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The Effect Of Nutritional Factor (*Rasaj Bhav*) On The Structural Changes In *Garbha*

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ABSTRACT

Probiotics are increasingly utilized in aquaculture to enhance fish health, yet their tissue-level effects remain underexplored in freshwater species. This study investigated the dose- and time-dependent effects of dietary *Bacillus coagulans* on liver and intestinal histology in *Cyprinus carpio* over a 60-day feeding trial. A total of 180 fingerlings were divided into four groups: control (basal diet) and three treatment groups receiving 0.05×10^9, 0.1×10^9, or 0.15×10^9 CFU g^{-1} of *B. coagulans*. Liver and intestinal tissues were sampled at 20, 40, and 60 days, fixed in formalin, processed, and examined using hematoxylin and eosin staining. Histological analysis revealed progressive, dose-dependent improvements in hepatocyte integrity, lobular organization, and sinusoidal structure, with T3 (highest dose) showing near-normal hepatic architecture by day 60. Similarly, intestinal morphology improved with increased villus height, goblet cell density, and epithelial continuity, particularly in higher-dose groups, indicating enhanced mucosal barrier function and absorptive capacity. No signs of necrosis, inflammation, or tissue degeneration were observed in probiotic-fed fish, confirming safety and biocompatibility. These findings align with previous reports on probiotics enhancing metabolic efficiency, mucosal immunity, and tissue health in freshwater fish. Overall, dietary supplementation with *B. coagulans* at 10^9 CFU g^{-1} over 60 days effectively promotes liver and intestinal health in common carp, supporting its application as a functional feed additive in sustainable aquaculture.

Keywords: *Cyprinus carpio*, *Bacillus coagulans*, liver histology, intestinal morphology, probiotics, aquaculture

1. Introduction

Aquaculture is one of the fastest-growing food production sectors globally, with a pressing need to optimize fish health, growth, and immune competence. Feed composition is a primary determinant of physiological performance and organ integrity, particularly in metabolically active tissues such as the liver and intestine. Malnutrition, stress, or pathogen exposure can compromise organ morphology, leading to hepatic vacuolation, sinusoidal congestion, and villus degeneration, which reduce nutrient absorption and immunity (Naie et al., 2022; Zhou et al., 2022. Dawood et al., 2020).

Probiotics, live microorganisms that confer health benefits on the host when administered in adequate amounts have emerged as sustainable alternatives to antibiotics. They improve gut microbiota balance, stimulate digestive enzyme activity, and enhance immune modulation (Dawood et al., 2020). Among various probiotic candidates, *Bacillus coagulans*, a spore-forming lactic acid bacterium, demonstrates resilience in aquaculture feed, survival under gastric conditions, and the ability to produce beneficial metabolites such as short-chain fatty acids, bacteriocins, and exopolysaccharides (Liang et al., 2024). Although probiotics are widely used in aquaculture to enhance growth and immune function, there is a significant gap in understanding their histological effects at the tissue level, particularly in freshwater species such as *Cyprinus carpio* (Xia et al., 2024; Meng et al., 2023). Previous studies have primarily focused on growth metrics, enzyme activity, and immune parameters, leaving a lack of systematic evaluation of liver and intestinal morphology over time and across different doses.

This study investigates the dose- and time-dependent effects of dietary *Bacillus coagulans* on liver and intestinal histology in *Cyprinus carpio* over a 60-day feeding period. The research focuses on how *B. coagulans* supplementation may enhance hepatic and intestinal structure by improving metabolic efficiency, antioxidant defense, and mucosal integrity. By linking gut–liver axis modulation with observable tissue-level changes, the study aims to provide a mechanistic understanding of probiotic action and determine optimal probiotic dosages for sustainable aquaculture health management.

2. Materials and Methods

2.1 Experimental Design

A total of 180 healthy *Cyprinus carpio* fingerlings (15 ± 1 g) were randomly distributed into four dietary groups, each with three replicates of 15 fish per tank. The groups included: Control (C), receiving a basal diet without probiotics; T1, receiving the basal diet supplemented with *Bacillus coagulans* at 0.05×10^9 CFU g^{-1}; T2, supplemented with 0.1×10^9 CFU g^{-1}; and T3, supplemented with 0.15×10^9 CFU g^{-1}. The feeding trial was conducted for 60 days, during which fish were fed twice daily at a rate of 3% of their body weight. Throughout the experimental period, water quality was closely maintained within optimal ranges to minimize environmental stress and ensure reliable results. Water temperature was maintained at 26 ± 2 °C, pH ranged between 7.0 and 7.5, dissolved oxygen levels were kept at or above 5.5 mg/L, and ammonia concentration was controlled below 0.02 mg/L. Regular monitoring ensured stability of these parameters, providing a consistent rearing environment necessary to evaluate the effects of dietary *B. coagulans*. This controlled setup enabled accurate assessment of growth performance, physiological responses, and tissue-level changes in relation to probiotic supplementation in common carp.

2.2 Probiotic Preparation

Commercial *Bacillus coagulans* (10^9 CFU/g), a Gram-positive, spore-forming, lactic acid-producing probiotic strain with high resistance to gastrointestinal stress, was procured from Sanzyme Biologics Pvt. Ltd., Hyderabad, India. The probiotic was incorporated into the basal diet to achieve the desired concentrations for each treatment group: T1 (0.05×10^9 CFU g^{-1} feed), T2 (0.1×10^9 CFU g^{-1} feed), and T3 (0.15×10^9 CFU g^{-1} feed). To ensure uniform distribution, the required amount of probiotic powder was suspended in a small volume of distilled water and sprayed evenly over the feed pellets. The coated pellets were then air-dried at room temperature under sterile conditions to prevent microbial contamination. After drying, the feed was sealed in airtight containers and stored at 4 °C to preserve the viability of the probiotic spores. To minimize loss of activity and ensure microbial stability, fresh feed was prepared every seven days throughout the experimental period. This method ensured that fish in each treatment group received accurate and consistent doses of *B. coagulans* during the feeding trial.

2.3 Sample Collection

Fish were sampled on the 20th, 40th, and 60th days of the feeding trial to assess histological, hematological, and growth responses. Prior to sampling, fish were anesthetized using tricaine methane sulfonate (MS-222) at a concentration of 200 mg/L to reduce handling stress and ensure humane treatment. At each interval, three fish were randomly selected from each replicate tank. Liver and intestinal tissues were carefully dissected, rinsed with ice-cold physiological saline to remove blood and gut contents, and immediately preserved in 10% neutral buffered formalin for histological processing. All procedures were conducted in accordance with institutional ethical guidelines and approved protocols for the care and use of aquatic animals.

2.4 Histological Procedures

Liver and intestinal tissues were fixed in 10% neutral buffered formalin for 24 hours, followed by dehydration in a graded ethanol series, clearing in xylene, and embedding in paraffin. Tissue blocks were sectioned at a thickness of 5–7 µm using a rotary microtome and mounted onto glass slides. The sections were stained with Hematoxylin and Eosin (H&E) to examine general tissue morphology. Histological observations were carried out using a Leica DM500 light microscope at 10× and 40× magnifications. Parameters evaluated included hepatocyte morphology, sinusoidal organization, villus height, crypt architecture, goblet cell density, and overall epithelial integrity.

3. Results

3.1 Liver Histology

Histological examination of liver tissues from *Cyprinus carpio* fed graded levels of *Bacillus coagulans* over 20, 40, and 60 days revealed progressive, dose-dependent improvements in hepatic architecture, confirming the hepatoprotective role of dietary probiotics.

Day 20

Liver sections of the control group (C) showed mild structural disturbances, including slightly dilated sinusoids, indistinct hepatocyte boundaries, and scattered cytoplasmic irregularities, suggesting early physiological stress. In T1, mild improvement was observed with better-defined hepatocyte organization and reduced sinusoidal dilation. More apparent structural enhancement occurred in T2, where hepatocytes displayed well-preserved cytoplasm, clearer cellular boundaries, compact hepatic cords, and improved tissue cohesiveness. The highest improvement was recorded in T3, exhibiting near-normal hepatic lobular architecture with a clearly visible central vein, orderly hepatic cords, intact hepatocytes, and absence of necrosis, vacuolation, or inflammation — indicating early probiotic-induced hepatoprotection.

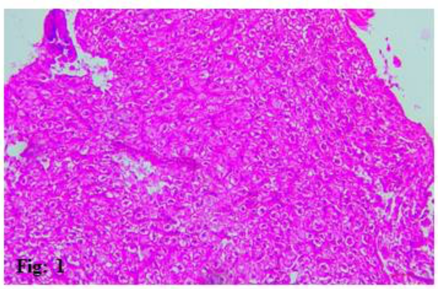
Day 40

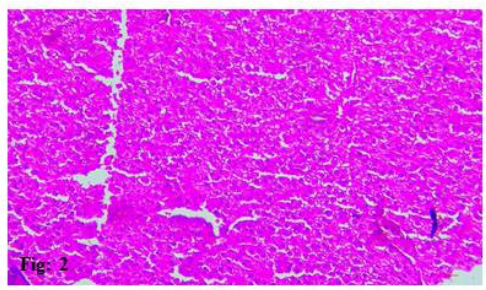
By day 40, cumulative benefits became more evident. The control group continued to display mild hepatocellular disorganization with irregular hepatocyte alignment and intermittent sinusoidal dilation. T1 showed improved tissue compactness and clearer hepatocyte structures. T2 demonstrated uniformly aligned hepatocyte plates, reduced intercellular spaces, and better-defined sinusoidal networks, indicating improved microcirculation and nutrient exchange.

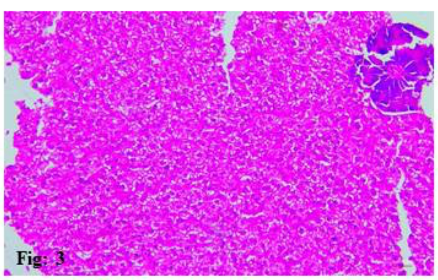
The T3 group exhibited ideal hepatic histoarchitecture a clearly defined central vein surrounded by radially arranged hepatocyte cords, intensely eosinophilic cytoplasm, and well-preserved nuclei with an increased nucleus-to-cytoplasm ratio. No signs of necrosis, congestion, or inflammatory infiltration were present.

Day 60

At the end of the trial, liver morphology clearly reflected the long-term influence of probiotic supplementation. The control group continued to show mild hepatocellular vacuolation, sinusoidal congestion, and poorly organized parenchyma, indicating unresolved hepatic stress.
T1 displayed moderate improvement with clearer cellular alignment and slightly better vascular clarity. T2 livers showed compact hepatic lobules, prominent central veins, strongly eosinophilic cytoplasm, and clearly visible nuclei — indicative of active metabolic function.
In the T3 group, hepatic tissue appeared near perfect. Hepatocytes were polygonal with round, centrally located nuclei, dense cytoplasm, and well-defined cell borders. Central veins and sinusoids were prominent and unobstructed, with no evidence of necrosis, fibrosis, fatty degeneration, or inflammatory cell infiltration. This indicates optimal liver health, maximal metabolic activity, and strong hepatoprotective and restorative effects of *B. coagulans* at 10^9 CFU/g and above (Figures 1–3).







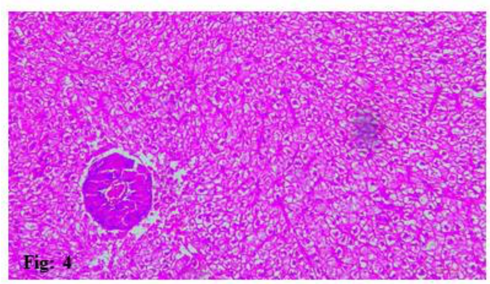


Fig 1: Control (20), **Fig 2:** Treatment 1(20), **Fig 3: T**reatment 2(20), **Fig 4:** Treatment 3(20)

3.2 Intestinal Histology

Histological assessment of intestinal tissues revealed marked improvements in mucosal integrity, villus morphology, goblet cell distribution, and epithelial organization in a probiotic dose- and time-dependent manner.

Day 20

In the control group, intestinal sections revealed shorter, irregular villi with flattened epithelial cells and poorly developed crypts of Lieberkühn. T1 showed moderate improvements with better villus organization and more uniform epithelial lining. T2 demonstrated taller and more regularly shaped villi with clearly formed crypts, indicating the onset of mucosal regeneration. The T3 group exhibited the most advanced intestinal structure with densely packed; elongated villi, intact epithelial cells, well-organized crypts, and absence of sloughing, necrosis, or inflammatory changes — suggesting early enteroprotection and enhanced absorptive efficiency (Figure 1-4).

Day 40

By this stage, intestinal improvements were more pronounced. Control fish still exhibited short villi and reduced goblet cell density. In T1, villi appeared slightly elongated, with improved epithelial continuity. T2 showed well-formed, closely packed villi, clear epithelial margins, and improved mucosal folding. T3 achieved near-optimal structure: tall and dense villi, continuous epithelial lining with prominent brush borders, and a well-developed lamina propria. Goblet cell distribution was markedly enhanced in T2 and T3, indicating improved mucus secretion and mucosal immunity. No inflammatory infiltration was observed (Figure 1-4).

Day 60

A clear divergence among treatments was observed. The control group maintained short, irregular villi with sparse goblet cells, suggesting limited adaptive response. T1 demonstrated modest improvements with elongated villi and more organized epithelial layers. T2 had significantly taller villi, higher goblet cell density, and deeper crypts of Lieberkühn. The most advanced intestinal microstructure was seen in T3. Villi were long, slender, and tightly arranged, resulting in an expanded absorptive surface area. Enterocytes displayed dense cytoplasm, clearly defined brush borders, and enlarged nuclei, indicating heightened metabolic and transport activity. Goblet cells were abundant and regularly interspersed, and crypts remained deep and functional. There were no signs of mucosal erosion, necrosis, or inflammation, highlighting the cumulative regenerative and protective effect of probiotic supplementation (Figure 1-4).

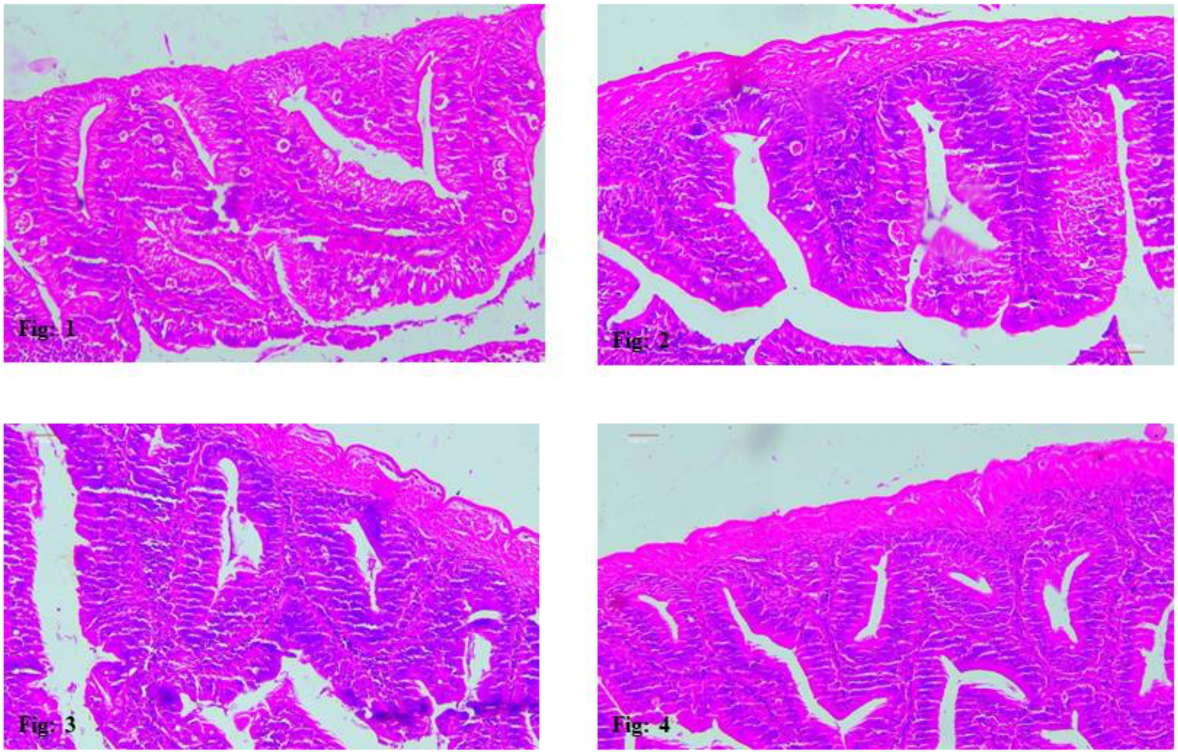


Fig 1: Control (40), **Fig 2:** Treatment 1(40), **Fig 3: T**reatment 2(40), **Fig 4:** Treatment 3(40)

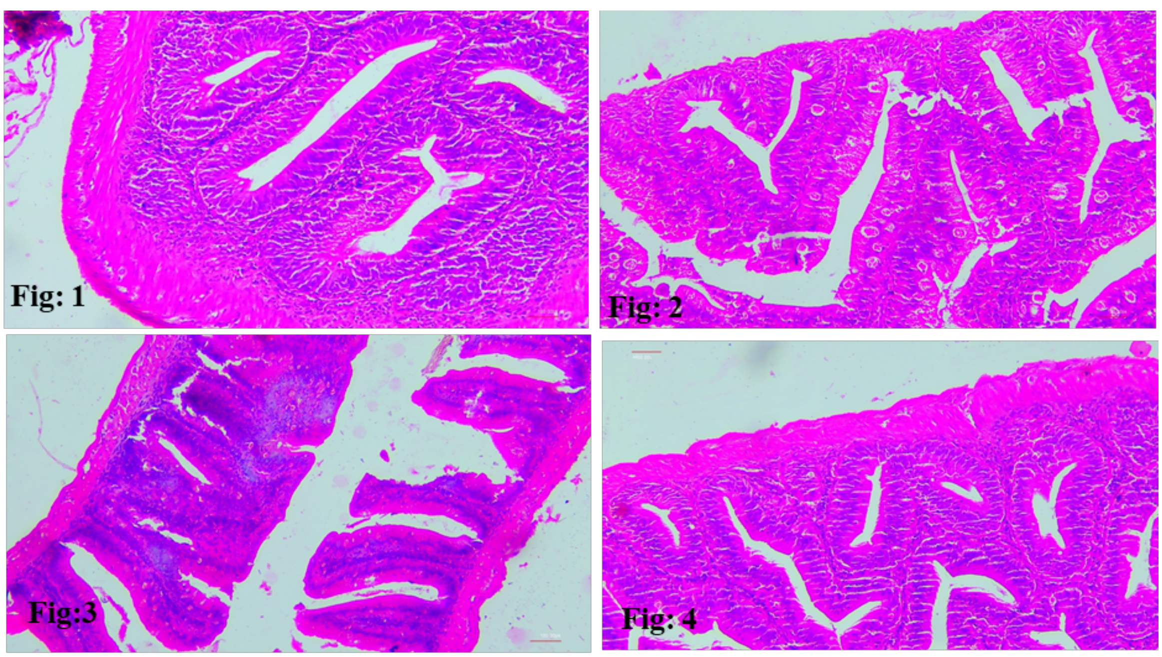


Fig 1: Control (60), **Fig 2:** Treatment 1(60), **Fig 3: T**reatment 2(60), **Fig 4:** Treatment 3(60)

4. Discussion

The present study evaluated the histological effects of dietary supplementation with *Bacillus coagulans* on liver and intestinal tissues of *Cyprinus carpio* over a 60-day period. Our results demonstrate a **dose- and time-dependent improvement** in both hepatic and intestinal microstructure, highlighting the physiological benefits of probiotics in freshwater aquaculture species.

4.1 Hepatic Responses

The liver, as a central organ for metabolism, detoxification, and nutrient processing, is highly responsive to dietary interventions. In this study, histological examination revealed that probiotic-fed groups, particularly T3 (highest dose), exhibited **well-preserved hepatocytes, compact hepatic lobules, defined central veins, and reduced vacuolation** compared to controls. These findings suggest **enhanced metabolic efficiency and hepatoprotective effects** induced by prolonged probiotic supplementation.

These results are consistent with previous research showing that probiotics such as *Bacillus velezensis* and *Lactobacillus rhamnosus* reduce hepatic lipid deposition, vacuolization, and injury scores in common carp and other freshwater species at optimal doses (0.3–0.4 g/kg) (Ghaly et al., 2023; Jaramillo-Torres et al., 2019; Chen et al., 2024, Meng et al., 2023). Markers of liver health, including ALT, AST, and triglycerides, are generally improved in probiotic-fed fish, and antioxidant and anti-inflammatory gene expression is upregulated, mitigating oxidative and metabolic stress (Chen et al., 2024, Meng et al., 2023). No histopathological signs of necrosis, steatosis, or inflammation were observed in T3 fish, aligning with reports that recommended probiotic doses are safe and effective in preserving liver tissue integrity (Mhanawi, et al., 2021, Chen et al., 2024,).

Moreover, the dose-dependent nature of hepatoprotective effects observed in this study mirrors trends reported in the literature: mid-to-high probiotic doses tend to yield maximal improvements in liver morphology and function, while lower doses induce moderate effects (Xia et al., 2024, Chen et al., 2024, Meng et al., 2023). The cumulative benefits observed at day 60 confirm that **long-term supplementation is crucial** for achieving full hepatoprotective and metabolic benefits.

4.2 Intestinal Responses

The intestine plays a pivotal role in nutrient absorption and immune defense. Histological evaluation revealed **significant improvements in villus height, width, goblet cell density, and epithelial integrity** in probiotic-supplemented groups. These effects were most pronounced in the T3 group by day 60, indicating **enhanced absorptive surface area and mucosal barrier function.**

These observations are consistent with prior studies demonstrating that probiotics, including *Bacillus subtilis*, *Bacillus velezensis*, *Lactobacillus plantarum*, and mixed formulations (Synbiozyme 500®), increase villus height and goblet cell numbers in common carp after 6–12 weeks of supplementation (Xia et al., 2018, Mahmodi et al., 2021). Enhanced epithelial thickness and goblet cell proliferation are critical for mucosal defense, as goblet cells secrete mucins that form a protective layer against pathogens (De Marco et al., 2023). Additionally, probiotic supplementation can modulate gut barrier-related genes such as ZO-1 and occludin, further supporting intestinal integrity and functional adaptation (Xia et al., 2024, Chen et al., 2024).

The dose-dependent improvements observed in the present study align with reports that mid-to-high doses of probiotics result in optimal intestinal health outcomes, whereas lower doses produce modest structural gains (Wu et al., 2023). The **time-dependent effects** also reflect previous findings: most histological benefits appear after 6–12 weeks of continuous probiotic feeding, with cumulative enhancements in villus morphology, goblet cell distribution, and epithelial continuity (Xia et al., 2018, Asha et al., 2024).

4.3 Integration of Liver and Gut Responses

The observed histological enhancements in liver and intestine suggest a **coordinated systemic benefit** of *B. coagulans* supplementation. Improved hepatic architecture may support more efficient nutrient metabolism, which, in combination with an enhanced intestinal absorptive surface and mucosal barrier, likely contributes to the overall improved growth performance and physiological resilience observed in probiotic-fed fish.

Our findings corroborate recent studies emphasizing the **importance of probiotics as functional feed additives** in freshwater aquaculture. Both the liver and intestinal tissues respond positively in a **dose- and time-dependent manner**, reinforcing the concept that probiotic supplementation should be optimized for both concentration and duration to achieve maximal physiological benefits (Ghaly et al., 2023; Jaramillo-Torres et al., 2019; Wu et al., 2023).

Furthermore, the lack of histopathological alterations in both liver and gut at the highest probiotic dose supports the **safety and biocompatibility** of *B. coagulans* as a dietary supplement in *C. carpio*, consistent with prior recommendations for aquaculture probiotics (Mhanawi, et al., 2021, Chen et al., 2024).

4.4 Conclusion

In summary, the present study demonstrates that **dietary supplementation with** *Bacillus coagulans* **significantly enhances the histological architecture of liver and intestine in** *Cyprinus carpio*. The improvements are **dose- and time-dependent**, with the highest dose over 60 days producing near-optimal tissue morphology, improved hepatocyte integrity, enhanced villus height, and increased goblet cell density. These findings align with both **recent and prior studies** in freshwater aquaculture species, confirming that *B. coagulans* is an effective probiotic for promoting metabolic efficiency, mucosal immunity, and tissue health in carp.

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