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

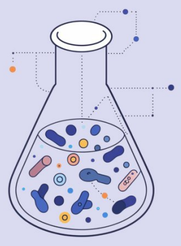
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Main Finding

Feeding juvenile milkfish with a single-strain *Bacillus coagulans* probiotic at **15 mL/kg feed** produced the highest growth, survival, hematological status, and intestinal villus development compared to control and *Streptococcus thermophilus*–based diets.

- Single-strain *B. coagulans* (15 mL/kg) yielded the highest specific growth rate (≈2.8%/day)
- Survival reached approximately 93% in probiotic-supplemented groups
- *B. coagulans* produced the tallest and widest intestinal villi, with no gastric pathology observed



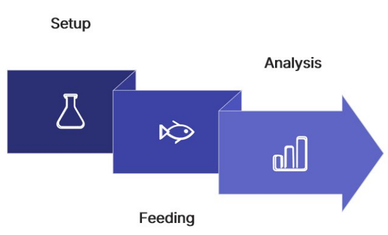
Introduction

Aquaculture Challenge
Milkfish (*Chanos chanos*) is a major aquaculture species across Southeast Asia, yet farms continue to struggle with suboptimal feed efficiency and growth performance.

Probiotic Candidates
Streptococcus thermophilus and *Bacillus coagulans* are promising probiotics shown to improve digestion, immunity, and gut health in finfish.

Study Objective
This study compared single-strain and combined probiotic strategies at a fixed dose of 15 mL/kg feed in juvenile milkfish over a 45-day trial.

Methods



A 45-day completely randomized feeding trial was conducted with 270 milkfish juveniles distributed across 18 tanks. Six dietary treatments were tested in triplicate, and responses were analyzed by one-way ANOVA with Tukey HSD post-hoc testing ($p < 0.05$).

Experimental Treatments

T1 – Control
Basal diet with no probiotic supplementation

T2 – *S. thermophilus*
Single-strain *S. thermophilus* at 15 mL/kg feed

T3 – *B. coagulans*
Single-strain *B. coagulans* at 15 mL/kg feed **(Best performer)**

T4–T6 – Mixed
Combined strains at 50:50, 25:75, and 75:25 (*S. thermophilus : B. coagulans*) ratios

Key Results: Growth Performance

≈2.8% — Specific Growth Rate: Highest SGR per day in *B. coagulans* group

93% — Survival Rate: Sustained in all probiotic-supplemented groups

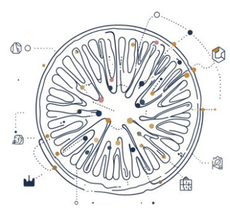
Lowest — Feed Conversion Ratio: Numerically lowest FCR and highest feed efficiency in *B. coagulans* diet

Interpretation

Single-strain *B. coagulans* produced the greatest weight gain and length gain over the 45-day trial. Although FCR differences were not statistically significant across groups, the *B. coagulans* diet showed the most favorable numerical trend. Water quality remained within acceptable ranges throughout the experiment.

All probiotic groups maintained high survival and suitable water quality conditions throughout the trial.

Key Results: Hematology & Intestinal Morphology



Hematological Parameters

- *B. coagulans* significantly increased erythrocyte counts and hematocrit values
- Enhanced oxygen-carrying capacity suggests improved systemic health and metabolic activity
- No adverse hematological changes observed in any treatment group

Intestinal Villus Morphology

- *B. coagulans*-fed fish developed the tallest and widest intestinal villi among all groups
- Greater villus surface area corresponds to enhanced nutrient absorption capacity
- Gastric histology remained normal and without pathological lesions across all treatments

Conclusions

Superior Single-Strain Performance
At 15 mL/kg feed, single-strain *Bacillus coagulans* outperformed *S. thermophilus* and all mixed-strain diets in juvenile milkfish across all measured parameters.

Multi-System Benefits
The probiotic improved growth performance, hematological profile, and intestinal villus morphology without inducing any detectable gastric pathology.

Future Directions
B. coagulans is a strong candidate for functional feeds in milkfish aquaculture. Dose–response studies and farm-scale trials are needed to confirm practical applicability.

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 (%)
T1	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
T2	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
T3	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
T4	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
T5	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
T6	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

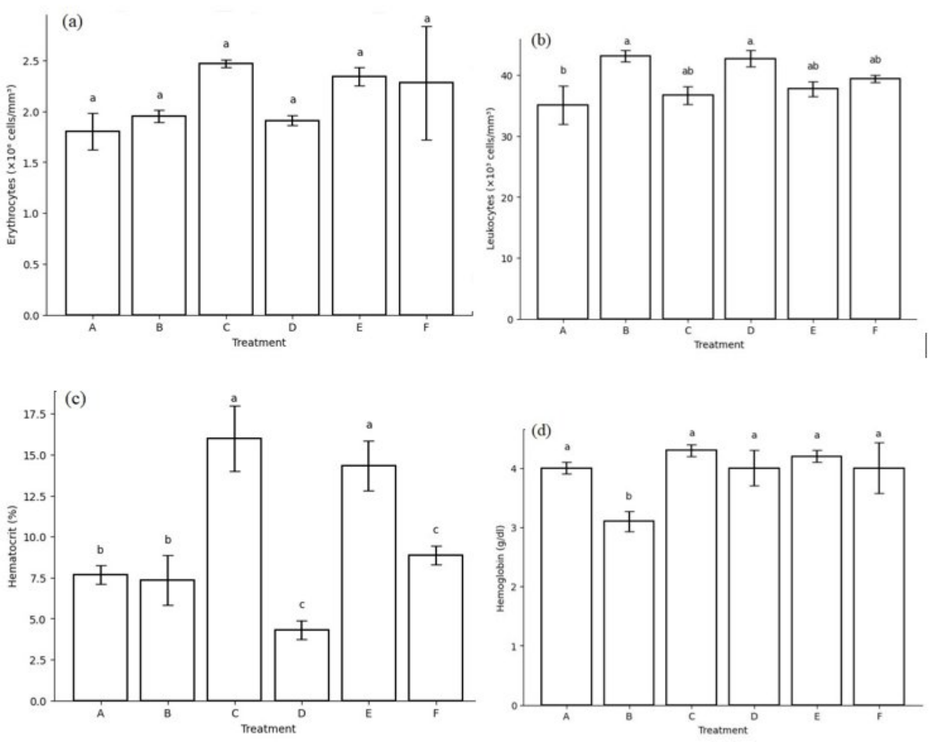


Figure 1. Hematological parameters of milkfish (*Chanos chanos*) across treatments, including erythrocytes (a), leukocytes (b), hematocrit (c), and hemoglobin (d). Data are presented as mean ± SD. Different superscript letters indicate significant differences among treatments: Hemoglobin ($F_{5,12} =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

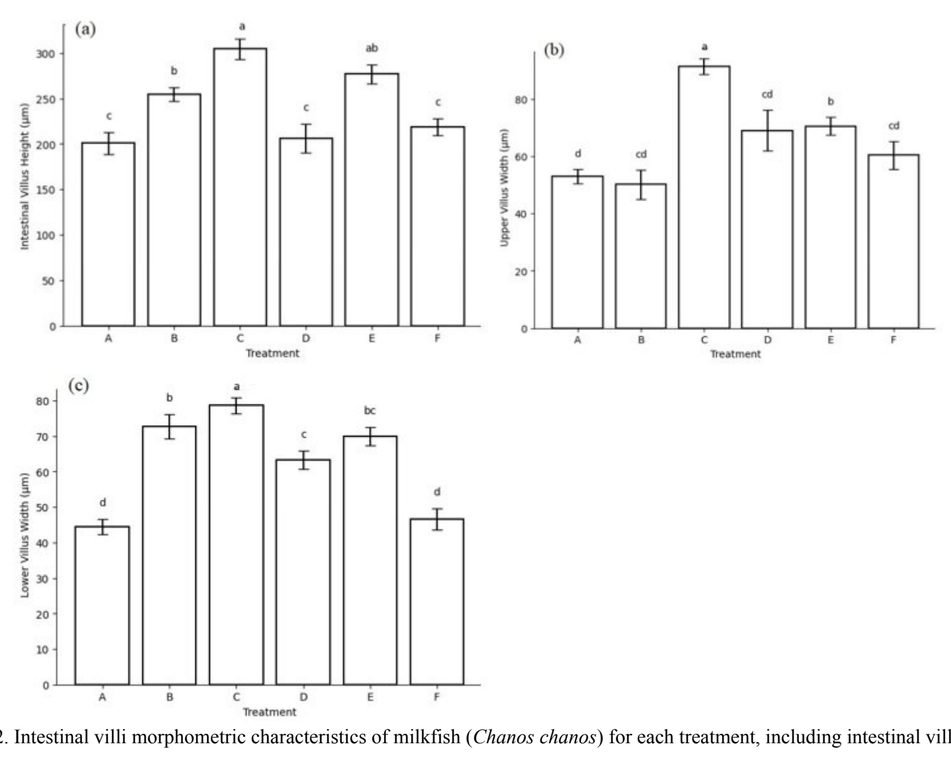


Figure 2. Intestinal villi morphometric characteristics of milkfish (*Chanos chanos*) for each treatment, including intestinal villus height (a), upper villus width (b), and lower villus width (c). Data are presented as mean ± SD. Different superscript letters indicate significant differences among treatments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

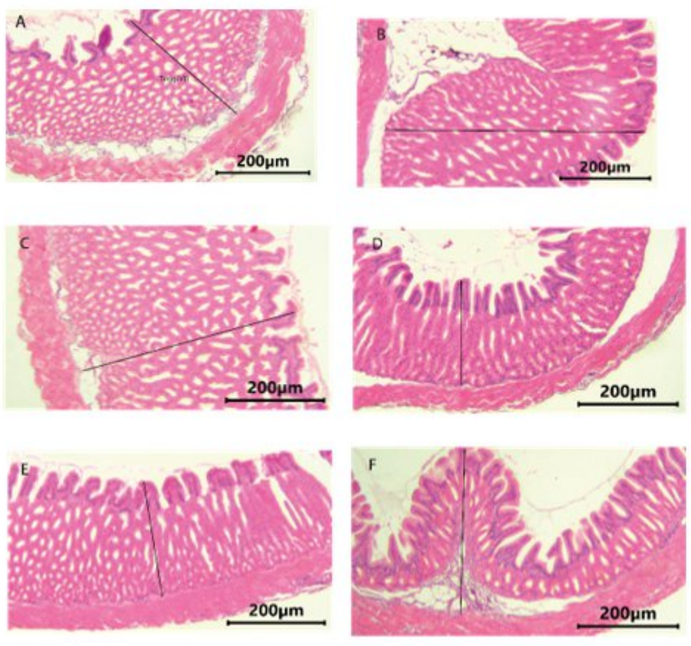


Figure 3. Representative photomicrographs of anterior intestinal cross-sections showing taller and wider villi in fish from Treatment C compared with the control group. Scale bar = 200 µm.

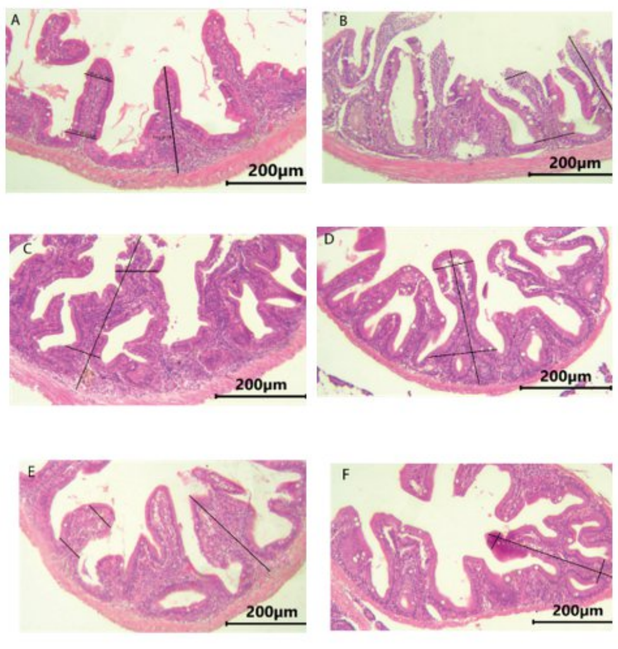


Figure 4. Gastric histo-morphology of milkfish (*Chanos chanos*) under different probiotic treatments showing the absence of histological abnormalities (Hematoxylin and Eosin, 200 µm).