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Effects of *Bacillus coagulans* on haematological parameters of *Cyprinus carpio*

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Key words: Probiotics, *Bacillus coagulans*, *Cyprinus carpio*, Haematological parameters, Recirculating aquaculture system

Abstract

This research assessed the impact of dietary *Bacillus coagulans* supplementation on hematological parameters and immunological state in common carp (*Cyprinus carpio*) cultivated in a recirculating aquaculture system. Fish were given diets enriched with different concentrations of *B. coagulans* for durations of 20, 40, and 60 days. Essential hematological parameters—such as red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC) count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH)—were evaluated to ascertain physiological and immunological responses. Results indicated substantial elevations in RBC, Hb, PCV, and WBC levels in the probiotic-treated groups, with the peak values seen in the T3 group at day 60. The MCV and MCH levels reached their peak early and stabilized by day 60, suggesting adaptive hematological control. The observations indicate that *B. coagulans* promotes hematopoiesis and immunological function in *C. carpio*, presenting a possible probiotic alternative to antibiotics for enhancing fish health and performance in sustainable aquaculture systems.

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Introduction

Aquaculture plays an important role in global food security and economic development, but sustainable techniques are essential to solve difficulties relating to fish health, growth performance, and environmental effect. Probiotics have emerged as viable alternatives to antibiotics in aquaculture, delivering advantages such as higher immunity, better digestion, and disease resistance (Troell *et al.*, 2023). *Cyprinus carpio*, often known as common carp, faces ecological threats from habitat loss, overexploitation, and migratory obstacles (Humphries and Winemiller, 2009), leading to heightened dependence on aquaculture.

Intensive agricultural techniques may adversely affect aquatic ecosystems and biodiversity (Saeedi *et al.*, 2024). Sustainable management measures, including protected areas and community-based conservation, are vital for balancing aquaculture expansion with ecological preservation (Zebua *et al.*, 2024).

Concurrent progress in sturgeon aquaculture, particularly with *Acipenser baerii*, illustrates the advantages of enhanced breeding and nutritional methodologies, while also underscoring analogous difficulties in disease management and ecological sustainability (Pashko *et al.*, 2024; Yurin *et al.*, 2024). Probiotic supplementation, particularly with strains such as *Bacillus* and *Lactobacillus*, markedly enhances specified growth rates (SGR), feed conversion ratios (FCR), and immunological responses (Fachri *et al.*, 2024; Calcagnile *et al.*, 2024). Species-specific advantages have been recorded in grey mullet, striped catfish, and others, demonstrating resistance to diseases such as *Nocardia seriolae* and *Edwardsiella ictaluri* (Chan *et al.*, 2024).

Bacillus coagulans, a resilient spore-forming probiotic, improves gastrointestinal health, nutritional assimilation, and immune function in aquaculture species. In *Cyprinus carpio*, it shows potential for enhancing hematological indices—total erythrocyte count (TEC), hemoglobin (Hb), and packed cell volume (PCV)—which are critical indications of health and stress (Mary and Raj, 2023; Naveenkumar *et al.*, 2017; Pradhan *et al.*, 2014). This research examines the impact of *Bacillus coagulans* on the growth and hematological parameters of *C. carpio* cultivated in a recirculating aquaculture system, with the objective of formulating probiotic-based approaches for sustainable and efficient fish farming.

Materials and methods

Collection and acclimatization of fish

Fingerlings of *Cyprinus carpio* (L.) were purchased from the Fishery Department at B.R. Project, situated around 5 km from the Jnana Sahyadri campus, Shankaraghatta. Upon arriving at the laboratory, the fingerlings were acclimatized under controlled environmental conditions for a predetermined length before to the initiation of the experiment. The chosen fish had an average body weight of 15 ± 1 g. During the acclimatization phase, they were given a commercially available pelleted floating carp feed, which also served as the usual baseline diet for the research. Feeding was performed twice daily to achieve optimum adaptation to the laboratory circumstances and to reduce handling-induced stress.

Commercial feed probiotic supplementation

The probiotic strain *Bacillus coagulans* (10^9 CFU/g), a gram-positive, spore-forming, lactic acid-producing bacterium recognized for its endurance in gastrointestinal settings, was received from Sanzyme Biologics Private Limited, Hyderabad, India. This commercially available formulation was added into the experimental diet provided for *Cyprinus carpio* fingerlings. The major purpose of its inclusion was to test its impact on important physiological parameters, including growth performance, biochemical indices, and digestive enzyme activities. The selection of *B. coagulans* was based on its proven stability under feed processing conditions and its established efficiency in altering gut microbiota, hence giving prospective benefits in aquaculture nutrition and health management.

Experimental design

A 60-day feeding study was undertaken using *Cyprinus carpio* fingerlings of uniform beginning weight to examine the physiological effects of probiotic administration. Fish were given a designed

meal comprising 32% crude protein at 3% of their body weight daily. The experiment comprised of four groups: a control (C) with no probiotics, and three treatment groups—T1, T2, and T3—supplemented with *Bacillus coagulans* at 0.05×10^9, 0.1×10^9, and 0.15×10^9 CFU g^{-1}, respectively. Sampling occurred on days 20, 40, and 60, during which fish were killed, and blood samples were obtained for haematological investigation. Blood was taken by cardiac puncture using 2 mL syringes prepared with 150–200 µL of EDTA to avoid coagulation. The puncture site was placed ventrally, between the anterior bases of the pectoral fins. Samples were kept in 1.5 mL sterile Eppendorf tubes and refrigerated until analysis. This strategy enabled regular and sterile sample for the study of hematological responses to probiotic administration.

Hematological examination

Hematological parameters were evaluated using established protocols to assess the physiological responses of *Cyprinus carpio* to various dietary regimens. Total red blood cell (RBC) and white blood cell (WBC) counts were obtained using an advanced Neubauer hemocytometer, following the technique published by Hesser (1960). Hemoglobin (Hb) concentration was determined using the cyanmethemoglobin technique according to Blaxhall and Daisley (1973), while hematocrit (Hct) values were measured using micro-hematocrit capillary tubes based on the methods of Satheeshkumar *et al.* (2012). Differential leukocyte counts were determined using Giemsa staining, following the methodology published by Shah *et al.* (2009). Furthermore, erythrocyte indices including mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) were determined utilizing formulas provided by Dacie and Lewis (2001), where MCHC (g/dL) = (Hb / Hct) × 100, MCH (pg) = (Hb / RBC) × 10, and MCV (fL) = (Hct/ RBC) × 10. These indicators supplied useful insights into the erythrocytic state and general health condition of the fish treated to probiotic-supplemented diets.

Statistical analysis

The results were represented as mean ± standard deviation (SD), and differences between groups were examined using one-way analysis of variance (ANOVA). Statistical significance was judged at levels of $p < 0.05$ and $p < 0.001$. All statistical analyses were done using SPSS software.

Results

The haematological examination of *Cyprinus carpio* fed with probiotic-supplemented diets over 20, 40, and 60 days demonstrated consistent, dose- and time-dependent improvements across all measured parameters compared to the control group (Tables 1–3). Red Blood Cell (RBC) counts substantially increased in the high-dose T3 group, increasing from $1.61 \pm 0.02 \times 10^6$/µL at day 20 to $2.50 \pm 0.02 \times 10^6$/µL at day 60, suggesting greater erythropoiesis and oxygen-carrying ability. Haemoglobin (Hb) concentrations showed a similar pattern, reaching 9.11 ± 0.06 g/dL in T3 by day 60, compared 5.69 ± 0.05 g/dL in the control, showing enhanced metabolic support.

Packed Cell Volume (PCV) significantly increased in the probiotic groups, with T3 reaching a high of 27.85 ± 0.14% at day 60, compared to 21.77 ± 0.53% in the control. Early rises in Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were seen, notably in T3, but values progressively stabilized, indicating a shift toward erythrocyte homeostasis. While Mean Corpuscular Haemoglobin Concentration (MCHC) changed somewhat, it remained within physiological limits, suggesting no deleterious impact on red cell integrity.

White Blood Cell (WBC) counts exhibited the most dramatic elevation, with T3 reaching 217.26 ± 1.87/µL by day 60 vs 176.47 ± 0.17/µL in the control, showing immune system activation. Overall, the findings demonstrate that *Bacillus coagulans* supplementation considerably improves hematological health, oxygen transport, and immunological function in *C. carpio*, indicating its potential as a useful probiotic in aquaculture.

Table 1. Hematological parameters of *C. carpio* treated with various amounts of probiotics throughout 20 days of trial

Parameters	Control	T1	T2	T3
RBC (×10⁶/µL)	1.28 ± 0.02	1.38 ± 0.02	1.47 ± 0.01	1.61 ± 0.02
Hb (g/dL)	5.69 ± 0.05	6.80 ± 0.08	7.82 ± 0.02	8.77 ± 0.02
PCV (%)	14.63 ± 0.12	17.35 ± 0.25	22.65 ± 0.31	25.14 ± 0.12
MCV (fL)	114.78 ± 1.65	125.73 ± 1.77	153.99 ± 2.95	155.90 ± 1.85
MCH (pg)	44.64 ± 0.74	49.27 ± 0.87	53.15 ± 0.39	54.36 ± 0.57
MCHC (g/dL)	38.85 ± 0.33	39.17 ± 0.52	34.54 ± 0.43	34.84 ± 0.31
WBC (/µL)	154.67 ± 1.86	167.65 ± 11.50	204.80 ± 2.26	212.43 ± 2.68

Table 2. Hematological parameters of *C. carpio* treated with various amounts of probiotics throughout 40 days of trial

Parameters	Control	T1	T2	T3
RBC (×10⁶/µL)	1.62 ± 0.02	1.85 ± 0.02	2.18 ± 0.02	2.34 ± 0.07
Hb (g/dL)	7.08 ± 0.03	8.70 ± 0.04	8.80 ± 0.02	9.13 ± 0.16
PCV (%)	16.55 ± 0.20	19.47 ± 0.14	24.70 ± 0.09	26.39 ± 0.24
MCV (fL)	102.15 ± 1.77	105.06 ± 1.49	113.55 ± 1.08	113.08 ± 3.22
MCH (pg)	43.75 ± 0.39	46.93 ± 0.49	40.47 ± 0.43	39.21 ± 1.15
MCHC (g/dL)	42.80 ± 0.43	44.68 ± 0.42	35.64 ± 0.15	34.61 ± 0.59
WBC (/µL)	174.03 ± 0.94	181.57 ± 1.46	204.30 ± 2.06	214.48 ± 1.63

Table 3. Hematological parameters of *C. carpio* treated with various amounts of probiotics throughout 60 days of trial

Parameters	Control	T1	T2	T3
RBC (×10⁶/µL)	2.12 ± 0.08	2.22 ± 0.01	2.33 ± 0.02	2.50 ± 0.02
Hb (g/dL)	7.23 ± 0.06	7.63 ± 0.03	8.94 ± 0.02	9.11 ± 0.06
PCV (%)	21.77 ± 0.53	23.82 ± 0.15	25.64 ± 0.12	27.85 ± 0.14
MCV (fL)	103.23 ± 3.72	107.52 ± 0.47	110.11 ± 1.26	111.08 ± 0.40
MCH (pg)	34.21 ± 1.04	34.46 ± 0.24	38.38 ± 0.46	36.40 ± 0.31
MCHC (g/dL)	33.16 ± 0.87	32.05 ± 0.20	34.87 ± 0.16	32.73 ± 0.18
WBC (/µL)	176.47 ± 0.17	183.07 ± 1.96	206.44 ± 0.56	217.26 ± 1.87

Discussion

Probiotic supplementation has emerged as a promising method in aquaculture, giving many advantages such as better growth, improved immunity, improved nutrient absorption, and increased economic efficiency. Several studies have proven that probiotics significantly affect specific growth rate (SGR) and feed conversion ratio (FCR), notably in freshwater fish such as Nile tilapia and African catfish (Ariyanto and Anika, 2024; Omar *et al.*, 2024; Hadijah *et al.*, 2024). These effects are mostly related to the synthesis of digestive enzymes and enhanced gut shape, which promote effective feed consumption (Ringø *et al.*, 2020; Mahmoodian *et al.*, 2024).

In addition to digestive efficiency, probiotics also strengthen the immune system by raising immunoglobulin M (IgM) levels and upregulating immune-related genes such as TNF and IL-6 (Choi *et al.*, 2024; Ferdous *et al.*, 2023). They improve gut health by altering the microbiota, promoting beneficial bacterial populations, and reducing harmful microorganisms (Cerezuela *et al.*, 2011; Mahmoodian *et al.*, 2024). Furthermore, enzymes like as amylase, protease, and lipase generated by probiotics increase nutrient digestion and reduce anti-nutritional components prevalent in plant-based diets (Amenyogbe *et al.*, 2024). These steps not only decrease mortality and antibiotic consumption but also increase sustainability and profitability in aquaculture operations (Fachri *et al.*, 2024; Vulla *et al.*, 2024; Han *et al.*, 2024).

The efficiency of probiotic genera such as *Bacillus* and *Lactobacillus* has been widely confirmed in diverse aquaculture species. In grass carp (*Ctenopharyngodon idella*), dietary *Bacillus subtilis* increased SGR, FCR, and gut enzyme activity, while modifying intestinal microbiota (Wu *et al.*, 2012). Similarly, *Lactobacillus plantarum* generated from grass carp greatly boosted growth performance, immunological responses, and digestive enzyme activity in *Labeo rohita* (Yasmin *et al.*, 2024).

Synbiotic combinations such *Bacillus licheniformis* with fructooligosaccharide (FOS) further enhanced immune responses and survival rates post-pathogen exposure (Sukul *et al.*, 2023).

The current research assessed the effects of dietary *Bacillus coagulans* supplementation on haematological markers of *Cyprinus carpio* across a 60-day trial period. Consistent improvements were reported across all haematological indicators in treated groups. RBC counts in the high-dose T3 group increased considerably from 1.28 ± 0.02 $\times 10^6$/µL (control) to 2.50 ± 0.02 $\times 10^6$/µL by day 60, while Hb concentration jumped from 5.69 ± 0.05 g/dL to 9.11 ± 0.06 g/dL. Similarly, PCV levels rose from 14.63 ± 0.12% to 27.85 ± 0.14%, suggesting better erythropoiesis and oxygen delivery. MCV and MCH peaked at day 20, followed by stability by day 60, indicating a return to erythrocyte homeostasis. The most noticeable change was in WBC count, which climbed from 154.67 ± 1.86/µL (control) to 217.26 ± 1.87/µL (T3, day 60), showing immunological enhancement.

These findings are consistent with earlier research revealing that *B. coagulans* enhances RBC, Hb, and hematocrit (HCT) levels, consequently boosting oxygen-carrying capacity and general physiological performance (Zhang *et al.*, 2023; Xu *et al.*, 2014). The rise in WBCs suggests an active immune system, which is critical for disease resistance in intensive aquaculture systems. Other studies have also underlined the importance of WBCs as indicators of immunological state, stress, and environmental circumstances (Sayed-Lafi *et al.*, 2023; Satkar *et al.*, 2024; Ahmed *et al.*, 2020; Haghparast *et al.*, 2020; Lataretu *et al.*, 2013).

Despite these hopeful results, heterogeneity in metrics like MCV, MCH, and MCHC across various research shows that the benefits of probiotics may rely on individual strains, dose, fish species, and culture conditions (Ayala *et al.*, 2008; Adriani *et al.*, 2013). Additionally, the immunomodulatory effects identified in the current research may also be ascribed to cytokine modulation, resulting to decreased stress and enhanced hematological stability (Zhang *et al.*, 2023; Xu *et al.*, 2014).

Given the difficulties associated with antibiotic usage in aquaculture—such as environmental pollution, antimicrobial resistance, and food safety concerns—probiotics provide a sustainable option for boosting fish health and production (Nagesh *et al.*, 2025). The outcomes of this research underline the potential of *Bacillus coagulans* as an effective dietary supplement for enhancing haematological parameters and increasing immunological function in *C. carpio*, contributing to sustainable aquaculture techniques.

Conclusion

The current research reveals that nutritional supplementation with *Bacillus coagulans* considerably boosts hematological parameters and immunological responses in *Cyprinus carpio* under a recirculating aquaculture system. Prolonged dosing for 60 days resulted in considerable improvements in RBC count, hemoglobin concentration, hematocrit value, and white blood cell levels, suggesting increased oxygen-carrying ability, erythropoiesis, and immunological state. The early increases in MCV and MCH followed by normalization imply adaptive physiological responses and hematological stability with ongoing probiotic consumption. These data demonstrate the potential of *B. coagulans* as a sustainable alternative to antibiotics, leading to enhanced fish health, survival, and production in aquaculture. Incorporating probiotics such as *B. coagulans* into aquafeeds might therefore play a crucial role in promoting eco-friendly and health-oriented aquaculture practices.

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