Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 1287-1296 | ISSN:2251-6727

Probiotic Efficacy of *Bacillus Coagulans* on Biochemical and Physiological Impacts in *Cyprinus Carpio* Across Developmental Stages

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(Received: 16 January 2025 Revised: 20 Feburary 2025 Accepted: 31 March 2025)

KEYWORDS

Bacillus coagulans, *Cyprinus carpio*, probiotics, biochemical response, enzymatic activity.

ABSTRACT:

Introduction: Probiotics are developing as a viable alternative to antibiotics in aquaculture, benefiting fish health, metabolism, and immunity. With rising concerns about antibiotic resistance, this research analyzes *Bacillus coagulans* supplementation in *Cyprinus carpio* (common carp) fingerlings. Over a 60-day feeding study, we assessed its impacts on biochemical and enzymatic responses, seeking to improve sustainable aquaculture methods by lowering dependency on antibiotics while boosting fish well-being.

Objectives: This study aims to investigate the influence of *Bacillus coagulans* supplementation on *Cyprinus carpio* fingerlings' biochemical and enzymatic responses throughout a 60-day feeding experiment. The research focused on metabolic alterations (glucose, cholesterol, protein, glycogen) and enzyme activity (phosphatases, aminotransferases) to assess the probiotic's potential in enhancing fish health and promoting sustainable aquaculture.

Methods: *Cyprinus carpio* fingerlings (15 ± 1 g) were allocated to four groups: a control (C) and three experimental groups receiving *B. coagulans* at 0.05×10^9 (T1), 0.1×10^9 (T2), and 0.15×10^9 CFU g^{-1} (T3). Fed a 32% crude protein diet at 3% body weight daily for 60 days, fish underwent biochemical analysis (liver, muscle, intestinal parameters) and enzymatic tests (ACP, ALP, AST, ALT). Statistical significance was established using P-values.

Results: In the T3 group (0.15×10^9 CFU g^{-1}), liver glucose and cholesterol decreased intensely ($P < 0.0001$), but protein and glycogen increased ($P < 0.0002$). Muscle glucose declined ($P < 0.0009$) with increasing protein, and intestine cholesterol lowered ($P = 0.0009$). Enzyme activities indicated greater ACP and ALP in T3 ($P < 0.0525$), with decreased hepatic AST and ALT ($P < 0.0002$), suggesting reduced liver stress and enhanced metabolic performance.

Conclusions: *B. coagulans* supplementation at 0.15×10^9 CFU g^{-1} substantially increased lipid metabolism, protein synthesis, and enzymatic activity in *C. carpio*. Lower glucose and cholesterol, combined more protein and glycogen, reflect effective food usage. Elevated ACP and ALP, with decreasing AST and ALT, suggest improved metabolic health and lower hepatic stress. These results underline *B. coagulans* potential as a probiotic, promoting fish health and sustainable aquaculture by lowering antibiotic reliance.

1. Introduction

Common carp (*Cyprinus carpio* L.) has been farmed widely in China for decades because to its nutritious properties, exquisite flavor, and affordability [1, 2]. However, fish reared in intensive systems have a substantial risk of bacterial illnesses [3, 4]. Several studies have established the efficacy of antibiotics as feed additives in animal husbandry [5, 6]. Nevertheless, antibiotics are not recommended for disease management in aquaculture owing to their environmental harm, the rise of bacterial resistance, pollution, and poor repercussions for fish health, presenting food safety problems for human consumption [7, 9]. Therefore,

finding alternatives to antibiotics is vital for both the aquaculture sector and public health [10].

Probiotics are living microorganisms comprised of nonpathogenic bacteria that give advantages to host animals by boosting growth, immunology, and gut microbial balance [11,14]. Probiotics are increasingly being employed as an alternative to antibiotics in aquaculture [13, 15, 16]. Several studies have proven the widespread use of probiotics in aquaculture, including lactic acid bacteria and yeast. Meanwhile, Bacillus spp. has emerged as a potential trend owing to its favorable qualities [17, 18].

In aquaculture, *Bacillus coagulans*, a spore-forming gram-positive bacterium, has often been employed as a feed addition owing to its combination of lactic acid bacteria and Bacillus characteristics [19, 20]. Moreover, *B. coagulans* is renowned for its ability to survive high temperatures, acidity, and bile salts, as well as its power to suppress enteropathogens [21, 24]. Several research have indicated that *B. coagulans* improves growth performance, boosts immunological function, controls gut microbiota, and protects against illness [25, 26].

Previous study has suggested that *Bacillus coagulans* has favorable benefits by colonizing the intestines of animals, enhancing intestinal health, and repelling infections [27]. This probiotic bacterium benefits the host's digestive system by supporting a healthy gut microbiota, increasing the immune response, and preventing dangerous microbe development. The capacity of *B. coagulans* to survive extreme circumstances such as high temperatures, acidity, and bile salts further adds to its usefulness as a probiotic addition. Therefore, in probiotic screening, significant focus has been devoted to the colonization potential of bacteria under *in vivo* settings [28]. Effective colonization is vital, since probiotics can only exhibit their health advantages when they effectively remain in the host's gastrointestinal system for a lengthy duration. The introduction of *B. coagulans* (10^9 CFU/g) as a feed supplement boosts its colonization capability by promoting interactions with the host's biochemical and physiological systems. These interactions contribute in the creation and maintenance of a stable and healthy microbial population in the gut. Consequently, adhesion ability and colonization capacity have become essential factors for probiotic selection, assuring the long-term efficacy of probiotics in enhancing gut health and disease resistance [29].

The increased focus on sustainable aquaculture underlines the potential of probiotics in improving fish health, minimizing environmental impact, and raising overall production in *Cyprinus carpio* farming. However, issues such as strain-specific effectiveness, appropriate doses, and long-term ecological consequences persist. This work intends to address these gaps by examining the function of *B. coagulans* (10^9 CFU/g) in *C. carpio* aquaculture, concentrating on its colonization capability in the gut and its biochemical and physiological impacts throughout developmental stages.

The study studies the influence of *B. coagulans* on growth performance, immunological function, and gut microbiota modification. Various biochemical assessments, including total protein, total cholesterol, glucose, and glycogen levels, were undertaken. Enzymatic activities were examined by measuring alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminase (ALT), and aspartate transaminase (AST). By studying probiotic uses in *C. carpio*, this work adds to the greater purpose of building sustainable and ecologically responsible aquaculture operations, assuring long-term sustainability in both food production and the ornamental fish business.

2. Objectives

The major purpose of this research is to examine the function of *Bacillus coagulans* (10^9 CFU/g) as a probiotic feed additive in *Cyprinus carpio* aquaculture, concentrating on its colonization capabilities, biochemical effects, and overall influence on fish health. This study intends to analyze its impact on growth performance, function, and gut microbiota modification. Specific biochemical markers, including total protein, cholesterol, glucose, and glycogen levels, will be evaluated with enzymatic activities such as alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminase (ALT), and aspartate transaminase (AST).

3. Methods

Collection and Acclimatization of Fish

Fingerlings of *Cyprinus carpio (L.)* (*C. carpio*) were purchased from the Fishery Department, B.R. Project, located around 5 km from the Jnana Sahyadri campus,

Shankaraghatta. Upon arrival, the fish were brought to the laboratory and acclimatized under controlled circumstances for the appropriate time before research. The chosen fingerlings had an average weight of 15 ± 1g. During the acclimation phase, the fish were kept on a commercially available pelleted floating carp feed, which served as the baseline diet for the experiment. Feeding was carried out twice daily to achieve adequate adaption to the laboratory settings.

Commercial Feed Probiotics

The gut probiotic *Bacillus coagulans* (*B. coagulans*) (10^9 CFU/g) was purchased from Sanzyme Biologics Private Limited, Hyderabad. This commercially available probiotic was introduced into the experimental meal to examine its effects on the growth, biochemical, and enzymatic parameters of *Cyprinus carpio* fingerlings.

Experimental Design

A 60-day feeding trial was done using *C. carpio* fingerlings of equal weight, which were fed at 3% of their body weight with a food containing 32% crude protein. The fish were randomly assigned to four treatment groups: a control group (C) that received a standard commercial diet without probiotics, and three experimental groups supplemented with *B. coagulans* at different concentrations—0.05×10^9 CFU g^{-1} (T1), 0.1×10^9 CFU g^{-1} (T2), and 0.15×10^9 CFU g^{-1} (T3).

To determine the impact of probiotic supplementation, fish from each group were slaughtered on the 20th, 40th, and 60th days, and tissue samples from the liver, muscle, and intestine were obtained for biochemical examination. Various biochemical measurements were done using recognized procedures, including total protein measurement by Lowry method [28], total cholesterol determination by Zak's method [29], and glucose and glycogen calculation using the Anthrone method [30]. Enzymatic activities were examined by measuring alkaline phosphatase (ALP) following method [31], acid phosphatase (ACP) by Bergmeyer method [32], and alanine transaminase (ALT) and aspartate transaminase (AST) using the IFCC method [31].

Biochemical Estimations

To analyze the biochemical composition of *C. carpio* fingerlings, essential parameters were assessed using well-established techniques. Total protein content was evaluated using the Lowrie's method [28], where peptide bonds react with the Folin-Ciocalteu reagent to create a detectable colorimetric response. Total cholesterol levels were determined utilizing Zak's method [29], which involves the interaction of cholesterol with ferric chloride and sulfuric acid for spectrophotometric measurement. Glucose and glycogen concentrations were evaluated utilizing the anthrone method [30], wherein carbohydrates are digested into furfural derivatives, yielding a measurable absorbance. To analyze enzyme activity, acid phosphatase (ACP) was quantified according to Bergmeyer method [32], while alkaline phosphatase (ALP) levels were evaluated [31], both relying on substrate hydrolysis and subsequent phosphate release. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed using the International Federation of Clinical Chemistry (IFCC) technique [31], which tracks NADH oxidation in a coupled reaction system. These biochemical assessments gave useful information into the metabolic and enzymatic responses of the fish to probiotic treatment.

Statistical Analysis

All experimental data were examined using one-way analysis of variance (ANOVA) to find significant differences across treatment groups. Post hoc comparisons were undertaken using Tukey's multiple range test to find particular group changes. Statistical significance was defined at $P < 0.05$ (5%), assuring robust and dependable results. Data analysis was performed using GraphPad Prism (Version 9.0) for precise computations and graphical depiction.

4. Results

Effects on Biochemical Constituents

The supplementation of *B. coagulans* considerably impacted the metabolic profiles of *C. carpio* fingerlings, with different alterations identified in the liver, muscle, and intestinal tissues across the 60-day feeding study. These modifications were notably obvious in glucose, cholesterol, glycogen, and protein levels, suggesting the potential metabolic benefits of probiotic treatment.

Liver Biochemical Response

In the liver, glucose concentrations changed considerably across the control (C) and probiotic-treated groups (T1, T2, T3) at each assessment point (20th, 40th, and 60th days). Notably, the highest probiotic dose (T3: 0.15×10^9 CFU g^{-1}) led to a significant drop in glucose levels compared to the lower-dose treatments and control (P < 0.0001, F = 51.88; Figure 1). A similar trend was found in cholesterol levels, which were considerably lower in T3 (P < 0.0005, F = 20.11), suggesting increased lipid metabolism. Conversely, glycogen concentration exhibited a steady increase across all time periods, with T3 having the greatest values (P < 0.0002, F = 25.18). Protein content in the liver also climbed consistently from T1 to T3, with T3 demonstrating the highest significant increase (P < 0.0023, F = 12.81), indicating enhanced protein synthesis and overall metabolic efficiency.

Muscle Biochemical Response

Muscle tissue demonstrated a comparable response to probiotic treatment, particularly in glucose metabolism. All probiotic-treated groups demonstrated a substantial drop in glucose concentration compared to the control (P < 0.0285, F = 5.149), with T3 recording the lowest levels (<1.0 mg/mL) by day 60 (Figure 2). Cholesterol and protein levels followed a parallel trend, with substantial reductions in the treated groups relative to the control (P < 0.5831, F = 0.573 for cholesterol; P < 0.0009, F = 17.01 for protein). However, unlike in the liver, glycogen levels in muscle remained essentially unaltered by probiotic administration, demonstrating no statistically significant variation across groups and time points (P = 0.1566, F = 2.294).

Intestinal Biochemical Response

The intestine demonstrated a specific biochemical response to probiotic therapy. Unlike in the liver and muscle, glucose (P = 0.9032, F = 0.103) and glycogen (P = 0.0529, F = 4.147) levels did not reveal statistically significant differences across treatment groups over time, albeit all probiotic-supplemented groups exhibited lower quantities compared to the control (Figure 3). Protein levels, while not substantially different across groups at individual time points, demonstrated notable fluctuations during the study period, as indicated by Tukey's post hoc analysis (P = 0.0031, F = 11.79). Meanwhile, cholesterol levels showed a steady drop among groups, with T3 displaying the lowest concentration (P = 0.0009, F = 16.83), demonstrating that probiotics may alter lipid metabolism in the gut environment.

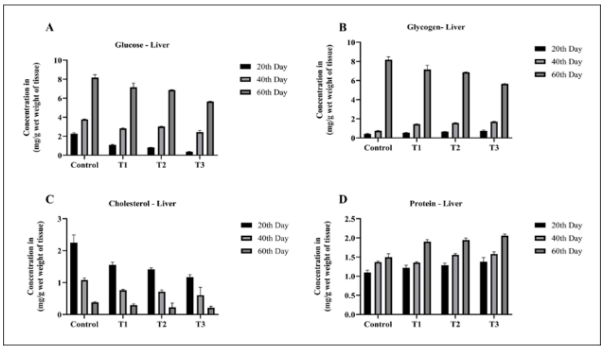

Figure 1: Effect of probiotics on liver glucose, glycogen, cholesterol, and protein levels in different treatment groups over 20, 40, and 60 days.

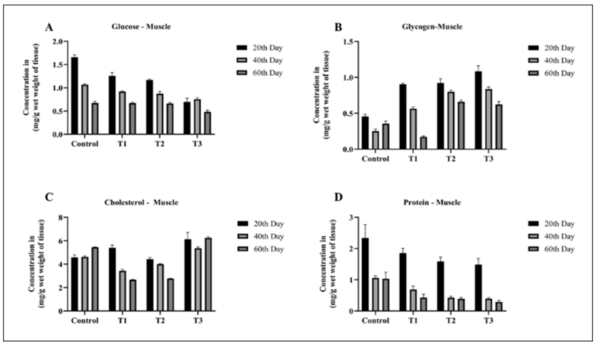

Figure 2: Probiotic effects on muscle glucose, glycogen, cholesterol, and protein levels across treatment groups at 20, 40, and 60 days.

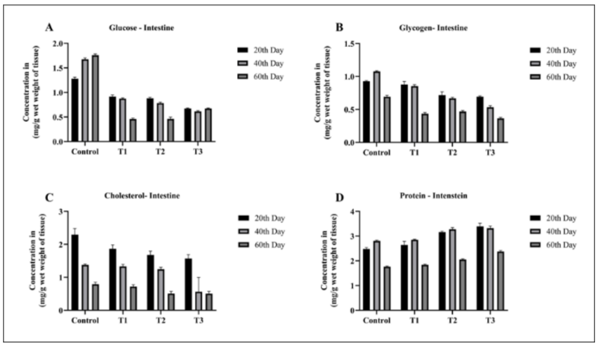

Figure 3: Probiotic effects on intestinal glucose, glycogen, cholesterol, and protein levels across treatment groups at 20, 40, and 60 days.

Effects on Enzyme Activities

The supplementation of *B. coagulans* significantly altered enzyme activities in the liver, muscle, and gut of *C. carpio* fingerlings across the 60-day feeding trial.

Notable changes were detected in acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels, suggesting a probiotic-induced modification of metabolic and physiological processes.

Liver Enzyme Activity

In the liver, ACP levels remained statistically stable throughout time intervals (P = 0.3571, F = 1.157), while groups T2 and T3 displayed a considerable increase by the 60th day (Figure 4A). ALP activity was steady at the 20th and 40th days across all groups, although a considerable elevation was noted in T2 and T3 by day 60 (P = 0.0525, F = 4.161; Figure 4B). AST levels exhibited a progressive drop from T1 to T3, while the differences between time points were not statistically significant (P = 0.5453, F = 0.6492; Figure 4C). However, ALT levels indicated a substantial drop in probiotic-treated groups compared to the control, with the most pronounced decline happening at the 40th and 60th days (P < 0.0002, F = 26.89; Figure 4D).

Muscle Enzyme Activity

In muscle tissue, ACP levels demonstrated an increasing trend from the control to T3, however the differences across time were not statistically significant (P = 0.0729, F = 3.552; Figure 5A). ALP levels, on the other hand, exhibited a significant drop in T1 to T3 by the 60th day (P < 0.0001, F = 55.45; Figure 5B), suggesting probiotic-mediated metabolic changes. AST levels in muscle demonstrated a large decrease in T3 throughout the experimental period (Figure 5C), while ALT levels declined progressively, with T3 display the most pronounced reduction by day 60 (P < 0.0016, F = 14.41; Figure 5D).

Intestinal Enzyme Activity

The gut revealed substantial variability in ACP levels between time intervals and treatments (P < 0.0001, F = 85.73), with major variances between the 20th and 40th days (Figure 6A). ALP levels increased progressively from T1 to T3, however the increases were not statistically significant (P = 0.2406, F = 1.676; Figure 6B). AST levels fell gradually from T1 to T3, with T3 exhibiting the most dramatic reduction compared to the control, suggesting a possible probiotic-induced hepatoprotective effect (Figure 6C). Similarly, ALT levels revealed a considerable dose-dependent drop in T3 across all time periods (Figure 6D), further emphasizing the potential metabolic advantages of *B. coagulans* supplementation.

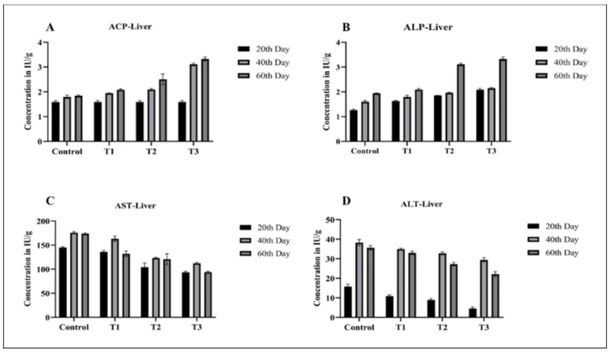

Figure 4: Probiotic effects on ACP, ALP, AST, and ALT levels in the liver across treatment groups at 20, 40, and 60 days.

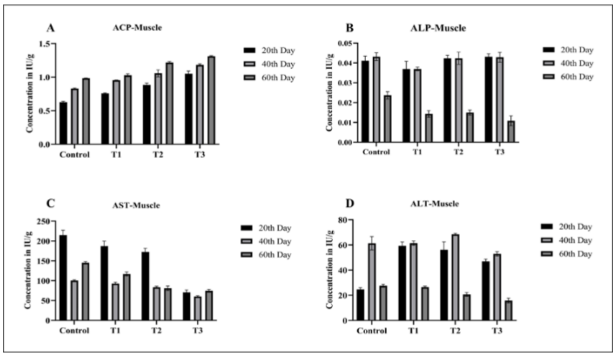

Figure 5: Effect of probiotics on ACP, ALP, AST, and ALT levels in muscle at 20, 40, and 60 days.

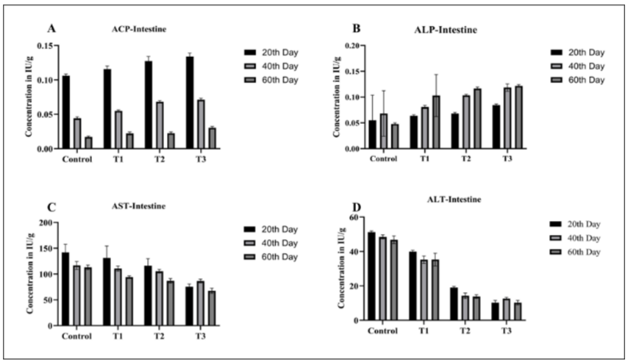

Figure 6: Effect of probiotics on ACP, ALP, AST, and ALT levels in the intestine across treatment groups at 20, 40, and 60 days.

5. Discussion

The health of fish is greatly impacted by both the kind and duration of probiotic therapy. To optimize the advantages of probiotic bacteria in their digestive system, the most effective strategy is via food supplementation. In aquaculture, probiotics may be easily introduced either as feed additives or directly into the water, guaranteeing optimum colonization and boosting overall fish health and resilience [33].

A functional nutritional supplement plays a critical role in enhancing farmed fish's defense against viral illnesses while also improving overall growth performance. Given the increased danger of antibiotic-resistant bacteria in aquaculture, which may severely affect not just fish but also consumers, terrestrial animals, and the environment, worries regarding the use of antimicrobials in aquaculture have risen dramatically. As a consequence, there is an increased focus on sustainable alternatives to antibiotics, such as probiotics and functional nutrition, to promote healthier and more robust fish populations [34].

The aquafeed business attempts to promote the sustainability and efficiency of aquaculture by maintaining optimum fish health, development, and resilience. This is done via nutritionally balanced meals that not only promote development but also boost immunological responses, lowering the need for medicinal treatments. The nutritional condition of farmed fish plays a vital role in determining their immunological health, directly impacting their capacity to survive illnesses and environmental stresses. A well-formulated diet gives a better degree of confidence in sustaining the general well-being and productivity of farmed fish species [35].

This work offers persuasive evidence that nutritional supplementation with *B. coagulans* considerably impacts the biochemical and enzymatic profiles of *C. carpio* fingerlings across a 60-day feeding experiment. The findings of our feeding experiment indicated that glucose levels fell considerably in the highest probiotic dosage group (T3, 0.15×10^9 CFU g^{-1} feed) compared to lower-dose groups (T1, T2) and the control (C) ($P < 0.0001$, $F = 51.88$). This pattern shows that *B. coagulans* increases glucose absorption and utilization, presumably via altering gut microbiota and enhancing insulin sensitivity [36]. The activation of glucose transporters and enhanced glycogenesis further explain this decrease [37].

Similarly, cholesterol levels in the liver declined dramatically in T3 ($P < 0.0005$, $F = 20.11$), confirming the idea that *B. coagulans* stimulates bile salt hydrolase (BSH) activity, which facilitates cholesterol breakdown and excretion [38]. Similar outcomes have been found in *Oreochromis niloticus* and *Labeo rohita* after probiotic administration [39]. Interestingly, liver glycogen levels rose in T3 ($P < 0.0002$, $F = 25.18$), indicating greater glycogenesis, possibly owing to better nutritional absorption and a faster conversion of glucose into glycogen [40]. Protein concentrations revealed a dose-dependent increase, peaking in T3 ($P < 0.0023$, $F = 12.81$), corroborating the probiotic's involvement in boosting protein synthesis. These findings accord with those of [41], who revealed higher hepatic protein concentration in probiotic-fed ornamental fish.

In muscle tissue, critical for fish development and motility, glucose levels dropped gradually across all probiotic-treated groups, with T3 displaying the lowest concentration by day 60 (<1.0 mg/mL; $P < 0.0285$, $F = 5.149$). This drop likely represents enhanced glucose absorption by muscle cells for energy generation, comparable with prior results on probiotic-fed fish [42]. Cholesterol levels followed a similar decreasing trend ($P < 0.5831$, $F = 0.573$), whereas protein content considerably increased ($P < 0.0009$, $F = 17.01$), suggesting better amino acid absorption and utilization, potentially owing to probiotic-induced activation of digestive enzymes [43]. However, muscle glycogen levels remained unaltered ($P = 0.1566$, $F = 2.294$), indicating that hepatic glycogen stores react more dynamically to probiotic intervention than muscle reserves.

In contrast, the gut revealed unique biochemical reactions. While glucose ($P = 0.9032$, $F = 0.103$) and glycogen ($P = 0.0529$, $F = 4.147$) levels exhibited no significant differences across treatment groups, probiotic-supplemented fish consistently reported lower values than the control. This supports improved glucose absorption and delivery to peripheral organs for metabolic use [44]. Protein levels changed throughout time, with significant fluctuations revealed using Tukey's post hoc analysis ($P = 0.0031$, $F = 11.79$), corroborating prior results that probiotics improve intestinal protein synthesis by regulating gut microbiota and nutritional bioavailability [45]. Progressive decreases in intestinal cholesterol levels, notably in T3 (P

= 0.0009, F = 16.83), further underscore the probiotic's involvement in enhancing lipid metabolism—a vital element in intestinal health and lipid homeostasis [46].

Furthermore, the enzymatic activity of acid phosphatase (ACP) and alkaline phosphatase (ALP) in the liver was also considerably influenced by probiotic administration. ACP levels were steady throughout time periods (P = 0.3571, F = 1.157) but rose in T2 and T3 by day 60. ALP levels, initially constant, rose dramatically in T2 and T3 by the conclusion of the experiment (P = 0.0525, F = 4.161), suggesting increased liver function and detoxification, which coincides with probiotic-induced enzyme activation found in other fish species [47].

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels dropped in probiotic-treated groups, with ALT exhibiting a considerable reduction at days 40 and 60 (P < 0.0002, F = 26.89). Lower AST and ALT levels imply lower hepatic stress and enhanced liver function, further supporting the concept that *B. coagulans* mitigates oxidative damage and promotes cellular resilience [48].

Muscle enzyme activity displayed tendencies comparable to those in the liver. ACP levels rose steadily but without statistical significance (P = 0.0729, F = 3.552), whereas ALP levels fell substantially by day 60 in all probiotic-fed groups (P < 0.0001, F = 55.45). AST and ALT levels also reduced, with the most considerable decreases reported in T3 (P < 0.0016, F = 14.41), indicating the probiotic's role in decreasing metabolic stress and improving muscular performance.

In the intestine, ACP levels fluctuated considerably over time (P < 0.0001, F = 85.73), suggesting dynamic enzymatic responses to probiotic therapy. ALP levels showed a consistent rise from T1 to T3 (P = 0.2406, F = 1.676), demonstrating increased gut health and nutritional absorption. AST and ALT levels revealed a steady drop, further demonstrating the probiotic's protective benefits on liver function.

Conclusion

These findings cooperatively highlight *B. coagulans* as a potential probiotic with strong metabolic and enzymatic effects in aquaculture. The observed benefits in glucose metabolism, lipid control, protein synthesis, and enzymatic activity show that dietary probiotics may be a sustainable method for increasing fish health, growth, and resilience. Future research should study the long-term benefits of *B. coagulans* supplementation and its application across various fish species to maximize probiotic-based aquaculture solutions.

Conflicts of Interest:

The authors declare that they have no conflict of interest.

Acknowledgment:

The authors express their deepest gratitude to Dr. Ashashree for the invaluable help provided. They extend their appreciation to the Department of Zoology at Sahyadri College, Kuvempu University, Shimoga, Karnataka, India, for giving the essential research amenities. The authors express gratitude to Stellixir Biotech Pvt. Ltd., Bangalore, for providing crucial laboratory facilities.

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