

EFFECTS OF DIETARY PROBIOTICS ON GROWTH AND HEMATOLOGICAL INDICES OF COMMON CARP (CYPRINUS CARPIO)

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Abstract

Probiotics are live beneficial microorganisms that, when administered in adequate amounts, enhance the host's health by maintaining and balancing the gut microbial community. The use of microbial probiotics is emerging in aquaculture as a sustainable strategy to enhance fish health without antibiotics. This study assessed the effects of dietary Lyophilized *Saccharomyces boulardii* and *Shewanella putrefaciens*, individually and in combination, on the growth and hematological profiles of juvenile *Cyprinus carpio*. A 60 day feeding trial was conducted using four treatments: a non supplemented control, *L. S. boulardii*, *S. putrefaciens*, and a combination of both strains at equal concentrations. Fish were fed twice daily, growth was recorded fortnightly, and final hematological parameters including hemoglobin, hematocrit, erythrocyte and leukocyte counts, erythrocyte indices, platelet levels, and differential leukocyte counts were evaluated. Data were statistically analyzed using one way ANOVA with post hoc testing at $p < 0.05$. Probiotic supplementation significantly improved growth relative to the control, with *S. putrefaciens* producing the highest weight gain and specific growth rate, followed by *L. S. boulardii*, while the combination group showed moderate improvement. Hematological assessments indicated enhanced hemoglobin, hematocrit, and red white blood cell counts in probiotic fed fish, with no abnormal deviations in erythrocyte indices or platelet values, confirming physiological safety. All treatments maintained 100% survival. In conclusion, single strain probiotic supplementation particularly *S. putrefaciens* positively influenced growth and hematological health in *C. carpio*. These findings support probiotics as effective, non antibiotic tools for improving fish performance and immune competence, contributing to sustainable aquaculture practices.

INTRODUCTION

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer measurable health benefits to the host by

maintaining or restoring intestinal microbial balance (FAO/WHO, 2001). In aquaculture, probiotics have gained considerable importance as sustainable alternatives to antibiotics for

improving fish growth, feed utilization, and immune competence (Merrifield et al., 2010). The intensification of aquaculture practices has led to frequent disease outbreaks and the excessive use of chemotherapeutic agents, resulting in antimicrobial resistance and concerns regarding antibiotic residues in aquatic products (Nayak, 2010). Therefore, the development of biologically safe and sustainable strategies to enhance fish health and productivity has become a priority for the industry (FAO, 2020).

Among the probiotic candidates investigated in aquatic systems, Lyophilized *Saccharomyces boulardii* and *Shewanella putrefaciens* have shown promising potential. *S. boulardii* is a probiotic yeast known for its ability to survive harsh gastrointestinal conditions and support intestinal health (McFarland, 2010). Its lyophilized (freeze dried) form ensures stability, prolonged shelf life, and maintained viability during feed processing and storage. Upon rehydration, the yeast becomes metabolically active, contributing to microbial balance and improved digestion (Czerucka et al., 2007). Similarly, *S. putrefaciens*, a Gram negative bacterium, has been reported to enhance nutrient assimilation, produce bioactive metabolites, and inhibit pathogenic microorganisms in aquatic environments (Kesarcode-Watson et al., 2008; Tapia-Paniagua et al., 2014). By supporting microbial equilibrium in both the gut and surrounding water, it may improve feed conversion efficiency and overall physiological stability in cultured fish.

Probiotics exert their beneficial effects through multiple mechanisms, including modulation of intestinal microbiota, competitive exclusion of pathogens, production of antimicrobial compounds, and stimulation of immune responses (Merrifield et al., 2010). They enhance innate immunity by increasing macrophage activity, lysozyme levels, and cytokine regulation, thereby strengthening primary defense mechanisms (Ringø et al., 2020). Improvements in digestive enzyme activity and nutrient absorption have also been associated with enhanced growth performance (Gatesoupe, 2007).

The common carp (*Cyprinus carpio*) is one of the most widely cultured freshwater fish species due

to its rapid growth, adaptability, and economic importance. However, intensive farming often leads to disease susceptibility and suboptimal feed conversion. Hematological parameters such as hemoglobin concentration, red blood cell count, and white blood cell count serve as reliable indicators of physiological status and immune function in fish (Roberts, 2012).

Although numerous studies have evaluated single strain probiotics, limited research has investigated the combined effects of yeast and bacterial probiotics, particularly the synergistic interaction between Lyophilized *S. boulardii* and *S. putrefaciens* in *C. carpio*. Therefore, the present study evaluates the effects of dietary inclusion of these probiotics on growth performance and hematological parameters of common carp under controlled freshwater conditions. The findings aim to provide empirical evidence supporting the use of combined probiotics as functional feed additives to enhance fish health, improve production efficiency, and promote sustainable aquaculture practices

MATERIALS AND METHODS

Experimental Fish and Husbandry

Juvenile common carp (*Cyprinus carpio*) with an average initial weight of approximately 10 g were obtained from Fateh Jang Fish Hatchery, Punjab, Pakistan. Fish were transported in oxygenated polyethylene bags to the Fisheries and Aquaculture Laboratory, Department of Zoology, Kohat University of Science and Technology (KUST), Kohat.

Upon arrival, fish were acclimatized for two weeks in aerated fiberglass tanks and fed a basal commercial diet twice daily. During acclimatization and throughout the experimental period, tanks were cleaned daily by siphoning to remove feces and uneaten feed. Water quality parameters were maintained within acceptable ranges for carp culture under laboratory conditions. All procedures involving fish handling and sampling were conducted in accordance with institutional ethical guidelines approved by the Research Ethical Committee of KUST.

Experimental Design and Feeding Trial

The experiment was conducted using a completely randomized design (CRD). A total of

60 fish were randomly allocated into four dietary treatment groups (15 fish per group). Each treatment was maintained under identical environmental and management conditions to ensure that dietary supplementation remained the only experimental variable.

The feeding trial lasted 60 days. The dietary treatments were as follows:

T1: Basal diet supplemented with lyophilized *Saccharomyces boulardii* at 1×10^8 CFU/g of feed.

T2: Basal diet supplemented with *Shewanella putrefaciens* at 1×10^8 CFU/g of feed.

T3: Basal diet supplemented with a combination of lyophilized *S. boulardii* and *S. putrefaciens*, each at 1×10^8 CFU/g of feed.

Control (T0): Basal diet without probiotic supplementation.

Fish were hand fed twice daily (08:00 and 16:00 h) at 3% of their body weight. Feeding rates were adjusted biweekly based on weight measurements. The experimental protocol followed established methodologies for probiotic evaluation in aquaculture nutrition studies (Merrifield et al., 2010; Nayak 2010).

Diet Preparation and Probiotic Incorporation

A commercial pelleted feed served as the basal diet. The probiotics lyophilized *S. boulardii* and *S. putrefaciens* were procured from PharmEvo Pvt. Ltd., Karachi, Pakistan. The required quantity of each probiotic was incorporated into the crushed basal feed to achieve a final concentration of 1×10^8 CFU/g.

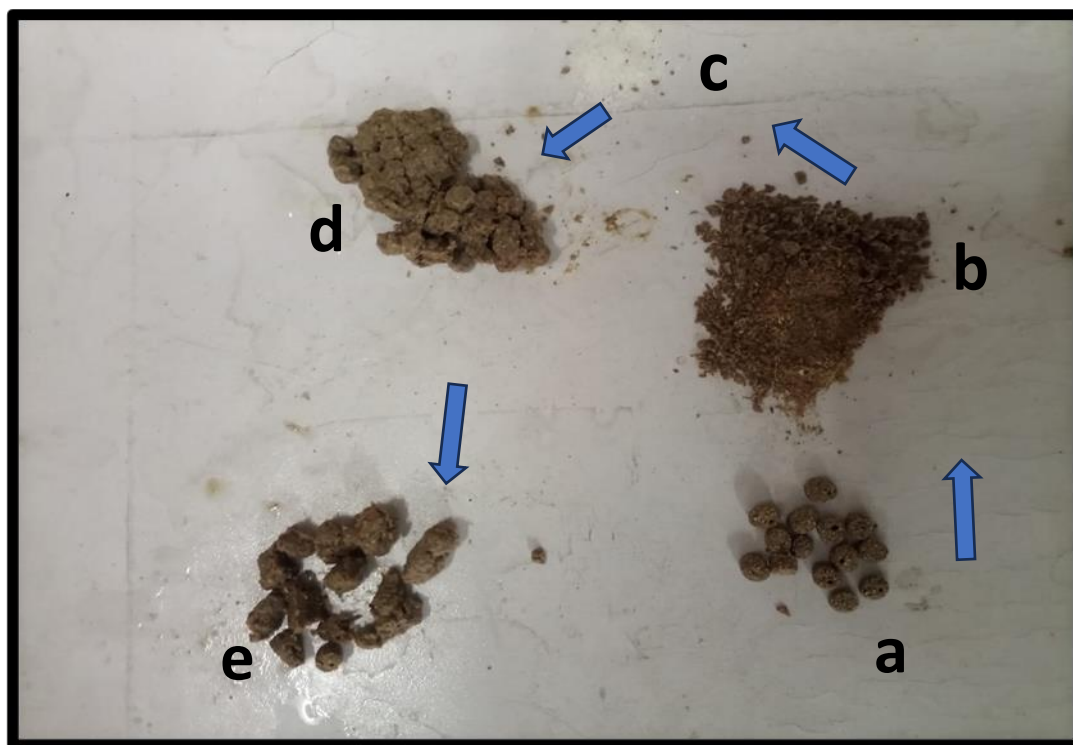


Figure 1: Preparation of medicated food. a) Normal fish diet (b) Crushed into powder (c) Probiotic (d) Incorporation of probiotic (e) Experimental diet

The probiotic cultures were mixed thoroughly with the feed to ensure uniform distribution. The supplemented diets were air dried at room temperature and stored in airtight containers until use. Fresh feed batches were prepared

periodically to maintain probiotic viability (Tapia-Paniagua et al., 2014).

Growth Performance Evaluation

Fish were batch weighed fortnightly after short term fasting to reduce variation due to gut content. Growth performance was assessed using the following parameters:

Weight Gain (WG)

WG = Final weight – Initial weight

Specific Growth Rate (SGR, % day⁻¹)

$$SGR = \frac{[\ln \text{ Final weight} - \ln \text{ Initial weight}] / \text{Duration (days)}}{\times 100}$$

These indices were used to evaluate growth efficiency and dietary effects on fish performance (Nayak 2010).

Hematological Analysis

At the end of the 60 day feeding period, six fish per treatment were randomly selected for hematological analysis. Fish were anesthetized

using MS 222 (tricaine methanesulfonate) prior to blood collection to minimize stress.

Blood samples were drawn aseptically from the caudal vein using sterile syringes and transferred into EDTA coated tubes. The following hematological parameters were determined:

Hemoglobin (Hb) concentration using the cyanmethemoglobin method

Hematocrit (Hct) by microhematocrit centrifugation

Red blood cell (RBC) and white blood cell (WBC) counts using a Neubauer hemocytometer

Differential leukocyte count (DLC) from Giemsa stained blood smears examined under a compound microscope

These parameters were selected as indicators of physiological and immunological status in fish (Roberts 2012; Lazado and Caipang 2014; Witeska et al., 2023).

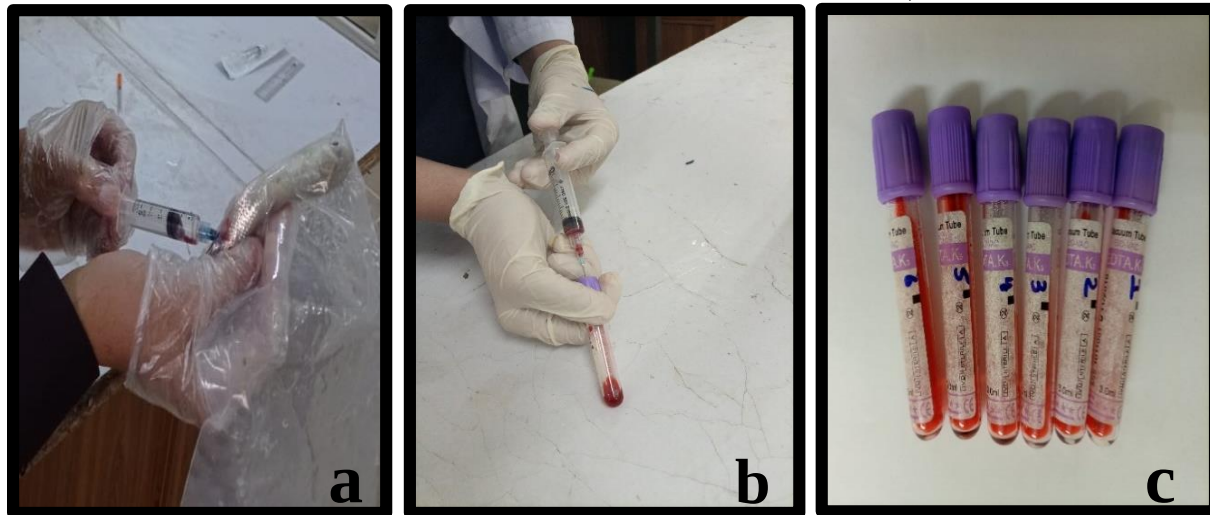


Figure 2: Blood collection. a) Extraction of blood from fish (b) Poring blood from syringe into EDTA tube (c) Blood sample in EDTA tube

Statistical Analysis

All data were analyzed using SPSS version 19. Results were expressed as mean $\pm$ standard deviation (SD). One way analysis of variance (ANOVA) was used to evaluate differences among treatment groups. When significant differences were detected ($p < 0.05$), Tukey's Least Significant Difference (LSD) test was applied for multiple comparisons. Graphical representations were generated to illustrate variations in growth and hematological parameters among treatments.

RESULTS

The effects of probiotic supplemented diets on growth performance and hematological parameters of juvenile *Cyprinus carpio* were evaluated over a 60 day feeding trial. Data were analyzed using one way ANOVA followed by Tukey's/LSD post hoc tests at a significance level of $p < 0.05$. Results are presented as mean $\pm$ standard deviation (SD). Different superscript

letters within the same row indicate statistically significant differences among treatments.

Growth Performance

Fortnightly Body Weight

Mean body weight increased progressively in all experimental groups throughout the trial (Days 1, 14, 28, 42, and 60). However, fish receiving probiotic supplemented diets exhibited significantly higher growth compared with the control group ($p < 0.05$).

Among treatments, Group B (*Shewanella putrefaciens*) demonstrated the strongest growth

response, followed by Group A (Lyophilized *Saccharomyces boulardii*) and the combined probiotic group (A + B). The control group consistently recorded the lowest mean body weight across all sampling intervals.

By Day 60, the highest final mean body weight was observed in Group B, whereas the control group maintained the lowest value. These findings confirm that probiotic supplementation significantly enhanced growth rate relative to non supplemented fish.

TABLE 3.1: Fortnightly mean body weight (g) of common carp fed experimental diets

Day	Control	Group A (<i>L. S. boulardii</i>)	Group B (<i>S. putrefaciens</i>)	Group C (Mix)
01	10.00 ± 0.03	10.20 ± 0.05	9.90 ± 0.04	10.10 ± 0.06
14	12.40 ± 0.90 ^d	12.80 ± 0.85 ^c	13.10 ± 0.80 ^a	12.30 ± 0.95 ^b
28	14.10 ± 1.00 ^d	15.20 ± 1.05 ^c	16.00 ± 0.95 ^a	14.20 ± 1.10 ^b
42	17.50 ± 1.20 ^d	19.00 ± 1.10 ^c	20.50 ± 1.25 ^a	18.00 ± 1.30 ^b
60	22.35 ± 0.83 ^d	26.05 ± 0.92 ^c	27.55 ± 0.88 ^a	24.55 ± 1.01 ^b

Different superscripts (a–d) indicate significant differences at $p < 0.05$ (Tukey-LSD).

Final Growth Parameters

All treatment groups consisted of at least 15 fish, ensuring adequate statistical power. Growth indices after 60 days showed significant improvement in probiotic fed groups compared with the control ($p < 0.05$).

Group B recorded the highest final weight and weight gain, followed by Group A and the combined treatment group. The control group

showed the lowest growth performance across all evaluated parameters. Specific growth rate (SGR) followed a similar trend, with probiotic treatments significantly outperforming the control. Survival remained 100% in all groups.

Although the combined probiotic treatment improved growth compared with the control, it did not surpass the performance of single strain treatments. These results indicate that individual probiotic supplementation was more effective than combined administration under the present experimental conditions.

TABLE 3.2: Growth Performance of *C. carpio* Fed Different Probiotic Diets for 60 Days

Treatment	No. of Fish/Group	Average Initial Weight (g)	Average Final Weight (g)	Weight Gain (g)	SGR (%/day)	Survival (%)
Group A (Probiotic A)	≥15	10.20 ± 0.05	26.05 ± 0.92 ^c	15.85 ± 0.92 ^c	1.47 ± 0.03 ^c	100
Group B (Probiotic B)	≥15	9.90 ± 0.04	27.55 ± 0.88 ^a	17.65 ± 0.88 ^a	1.59 ± 0.02 ^a	100
Group C (A + B Combination)	≥15	10.10 ± 0.06	24.55 ± 1.01 ^b	14.45 ± 1.01 ^b	1.41 ± 0.04 ^b	100
Group D (Control)	≥15	10.00 ± 0.03	22.35 ± 0.83 ^d	12.35 ± 0.83 ^d	1.27 ± 0.03 ^d	100

Means followed by different superscript letters within the same column differ significantly ($p < 0.05$).

Hematological Parameters**Hemoglobin (Hb)**

Hemoglobin concentration differed significantly among treatments ($p < 0.05$). The control group exhibited a mean Hb value of 8.27 ± 0.24 g/dL, within the normal physiological range (6.5–9.5 g/dL).

Group A showed a moderate increase (8.99 ± 0.28 g/dL), whereas Group B recorded the

highest Hb concentration (9.47 ± 0.27 g/dL), slightly exceeding the upper physiological limit. In contrast, the combined treatment group showed a lower mean value (7.63 ± 1.06 g/dL), with greater variability.

These results demonstrate that single probiotic supplementation, particularly Group B, significantly elevated hemoglobin levels compared with the control.

TABLE 3.3: Comparative Analysis of Haemoglobin Concentrations Across Experimental Groups

Groups	Hemoglobin (g/dL) (Mean $\pm$ SD)	Physiological Range (6.5–9.5 g/dL)	Remarks
Control	8.27 ± 0.24	Within normal limits	✓ Normal
Experimental Group 1 (Probiotic A)	8.99 ± 0.28	Slightly above normal mean	↑ Mild increase
Experimental Group 2 (Probiotic B)	9.47 ± 0.27	Above normal range	↑ Significantly higher
Experimental Group 3 (Probiotic A + B)	7.63 ± 1.06	Below control and lower normal limit	↓ Decrease observed

Hematocrit (Packed Cell Volume, PCV)

Hematocrit values remained within the normal range (30–38%) in all groups. The control group recorded $34.37 \pm 0.96\%$. Group A showed a slight increase ($36.11 \pm 0.67\%$), while Group B exhibited the highest PCV ($37.43 \pm 1.22\%$). The

combined treatment group displayed a value of $33.87 \pm 2.17\%$, slightly lower than the control and with higher variability.

Group B demonstrated a significantly higher hematocrit compared with the control ($p < 0.05$), whereas the combined treatment did not provide additional improvement.

TABLE 3.4: Comparative Analysis of Hematocrit (Packed Cell Volume) Across Experimental Groups

Groups	Hematocrit (PCV%) (Mean $\pm$ SD)	Physiological Range (30–38%)	Remarks
Control	34.37 ± 0.96	Within normal limits	✓ Normal
Experimental Group 1 (Probiotic A)	36.11 ± 0.67	Within normal limits	✓ Normal
Experimental Group 2 (Probiotic B)	37.43 ± 1.22	Upper physiological limit	↑ Higher than control
Experimental Group 3 (Probiotic A + B)	33.87 ± 2.17	Within normal limits	≈ Normal

Red Blood Cell (RBC) Count

RBC counts differed significantly among treatments ($p < 0.05$). The control group recorded $1.90 \pm 0.05 \times 10^6/\mu\text{L}$. Group A showed a moderate increase ($2.09 \pm 0.06 \times 10^6/\mu\text{L}$), and Group B exhibited the highest value ($2.23 \pm 0.08 \times 10^6/\mu\text{L}$).

The combined treatment group ($1.88 \pm 0.36 \times 10^6/\mu\text{L}$) did not differ significantly from the control and displayed higher variability. These findings indicate that single probiotic supplementation, particularly Group B, enhanced erythropoiesis more effectively than the combined treatment.

Table 3.5: Red Blood Cell (RBC) Count

Groups	RBC Count ($\times 10^6/\mu\text{L}$) (Mean $\pm$ SD)	Physiological Range ($1.2 - 2.5 \times 10^6/\mu\text{L}$)	Remarks
Control	1.90 ± 0.05	Within normal range	✓ Normal
Experimental Group 1 (A)	2.09 ± 0.06	Above control	↑ Mild increase
Experimental Group 2 (B)	2.23 ± 0.08	Significantly higher	↑↑ Significant increase
Experimental Group 3 (A + B)	1.88 ± 0.36	Within normal limits	≈ Stable

White Blood Cell (WBC) Count

WBC counts were significantly elevated in probiotic treated groups ($p < 0.05$). The control group showed $28.52 \pm 0.49 \times 10^3/\mu\text{L}$. Group A

and Group B recorded higher values (33.58 ± 0.87 and $35.33 \pm 0.73 \times 10^3/\mu\text{L}$, respectively).

The combined treatment group showed $26.20 \pm 8.50 \times 10^3/\mu\text{L}$, remaining within normal limits but with greater variability. Group B demonstrated the strongest leukocytic response.

Table 3.6: White Blood Cell (WBC) Count

Groups	WBC Count ($\times 10^3/\mu\text{L}$) (Mean $\pm$ SD)	Physiological Range ($15 - 40 \times 10^3/\mu\text{L}$)	Remarks
Control	28.52 ± 0.49	Within normal range	✓ Normal
Experimental Group 1 (A)	33.58 ± 0.87	Higher than control	↑ Enhanced leukocytosis
Experimental Group 2 (B)	35.33 ± 0.73	Higher end of normal	↑ Strong immune activation
Experimental Group 3 (A + B)	26.20 ± 8.50	Within normal range	↓ Broad variation observed

Erythrocyte Indices (MCV, MCH, MCHC)

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) remained within physiological ranges in all

treatments. No pathological alterations were observed.

Minor variations were detected among groups, but probiotic supplementation did not induce significant adverse changes in erythrocyte morphology or hemoglobin characteristics.

TABLE 3.7: Effect of Probiotic Supplementation on Erythrocyte Indices (MCV, MCH, MCHC)

Groups	MCV (fL)	Physiological Range (120 – 150)	MCH (pg)	Physiological Range (30 – 40)	MCHC (g/dL)	Physiological Range (26 – 33)
Control	137.67 ± 4.17	✓ Normal	34.03 ± 0.66	✓ Normal	30.60 ± 0.63	✓ Normal
Exp. Group 1 (A)	133.29 ± 3.62	✓ Normal	35.26 ± 1.15	✓ Normal (↑ High)	30.83 ± 1.03	✓ Normal
Exp. Group 2 (B)	129.82 ± 4.67	✓ Normal (Slight ↓)	33.67 ± 0.92	✓ Normal	30.60 ± 0.85	✓ Normal

Exp. Group 3 (A + B)	134.68 ± 10.22	✓ Normal	35.10 ± 2.41	✓ Normal	29.18 ± 1.89	✓ Normal
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Platelet Count

Platelet counts in all groups remained within the normal physiological range ($80\text{--}120 \times 10^3/\mu\text{L}$). The control group recorded $96.00 \pm 3.20 \times 10^3/\mu\text{L}$. Group A and Group B showed modest

increases (99.40 ± 1.70 and $107.75 \pm 1.83 \times 10^3/\mu\text{L}$, respectively). The combined treatment group recorded $103.30 \pm 11.40 \times 10^3/\mu\text{L}$, with higher variability. No abnormal thrombocytosis or thrombocytopenia was observed in any group.

TABLE 3.8: Effect of Probiotic Supplementation on Platelet Count

Groups	Platelet Count ($\times 10^3 \mu\text{L}^{-1}$) (Mean ± SD)	Physiological Range (80 – 120)	Remarks
Control	96.00 ± 3.20	✓ Normal	—
Exp. Group 1 (A)	99.40 ± 1.70	✓ Normal	Slight elevation
Exp. Group 2 (B)	107.75 ± 1.83	✓ Normal (High end)	↑ Mild increase
Exp. Group 3 (A + B)	103.30 ± 11.40	✓ Normal	Variable values

Differential Leukocyte Count

All leukocyte types remained within established physiological ranges.

Lymphocytes: Values ranged from 70.22% to 77.12%, with Group B showing the highest proportion.
Neutrophils: All groups remained within 5–15%, with the combined treatment group recording the lowest percentage.

Monocytes, Eosinophils, and Basophils: All remained within normal limits across treatments.

Single probiotic treatments, particularly Group B, demonstrated more consistent immune enhancement compared with the combined supplementation.

TABLE 3.9: Differential Leukocyte Count (% Mean ± SD)

Cell Type	Normal Range (%)	Control	Exp. Group 1 (A)	Exp. Group 2 (B)	Exp. Group 3 (A + B)
Lymphocytes	60 – 80	72.78 ± 2.6	72.87 ± 0.8	77.12 ± 1.9	70.22 ± 5.0
Neutrophils	5 – 15	12.07 ± 0.43	11.08 ± 0.26	11.13 ± 0.37	9.06 ± 2.9
Monocytes	5 – 10	7.98 ± 0.29	6.97 ± 0.18	6.92 ± 0.19	7.28 ± 1.56
Eosinophils	1 – 5	3.00 ± 0.09	4.08 ± 0.13	4.08 ± 0.08	3.92 ± 1.26
Basophils	0 – 1	0.98 ± 0.04	1.00 ± 0.00	1.00 ± 0.00	0.78 ± 0.25

DISCUSSION

The present investigation evaluated the influence of dietary supplementation with two probiotics, Lyophilized *Saccharomyces boulardii*

and *Shewanella putrefaciens*, administered individually and in combination, on growth performance and hematological health in juvenile *Cyprinus carpio*. These findings

demonstrate that probiotic inclusion significantly improved growth indices, erythropoietic activity, immune related blood parameters, and hematological stability when compared with the control group. These outcomes support the growing body of evidence recognizing probiotics as effective functional feed additives that enhance nutrient utilization, stabilize intestinal microflora, and improve physiological adaptability in freshwater aquaculture species. Growth performance was markedly enhanced in fish receiving probiotic supplemented diets, with the most pronounced improvements observed in the group fed *Shewanella putrefaciens*. The significantly higher weight gain and specific growth rate recorded in this group indicate superior feed utilization efficiency and metabolic performance. These findings are consistent with earlier studies reporting that *Shewanella* species produce extracellular digestive enzymes, including proteases, lipases, and amylases, which enhance nutrient digestion and absorption in fish (Zhou et al., 2009). In addition, *S. putrefaciens* has been shown to improve gut microbial balance through competitive exclusion of pathogenic bacteria and siderophore mediated nutrient competition, thereby creating a favorable intestinal environment for nutrient assimilation (Balcázar et al., 2006). Improved intestinal microbial composition has been linked to enhanced intestinal morphology and absorptive capacity, mechanisms widely reported in carp and tilapia fed probiotic enriched diets (Huynh et al., 2017). The superior growth performance observed in the present study therefore appears biologically justified and in agreement with previous research. Fish supplemented with Lyophilized *Saccharomyces boulardii* also exhibited significant improvements in growth performance relative to the control group, as reflected by increased final body weight, weight gain, and specific growth rate. Yeast based probiotics are known to promote gut microbial stability and enhance digestive efficiency, resulting in improved nutrient availability for growth (Gatesoupe, 2007). Earlier studies have demonstrated that *Saccharomyces* species enhance feed digestion by increasing enzyme activity and improving intestinal function, ultimately supporting somatic growth in freshwater fish (El-Haroun et al., 2006; Lara-Flores et al., 2010). The

significant growth enhancement observed in the present study is therefore consistent with the established growth promoting properties of yeast probiotics. However, the comparatively lower growth response observed with *L. S. boulardii* relative to *S. putrefaciens* may be attributed to differences in strain-specific functionality and host microbe interactions, as highlighted by (Hai, 2015). Despite this variation, the results confirm the suitability of *L. S. boulardii* as a natural growth enhancing probiotic for sustainable aquaculture practices. In contrast, the combined probiotic treatment resulted in lower growth performance compared with single strain treatments, although growth indices remained higher than those of the control group. The absence of synergistic effects suggests that concurrent supplementation with bacterial and yeast probiotics did not enhance growth efficiency beyond individual applications. Previous studies have reported that mixed probiotic formulations may lead to microbial competition within the gastrointestinal tract, including competition for adhesion sites and nutrients, thereby reducing functional efficiency (Hai, 2015). (Balcázar et al., 2006) further emphasized that probiotic strains differ in their modes of action, and inappropriate combinations may interfere with metabolic activity and gut homeostasis. Additionally, (Gatesoupe, 2007) noted that yeast probiotics primarily enhance digestion through enzyme stimulation, whereas bacterial probiotics often exert their effects through microbial modulation. When these mechanisms operate simultaneously without proper compatibility, intestinal balance may be disrupted, resulting in inconsistent nutrient assimilation and reduced growth performance. Similar observations have been reported by (Mohapatra et al., 2012) who found that single strain probiotics frequently produce more consistent growth responses in carp species than mixed formulations. These findings support the growth patterns observed in the present study. Hematological parameters provided valuable insights into the physiological and immunological status of fish subjected to probiotic supplementation. Fish receiving single strain probiotics, particularly *Shewanella putrefaciens*, exhibited significant increases in hemoglobin concentration, red blood cell count,

and hematocrit values. These changes indicate enhanced erythropoietic activity and improved oxygen carrying capacity, which are essential for sustaining higher metabolic rates and supporting growth. The observed improvements in erythrocytic parameters are consistent with reports by (Irianto and Austin, 2002) who demonstrated that probiotics stimulate hematopoiesis by improving nutrient absorption and metabolic efficiency in fish. Enhanced bioavailability of iron and amino acids resulting from improved gut function may contribute to increased hemoglobin synthesis and red blood cell production, thereby supporting aerobic metabolism (Aly et al., 2008). White blood cell counts were also elevated in probiotic fed fish, indicating enhanced immune activation and improved disease resistance potential. The most pronounced increase was observed in fish receiving *L. S. putrefaciens*, suggesting strong stimulation of innate immune mechanisms. Bacterial probiotics have been shown to enhance leukocyte proliferation, macrophage activity, and cytokine production, thereby strengthening nonspecific immune defenses in freshwater fish (Balcázar et al., 2006). Fish fed *L. S. boulardii* also showed a significant rise in white blood cell counts, although the magnitude of increase was lower. Yeast probiotics are recognized for their immunostimulatory properties, which include activation of leukocytes and enhancement of phagocytic activity (Lara-Flores et al., 2010). In contrast, the combined probiotic group exhibited more variable immune responses, suggesting that mixed formulations may reduce immune efficiency due to microbial competition or altered immune signaling pathways (Hai, 2015). Erythrocyte indices, including MCV, MCH, and MCHC, remained within normal physiological ranges across all treatments, indicating that probiotic supplementation did not induce hematological stress. Slight improvements observed in probiotic fed groups reflect enhanced red blood cell quality and hemoglobin incorporation, contributing to improved oxygen transport. Similar stability in erythrocyte indices following probiotic supplementation has been reported in freshwater and marine fish species and is associated with balanced erythropoiesis and reduced physiological stress (Merrifield et al., 2010). Platelet counts also remained within normal limits, indicating stable hematopoietic

balance and circulatory health. Improved platelet stability observed in single probiotic treatments may reflect enhanced hematopoietic regulation and immune metabolic integration (Abdel Tawwab et al., 2008). Differential leukocyte analysis revealed increased lymphocyte proportions in probiotic fed fish, suggesting enhanced adaptive immune capacity and immune readiness. Similar increases in lymphocyte percentages have been reported in probiotic supplemented carp and tilapia and are associated with improved disease resistance (Panigrahi et al., 2007). Neutrophil and monocyte count remained within normal ranges, indicating immune balance rather than inflammatory stress. The more consistent leukocyte profiles observed in single strain treatments further emphasize the importance of probiotic compatibility in immune modulation. Correlation analysis revealed strong positive relationships between growth performance and hematological parameters, particularly hemoglobin concentration, red blood cell count, and white blood cell levels. Improved blood physiology enhances oxygen transport and metabolic efficiency, supporting faster growth, while enhanced immune function reduces the energy costs associated with subclinical infections (Aly et al., 2008). Probiotics further contribute by stabilizing gut microbiota, reducing endotoxin load, and improving systemic physiological performance (Ringø et al., 2010). Together, these local and systemic effects explain the concurrent improvements in growth and health observed in probiotic-fed fish. These findings of the present study demonstrate that dietary probiotics, particularly single strain *Shewanella putrefaciens*, effectively enhance growth performance, hematological health, and immune competence in juvenile *Cyprinus carpio*. The results support the use of probiotics as safe, natural, and sustainable alternatives to antibiotics in freshwater aquaculture and emphasize the importance of selecting compatible probiotic strains to maximize production efficiency and fish health.

CONCLUSION

The present study demonstrated that dietary supplementation with probiotics significantly improved the growth performance and hematological status of juvenile *Cyprinus carpio*

during the 60 day feeding trial. Fish fed probiotic supplemented diets exhibited higher body weight, greater weight gain, and improved specific growth rate compared with the control group, indicating the positive influence of probiotics on nutrient utilization and overall growth. Among the tested treatments, the diet supplemented with *Shewanella putrefaciens* (Group B) produced the most pronounced improvement in growth parameters, followed by *L. Saccharomyces boulardii* (Group A) and the combined probiotic treatment (Group C). Although the mixed probiotic diet enhanced growth relative to the control, it did not outperform the single strain treatments, suggesting that individual probiotic strains may exert more efficient physiological effects under the conditions of this experiment.

The hematological analysis further confirmed the beneficial impact of probiotic supplementation on fish health. Probiotic fed groups exhibited higher hemoglobin concentration, hematocrit values, red blood cell counts, and white blood cell counts compared with the control group, reflecting improved oxygen carrying capacity and enhanced immune responsiveness. The most notable hematological enhancement was observed in fish fed *Shewanella putrefaciens*, which consistently showed the highest values for hemoglobin, hematocrit, RBC, and WBC counts. Meanwhile, erythrocyte indices (MCV, MCH, and MCHC), platelet counts, and differential leukocyte profiles remained within normal physiological ranges across all treatments, indicating that probiotic supplementation did not produce any adverse physiological effects. Overall, the findings suggest that probiotic inclusion in the diet of common carp juveniles can promote better growth performance and strengthen hematological health without causing pathological alterations.

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