P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2024.273

Effects of Synbiotic Supplementation on Growth Performance, Hematological Parameters and Gut Health in Nile Tilapia (*Oreochromis niloticus*)

Rahmi [1,*], Nur Insana Salam [1], Andi Ninnong Renita Relatami [2], Amelia Ramadhani Anshar [2], Akmal [3], Mohammad Syaichudin [3], Hidayat Suriyanto Suwoyo [3], Yuani Mundayana [3], Khairun Nisaa [3], Fitri Indah Yani [4] and Emienour Muzalina [5]

[1]Aquaculture Study Program, Faculty of Agriculture, University of Muhammadiyah Makassar, Jl. Sultan Alauddin No. 259, Makassar, 90234, South Sulawesi, Indonesia
[2]Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Jl Perintis Kemerdekaan Km 10 Makassar, 90245, South Sulawesi, Indonesia
[3]National Research and Innovation Agency, Jl. Raya Jakarta-Bogor No. 32, Pakansari, Bogor Regency, 16915, East Java, Indonesia
[4]Aquaculture Study Program, Faculty of Agriculture, University of Muhammadiyah Pare-pare, Jl. Jend. Ahmad Yani No.Km. 6, Bukit Harapan, Soreang, Parepare city, 91112, South Sulawesi
[5]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
***Corresponding author:** rahmiperikanan@unismuh.ac.id

Article History: 24-680 Received: 17-Oct-24 Revised: 04-Dec-24 Accepted: 12-Dec-24 Online First: 27-Dec-24

ABSTRACT

This study aimed to examine the impact of synbiotic supplementation, precisely the combination of the probiotic *Bacillus subtilis* with banana flour as a prebiotic, on Nile tilapia's growth performance, immune response, and gut health. The juvenile tilapia obtained from a regional aquaculture facility were randomly assigned to experimental groups that received different concentrations of the probiotic *B. subtilis* in conjunction with prebiotics derived from banana flour. Throughout the 63-day study period, growth metrics, including weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER), were evaluated regularly. The findings indicated that synbiotic supplementation markedly enhanced growth performance compared to the control group. The results of the hematological evaluations demonstrated elevated red blood cell (RBC) counts, hemoglobin concentrations and white blood cell (WBC) counts, which suggests an enhanced immune response. The histopathological examination of the intestinal tissues indicated that the synbiotic-fed fish displayed increased villus height, crypt depth, and goblet cell density, which suggests that their gut health and nutrient assimilation were improved. In summary, incorporating synbiotics in tilapia diets has resulted in enhanced growth performance, immune functionality, and gastrointestinal health, which presents a promising strategy for advancing sustainable aquaculture practices.

Key words: *Bacillus subtilis.*, Growth, Synbiotic, Tilapia.

INTRODUCTION

Aquaculture has been identified as a rapidly growing sector within the global food production industry. Its expansion can be attributed to two principal factors: the increasing demand for fish protein driven by population growth and shifting dietary preferences. Among the species cultivated within this domain, *Oreochromis niloticus* (Nile tilapia) is particularly notable for its rapid growth rate, adaptability to various environments, and superior nutritional profile (Suwannatrai 2023). Nevertheless, the intensification of aquaculture practices has increased awareness of the importance of fish health, disease outbreaks' prevalence, and environmental practices' sustainability. The extensive application of antibiotics to manage diseases has precipitated the rise of antibiotic-resistant bacteria, which presents significant risks to aquatic organisms and human health (Eor et al. 2021; Gewaily et al. 2021). As a result, exploring alternative strategies becomes imperative to foster sustainable fish growth, health, and immunity (Zhu et al. 2022; Mahmoud et al. 2022; Yostawonkul et al. 2023).

Cite This Article as: Rahmi, Salam NI, Relatami ANR, Anshar AR, Akmal, Syaichudin M, Suwoyo HS, Mundayana Y, Nisaa K, Yani FI and Muzalina E, 2025. Effects of Synbiotic Supplementation on Growth Performance, Hematological Parameters and Gut Health in Nile Tilapia (*Oreochromis niloticus*). International Journal of Veterinary Science 14(3): 470-477. https://doi.org/10.47278/journal.ijvs/2024.273

Recent scholarly inquiry highlights the potential of probiotics and prebiotics as beneficial dietary supplements for improving fish health and promoting sustainable aquaculture practices. Symbiotics, defined as a combination of probiotics and prebiotics, have garnered scholarly interest due to their potential to enhance and augment nutrient absorption, immune responses and gastrointestinal health in farmed fish species (Afrose et al. 2022; Magouz et al. 2023; Pardede et al. 2024). Probiotics, defined as live microorganisms, confer health benefits to the host organism, whereas prebiotics serve as a source of nutrition for beneficial gut microbiota, promoting their proliferation and functional activity (Zhu et al. 2022). In light of the aquaculture industry's pursuit of alternatives to antibiotic use, synbiotics emerge as a viable solution to enhance fish productivity and sustainability (El-Nobi et al. 2021; Sîrbu et al. 2022; Munni et al. 2023).

Despite the promising advantages associated with synbiotics, considerable gaps persist in understanding the specific mechanisms through which they affect fish growth and health, particularly in commercially significant species such as Nile tilapia. While some studies have indicated favorable outcomes, including enhancements in growth performance, immune functionality, and gut morphology, others have documented variability contingent upon the formulation and dosage of the synbiotics employed (Dehaghani et al. 2015; Gewaily et al. 2021; Mogouz et al. 2023). This inconsistency challenges the pragmatic application of synbiotics within commercial aquaculture, where dependable and predictable results are critical for optimizing production processes (Sîrbu et al. 2022). Therefore, further comprehensive investigations are required to elucidate specific synbiotic combinations' effects on tilapia's growth and health.

This study aims to evaluate the effects of a synbiotic formulation that integrates *B. subtilis* and banana flour on the growth performance, immune response, and histological characteristics of the gut in Nile tilapia. The objective is to enhance understanding regarding how varying concentrations of synbiotics impact these vital parameters, thus providing insights into the practical applications of synbiotics within tilapia aquaculture. Prior research has proposed that synbiotics may facilitate enhanced nutrient absorption by improving intestinal morphology, including the augmentation of villus height and crypt depth, which subsequently enhances digestive efficiency and growth performance (Mohammadi et al. 2020; El-Nobi et al. 2021). Furthermore, synbiotics have been demonstrated to elevate immune responses, a critical factor for disease resistance in aquaculture environments (Yang et al. 2021).

MATERIALS AND METHODS

Ethical approval statements

The *in vivo* study was conducted per the ethical standards set forth by the Ethical Principles of Experimentation and the Health and Animal Welfare Ethics Committee of the Public Health Faculty at Hasanuddin University (No. 29824091190). The methodology was approved by the Committee on Research Ethics of the Health and Animal Ethics Department. Throughout the study, every effort was made to reduce the pain and suffering of the experimental fish.

Materials

The juvenile Nile tilapia in this study were maintained in 500-liter tanks for a two-week acclimation period to ensure consistent health and adaptation. Critical water quality parameters were monitored and recorded at three stages: the start (day 0), middle (day 31) and end (day 63) of the experiment. The parameters included temperature (28–30°C), pH (6.5–7.5), dissolved oxygen (>5mg/L), and ammonia (<0.5mg/L), all maintained at optimal levels to support health and growth. Physico-chemical water analyses was conducted weekly to ensure environmental stability. During the acclimation phase, the fish were fed a synbiotic-free commercial diet (MS Prima Feed PF 1000, protein content: 39–41%) to standardize baseline health. After acclimatization, experimental diets were introduced, incorporating *B. subtilis* as a probiotic and banana flour as a prebiotic, which were blended into the commercial feed. Fish were fed three times daily at 3% of their body weight, with feeding rates adjusted weekly based on average weight. These controlled stages of acclimatization, rigorous water quality monitoring, and precise dietary preparation ensured optimal experimental conditions to evaluate the effects of synbiotic supplementation on growth performance, hematological parameters, and gut health in Nile tilapia.

Experimental design

The experimental study was designed to evaluate the effects of synbiotic supplementation on Nile tilapia over a period of 63 days. A randomized design was employed, incorporating four treatment groups and a control group, each replicated three times. The treatment groups were provided with diets supplemented with varying concentrations of a synbiotic formulation consisting of *B. subtilis* as the probiotic and banana flour as the prebiotic. In contrast, the control group received a basal diet devoid of synbiotics. This design aimed to investigate the optimal concentration of synbiotics for enhancing growth, immune response, and gut health.

The treatment groups were distinguished based on the concentrations of *B. subtilis* included in the diets. The groups were designated as follows: A (control), B (1×10^5 CFU/mL), C (1×10^7 CFU/mL) and D (1×10^9 CFU/mL). These concentrations were carefully prepared by culturing *B. subtilis* in a sterile nutrient broth medium under aerobic conditions at 37°C for 24 hours. The bacterial cultures were diluted with sterile distilled water to achieve the desired CFU levels. To prepare the prebiotic component, banana flour was produced by drying unripe bananas, grinding them into powder, and sterilizing them (Rahmi et al. 2024). The synbiotic formulations were blended with a commercial feed to ensure uniformity. The prepared feed was air-dried and stored under refrigeration to preserve its quality.

Fish from each group were housed in aquariums measuring 50×30×30cm, with 20 fish per aquarium, following established stocking density guidelines. Feeding was done thrice daily at 08:00, 15:00, and 18:00 WITA. The feeding rate was set at 3% of the fish's body weight and adjusted weekly based on the average weight of the fish in each group. Water exchanges were performed weekly, with 50% of the water volume replaced to maintain optimal hygiene and environmental conditions.

Water quality parameters were closely monitored throughout the experiment to ensure the health and well-being of the fish. Key parameters included water temperature, maintained at 28–30°C using a digital water heater; pH levels, stabilized between 6.5 and 7.5 with a calibrated pH meter; and dissolved oxygen (DO), kept above 5mg/L using a portable DO meter. Ammonia levels were also assessed weekly using a colorimetric ammonia test kit, ensuring concentrations remained below the harmful threshold of 0.5mg/L. Weekly averages for these parameters were calculated to ensure consistent and reliable conditions across all groups, supporting the validity of the experimental results.

Parameters measured

The growth performance of the fish was evaluated using established criteria, including Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER). These parameters were calculated as follows:

- WG: Determined by subtracting the initial weight (Wi) from the final weight (Wf) of the fish: WG=Wf−Wi, where Wf represents the final weight, and Wi represents the initial weight (Radwan et al. 2023).
- SGR: Calculated as the percentage increase in body weight per day: SGR (%)=(ln (Wf)−ln (Wi))/t × 100, where t represents the duration of the experimental procedure (Ricker 1979).
- FCR: Represents the efficiency of feed utilization, calculated as the total feed intake divided by the total weight gain: FCR=Total Feed Intake/Total Weight Gain (Tacon 1990).
- PER: Evaluates the efficiency of dietary protein conversion into body weight gain: PER=Weight Gain/Protein Consumed (Mitchell 1989).

These parameters facilitate a comprehensive evaluation of the growth performance of tilapia subjected to various dietary interventions (Urach et al. 2020; Magouz et al. 2023).

Hematological analysis was performed to assess blood composition and evaluate the immune response of the fish. At the end of the experimental period, blood samples were collected from the caudal vein using sterile syringes and transferred into tubes coated with ethylenediaminetetraacetic acid (EDTA) to prevent clotting (Argungu et al. 2017). Key hematological parameters, including Red blood cell (RBC) count, hemoglobin (Hb) concentration, and white blood cell (WBC) count, were measured using an automated hematology analyzer (Hrubec et al. 2000). These parameters provide insights into oxygen transport capacity and immune functionality.

Histopathological analysis was conducted to assess the microscopic structure of the intestine. Intestinal tissue specimens were collected from three fish's anterior, middle, and posterior regions per treatment group (Fig. 3). The specimens were preserved in 10% formalin for 24 hours, dehydrated with graded ethanol solutions, and embedded in paraffin wax. Tissue sections of 5µm thickness were prepared and stained with hematoxylin and eosin (H&E). The histological features analyzed included villus height, crypt depth, and goblet cell density. These features were quantified using a light microscope with a digital camera and image analysis software to ensure accuracy and reproducibility (Liu et al. 2016; Sahiti et al. 2018; Dawood et al. 2020; Wakkas 2023).

Statistical analysis

The data on growth performance, hematological assessments, and histopathological investigations were subjected to statistical analysis using the SPSS software (version 25.0) (IBM Corp 2017). The normality of the data was evaluated using the Shapiro-Wilk test and the homogeneity of variances was assessed using Levene's test to ensure the validity of the parametric analysis. Differences between the treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons to identify significant differences among groups. A statistical significance threshold was set at $P<0.05$. The results are expressed as means ± standard deviation (SD), providing clarity and precision in the presentation of the measured parameters. The statistical methodologies employed followed established practices for biological and aquaculture research (Zar 2010; Field 2017).

RESULTS & DISCUSSION

Growth performance

The growth performance of Nile tilapia (*O. niloticus*) was subjected to comprehensive evaluation over a 63-day experimental period, employing scientifically established metrics, including weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER) (Fig. 1). The finding indicated that dietary supplementation with synbiotics, particularly *B. subtilis*, led to a notable enhancement in growth performance across all measured metrics in comparison to the control group. Of the treatment groups, treatment B (1×10^5 CFU/mL) yielded the most notable results, recording the highest WG, SGR, and PER values while maintaining the lowest FCR, indicating improved feed efficiency and nutrient utilization. Following treatment B and treatment D (1×10^9 CFU/mL), substantial but slightly reduced improvements were demonstrated. Conversely, treatment C (1×10^7 CFU/mL) showed only moderate enhancements. The control group (A) exhibited the lowest growth performance, confirming the absence of benefits from symbiotic supplementation. By designating groups with alphabetical identifiers, data trends were more effectively analyzed, underscoring the critical role of symbiotic concentration in maximizing growth efficiency. This study highlights the potential of *B. subtilis* as a dietary supplement to support sustainable aquaculture practices by enhancing growth metrics and feed efficiency in Tilapia.

The fish in the treatment group that received supplementation with treatment C of *B. subtilis* exhibited the highest mean weight gain, indicating that probiotics effectively enhanced nutrient absorption and utilization, thereby fostering superior growth. The feed conversion ratio (FCR) was markedly diminished in this cohort, indicative of enhanced feed efficiency compared to the control group. A lower FCR indicated a reduced amount of feed required for weight gain, offering economic and

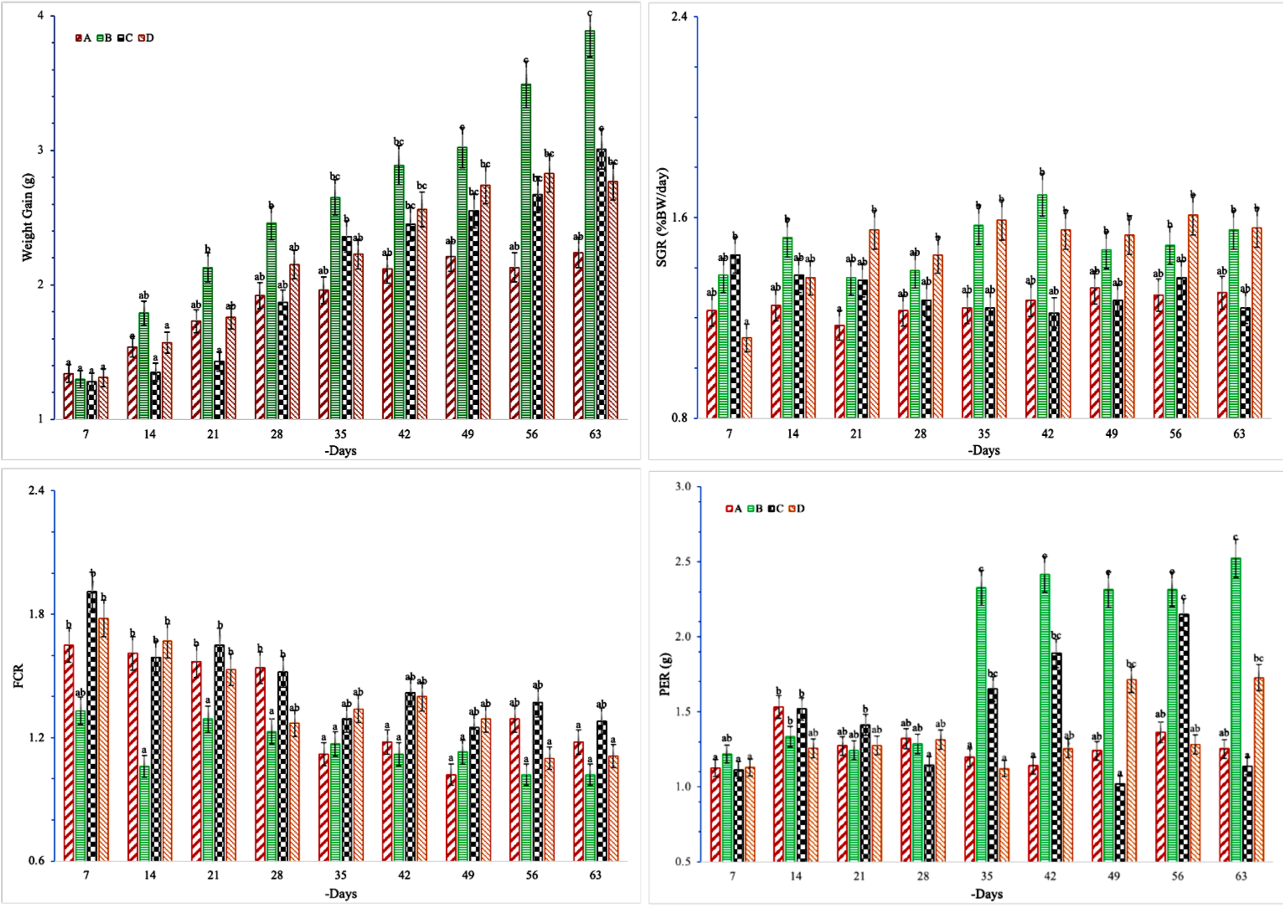

Fig. 1: Growth performance metric, including weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR), and protein efficiency ratio (PER) of Nile tilapia cultured over a 63-day period. The X-axis represents the experimental groups: control (A), 1×10^5 CFU/mL (B), 1×10^7 CFU/mL (C), and 1×10^9 CFU/mL (D), while the Y-axis displays the respective values for each treatment.

environmental benefits. These findings are consistent with those reported by Panase et al. (2023), who observed comparable improvements in fish growth metrics through probiotic supplementation, thereby reinforcing its role in sustainable aquaculture.

The specific growth rate (SGR) reached its maximum value in the treatment group receiving treatment C, exhibiting a markedly accelerated growth trajectory compared to the other groups. These findings indicate that the optimal dosage of *B. subtilis* probiotics enhanced the fish's ability to gain body weight efficiently over the experimental period. Similarly, the Protein Efficiency Ratio (PER) was highest in the same group, highlighting the improved conversion of dietary protein into body mass. These results are consistent with those reported by Wang (2023), who noted that probiotics optimize nutrient metabolism and protein utilization in fish, thereby supporting their role in sustainable aquaculture practices.

The results of the statistical analysis indicated that there were statistically significant differences ($P<0.05$) between the control and treatment groups. The treatment group that received treatment C demonstrated the most pronounced improvements across all growth metrics. This concentration of *B. subtilis* markedly enhanced growth performance in Nile tilapia, underscoring its effectiveness as a dietary supplement. These results align with those of previous studies by Panase et al. (2023) and Wang (2023), which demonstrated that probiotics improve growth parameters, nutrient absorption, and feed efficiency in aquaculture. This highlights the potential of probiotics for sustainable fish farming practices.

Hematological parameters

Hematological Analysis Hematological indices, including red blood cell (RBC) count, hemoglobin (Hb) concentration, and white blood cell (WBC) count, were monitored regularly across various treatment groups to determine the impact of synbiotic supplementation on the immune response of Nile tilapia (Fig. 2).

The experimental group of fish that received supplementation with *B. subtilis* exhibited markedly elevated RBC counts compared to the control group. The group administered 1×10^5 CFU/mL of *B. subtilis* exhibited the most pronounced RBC count, closely followed by those groups exposed to higher dosages (1×10^9 CFU/mL and 1×10^7 CFU/mL). These augmented RBC counts imply an enhanced capacity for oxygen transport, which is essential for accommodating the metabolic requirements of developing fish.

A comparable pattern was observed in the hemoglobin concentrations, whereby in the 1×10^5 CFU/mL group exhibited the highest Hb levels, which were significantly higher than those of the control group. The observed increase in hemoglobin concentrations indicated an enhanced capacity for oxygen transport and a general improvement in health status, thereby reflecting the favorable impact of symbiotic supplementation on the overall well-being of the fish.

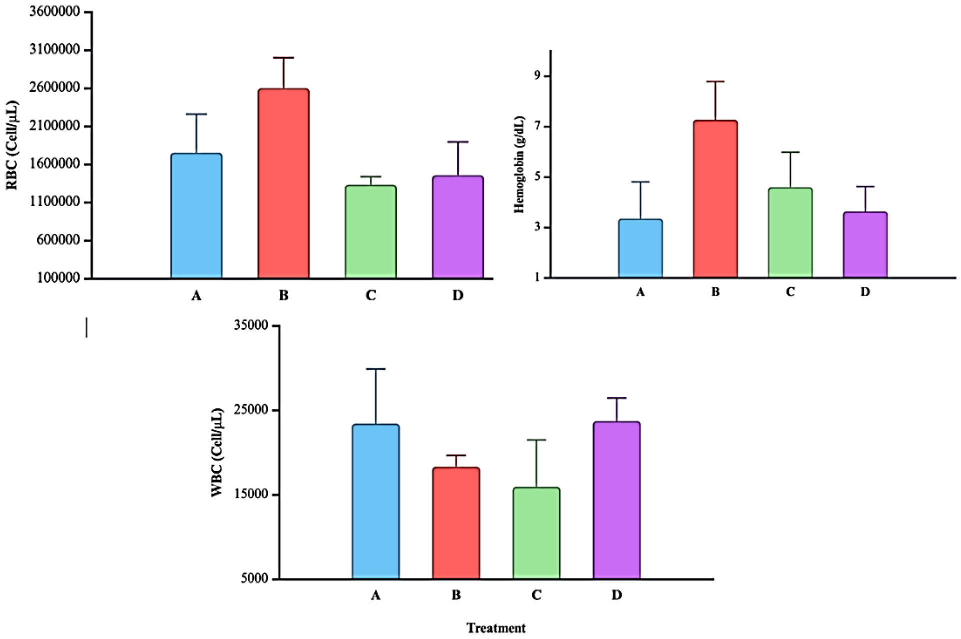

Fig 2: Red Blood Cell (RBC), Hemoglobin, and White Blood Cell (WBC) of Tilapia fish cultured over a duration of 63 days. The X-axis represents the experimental groups: control (A), 1×105 CFU/mL (B), 1×107 CFU/mL (C), and 1×109 CFU/mL (D), while the Y-axis displays the respective values for each treatment

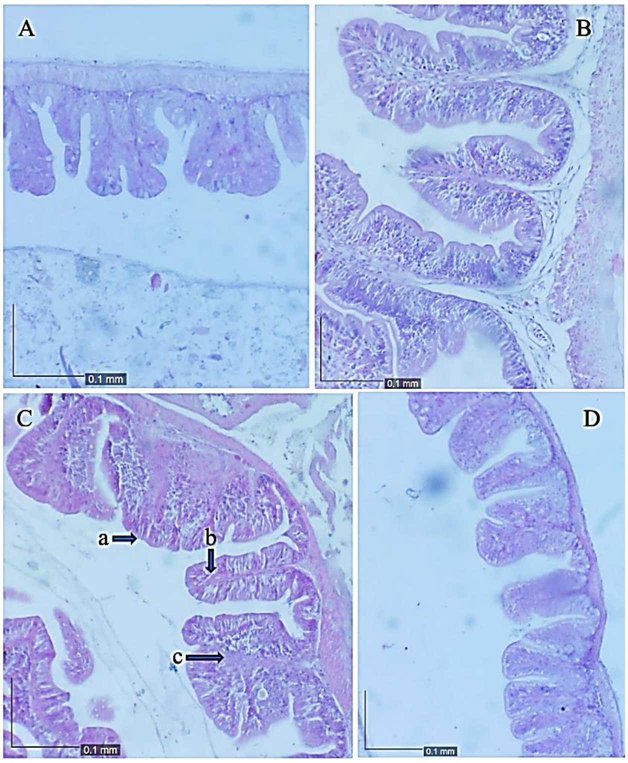

Fig 3: Histopathology of Tilapia Fish Intestine at 40× 10× magnification measuring changes in the structure and integrity of intestinal tissue. A= control (anterior region); B=1×10^5 CFU/mL probiotic (posterior region); C=1×10^7 CFU/mL probiotic (middle region); and D=1×10^9 CFU/mL probiotic: (a) Villus height; (b) Crypt depth; (c) Goblet cell density. Scale Bar=0.1mm.

The WBC count, a vital parameter indicative of immune functionality, was significantly elevated across all groups receiving synbiotics compared to the control group. The treatment group that received 1×10^5 CFU/mL exhibited the highest WBC count, indicating an enhanced immune response. The observed elevation in WBC levels indicates enhanced protection against pathogens, thereby suggesting that symbiotic supplementation has augmented the fish's immune system. The statistical analysis revealed significant disparities between the groups treated with synbiotics and the control group across all hematological metrics ($P<0.05$). Fish subjected to the 1×10^5 CFU/mL dosage of *B. subtilis* consistently exhibited superior RBC, Hb, and WBC levels, suggesting that this particular dosage of symbiotics was most effective.

These findings corroborate those of previous investigations which have illustrated the advantageous effects of probiotics and synbiotics on hematological parameters in fish, contributing to enhanced immune functionality and increased resilience to stressors and diseases (Dawood et al. 2019; Munni et al. 2023).

Histopathology

The histopathological examination of the intestines of Nile tilapia focuses on key indicators of gastrointestinal health, including villus height, crypt depth, and goblet cell density. These parameters are fundamental to evaluating nutrient absorption efficacy and the overall integrity of the intestinal tract (Fig. 3). Villus height was quantified. The administration of synbiotic supplementation resulted in a statistically significant increase of villus height within the intestinal tissues of tilapia. The cohort of fish that received the treatment at a concentration of treatment C exhibited the most pronounced increase in villus height compared to the control cohort. The increase in villus height is associated with expanding the surface area available for nutrient absorption, facilitating enhanced growth performance.

Notable increases in crypt depth were observed in the experimental groups, particularly among those who received concentrations of *B. subtilis* treatment C and treatment D. An increase in crypt depth serves as an indicator of enhanced cellular turnover, which promotes the accelerated regeneration of intestinal cells, a factor crucial for maintaining gut health and overall efficiency (Dawood et al. 2020).

The groups that were administered synbiotics exhibited a notable increase in goblet cell density, with the treatment group receiving treatment C demonstrating the highest density. Goblet cells play a vital role in synthesizing mucus, which protects the gut lining against pathogens and mechanical injury. The increase in goblet cell density indicates that mucosal protection has been strengthened, thereby enhancing the fish's ability to combat intestinal infections and maintain gut integrity (Liu et al. 2016).

The histopathological increases in villus height, crypt depth, and goblet cell density observed in the synbiotic-treated cohort were significantly (P<0.05) superior to those documented in the control group (Table 1). These findings indicate that synbiotic supplementation, particularly at the concentration of treatment C, exerts a beneficial influence on the intestinal morphology of tilapia, thereby contributing to enhanced nutrient absorption and gut health (Adeoye et al. 2016; Ciptaan et al. 2024).

Table 1: Histopathological Measurements of Nile Tilapia (*O. niloticus*)

Treatment Groups	Villus Height (mm)	Crypt Depth (mm)	Goblet Cell Density (mm)
A	0.300±0.84a	0.100±0.38a	0.075±0.62a
B	0.500±0.79c	0.180±0.55c	0.120±6.38b
C	0.400±1.26b	0.150±0.67b	0.100±3.14b
D	0.450±4.96b	0.160±1.47b	0.110±0.08b

Different superscripts in each column indicate significant (P<0.05) differences.

These findings align with those of previous research, which has demonstrated the benefits of probiotics and synbiotics in promoting gut health in aquaculture species. The improvement of intestinal morphology is associated with enhanced nutrient utilization, growth performance, and disease resistance (Adeoye et al. 2016; Dawood et al. 2020; Sultana et al. 2024).

The results of this study demonstrate that synbiotic supplementation, specifically the combination of *B. subtilis* and banana flour, significantly enhances Nile tilapia's growth performance, immune functionality, and gut health. These findings are consistent with previous studies that have demonstrated the positive influence of probiotics and prebiotics on fish health and the sustainability of aquaculture (Afrose et al. 2022; Sîrbu et al. 2022).

The administration of synbiotic supplementation resulted in significant improvements across several growth parameters, including WG, FCR, SGR, and PER. The group that received the treatment at treatment C of *B. subtilis* exhibited the highest metrics across all evaluated growth parameters, indicating that this concentration optimally enhances nutrient absorption and feed utilization. The observed improvements in FCR and PER indicate that synbiotics augment digestive efficiency, enabling fish to utilize feed more effectively, culminating in enhanced growth outcomes. This observation is consistent with previous studies that have demonstrated that *B. subtilis* enhances intestinal morphology and nutrient metabolism in fish (Panase et al. 2023; Wang 2023).

It is proposed that the administration of synbiotics has facilitated these enhancements through the augmentation of the gastrointestinal milieu, thereby fostering the proliferation of probiotic microorganisms and ameliorating enzymatic functionality. Such modifications are instrumental in optimizing the assimilation of nutrients and fostering superior growth trajectories. Investigations on alternative piscine species, such as the red drum and hybrid striped bass, have similarly corroborated that synbiotics augment digestive enzymes and intestinal morphology, culminating in enhanced growth efficacy (Anguiano et al. 2012).

The results of this investigation indicate that the administration of symbiotics has a beneficial impact on hematological parameters, especially the erythrocyte count, hemoglobin levels, and leukocyte count. The group that received treatment C exhibited the most significant enhancements, indicative of augmented oxygen transport capacity and immune competency. The observed elevation in erythrocyte and hemoglobin concentrations implies improved overall vitality and metabolic proficiency. The surge in leukocyte counts, in turn, denotes a fortified immune apparatus, thereby enabling the fish to exhibit greater resilience against pathogenic incursions. These findings are consistent with previous research indicating that symbiotics enhance fish immunity by elevating immune-related metrics, including lysozyme activity and phagocytic response (Dawood et al. 2019). The enhanced hematological profile suggests that synbiotics promote growth and improve fish's health and resilience, thereby reducing the reliance on antibiotics in aquaculture settings (Dawood et al. 2016).

Histopathological examinations of the intestinal tissues revealed that fish subjected to synbiotic feeding demonstrated significantly enhanced villus height, crypt depth, and goblet cell density.

The treatment C cohort showed the most significant improvements in all three of these parameters. The augmentation of villus height increases the absorptive surface area for nutrients. In contrast, deeper crypts facilitate accelerated regeneration of intestinal cells, both of which contribute to improved growth and health outcomes. The increased density of goblet cells indicates enhanced mucosal defense, vital for preventing infection and preserving gut integrity. These observations align with previous research indicating that probiotics enhance intestinal morphology and mucosal protection in aquaculture species, thereby improving nutrient utilization and disease resistance (Adeoye et al. 2016; Liu et al. 2021). The enhanced intestinal health documented in this inquiry suggests that the supplementation of synbiotics can bolster nutrient absorption and gut barrier functionality, thereby supporting optimal growth and immune responsiveness (Dawood et al. 2018).

The findings of this study have significant implications for the advancement of sustainable aquaculture methodologies. Incorporating synbiotics, particularly those derived from *B. subtilis*, presents a feasible alternative to the application of antibiotics to enhance fish health and productivity. By simultaneously improving growth performance and immune functionality, synbiotics may contribute to attenuating disease incidents and minimizing antibiotic usage, thus addressing the pressing issue of antibiotic resistance within aquaculture. Future research should optimize synbiotic formulations and dosages tailored to various aquaculture species and production paradigms. Additionally, further investigations are required to elucidate how synbiotics enhance fish health, particularly the interplay between gut microbiota, immune responses, and nutrient metabolism.

Conclusion

The administration of synbiotic supplements, particularly those containing *Bacillus subtilis*, has markedly enhanced growth performance, immune functionality, and gastrointestinal health in Nile tilapia (*Oreochromis niloticus*). This methodology presents a viable approach for advancing sustainable aquaculture practices by promoting fish health and productivity while minimizing the dependence on antibiotic interventions. Subsequent research efforts should concentrate on optimizing synbiotic formulations and their respective dosages tailored for various aquaculture species and production systems, in addition to elucidating the specific mechanisms through which synbiotics contribute to enhancing fish health.

Acknowledgment: The authors express profound gratitude for the assistance rendered by the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia, via grant number 0536/E5/PG.02.00/2023. This aid was essential for the successful realization of this research project.

Author Contribution: R: The first author conceptualized, supervised, and designed the study, conducted literature searches, prepared figures, managed administration, secured funding, and revised the manuscript. A: Contributed to conceptualization, study design, supervision, literature search, figure preparation, and writing and revising the manuscript. NIS: Designed the study, performed software tasks, conducted validation, data analysis, and data collection, and wrote and revised the manuscript. ANRR: Conducted software tasks and data collection and managed resources. ARA: Performed software tasks, conducted validation, data analysis, and data collection. MS: Validated the study and contributed to data collection and resources. HSS: Contributed to data collection and resources. YM: Assisted with data collection and resource management. KN: Conducted literature search and data collection, prepared figures, and wrote and revised the manuscript. FIY: Contributed to data collection and resource management. EM: Assisted with data collection and resource management.

REFERENCES

Adeoye A, Yomla R, Jaramillo-Torres A, Rodiles A, Merrifield D and Davies S, 2016. Combined effects of exogenous enzymes and probiotic on Nile tilapia (*Oreochromis niloticus*) growth, intestinal morphology and microbiome. Aquaculture 463: 61-70. https://doi.org/10.1016/j. aquaculture.2016.05.028

Afrose F, Islam MR, Nasren S, Hossain MA and Iqbal MM, 2022. Do dietary synbiotics modulate growth performance and haematological properties of tilapia, *Oreochromis niloticus*? Journal of Limnology and Freshwater Fisheries Research 8(2): 131-139. https://doi.org/10.17216/limnofish.855306

Anguiano M, Pohlenz C, Buentello A and Gatlin D, 2012. The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*). British Journal of Nutrition 109(4): 623-629. https://doi.org/10.1017 /s0007114512001754

Argungu LA, Siraj SS, Christianus A, Amin MSN, Daud SK, Abubakar MS, Abubakar IA and Aliyu-Paiko M, 2017. A simple and rapid method for blood collection from walking catfish, *Clarias batrachus* (Linneaus, 1758). Iranian Journal of Fisheries Sciences 17(3): 935-944. https://doi.org/10. 22092/ijfs.2018.114710

Ciptaan G, Mirnawati, Martaguri I, Fajrona K and Srifani A, 2024. Enhancing the quality and nutrient content of soybean milk waste as poultry feed through fermentation with Bacillus subtilis. International Journal of Veterinary Science 13(2): 154-159. https://doi.org/10.47278/journal.ijvs/2023.076

Dawood MAO and Koshio S, 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. Aquaculture 454: 243-251. https://doi.org/10.1016/j .aquaculture.2015.12.033

Dawood MAO, Koshio S and Esteban MÁ, 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: A review. Reviews in Aquaculture 10(4): 950–974. https://doi.org/10.1111/raq.12209

Dawood MA, Eweedah NM, Moustafa Moustafa E and Shahin MG, 2019. Effects of feeding regimen of dietary Aspergillus oryzae on the growth performance, intestinal morphometry and blood profile of Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition 25(5): 1063-1072. https://doi.org/10. 1111/anu.12923

Dawood M, 2023. Combined effects of water salinity and ammonia exposure on the antioxidative status, serum biochemistry and immunity of Nile tilapia (*Oreochromis niloticus*). Fish Physiology and Biochemistry 49(6): 1461-1477. https://doi.org/10.1007/s10695-023-01267-5

Dawood M, Gewaily M, Soliman A, Shukry M, Amer A, Younis E and Fadl S, 2020. Marine-derived chitosan nanoparticles improved the intestinal histo-morphometrical features in association with the health and immune response of grey mullet (*Liza ramada*). Marine Drugs 18(12): 611. https://doi.org/10.3390/md18120611

Dehaghani P, Baboli M, Moghadam A, Ziaei-Nejad S and Pourfarhadi M, 2015. Effect of synbiotic dietary supplementation on survival, growth performance, and digestive enzyme activities of common carp (*Cyprinus carpio*) fingerlings. Czech Journal of Animal Science 60(5): 224-232. https://doi.org/10.17221/8172-cjas

El-Nobi G, Hassanin M, Khalil A and Mohammed A, 2021. Comparative efficiency of the dietary addition of synbiotic "curazol-m" and norfloxacin on the growth performance, body composition, and histological alteration of the Nile tilapia. Egyptian Journal of Aquatic Biology and Fisheries 25(1): 571-582. https://doi.org/10.21608/ejabf.2021.146710

Eor J, Son Y and Kim S, 2021. The anti-inflammatory and anti-oxidative potential of synbiotics in two independent cell lines. International Journal of Dairy Technology 74(3): 518-527. https://doi.or g/10.1111/1471-0307.12777

Field A, 2017. Discovering Statistics Using IBM SPSS Statistics (5th Edition). SAGE Publications.

Gewaily M, Abdo S, Moustafa E, AbdEl-Kader M, El-Razek I, El-Sharnouby M and Dawood M, 2021. Dietary synbiotics can help relieve the impacts of deltamethrin toxicity of Nile tilapia reared at low temperatures. Animals 11(6): 1790. https://doi.org/10.3390/ani11061790.

Hrubec TC, Cardinale JL and Smith SA, 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). Veterinary Clinical Pathology 29(1): 7-12. https://doi.org/10.1111/j.1939-165X.2000.tb00389.x

IBM Corp. Released, 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Liu Q, Wen L, Pan X, Huang Y, Du X, Qin J and Lin Y, 2021. Dietary supplementation of *Bacillus subtilis* and enterococcus faecalis can effectively improve the growth performance, immunity, and resistance of tilapia against *Streptococcus agalactiae*. Aquaculture Nutrition 27(4):

1160-1172. https://doi.org/10.1111/anu.13256
Liu W, Ran C, Liu Z, Gao Q, Xu S, Ringø E and Zhou Z, 2016. Effects of dietary *Lactobacillus plantarum* and ahllactonase on the control of *Aeromonas hydrophila* infection in tilapia. Microbiologyopen 5(4): 687-699. https://doi.org/10.1002/ mbo3.362
Magouz F, Radwan I, Soltan H and El-Keredy A, 2023. Synbiotic lactic dry® enhanced the growth performance, growth-related genes, intestinal health, and immunity of Nile tilapia reared in inland brackish groundwater. Annals of Animal Science 23(2): 495-504. https://doi.org/10.2478/aoas-2022-0066
Mahmoud H, Farag M, Reda F, Alagawany M and Abdel-Latif H, 2022. Dietary supplementation with *Moringa oleifera* leaves extract reduces the impacts of sub-lethal fipronil in Nile tilapia, *Oreochromis niloticus*. Scientific Reports 12(1). https://doi.org/10.1038/s41598-022-25611-6
Mitchell GV, Jenkins MY and Grundel E, 1989. Protein efficiency ratios and net protein ratios of selected protein foods. Plant Foods for Human Nutrition 39: 53–58. https://doi.org/10.1007/BF01092401
Mohammadi G, Adorian T and Rafiee G, 2020. Beneficial effects of *Bacillus subtilis* on water quality, growth, immune responses, endotoxemia and protection against lipopolysaccharide-induced damage *Oreochromis niloticus* under biofloc technology system. Aquaculture Nutrition 26(5): 1476-1492. https://doi.org/10.1111/anu.13096
Munni M, Akther K, Ahmed S and Roy, 2023. Effects of probiotics, prebiotics and synbiotics as an alternative to antibiotics on growth and blood profile of Nile tilapia (*Oreochromis niloticus*). Aquaculture Research 2023: 1-12. https://doi.org/10.1155/2023/2798279
Panase A, Thirabunyanon M, Promya J and Chitmanat C, 2023. Influences of *Bacillus subtilis* and fructooligosaccharide on growth performances, immune responses, and disease resistance of Nile tilapia, *Oreochromis niloticus*. Frontiers in Veterinary Science 9: 1094681. https://doi.org/10.3389/fvets .2022.1094681
Pardede M, Widanarni W, Sukenda S and Yuhana M, 2024. Evaluation of dietary microencapsulated synbiotic *Pseudoalteromonas piscicida* 1ub, bacillus np5, and mannan-oligosaccharides to prevent *Vibrio parahaemolyticus* infection in pacific white shrimp *Penaeus vannamei*. Aquaculture Internation 32: 2077-91 https://doi.org/10.21203/rs.3.rs-2568513/v1
Radwan M, El-Sharkawy MA, Alabssawy AN, Ghanem SF, Mohammadein A, Al-Malki JS, Al-Thomali AW, Manaa EA, Soliman RA, Yassir S, Mekky AE, Bashar MAE and Kareem F, 2023. The synergy between serious parasitic pathogens and bacterial infestation in the cultured Nile tilapia (*Oreochromis niloticus*): a severe threat to fish immunity, causing mass mortality and significant economic losses. Aquaculture Internation 31: 2421–2449. https://doi.org/10.1007/s10499-023-01093-9
Rahmi, Relatami ANR, Anshar AR, Akmal, Syaichudin M, Firman SW, Tampangallo BR, Mundayana Y, Chadijah A, Nisaa K, Salam NI, Masriah A, Ikbal M, Indahyani F, Hoven ID and Muzalina E, 2024. Growth analysis and innate immune response of tilapia (*Oreochromis niloticus*) fed with synbiotic feeds in brackish water. International Journal of Veterinary Science 13(3): 291-299. https://doi.org/10.47278 /journal.ijvs/2023.100
Ricker WE, 1979. Growth rates and models. In: Fish Physiology, Academic Press 8: 677–743. https://doi.org/10.1016/S1546-5098(08)60126-7
Sahiti H, Bislimi K, Dalo E and Murati K, 2018. Effect of water quality in hematological and biochemical parameters in blood of common carp (*Cyprinus carpio*) in two lakes of Kosovo. Natural and Engineering Sciences 3(3): 323-332. https://doi.org/10.28978/nesciences.468987
Sîrbu E, Dima M, Tenciu M, Crețu M, Coadă M, Țoțoiu A and Patriche N, 2022. Effects of dietary supplementation with probiotics and prebiotics on growth, physiological condition, and resistance to pathogens challenge in Nile tilapia (*Oreochromis niloticus*). Fishes 7(5): 273. https://doi.org/10. 3390/fishes7050273
Sultana MA, Habib MA, Amin MN, Sabuz SH, Salma U, Begum MD and Haque MA, 2024. Effects of yogurt on growth performance, carcass traits, lipid profile and fecal microbial load of broiler chickens. International Journal of Agriculture and Biosciences. https://doi.org/10.47278/journal.ijab/2024.196
Suwannatrai K, 2023. Analysis of the Nile tilapia fish's (*Oreochromis niloticus* l.) proximate composition in Sakon Nakhon province, Thailand. Creative Science 15(2): 251073. https://doi.org/10.55674/cs.v15i2.251073
Tacon AGJ, 1990. Standard Methods for the Nutrition and Feeding of Farmed Fish and Shrimp. Argent Laboratories Press.
Urach B, Ramos A, Luz J, Júnior F, Schorer M and Braga L, 2020. Sodium butyrate improves the performance of juvenile tilapia, *Oreochromis niloticus* (linneaus, 1758). Research Society and Development 9(9): e551997535. https://doi.org/10.33448/rsd-v9i9.7535
Wakkas S, 2023. Impacts of food and habitat on blood profile of rohu *Labeo rohita* (hamilton, 1822). Asian Journal of Medical and Biological Research 9(2): 23-29. https://doi.org/10.3329/ajmbr.v9i2.65041
Wang L, 2023. Effects of different water quality regulators on growth performance, immunologic function, and domestic water quality of gift tilapia. Plos One 18(8): e0290854. https://doi.org/10.1371/journal.pone.0290854
Wang Z, 2023. Effects of fermented cottonseed meal substitution for fish meal on intestinal enzymatic activity, inflammatory and physical-barrier-related gene expression, and intestinal microflora of juvenile golden pompano (*Trachinotus ovatus*). Fishes 8(9): 466. https://doi.org/10.3390/ fishes8090466
Yang C, Jiang M, Lü X and Huang W, 2021. Effects of dietary protein level on the gut microbiome and nutrient metabolism in tilapia (*Oreochromis niloticus*). Animals 11(4): 1024. https://doi.org/10.3390/ani11041024
Yostawonkul J, Kamble M, Sakuna K, Madyod S, Sukkarun P, Medhe S and Sewaka M, 2023. Effects of mangosteen (*Garcinia mangostana*) peel extract loaded in nanoemulsion on growth performance, immune response, and disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas veronii* infection. Animals 13(11): 1798. https://doi.org/10.3390/ani13111798
Zar JH, 2010. Biostatistical Analysis. 5th Ed., Pearson Education.
Zhu Q, Song M, Azad M, Cheng Y, Liu Y, Li Y and Kong X, 2022. Probiotics or synbiotics addition to sows' diets alters colonic microbiome composition and metabolome profiles of offspring pigs. Frontiers in Microbiology 13: 934890. https://doi.org/10.3389/fmicb.2022.934890