvements in growth and survival using probiotic supplementation (Abubakar & Ipinjolu, 2020), others found negligible changes in blood



parameters despite improved feed utilization (Sîrbu *et al.*, 2022). This inconsistency underscores the need for species-specific evaluation of probiotic formulations, particularly in African catfishes, to optimize their potential for sustainable aquaculture.

The present study, therefore, seeks to evaluate the hematological and growth responses of *Heterobranchus longifilis* fingerlings fed probiotic-supplemented diets. Understanding how probiotics influence blood health and growth performance will provide insights into their physiological roles and guide their effective use in aquafeed formulation. The findings are expected to contribute to sustainable aquaculture practices by reducing dependence on antibiotics, improving fish welfare, and enhancing productivity.

The study therefore aims at evaluating the impact of probiotic-supplemented diets on the growth and hematological parameters of *Heterobranchus longifilis* fingerlings, establishing the correlation between enhanced blood health and overall growth performance.

MATERIALS AND METHODS

Experimental Site

The study was conducted at the aquarium of the Department of Biological Sciences, Bayero University, Kano, Nigeria, situated between latitude 11°58'30"–11°59'0" N and longitude 8°28'30"–8°28'50" E. The area has a typical Sudan savannah climate characterized by a mean annual temperature of about 34°C and an annual rainfall ranging from 800 to 1,000 mm (Rabiu *et al.*, 2018).

Experimental Fish and Acclimation

A total of 280 *Heterobranchus longifilis* fingerlings were procured from Manissad Farm, Lagos State, Nigeria. Fish were transported in 50 L plastic containers containing aerated water and acclimated in a 3,000 L capacity concrete tank for two weeks. During acclimation, they were fed a commercial diet containing 40% crude protein at 3% body weight twice daily (07:00-09:00 and 17:00-19:00). Prior to the feeding trial, the fish were starved for 24 hours to empty their stomachs and enhance feed acceptance (Okoye and Sule, 2002).

Experimental Design and Diet Formulation

The feeding trial lasted for seven months, using a completely randomized design (CRD) consisting of four treatments with three replicates each. A total of 10 fish were stocked per 110 L plastic basin, giving 30 fish per treatment. The experimental diets were formulated using locally available ingredients yellow maize, soybean meal, groundnut cake, and fish meal as major ingredients. The probiotics Aquapro and CHC were included at varying inclusion levels to evaluate their effects on hematological and growth responses. Diet formulation was done using the Pearson square method to achieve 40% crude protein. Feed ingredients were milled, thoroughly mixed, pelleted, and air-dried before storage in airtight containers until use.

Fish Rearing and Water Quality Management

Each basin was filled with fresh borehole water and covered with wire mesh to prevent fish escape. Water was changed twice weekly by siphoning before feeding to remove waste and maintain optimal quality. Temperature, dissolved oxygen, and pH were monitored daily using portable meters following the methods of the American Public Health Association (APHA, 2006). All parameters were maintained within optimal limits for tropical catfish culture.

Growth and Nutrient Utilization Parameters

Fish were weighed biweekly using a digital scale, and their lengths were measured with a measuring board. Feed were adjusted according to the new biomass. Growth and feed utilization indices were computed as follows (Abdel-Tawwab *et al.*, 2008):

Weight Gain (WG) = $W_2 - W_1$, Where W_2 = Final body mean weight (g) and W_1 = Initial body mean weight (g)

Specific Growth Rate (SGR) = $SGR = \frac{\ln W_2 - \ln W_1}{t} \times 100$

Where W_2 = Final weight, W_1 = Initial weight and T = period of experiment in days (212 days)

Feed Conversion Ratio (FCR) is calculated using the formal below



$$FCR = \frac{\text{Amount of feed fed (g)}}{\text{Weight gain (g)}} \times 100$$

Protein efficiency ratio (PER) is calculated to show the relationship between the weight gain in fish and the protein consumed.

$$PER = \frac{\text{Weight gain}}{\text{Protein fed}}$$

$$\text{Where Protein fed} = \frac{\text{protein in the diet} \times \text{diet consumed}}{100}$$

Mean Increase in Standard Length (SL)

$$\text{Mean increase in SL} = L_2 - L_1$$

Where L_2 = Final Standard Length (cm)

L_1 = Initial Standard Length (cm)

Mean Increase in Total Length (TL) = $L_2 - L_1$

Where L_2 = Final Total Length (cm) and L_1 = Initial Total Length (cm).

Survival Rate (SR) was determined using the formular below

$$SR = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

Blood Sampling and Hematological Analysis

At the end of the trial, blood samples were collected in the morning from the caudal vein of three fish per replicate using 5 mL heparinized syringes. Samples were transferred into EDTA bottles and transported to Aminu Kano Teaching Hospital (AKTH) for hematological analysis, following the procedures of Lawrence *et al.*, 2020.

The parameters analyzed included red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) (Joshna *et al.*, 2023; George *et al.*, 2017).

Erythrocyte indices were calculated using the following formulas:

$$MCH = \frac{Hb}{RBC} \times 100; MCHC = \frac{Hb}{PCV} \times 100; MCV = \frac{PCV}{RBC} \times 100$$

Percentage Composition of Experimental Diets

The percentage composition of the experimental diets formulated for the study is presented in Tables 1a, 1b, and 1c. Across all dietary treatments (Aquapro, CHC, and combined probiotic inclusions), soybean meal (33%) contributed the highest proportion of crude protein, followed by groundnut cake (30%), fish meal (21%), and yellow maize (10%) as the major energy source. Lysine, salt, palm oil, starch, and vitamin-mineral premix (VMP) had relatively minor but constant proportions across treatments.

The diets were formulated to meet the 40% crude protein requirement for *Heterobranchus longifilis* fingerlings (Fagbenro *et al.*, 1992), with an approximate protein-to-energy ratio of 2:1:1. Probiotic inclusion levels (0.00%, 0.50%, 1.00%, and 1.50%) were maintained uniformly across formulations, ensuring that any observed differences in growth or hematological responses could be attributed to the probiotic effects rather than macronutrient imbalances.



Table 1a: Percentage Composition of the Experimental Diets with Aquapro Inclusions

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
Fish meal	21.00	21.00	21.00	21.00
GNC	30.00	30.00	30.00	30.00
SBM	33.00	33.00	33.00	33.00
Yellow maize	10.00	10.00	10.00	10.00
Lysine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
P/oil	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00
VM/Premix	1.00	1.00	1.00	1.00
Probiotic	0.00	0.50	1.00	1.50
Total	100.00	100.00	100.00	100.00

KEY: Soybean meal (SBM), Groundnut cake (GNC), vitamin mineral premix (VMP)

Table 1b: Composition of the Experimental Diets with CHC Inclusions

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
Fish meal	21.00	21.00	21.00	21.00
GNC	30.00	30.00	30.00	30.00
SBM	33.00	33.00	33.00	33.00
Yellow maize	10.00	10.00	10.00	10.00
Lysine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
P/oil	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00
VM/Premix	1.00	1.00	1.00	1.00
Probiotic	0.00	0.50	1.00	1.50
Total	100.00	100.00	100.00	100.00

KEY: Soybean meal (SBM), Groundnut cake (GNC), vitamin mineral premix (VMP)

Table 1c: Percentage Composition of the Experimental Diets with Combined Inclusions

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
Fish meal	21.00	21.00	21.00	21.00
GNC	30.00	30.00	30.00	30.00
SBM	33.00	33.00	33.00	33.00
Yellow maize	10.00	10.00	10.00	10.00
Lysine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
P/oil	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00
VM/Premix	1.00	1.00	1.00	1.00
Probiotic	0.00	0.50	1.00	1.50
Total	100.00	100.00	100.00	100.00

KEY: Soybean meal (SBM), Groundnut cake (GNC), vitamin mineral premix (VMP)



Statistical Analysis

Data generated were subjected to one-way analysis of variance (ANOVA) using R statistical software (version 4.2). Duncan’s Multiple Range Test (DMRT) was applied to determine significant differences among treatment means at $p < 0.05$. All results were expressed as mean \pm standard error of the mean (SEM).

RESULTS AND DISCUSSION

Proximate Composition of Experimental Diets

Tables 2 show the proximate composition results for diets containing Aquapro, CHC, and their combination, respectively. The parameters measured included moisture, crude protein (CP), crude lipid (CL), crude fiber (CF), and ash content. Across all diets, the inclusion of probiotics resulted in marginal increases in crude protein and reductions in ash content, suggesting that probiotic additives may have influenced nutrient retention and stability during feed formulation.

For the Aquapro diets, the highest moisture content (8.95%) was recorded in T₂ (1.0%), while the highest crude protein content (30.45%) occurred in T₃ (1.5%). Crude lipid content slightly declined with higher probiotic inclusion, ranging from 5.54% (T₁) to 5.43% (T₃), while ash content decreased from 7.27% in the control to 6.82% in T₃.

Similar trend was observed in the CHC diets, where the 1.0% inclusion level (T₂) yielded the highest moisture (8.89%) and protein (30.39%) values, with ash content decreasing as probiotic inclusion increased. The reduction in ash suggests improved nutrient assimilation and reduced mineral loss during formulation.

For the combined Aquapro + CHC diets, T₃ (1.5%) recorded the highest crude protein (30.51%) and the least ash (6.81%), demonstrating that combined probiotics may enhance protein stability and reduce inorganic residue accumulation. These results are consistent with findings by Soheli *et al.* (2023) and Tanveer *et al.* (2024), who reported that probiotic inclusion enhances feed digestibility and protein retention in catfish species.

Table 2: Proximate Composition of Experimental Diets Supplemented with Varying Levels of Aquapro, CHC, and their Combination.

Parameters	Probiotic Inclusion			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
Moisture (%)	8.74	8.68	8.95	8.92
CP (%)	30.14	30.27	30.41	30.45
CL (%)	5.51	5.54	5.50	5.43
CF (%)	5.44	5.40	5.47	5.32
ASH (%)	7.27	7.16	6.85	6.82
CHC Inclusion				
Moisture (%)	8.74	8.59	8.89	8.87
CP (%)	30.14	30.36	30.37	30.39
CL (%)	5.51	5.49	5.47	5.42
CF (%)	5.44	5.41	5.42	5.40
ASH (%)	7.27	7.14	6.93	6.91
Combined (Aquapro and CHC)				
Moisture (%)	8.90	8.65	8.92	8.90
CP (%)	30.14	30.47	30.49	30.51
CL (%)	5.51	5.48	5.45	5.41
CF (%)	5.44	5.42	5.47	5.30
ASH (%)	7.27	7.21	6.83	6.81

KEY: Crude protein (CP), crude lipid (CL), crude fibre (CF)

Aquapro, CHC Diets and Combined Effects on Growth Performance and Feed Utilization

Growth performance results (Table 3a) showed no significant ($p > 0.05$) difference in mean initial weight (MIW) across treatments, indicating uniform baseline conditions. However, significant differences ($p < 0.05$) were recorded in mean final weight (MFW), mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER).

Fish fed 1.5% Aquapro (T_3) achieved the highest MFW (293.70 g) and SGR (1.94%), while the control recorded the lowest (166.81 g; 1.50%). The FCR improved progressively from 1.95 (control) to 1.63 (T_3), confirming more efficient feed utilization in probiotic-fed groups.

Condition factor (K), mean standard length (MSL), and mean total length (MTL) also increased with probiotic inclusion, indicating improved body conformation and growth balance. The survival rate (SR) was 100% in all probiotic-fed groups, compared to 93% in the control, reflecting the immunomodulatory benefits of Aquapro supplementation. These findings align with Akanmu and Omitoyin (2022), who observed enhanced growth and feed efficiency in *Heterobranchus bidorsalis* juveniles fed probiotic-supplemented diets.

Table 3a: Mean Growth Performance, Feed Utilization and Survival Rate of *Heterobranchus longifilis* Fed Aquapro Experimental Diets

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T_1 (0.50%)	T_2 (1.00%)	T_3 (1.50%)
MIW (g)	5.35±0.11 ^a	5.38±0.06 ^a	5.27±0.05 ^a	5.28± 0.07 ^a
MFW (g)	166.81±2.37 ^c	253.22±1.37 ^b	256.90±0.39 ^b	293.70±0.31 ^a
MWG (g)	161.45±2.26 ^c	247.94± 1.41 ^b	251.63±0.35 ^b	288.38±0.36 ^a
SGR (%)	1.50±0.00 ^b	1.93±0.09 ^a	1.90±0.47 ^a	1.94±0.14 ^a
FCR	1.95±0.01 ^c	1.67±0.00 ^b	1.65±0.00 ^b	1.63±0.00 ^a
PER	4.43±0.01 ^b	4.60±0.02 ^a	4.63±0.01 ^a	4.67±0.00 ^a
Condition Factor (K)	1.12±0.02 ^c	1.17±0.43 ^b	1.23± 0.69 ^a	1.28±0.28 ^a
MSL (cm)	27.2±1.24 ^c	35.0±1.27 ^b	34.4±0.97 ^b	40.41±1.62 ^a
MTL (cm)	39.73±1.15 ^c	41.37± 0.81 ^b	43.66±1.8 ^b	49.84±3.31 ^a
SR (%)	93.00 ^b	100.00 ^a	100.00 ^a	100.00 ^a

Means with similar superscripts in the same row are not significantly different at $p < 0.05$.

KEY: Initial weight (IWG), final weight gain (FWG), mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K), mean standard length (MSL), mean total length (MTL), survival rate (SR).

Table 3b presents the CHC probiotic diets as it affects growth parameters. Results followed a similar pattern. Final weight, MWG, and SGR increased significantly ($p < 0.05$) with probiotic inclusion. The best growth responses were recorded at 1.5% inclusion (T_3), with MFW of 226.49 g and SGR of 1.89%. The FCR and PER values reflected higher feed conversion efficiency compared to the control.

Although growth indices under CHC were slightly lower than those under Aquapro, the differences remained biologically significant. These variations may be due to strain-specific metabolic activity and enzyme secretion differences, as previously reported by Guo *et al.* (2025).

Table 3b: Mean Growth and Feed Utilization Performance of *Heterobranchus longifilis* Fed CHC Diets

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
MIW (g)	5.35±0.11 ^a	5.30±0.06 ^a	5.30± 0.07 ^a	5.28±0.04 ^a
MFW (g)	166.81±2.37 ^d	166.94±0.89 ^c	196.01±8.03 ^{ab}	226.49±5.0 ^a
MWG (g)	161.45±2.26 ^d	161.76± 0.86 ^c	190.79±89.08 ^{ab}	221.19±5.05 ^a
SGR (%)	1.50±0.00 ^d	1.82±0.00 ^c	1.85±0.00 ^{ab}	1.89±0.00 ^a
FCR	1.95±0.01 ^d	1.87±0.00 ^c	1.86±0.00 ^{ab}	1.84±0.00 ^a
PER	4.43±0.01 ^d	4.55±0.33 ^c	4.58±0.01 ^{ab}	4.62±0.01 ^a
Condition Factor (K)	1.12±0.02 ^d	1.14± 0.43 ^c	1.18± 0.69 ^{ab}	1.21±0.02 ^a
MSL (cm)	27.2±1.24 ^d	36.63±0.48 ^c	30.53±2.77 ^{ab}	33.74±3.62 ^a
MTL (cm)	32.93±2.61 ^d	39.37± 0.81 ^c	33.84±3.31 ^{ab}	37.54±3.58 ^a
SR (%)	93.00 ^b	100.00 ^a	100.00 ^a	100.00 ^a

Means with similar superscripts in the same row are not significantly different at $p < 0.05$.

KEY: Initial weight (IWG), final weight gain (FWG), mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K), mean standard length (MSL), mean total length (MTL), survival rate (SR).

Table 3c presents the growth and feed utilization performance of *Heterobranchus longifilis* fed combined probiotic diets. There was no significant ($p > 0.05$) difference in mean initial weight (MIW) across treatments, confirming homogeneous starting conditions. However, all growth indices mean final weight (MFW), mean weight gain (MWG), specific growth rate (SGR), and protein efficiency ratio (PER) improved significantly ($p < 0.05$) with increasing probiotic inclusion.

The best performance was observed in the 1.5 % inclusion group (T₃), with the highest MFW (348.79 g), MWG (342.46 g), and SGR (1.97 %), compared with the control (MFW = 166.81 g; SGR = 1.50 %). The feed conversion ratio (FCR) improved markedly from 1.95 (control) to 1.61 (T₃), while PER increased from 4.43 to 4.77, indicating superior feed and protein utilization.

Fish fed the combined probiotic diets also exhibited better somatic and morphometric performance. Condition factor (K) improved progressively, peaking at 1.30 in T₃, while mean standard length (MSL = 39.85 cm) and mean total length (MTL = 47.10 cm) were both significantly higher ($p < 0.05$) than in the control. Survival rate (SR) was 100 % in all probiotic-fed treatments versus 93 % in the control, confirming the positive health and immunomodulatory effects of combined probiotic supplementation.

These results demonstrate the synergistic benefits of Aquapro and CHC, as the combined inclusion produced greater improvements in growth and nutrient conversion than single-probiotic diets. The enhanced performance may result from complementary microbial interactions where one probiotic strain facilitates carbohydrate breakdown and another enhances amino acid assimilation leading to improved nutrient availability and metabolic efficiency. Comparable outcomes have been reported in *Clarias batrachus* and *Heterobranchus bidorsalis* when fed multi-strain probiotics that enhance both feed conversion and antioxidant function (Tanveer *et al.*, 2024; Tufarelli *et al.*, 2025).

Table 3c: Mean Growth and Feed Utilization Performance of *Heterobranchus longifilis* fed Combined Experimental Diets

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (0.10%)	T ₃ (1.50%)
MIW (g)	5.35±0.11 ^a	5.37± 0.16 ^a	5.34±0.07 ^a	5.33±0.08 ^a
MFW (g)	166.81±2.37 ^d	251.17±2.23 ^c	344.52±0.86 ^{ab}	348.79±4.33 ^a
MWG (g)	161.45±2.26 ^d	245.80±1.36 ^c	338.18±0.74 ^{ab}	342.46±4.39 ^a
SGR (%)	1.50±0.00 ^d	1.95±0.00 ^c	1.97±0.47 ^{ab}	1.97±0.41 ^a
FCR	1.95±0.01 ^d	1.63±0.00 ^c	1.62±0.00 ^{ab}	1.61±0.00 ^a
PER	4.43±0.01 ^d	4.76± 0.00 ^{ab}	4.72±0.01 ^c	4.77±0.00 ^a
Condition Factor (K)	1.12±0.28 ^d	1.18± 0.06 ^c	1.26±0.03 ^{ab}	1.30± 0.02 ^a
MSL (cm)	21.53±1.20 ^d	38.73±1.50 ^{ab}	36.12±0.53 ^c	39.85±2.17 ^a
MTL (cm)	26.26±2.35 ^d	41.8±0.97 ^c	45.91± 5.17 ^{ab}	47.10±3.92 ^a
SR (%)	93.00 ^b	100.00 ^a	100.00 ^a	100.00 ^a

Means with similar superscripts in the same row are not significantly different at $p < 0.05$.

KEY: Initial weight (IWG), final weight gain (FWG), mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K), mean standard length (MSL), mean total length (MTL), survival rate (SR).

Hematological Parameters of *Heterobranchus longifilis* Fingerlings Fed Probiotic-Supplemented Diets

Table 4a displays the hematological parameters of fish fed Aquapro-supplemented diets. The red blood cell (RBC) count increased from $2.95 \times 10^6 \text{ mm}^{-3}$ in the control to $3.09 \times 10^6 \text{ mm}^{-3}$ in T₃ (1.5%), with significant ($p < 0.05$) improvement compared to the control. This increase reflects enhanced erythropoiesis, likely due to improved nutrient absorption and intestinal microbial balance induced by probiotic activity.

Similarly, white blood cell (WBC) count increased from $8.54 \times 10^9 \text{ L}^{-1}$ in the control to $9.64 \times 10^9 \text{ L}^{-1}$ in T₃, indicating improved immune responses and leukocyte proliferation. Packed cell volume (PCV) and hemoglobin (Hb) followed a similar pattern, showing slightly higher values in probiotic-fed groups than in the control (PCV: 33.00-33.61%; Hb: 10.17–10.55 g/dL), suggesting more efficient oxygen-carrying capacity.

In contrast, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) decreased marginally with increasing probiotic levels. The decline in these erythrocyte indices may indicate faster red cell turnover and improved oxygen utilization efficiency.

These findings are consistent with Zhang *et al.* (2022), who reported that *Heteropneustes fossilis* fed probiotic-based diets exhibited elevated RBC and WBC counts, reflecting enhanced hematopoietic and immunological responses.

Table 4a: Mean Hematological Parameters of *Heterobranchus longifilis* fed with Aquapro Probiotics Supplemented Diets

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
RBC (10^6 mm^{-3})	2.95±0.03 ^b	3.09±1.01 ^a	3.09±1.04 ^a	3.09± 1.09 ^a
WBC (10^9 L)	8.54±0.00 ^b	9.60±0.00 ^a	9.62±0.03 ^a	9.64±0.05 ^a
PCV (%)	33.00±1.42 ^a	33.58±1.53 ^a	33.64±1.55 ^a	33.61±1.58 ^a
HB (g/dl)	10.17±0.02 ^a	10.48±0.10 ^a	10.50±0.22 ^a	10.55±0.31 ^a
MCH (pg)	42.19±0.26 ^a	40.38±0.31 ^{ab}	38.06±0.22 ^b	37.32±0.01 ^b
MCHC (g/dl)	33.40±0.32 ^a	33.38±0.42 ^b	33.35±0.04 ^b	33.30±0.38 ^b
MCV (fl)	115.57±4.32 ^a	112.35±2.12 ^b	108.20±2.15 ^b	110.04±2.18 ^b

Means with similar superscripts in the same row are not significantly different at $P < 0.05$.

KEY: Red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin: (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV).

The hematological responses of *H. longifilis* fed diets supplemented with CHC probiotics is shown in Table 4b. The results exhibited a similar trend to the Aquapro-fed group. RBC counts increased significantly from $3.95 \times 10^6 \text{ mm}^{-3}$ in the control to $3.09 \times 10^6 \text{ mm}^{-3}$ in treated groups, while WBC increased from $8.54 \times 10^9 \text{ L}^{-1}$ (control) to $9.55 \times 10^9 \text{ L}^{-1}$ (T₃).

Although PCV and Hb values did not differ significantly ($p > 0.05$), probiotic-fed fish maintained slightly higher values (PCV: 33.54-33.64%; Hb: 10.43-10.49 g/dL) than the control, reflecting better hematopoietic performance.

The MCH and MCHC values showed slight reductions with increasing CHC inclusion, from 42.19 pg and 33.40 g/dL in the control to 34.58 pg and 34.36 g/dL in T₂, respectively, while MCV declined from 115.57 to 104.60 fl. These patterns suggest increased oxygen transport efficiency through smaller, more active erythrocytes, possibly due to enhanced erythropoiesis stimulated by probiotic enzymes and metabolites (Abubakar & Ipinjolu, 2020). Overall, the CHC probiotic diets produced modest hematological improvements, supporting the hypothesis that probiotics stimulate immune modulation and enhance hematological stability in catfish.

Table 4b: Mean Hematological Parameters of *Heterobranchus longifilis* Fed with CHC Probiotics Supplemented Diets

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
RBC (10^6 mm^{-3})	3.95±0.03 ^b	3.03±1.00 ^a	3.09± 1.08 ^a	3.09±1.05 ^a
WBC ($10^9/\text{L}$)	8.54±0.00 ^b	9.50±4.17 ^a	9.59±5.18 ^a	9.55±7.12 ^a
PCV (%)	33.00±1.42 ^a	33.54±1.60 ^a	33.62±1.63 ^a	33.64±1.40 ^a
HB (g/dl)	10.17±0.02 ^a	10.43±2.24 ^a	10.47±1.98 ^a	10.49±7.31 ^a
MCH (pg)	42.19±0.26 ^a	37.75±0.28 ^b	34.58±0.00 ^b	35.92±1.02 ^b
MCHC (g/dl)	33.40±0.32 ^b	34.39±2.19 ^a	34.36±4.21 ^a	34.37±1.11 ^a
MCV (fl)	115.57±4.32 ^a	104.38±2.10 ^b	104.63±5.19 ^b	104.60±4.22 ^b

Means with similar superscripts in the same row are not significantly different at $P < 0.05$.

KEY: Red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin: (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV).

The hematological indices of *H. longifilis* fed combined Aquapro + CHC diets are presented in Table 4c. Results showed marked enhancement across all major blood parameters. RBC and WBC counts increased significantly ($p < 0.05$) with probiotic inclusion, peaking at $4.05 \times 10^6 \text{ mm}^{-3}$ and $9.62 \times 10^9 \text{ L}^{-1}$ in T₁ and T₃, respectively.

PCV and Hb values rose notably from 33.00% and 10.17 g/dL in the control to 35.80% and 13.58 g/dL in T₃, confirming a stimulatory effect on erythropoiesis and oxygen-carrying potential. The combined probiotics likely promoted nutrient absorption and iron metabolism, leading to enhanced red blood cell synthesis (Akanmu and Omitoyin, 2022).

Although MCH, MCHC, and MCV values decreased slightly at higher inclusion levels, these changes remained within the normal physiological range for healthy *H. longifilis* (Wang *et al.*, 2021). The slight decline suggests more efficient red blood cell activity and rapid turnover, typical in fish exhibiting accelerated growth and improved metabolic function.

This result corroborates findings by Tanveer *et al.* (2024), who demonstrated that multi-strain probiotics enhanced hematological and antioxidant responses in *Clarias batrachus*, attributing the

improvements to synergistic effects of microbial metabolites that optimize erythrocyte health and immune resilience.

Table 4c: Mean Hematological Parameters of *Heterobranchus longifilis* Fed with Combined Probiotics Supplemented Diets

Parameters	Probiotic Inclusion levels			
	Control (0.00%)	T ₁ (0.50%)	T ₂ (1.00%)	T ₃ (1.50%)
RBC (10 ⁶ mm ⁻³)	3.95±0.03 ^b	4.05±0.02 ^a	4.03±0.05 ^a	4.01± 0.08 ^a
WBC (10 ⁹ /L)	8.54±0.00 ^b	9.61±0.04 ^a	9.61±0.06 ^a	9.62±0.00 ^a
PCV (%)	33.00±1.42 ^b	35.20±3.01 ^a	35.40±2.00 ^a	35.80±1.66 ^a
HB (g/dl)	10.17±0.02 ^b	13.53±1.43 ^a	13.55±1.51 ^a	13.58±0.20 ^a
MCH (pg)	42.19±0.26 ^a	38.56±0.63 ^a	40.42±0.31 ^a	35.86±0.24 ^b
MCHC (g/dl)	33.40±0.32 ^a	33.30±3.64 ^a	33.24±1.22 ^a	33.20±0.10 ^a
MCV (fl)	115.57±4.32 ^a	106.72±0.48 ^b	106.98±4.87 ^b	106.93±6.23 ^b

Means with similar superscripts in the same row are not significantly different at P<0.05.

KEY: Red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin: (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV).

Comparative effects of Growth and Hematological Responses of *Heterobranchus longifilis* Fingerlings to Probiotic-Supplemented Diets

The integration of growth and hematological responses provides a holistic understanding of the physiological influence of probiotics on *Heterobranchus longifilis*. In this study, the administration of Aquapro, CHC, and their combination markedly enhanced both growth performance and blood health indicators relative to the control group, suggesting a strong link between probiotic supplementation, nutrient utilization, and improved metabolic function.

Growth performance indices mean final weight (MFW), mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) showed clear improvement with probiotic inclusion. Fish fed combined probiotic diets achieved the highest MFW (348.79 g) and SGR (1.97%), while also exhibiting the most efficient FCR (1.61) and PER (4.77). These outcomes indicate enhanced feed digestion and nutrient absorption, likely due to microbial enzyme secretion (amylase, protease, and lipase) that improved nutrient bioavailability (Osungbemi *et al.*, 2023; Tanveer *et al.*, 2024).

Probiotics are known to modulate gut microbiota, improve intestinal morphology, and suppress pathogenic bacteria, leading to better feed conversion and growth (Wang *et al.*, 2023). The 100% survival rate recorded in all probiotic-fed groups further reflects improved resilience and reduced stress susceptibility, consistent with findings in *Clarias gariepinus* and *Heteropneustes fossilis* fed multi-strain probiotic diets (Idowu *et al.*, 2025).

Hematological Responses and Their Physiological Implications

The hematological results mirrored the growth improvements, demonstrating elevated red blood cell (RBC) counts, white blood cell (WBC) counts, packed cell volume (PCV), and hemoglobin (Hb) concentrations in probiotic-fed fish. These indices are direct indicators of oxygen transport capacity, immune competence, and general physiological status.

Fish fed combined probiotics exhibited the highest RBC (4.05 × 10⁶ mm⁻³), WBC (9.62 × 10⁹ L⁻¹), PCV (35.80%), and Hb (13.58 g/dL), reflecting robust erythropoietic activity and enhanced immune modulation. These hematological enhancements suggest that probiotics stimulate hematopoietic organs, improving the synthesis of blood cells and boosting immunity through the production of metabolites such as short-chain fatty acids and vitamins as reported by Paico-Ruiz *et al.* (2024).



Lower mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) observed at higher probiotic inclusions indicate efficient oxygen use and higher erythrocyte turnover, which are characteristic of metabolically active and healthy fish populations (Witeska, 2023).

Interrelationship Between Growth and Hematological Performance

The concurrent improvement in growth and hematological indices reveals a physiological synergy between nutritional enhancement and systemic health. Improved RBC and Hb values correlate positively with better oxygen supply to tissues, thereby supporting higher metabolic activity and faster growth rates. Likewise, elevated WBC levels reflect a strengthened immune system capable of minimizing disease stress allowing more energy to be channeled toward growth rather than immune defense (Paico-Ruiz, 2024; Tanveer *et al.*, 2024).

The observed improvements in both growth and hematological parameters underscore the dual nutritional and physiological benefits of probiotic supplementation in aquaculture. Enhanced hematological profiles ensure oxygen transport and immune balance, which translate into superior growth performance and survival. Therefore, probiotics particularly when combined can serve as sustainable, non-antibiotic growth enhancers that promote health, feed efficiency, and disease resistance in *Heterobranchus longifilis* culture systems.

This integrative improvement aligns with the broader objective of eco-friendly aquaculture: maximizing productivity while maintaining the physiological welfare of cultured species. Consequently, probiotic supplementation at 1.0-1.5% inclusion levels is recommended for optimal growth and hematological stability in *H. longifilis* fingerlings.

CONCLUSION AND RECOMMENDATION

The findings from this study demonstrated that dietary supplementation with probiotics either singly (Aquapro or CHC) or in combination significantly improved both the growth performance and hematological health of *Heterobranchus longifilis* fingerlings when compared to the control diet. Fish fed probiotic-supplemented diets exhibited higher mean weight gain, specific growth rate, protein efficiency ratio, and survival rate, alongside enhanced hematological indices such as red and white blood cell counts, hemoglobin concentration, and packed cell volume. These improvements indicate that probiotics contribute to better nutrient utilization, enhanced immune competence, and overall physiological resilience in *H. longifilis*.

The combined inclusion of Aquapro and CHC at moderate levels (particularly 1.0-1.5%) produced the most pronounced positive effects, suggesting possible synergistic interactions between probiotic strains. The study, therefore, establishes probiotics as viable dietary additives for promoting health and sustainable growth in aquaculture species, particularly African catfish.

It is recommended that *Heterobranchus longifilis* farmers adopt probiotic-supplemented diets, preferably combined formulations at inclusion levels between 1.0-1.5%, to optimize fish growth performance, hematological health, and survival under controlled aquaculture systems. Further studies are encouraged to investigate the long-term effects of probiotics on reproductive performance and disease resistance in *H. longifilis*.

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