OPEN ACCESS Advances in Animal and Veterinary Sciences

Research Article

Single-Strain *Bacillus coagulans* Improves Growth, Hematology, and Intestinal Morphology in Milkfish (*Chanos chanos*) Juveniles

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Abstract | Background: Milkfish (*Chanos chanos*) is an important aquaculture commodity, *yet* it continues to face major cultivation challenges, primarily suboptimal feed efficiency, limiting growth and outcomes. Probiotic-based nutritional strategies, particularly using *Streptococcus thermophilus* and *Bacillus coagulans*, offer promising avenues to improve digestive function, nutrient utilization, and immune competence in cultured fish. Aim: This study evaluated the effects of single and combined probiotic supplementation on growth performance, hematological profiles, and the histological structure of the gastric and intestine of milkfish juveniles. Methods: We conducted a 45-day feeding trial using a completely randomized design with six treatments and three replications, involving 270 fish. Experimental diets consisted of a non-supplemented control and five diets containing 15 mL/kg feed of *S. thermophilus, B. coagulans*, or both in varying ratios, thereby representing single-strain and co-probiotic strategies at a fixed inclusion level. Response variables included growth indices (absolute weight and length, specific growth rate, feed conversion ratio, survival rate, and efficiency of protein), hematological, and histomorphometric characteristics of the digestive tract. Result: The single-strain *B. coagulans* treatment consistently produced the best overall response, yielding the highest specific growth rate, weight gain, survival rate, erythrocyte and hematocrit data, and the greatest intestinal villi dimensions, with significant improvements compared to the control group. Conclusion: Within the tested dose and strain proportions, *B. coagulans* alone produced better responses in specific growth rate, survival, hematocrit, and intestinal villi dimensions compared to control and co-probiotic treatments.

Keywords | Aquaculture, industry, probiotics, growth, health, digestive

Received | December 02, 2025; **Accepted** | April 28, 2026; **Published** | June 22, 2026
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Citation | Putra DF, Hamdi A, Akbar SA, Abbas MA, Ismail N, Ismarica I (2026). Single-strain *bacillus coagulans* improves growth, hematology, and intestinal morphology in milkfish (*chanos chanos*) juveniles. Adv. Anim. Vet. Sci., 14(6):1332-1343.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.6.1332.1343
ISSN (Online) | 2307-8316

INTRODUCTION

Milkfish (*Chanos chanos*) is one of leading aquaculture commodities possessing significant nutritional value and making an essential contribution to animal protein security (Asfar *et al.*, 2025). As a high-value brackish-water species, milkfish has biological and nutritional characteristics that support the development of intensive aquaculture systems (Hafiludin, 2015; Putra *et al.*, 2025).

National milkfish production in 2024 reached approximately 792,863.87 tons, which represents a 3.81%

decline compared to 2019 production level of 824,239.11 tons (MMAF, 2025). This downward trend is likely influenced by multiple interacting factors, including environmental variability, disease outbreaks, economic pressures, and on-farm management constraints. Within this broader context, low feed efficiency remains one of the key technical challenges at the farmer level because it leads to suboptimal feed utilization and reduced growth performance (Putra *et al*., 2021; Putra *et al*., 2022).

A practical solution to address this issue is the application of probiotics in feed. Probiotics such as *S. thermophilus* and *B. coagulans* produce enzymes and bioactive compounds that can enhance digestion, nutrient absorption, growth performance, and immune responses (Amoah *et al*., 2019; Supono *et al*., 2020; Kolanchinathan *et al*., 2022; Pan *et al*., 2024; Priya *et al*., 2025). Previous studies have shown that probiotics can enhance digestive activity by producing enzymes such as amylase, protease, lipase, and cellulase, as well as improving intestinal microbial balance (El-Saadony *et al*., 2021; Mujeeb *et al*., 2022; Abbas *et al*., 2023; Amenyogbe, 2023; Leong *et al*., 2023). Furthermore, *S. thermophilus* and *B. coagulans* have been reported to improve fish growth and physiological conditions through the production of lactic acid and beneficial nutrients (Ayyat *et al*., 2014; Fath El-Bab *et al*., 2022; Karpunina *et al*., 2022; Jabbar and Salman, 2023; Priya *et al*., 2025). At the same time, the physiological function of the stomach and intestine, where mechanical digestion, chemical digestion, and nutrient absorption occur, is highly dependent on healthy histological structures that support optimal fish growth (Hou *et al*., 2017; Fioramonti *et al*., 2003; Ruiz *et al*., 2020; Putra *et al*., 2024).

We hypothesized that co-supplementation of *S. thermophilus* and *B. coagulans* at fixed total dose would leverage *S. thermophilus* fermentation and *B. coagulans* enzymatic/sporulation advantages for superior outcomes versus single strains, tested via strain ratio gradients. *S. thermophilus*, a lactic acid bacterium, excels at carbohydrate fermentation and produces lactic acid plus exopolysaccharides that support gut mucosal integrity and initial pathogen exclusion (Karpunina *et al*., 2022; Ayyat *et al*., 2014). Meanwhile, *B. coagulans*, a spore-forming lactic acid producer, survives gastric transit to secrete proteases, amylases, and lipases while enhancing intestinal villus architecture and oxygen-carrying hematological capacity (Amoah *et al*., 2019; Fath El-Bab *et al*., 2022). However, studies evaluating the effects of these two bacteria on growth performance, hematological parameters, and histological structures of the gastric and intestine in milkfish remain limited.

This study is important because improved intestinal digestibility directly influences growth performance in milkfish. Hematological conditions and digestive tract histology not only reflect gastrointestinal health but also serve as key indicators for assessing the effectiveness of probiotics in enhancing feed efficiency (Amenyogbe, 2023; Zhao *et al*., 2018). Therefore, investigating the effects of *S. thermophilus* and *B. coagulans* on growth performance, hematological profiles, and the histological structure of the stomach and intestine will provide essential scientific insights for determining effective probiotic doses to improve productivity in milkfish aquaculture. Accordingly, this research aims to evaluate the influence of these probiotic bacteria on growth, hematological responses, and histological structures of the stomach and intestine, as well as to provide relevant scientific information for the development of functional feed technologies.

MATERIALS AND METHODS

Preparation of rearing containers and experimental design

Healthy milkfish (*Chanos chanos*) juveniles (0.77–0.95 g; 4.49–4.68 cm) were sourced from the Brackishwater Aquaculture Development Center, Ujung Batee, Aceh Besar, Indonesia (5.650935698129564, 95.42207107975949), a government-operated hatchery that implements best aquaculture practices and holds licenses and certification from the Ministry of Marine and Fisheries of the Republic of Indonesia. A total of 270 juveniles were used in the trial. Eighteen plastic containers (25 L) were washed with detergent, rinsed, and air-dried. Each container was labeled according to the treatment and filled with 15 L of rearing water at 25 ppt salinity. Fifteen juveniles were stocked per container, and stocking was conducted in the morning or late afternoon to minimize handling stress.

The experiment followed a completely randomized design with six treatments and three replicates, modified from Kumar *et al.* (2014). Key modifications included the use of milkfish (*Chanos chanos*) as the model species and the implementation of a fixed total probiotic volume (15 mL/kg) across all experimental diets to test specific strain ratios (1:0, 0:1, 1:1, 1:3, and 3:1), whereas the original study utilized different bacterial species and dosages for *L. vannamei*. The treatment structure was arranged to test the hypothesis that dietary co-supplementation of *S. thermophilus* and *B. coagulans* at a fixed total dose (15 mL/kg feed) could provide complementary effects and potentially match or exceed the responses obtained with single-strain diets. Accordingly, the treatments included: (A) control feed without probiotics; (B) feed with 100% *S. thermophilus* at 15 mL/kg feed (1.2×10^7 CFU/mL; final dose 1.8×10^8 CFU/kg feed); (C) feed with 100% *B. coagulans* at 15 mL/kg feed (1.8×10^8 CFU/kg feed); (D) feed with a 50% *S. thermophilus* + 50% *B.*

coagulans combination (total 15 mL/kg feed; 9 × 10^7 CFU of each strain/kg feed), representing a balanced co-probiotic strategy; (E) feed with 25% *S. thermophilus* + 75% *B. coagulans* (total 15 mL/kg feed; 4.5 × 10^7 CFU *S. thermophilus* + 1.35 × 10^8 CFU *B. coagulans*/kg feed); and (F) feed with 75% *S. thermophilus* + 25% *B. coagulans* (total 15 mL/kg feed; 1.35 × 10^8 CFU *S. thermophilus* + 4.5 × 10^7 CFU *B. coagulans*/kg feed), allowing evaluation of how shifting the dominance toward either strain within the same overall dose influences the biological response.

Growth performance monitoring

The trial lasted for 45 days. We measured body weight and length every 7 days. Commercial feed, Hi-Pro-Vite 783-2 (27% crude protein) was provided at 5% of the total biomass per day and divided into three equal portions administered at 08:00, 12:00, and 16:00. For each feeding, the required volume of probiotic suspension was first diluted in a small amount of dechlorinated freshwater to reach the target dose of 15 mL/kg feed, then thoroughly mixed with the dry pellets until all pellets appeared uniformly moistened, ensuring an even distribution of the bacteria on the feed surface according to the respective treatment. The coated feed was subsequently left to stand for approximately 15 minutes at room temperature to allow better adhesion and absorption of the probiotic suspension into the pellet matrix before being offered to the fish, rather than to achieve true fermentation. During feeding, aerators were turned off to minimize feed dispersion and potential leaching and were reactivated 15 minutes after feeding was completed; however, the exact proportion of the administered probiotic dose that was ingested versus lost to the water column was not quantified and is acknowledged as a limitation of the protocol.

Preparation of probiotic bacteria

Commercial probiotic products containing *S. thermophilus* and *B. coagulans* at 1.2 × 10^7 CFU/ml were obtained from the Freshwater Aquaculture Center, Sungai Gelam, Jambi, Indonesia, for use as feed additives.

Water siphoning and water quality monitoring

Water siphoning was performed every 3–5 days by removing 50–60% of the water volume and replacing it with 7 L of freshwater mixed with seawater to maintain environmental quality. Water quality parameters, including pH, salinity, and temperature, were measured three times daily to ensure optimal rearing conditions.

Histological preparation of the intestine and stomach

After 45 days of probiotic supplementation, the stomach and intestine were fixed in 10% Neutral Buffered Formalin, trimmed, and placed in specimen containers. Tissue dehydration followed a graded ethanol series (80%, 90%, 95% for 2 h each), absolute ethanol I and II (1 h each), and clearing with xylol I (1 h), followed by xylol II and III (45 min each). Tissues were infiltrated and embedded in paraffin blocks (I–III). Sections of 5–6 µm were sliced using a microtome, gently floated on a warm water bath at approximately 40–45°C to flatten, mounted on glass slides, dried for about 24 h on a slide warmer or at room temperature, and then stained with Hematoxylin–Eosin (HE) before microscopic examination (Olympus SZ61, Japan) (Putra *et al*., 2012).

Blood sampling and hematological analysis

Blood samples were collected from the caudal artery on day 45 using a 1-mL syringe containing 0.01 mL of 10% (w/v) EDTA solution as anticoagulant. The Blood was transferred into microtubes for erythrocyte, leukocyte, hematocrit, and hemoglobin analysis. Erythrocyte and leukocyte counts followed Hartika *et al*. (2014). Hematocrit was measured according to Anderson and Siwicki (1993). Hemoglobin concentration was analyzed using the Sahli method (Yanto et al., 2015).

Research parameters

Growth and eeed utilization

Absolute weight gain:

$$W\_ = W\_t - W\_o$$

Absolute length gain:

$$L \; = L_t - L_o$$

Feed Conversion Ratio (FCR):

$$FCR = \frac{\Sigma F}{(W_t - W_o) + W_d}$$

Survival Rate (SR):

$$SR = \frac{N_t}{N_o} \times 100$$

Feed Efficiency:

$$\text{Feed Efficiency} = \frac{(W_t + d) - W_o}{F} \times 100$$

Specific Growth Rate (SGR):

$$SGR = \frac{\ln W_t - \ln W_o}{t} \times 100$$

Intestinal and gastric villus morphometry

Villus height was measured at 40× magnification:

$$\text{Average villus height} = \frac{Pv_1 + Pv_2 + Pv_3}{3}$$

Where; Pv_1+Pv_2+Pv_3: Measurements of individual intestinal villi (replicates)

Data analysis and animal welfare ethics

All data were analyzed using one-way ANOVA according to the Completely Randomized Design. When significant treatment effects were detected, Tukey's Honestly Significant Difference (HSD) test was applied as a post-hoc procedure at a significance level of $P < 0.05$. This in vivo research complied with the ethical guidelines of the Experimental Animal Welfare Ethics Committee, Universitas Syiah Kuala. All procedures were conducted to minimize stress, pain, and discomfort to the experimental fish.

RESULTS AND DISCUSSION

Growth performance and feed utilization

Growth performance in Table 1 indicates that each treatment produced varying biological responses in the growth performance and survival of milkfish. All groups started the trial with a mean initial weight of 0.77–0.95 g and length of 4.49–4.68 cm, and the absolute length and weight values presented in Table 1 represent final measurements minus these initial data, allowing final body size and relative growth to be readily derived by readers. Treatment C exhibited the most improved performance among all treatments, as shown by the highest absolute length (1.66 ± 0.07 cm), which differed significantly from Treatment D, and the greatest absolute weight (1.84 ± 0.13 g), which was statistically higher than Treatment A. The specific growth rate (SGR) of Treatment C (2.80 ± 0.04% day^{-1}) was also the highest and significantly different from Treatment A, confirming that dietary supplementation of the two lactic acid bacteria, *S. thermophilus* and *B. coagulans*, during the rearing period generally improved growth performance and survival in milkfish compared to the non-supplemented control diet, with the single supplementation of *B. coagulans* (Treatment C: 100% *B. coagulans*, 15 mL/kg feed) resulting in the highest growth performance compared to all other treatments.

Although feed conversion ratio (FCR) did not differ significantly among treatments, Treatment C still demonstrated the most efficient feed conversion (1.41 ± 0.17), and in this study it yielded the lowest FCR (1.41) and highest FE (69.38%); however, these improvements were not statistically significant ($p > 0.05$).

This may be attributed to the inherent biological variability among individuals and the modest replication size ($n = 3$), which potentially limited the statistical power to detect smaller effect sizes in nutrient utilization parameters. Similar findings have been reported in other probiotic studies where growth performance is significantly enhanced before feed conversion indices reach statistical thresholds. Interestingly, the 50:50 probiotic combination (Treatment D) did not result in the expected synergistic benefit; its performance in weight gain and SGR was significantly lower than that of the single-strain *B coagulans* group (Treatment C) ($p < 0.05$) and did not differ significantly from the control group ($p > 0.05$). This suggests a potential competitive interaction between *S. thermophilus* and *B. coagulans* when administered in equal proportions, or perhaps a dilution effect where neither strain reached the threshold concentration required to trigger a significant growth response in milkfish juveniles.

Table 1: Growth performance of milkfish (*Chanos chanos*) across treatments, including absolute length, absolute weight, feed conversion ratio (FCR), specific growth rate (SGR), survival rate (SR), and feed efficiency (FE).

Treatment	Absolute length gain (cm)	Absolute weight gain (gr)	FCR	SGR (%/day)	SR (%)	FE (%)
A	1.14 ± 0.36^{ab}	0.85 ± 0.23^{b}	1.88 ± 0.38	1.84 ± 0.39^{b}	84.46 ± 3.85^{b}	50.94 ± 7.33
B	1.37 ± 0.15^{ab}	1.19 ± 0.16^{ab}	1.61 ± 0.44	2.19 ± 0.36^{ab}	91.10 ± 3.85^{a}	65.12 ± 17.32
C	1.66 ± 0.07^{a}	1.84 ± 0.13^{a}	1.41 ± 0.17	2.80 ± 0.04^{a}	93.30 ± 0.00^{a}	69.38 ± 6.48
D	0.94 ± 0.13^{b}	1.09 ± 0.47^{ab}	1.70 ± 0.61	1.90 ± 0.52^{ab}	91.10 ± 3.85^{a}	63.55 ± 21.33
E	1.43 ± 0.04^{ab}	1.68 ± 0.13^{a}	1.46 ± 0.04	2.32 ± 0.19^{ab}	91.10 ± 3.85^{a}	73.41 ± 9.89
F	1.30 ± 0.30^{ab}	1.28 ± 0.39^{ab}	1.61 ± 0.27	1.97 ± 0.30^{ab}	88.90 ± 3.85^{ab}	65.02 ± 3.31

Data are presented as mean ± SD. Different superscript letters within the same column indicate significant differences among treatments ($P < 0.05$). One-way ANOVA indicated that probiotic supplementation significantly affected absolute weight gain ($F_{5,12} = 5.09$, $p = 0.010$) and absolute length ($F_{5,12} = 3.42$, $p = 0.037$). The effect on Specific Growth Rate (SGR) was near the threshold of significance ($F_{5,12} = 3.08$, $p = 0.051$). No significant differences were found for Survival Rate ($F_{5,12} = 2.28$, $p = 0.112$), Feed Conversion Ratio ($F_{5,12} = 0.63$, $p = 0.680$), or Feed Efficiency ($F_{5,12} = 1.12$, $p = 0.148$)Note: A = control; B = 100% *S. thermophilus* (15 mL/kg feed); C = 100% *B. coagulans* (15 mL/kg feed); D = 50% *B. coagulans* + 50% *S. thermophilus* (15 mL/kg feed); E = 25% *S. thermophilus* + 75% *B. coagulans* (15 mL/kg feed); F = 75% *S. thermophilus* + 25% *B. coagulans* (15 mL/kg feed).

The survival rate (SR) varied across treatments, with Treatment A at 84.46%, Treatment F at 88.90%, and the remaining treatments (B, C, D, and E) showing higher rates of 91.10% or 93.30% (Table 1). These findings are consistent with previous studies demonstrating that both bacterial species can function as growth promoters in various aquaculture species, including Nile tilapia (*Oreochromis niloticus*), Lena sturgeon, *Sillago sihama* Forsskål (1775), and white shrimp (*Litopenaeus vannamei*) (Ayyat *et al*., 2014; Amoah *et al*., 2019, 2021; Fath El-Bab *et al*., 2022; Karpunina *et al*., 2022). Probiotics enhance fish growth primarily through two mechanisms: improving digestive health and enhancing water quality (Dachi *et al*., 2019; Leong *et al*., 2023; Qiu *et al*., 2023). Probiotic bacteria stimulate digestive enzymes that catalyze nutrient assimilation and degrade ammonia from fish waste, leading to healthier rearing environments (Muhammadar *et al*., 2018; El-Saadony *et al*., 2021; Mujeeb *et al*., 2022), although these water-quality effects were not directly quantified in the present experiment.

Hematological parameters

Hematological analysis (Figure 1) showed that erythrocyte counts were broadly similar among treatments, with mean data ranging from 1.80 ± 0.18 to 2.47 ± 0.04 × 10^6 cells/mm^3 and all groups sharing the same statistical letter. Although Treatment C exhibited the numerically highest erythrocyte concentration, it did not differ significantly from the control (A) or any other probiotic treatment (Figure 1a). Hematological parameters are effective indicators of fish health and are widely used to assess both metabolic and immune-related physiological responses (Esmaeili, 2021). The present study confirmed that probiotic supplementation significantly influenced erythrocyte, leukocyte, hematocrit, and hemoglobin levels, and these changes can be interpreted as reflecting modifications in both oxygen-carrying capacity and immune competence in milkfish. We observed the highest erythrocyte count in Treatment C, corresponding to dietary supplementation with *B. coagulans* at 15 mL/kg feed, suggesting an enhanced ability to transport oxygen to support tissue metabolism and growth. Erythrocytes transport hemoglobin-bound oxygen from the gills to body tissues (Ortega-Villaizan *et al*., 2022) and facilitating carbon dioxide transport through carbonic anhydrase activity (Shen *et al*., 2018). Given their dominant proportion in blood volume, erythrocyte levels are commonly used as physiological health indicators, including responses to pathogenic stress (Shen *et al*., 2018; Al-Sulivany, 2023; Mahapatra *et al*., 2023; Mahmoodian *et al*., 2025).

Leukocyte counts indicated that Treatments B and D yielded the highest data (43.13 × 10^3 and 42.73 × 10^3 cells/mm^3, respectively), both significantly different from several other treatments (Figure 1b). Probiotic supplementation also significantly increased leukocyte counts, indicating stronger immunity and a heightened state of non-specific

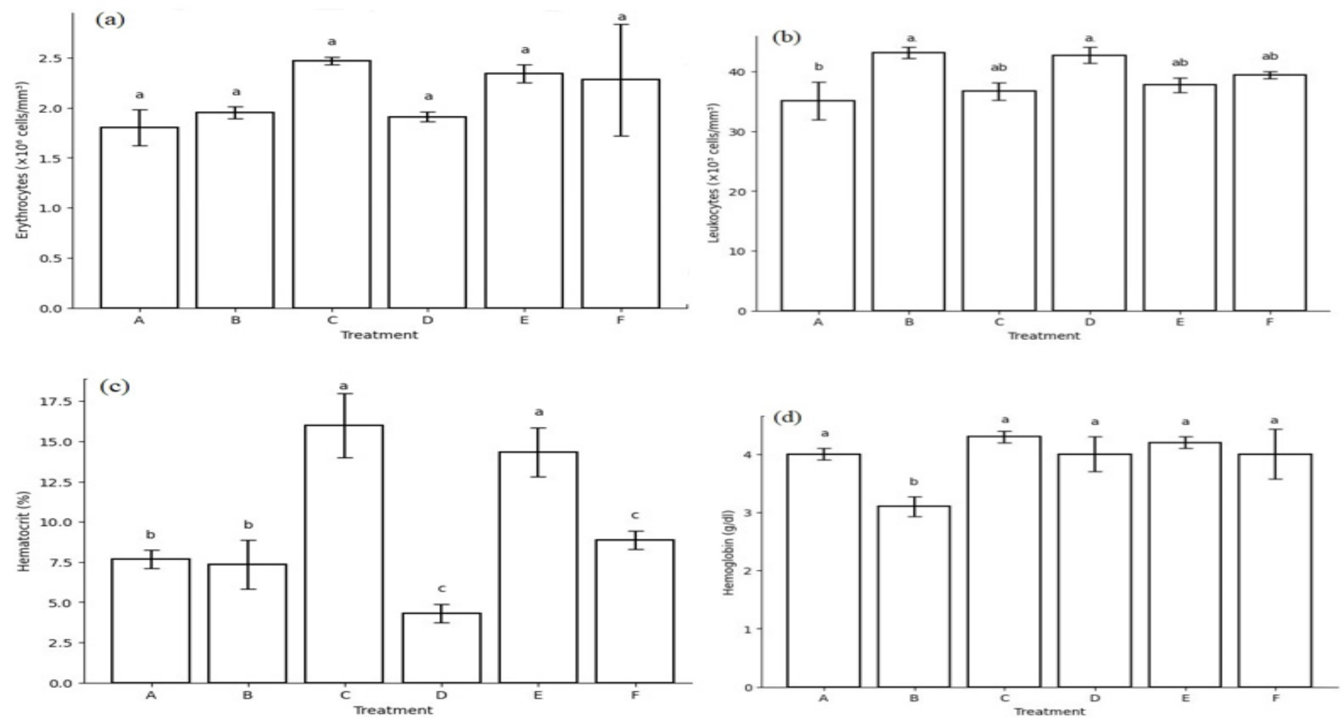

Figure 1: Hematological parameters of milkfish (*Chanos chanos*) across treatments, including erythrocytes (a), leukocytes (b), hematocrit (c), and hemoglobin (d). Data are presented as mean ± SD. Different superscript letters indicate significant differences among treatments: Hemoglobin ($F_{5,12}$ = 13.70, p < 0.001), hematocrit ($F_{5,12}$ = 49.21, p < 0.001), leukocytes ($F_{5,12}$ = 11.78, p < 0.001), and Erythrocyte ($F_{5,12}$ = 3.67, p = 0.030).

defense rather than growth alone (Abarike *et al*., 2020; Rashidah *et al*., 2023). In this context, the higher leukocyte data observed in some *S. thermophilus*–containing treatments may indicate a relatively stronger immunostimulatory influence of this species, whereas *B. coagulans* appears more closely associated with improvements in erythrocyte-related oxygen transport, although this functional differentiation remains hypothetical and requires targeted confirmation. Elevated leukocyte levels indicate strengthened cellular and humoral defense mechanisms against pathogens (Aznan *et al*., 2017; Jebashiny and Lakshmanan, 2022; Hasan *et al*., 2023; Emam *et al*., 2024).

Hematocrit data expressed that treatments C and E recorded the highest percentages (16.00% and 14.33%), and both differed significantly from the remaining treatments (Figure 1c). Hematocrit, the ratio of red blood cells to plasma, reflects oxygen-carrying capacity and overall physiological condition (Ahmed *et al*., 2022). In the present study, probiotic supplementation increased hematocrit data, indicating improved physiological health and circulatory function (Dias *et al*., 2020). Meanwhile, hemoglobin concentrations were generally similar among treatments except for the treatment B, which displayed a noticeably lower mean (3.10 ± 0.17 g/dL) than the other groups (around 4.0–4.3 g/dL) (Figure 1d). Hemoglobin, responsible for oxygen transport in cellular metabolism, is closely associated with erythrocyte levels; thus, increased erythrocytes typically correspond to elevated hemoglobin concentrations and greater oxygen-carrying capacity (Arnaudov and Arnaudova, 2022). Abnormal hemoglobin levels may signal inadequate nutrition, poor water quality, or infection (de Souza and Bonilla-Rodriguez, 2007).

Hematocrit also correlates positively with feed intake and nutritional status (Abarike *et al*., 2020; Tippayadara *et al*., 2021), supporting the interpretation that the hematological profile observed in probiotic-treated fish, particularly in the *B. coagulans* group, represents a combined enhancement of nutrient utilization, oxygen delivery, and and immune readiness. At the same time, the higher leukocyte counts in *S. thermophilus*–containing treatments suggest a testable hypothesis that *S. thermophilus* may exert comparatively stronger effects on leukocyte-mediated immune responses, whereas *B. coagulans* has a more pronounced influence on red-cell–related parameters.

Intestinal and gastric morphology

Intestinal villi morphometrics (Figure 3) were significantly influenced by the dietary interventions, with Treatment C consistently yielding the highest data for villus

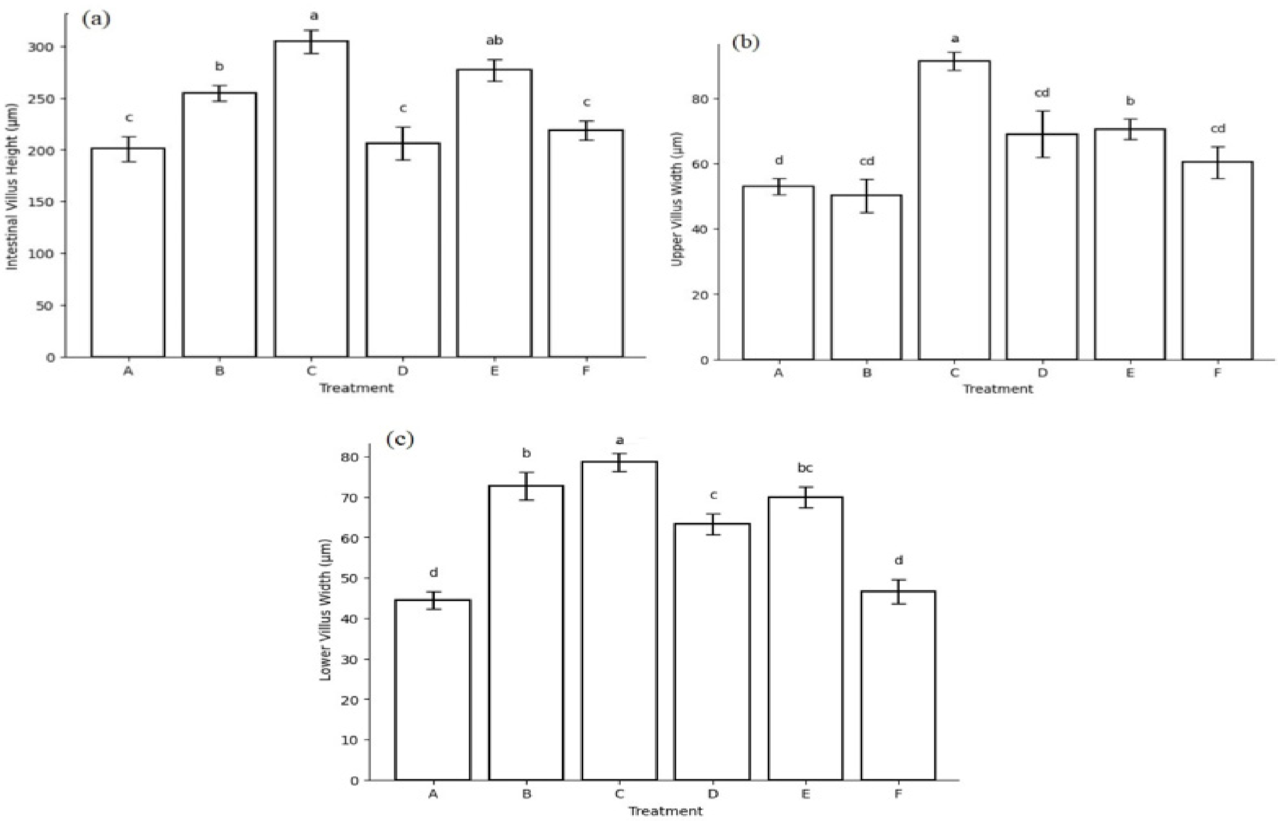

Figure 2: Intestinal villi morphometric characteristics of milkfish (*Chanos chanos*) for each treatment, including intestinal villus height (a), upper villus width (b), and lower villus width (c). Data are presented as mean ± SD. Different superscript letters indicate significant differences among treatments ($p < 0.001$). Specifically, significant variations were observed in intestinal villus height ($F_{5,12} = 40.25$), upper villus width ($F_{5,12} = 27.38$), and lower villus width ($F_{5,12} = 80.90$).

height (304.58 ± 11.30 µm), upper width (91.36 ± 2.77 µm), and lower width (78.67 ± 2.21 µm). These data were significantly greater than those of the lowest-performing groups, specifically Treatments A, D, and F. Treatments B and E showed intermediate responses, particularly regarding height and lower width, while Treatment A consistently exhibited the minimum data across all parameters (Figure 2a-c). Dietary probiotic supplementation significantly influenced the intestinal and gastric morphology of milkfish, with single-strain *B. coagulans* producing the most pronounced improvements in villus architecture and, by implication, nutrient absorption capacity. Because we did not quantify digestive enzyme activities or nutrient transport, this interpretation is necessarily indirect and relies on the well-established association between increased villus height/width and improved absorptive function in fish intestines (Margaret *et al*., 2021; Gonçalves *et al*., 2024; Totewad and Gyananath, 2024).

Nevertheless, in herbivorous–detritivorous species such as milkfish, which possess long intestines and depend heavily on efficient extraction of nutrients from plant and detrital material, an expanded villus surface area is likely to confer a functional advantage and is consistent with the higher growth observed in the *B. coagulans* group. Previous studies on various fish species have similarly reported improved intestinal morphology and function following probiotic administration, including in Nile tilapia (Wang *et al*., 2020; Fath El-Bab *et al*., 2022), stinging catfish (*Heteropneustes fossilis*) (Sohel *et al*., 2023), Gangetic mystus (*Mystus cavasius*) (Gaffar *et al*., 2023), rainbow trout (*Oncorhynchus mykiss*) (Simakov *et al*., 2022), and Atlantic salmon (*Salmo salar*) (Nimalan *et al*., 2023). In addition, probiotics contribute to digestive health through the secretion of digestive enzymes that break down complex feed molecules into simpler forms, enabling faster absorption by the villi (Afrilasari *et al*., 2016; Vasyliuk *et al*., 2023; Bakky *et al*., 2025). Colonization of beneficial bacteria also promotes microbial balance, enhances tissue repair, and increases goblet cell density as a protective mucosal response (Zheng *et al*., 2022; Hu *et al*., 2024; Du *et al*., 2025). These effects likely leading to more complete nutrient absorption and a stable gastric environment (Butt and Volkoff, 2019; De Marco *et al*., 2023).

Histological analysis of the stomach morphology further indicated that the structural integrity of the gastric tissues remained normal in all probiotic-supplemented treatments, as evidenced by the absence of any histological abnormalities (Figure 4A–F) Although the present study provides qualitative evidence

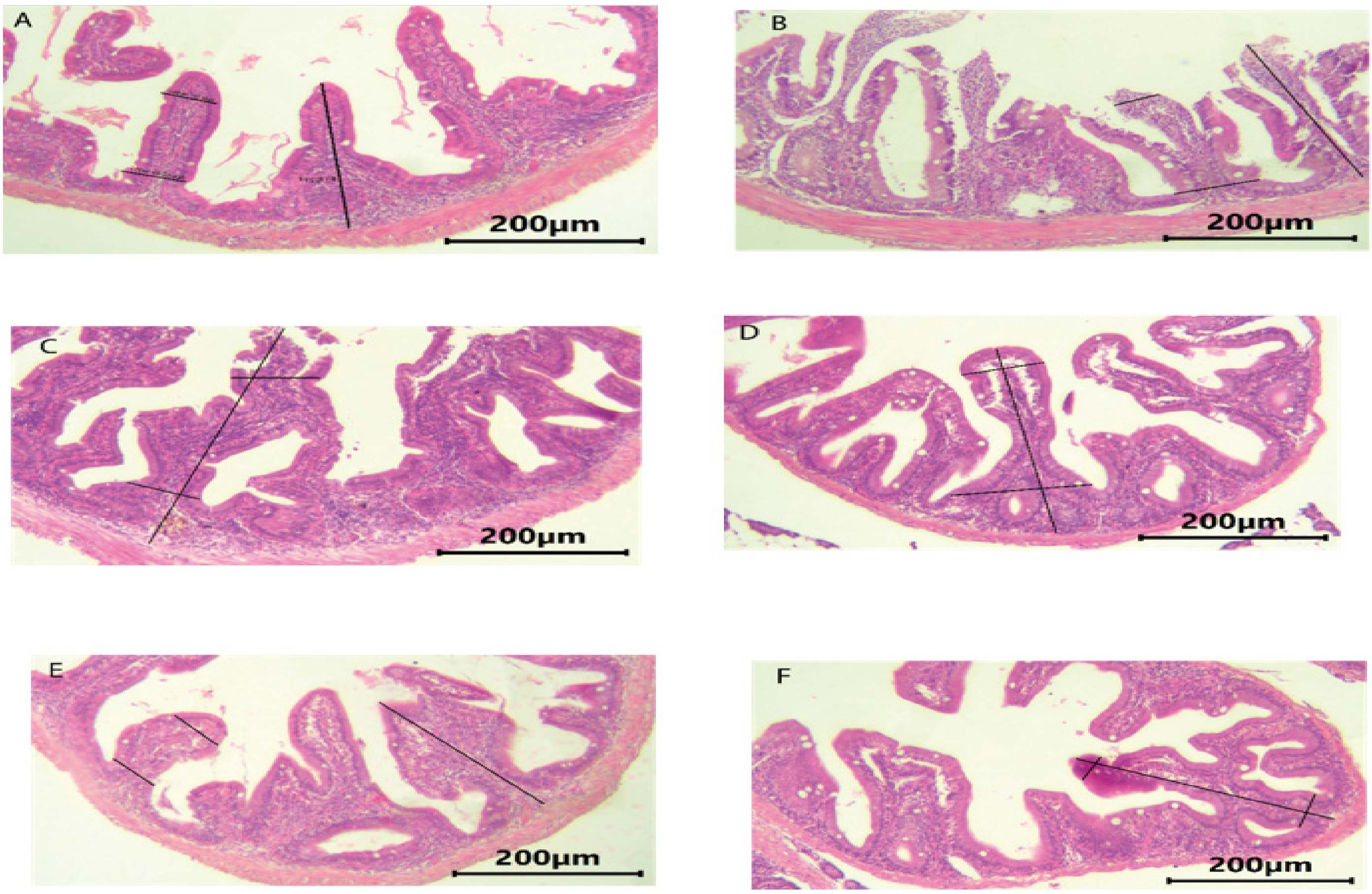

Figure 3: Representative photomicrographs of anterior intestinal cross-sections showing taller and wider villi in fish from Treatment C compared with the control group. Scale bar = 200 µm.

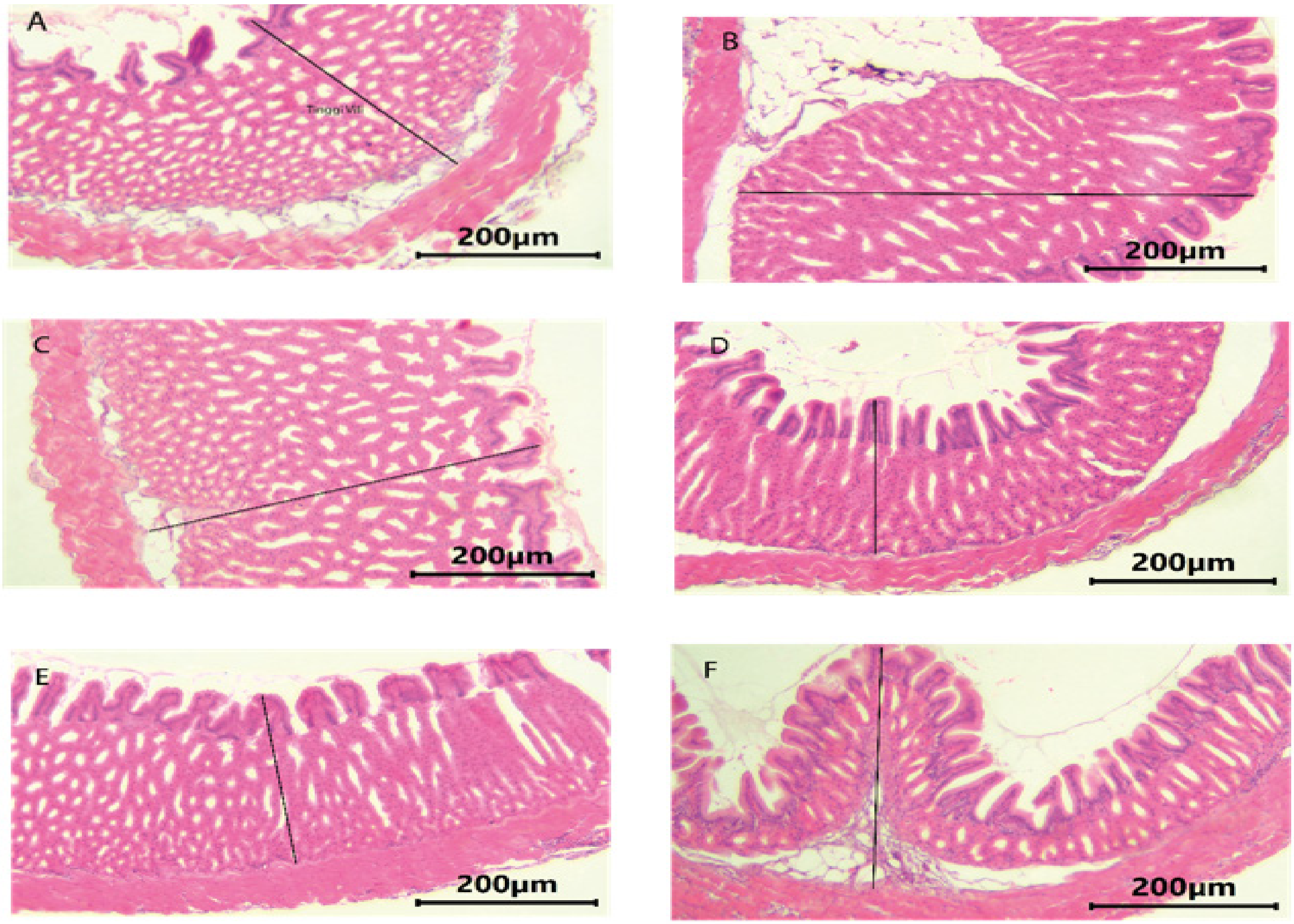

Figure 4: Gastric histo-morphology of milkfish (*Chanos chanos*) under different probiotic treatments showing the absence of histological abnormalities (Hematoxylin and Eosin, 200 µm).

Table 2: Water quality parameters during the rearing of milkfish (*Chanos chanos*) across treatments, including temperature, dissolved oxygen (DO), pH, and salinity.

Parameters	Temperature (°C)	DO (ppm)	pH	Salinity (ppt)
A	27.84 ± 0.17	7.15 ± 0.25	7.55 ± 0.11	24.83 ± 0.03
B	27.84 ± 0.17	7.11 ± 0.26	7.60 ± 0.11	24.83 ± 0.03
C	27.84 ± 0.17	7.21 ± 0.25	7.57 ± 0.13	24.83 ± 0.03
D	27.84 ± 0.17	7.09 ± 0.24	7.52 ± 0.11	24.83 ± 0.03
E	27.84 ± 0.17	7.14 ± 0.28	7.53 ± 0.1	24.83 ± 0.03
F	27.84 ± 0.17	7.11 ± 0.26	7.43 ± 0.11	24.83 ± 0.03

that probiotic supplementation did not induce gross or microscopic abnormalities in stomach morphology, a key limitation is that the gastric histology was assessed descriptively rather than through quantitative measurements. The future work should incorporate quantitative histomorphometry (measurements of mucosal fold height, epithelial thickness, and glandular density) to more rigorously evaluate how probiotics may influence stomach structure and to strengthen mechanistic inferences linking morphological changes with growth and health outcomes.

Water quality

Water quality parameters during the rearing period remained stable and within suitable ranges for milkfish growth. The recorded temperature ranged from 27–28.5°C, while dissolved oxygen levels were between 4.4–6.2 ppm across treatments. The overall minimum and maximum pH values observed across all tanks and sampling times were 6.8 and 8.4, respectively, with individual tanks typically fluctuating within a narrower band around neutral–slightly alkaline conditions; salinity remained stable between 24–25 ppt. These parameters were within optimal ranges, supporting the survival and growth performance of the fish, and indicate that none of the probiotic treatments had adverse effects on the culture environment.

Water quality parameters recorded in this study remained within normal tolerance limits for milkfish and supported acceptable survival and growth across all treatments. Probiotics play an important role in maintaining and improving water quality by metabolizing organic and inorganic waste materials (Sunitha and Krishna, 2016; Tabassum *et al*., 2021; El-Kady *et al*., 2022). These microbial activities reduce organic accumulation and support bioremediation by transforming toxic nitrogen compounds such as ammonia and nitrite into less harmful nitrate forms (Ren *et al*., 2021; Qiu *et al*., 2023). However, in the present study ammonia, nitrite, and nitrate were not measured, and the specific effects of the applied probiotics on these nitrogenous wastes and on the broader microbial community were therefore not directly quantified; any discussion of such mechanisms is based on the literature rather than on our own water chemistry data. The effectiveness of probiotics in modulating water quality depends on microbial species, dosage, application method, and physicochemical conditions of the water (Bazar *et al*., 2022), and detailed evaluation of these aspects, including routine monitoring of inorganic nitrogen species in milkfish culture, is highlighted as an important direction for future research.

CONCLUSION

Within this 45-day tank trial in juvenile milkfish (*Chanos chanos*), the diet supplemented with single-strain *B. coagulans* at 15 mL/kg feed (Treatment C) produced the best overall responses among the treatments tested, with higher specific growth rate, weight gain, survival, erythrocytes and hematocrit, and larger intestinal and gastric villi than the non-supplemented control. These results indicate that *B. coagulans* at this inclusion level is more effective than *S. thermophilus* or mixed-strain diets under our experimental conditions, but they do not establish this dose as optimal, nor do they demonstrate long-term or economic benefits over a full grow-out cycle. We therefore propose *B. coagulans* at 15 mL/kg feed as a promising candidate for further dose-response, farm-scale, and cost-effectiveness studies in milkfish aquaculture, rather than as an immediate recommendation for commercial use

ACKNOWLEDGMENT

We are grateful to LPPM USK for providing funding the Lektor scheme research fund with contract number: No. 329/UN11.2.1/PT.01.03/PNBP/2023 Tanggal 03 Mei 2023. This paper is also presented at the World Aquaculture Society (WAS) Conference 2026 in Singapore to support the EQUITY-LPDP program of Universitas Syiah Kuala (SK Rector 1087.UN11/KPT/2026).

NOVELTY STATEMENT

This study is the first to systematically compare single-strain and combined supplementation of *S. thermophilus* and *B. coagulans* at a fixed total dose in milkfish (*Chanos chanos*) juveniles. Using graded strain ratios and integrating growth, hematological, and intestinal histomorphometric endpoints, we demonstrate that *B. coagulans* alone at 15 mL/kg feed outperforms all mixed-strain formulations and the non-supplemented control, empirically challenging the assumption that multi-strain lactic acid bacteria combinations confer synergistic benefits in this species.

AUTHOR'S CONTRIBUTION

All authors contributed to the conception and design of the study. AH and DFP performed the fish rearing, probiotic supplementation, and growth monitoring. MAA and II conducted the hematological analyses and histological preparations. DFP and NI supervised the histomorphometric measurements and data interpretation. All authors participated in drafting, revising, and approving the final version of the manuscript for submission.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Data availability statement

The datasets generated and analyzed during the current study, including raw growth performance measurements, hematological data, histomorphometric measurements, and water quality records, are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This in vivo research complied with the ethical guidelines of the Experimental Animal Welfare Ethics Committee, Universitas Syiah Kuala. All procedures were conducted to minimize stress, pain, and discomfort to the experimental milkfish (*Chanos chanos*) juveniles. This study was performed exclusively on healthy hatchery-sourced fish using standard aquaculture practices, with no experimental disease challenges or invasive procedures beyond routine biometric sampling and terminal tissue collection under anesthesia.

Conflicts of interest

The authors declare no conflict of interest.

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