Commercial Probiotics Improve Growth, Feed Efficiency, Nitrogen Removal, Hemocyte Count and Suppression of *Vibrio* Population in Pacific White Shrimp Culture

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Abstract

The Pacific white shrimp (Penaeus vannamei) is a key species in global aquaculture, particularly under intensive farming systems where high stocking densities often lead to deteriorating water quality, disease outbreaks, and reliance on antibiotics. While probiotics are increasingly applied to enhance shrimp health and performance, comparative evidence on the efficacy of different commercial probiotic formulations remains limited. This study aimed to evaluate the effects of three commercial probiotic products on growth performance, water quality, microbial populations, and immune response in intensively reared Pacific white shrimp. Shrimp were fed diets supplemented with one of three probiotics (PB, PL, and PMB) or a control diet for 40 days. Growth parameters, nitrogenous waste levels, bacterial counts in water and organs, and total hemocyte count (THC) were analyzed. The PB treatment (multi-strain Bacillus) significantly enhanced specific growth rate (2.99±0.0027% day $^{-1}$), feed efficiency (57.5±0.09%), weight gain, and THC (3.80 × 10 ^{5} cells mm $^{-3}$) compared to control (P<0.05). The PB and PMB treatments also reduced total ammonia nitrogen, nitrite, nitrate, and Vibrio populations in water and shrimp tissues. Intestinal colonization by beneficial bacteria was highest in PB and PL groups. These results demonstrate that specific probiotic formulations, particularly PB, can improve growth, immunity, and water quality while reducing reliance on antibiotics and water exchange. The findings support the integration of targeted probiotics in sustainable shrimp aquaculture, including biofloc and recirculating aquaculture systems (RAS).

Keywords: *Bacillus, Lactobacillus, mixed bacteria, Penaeus vannamei, probiotics*

Introduction

The global demand for shrimp has made shrimp farming one of the most profitable aquaculture sectors, with the Pacific white shrimp (*Penaeus vannamei*) as the dominant cultivated species (Zhou *et al.*, 2020). To meet market demands and remain competitive, the shrimp industry has shifted from extensive to intensive farming systems, aiming for higher productivity (Utami *et al.*, 2024). This intensification has, however, increased the risk of disease outbreaks and environmental degradation due to high stocking densities and elevated feed inputs. Accumulated organic waste and nitrogenous compounds such as ammonia and nitrite deteriorate water quality, induce physiological stress, and promote pathogen proliferation, all of which contribute to reduced survival and economic losses (Lieke *et al.*, 2020; Amenyogbe, 2023; Tamilselvan and Raja, 2024).

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Sustainable and environmentally friendly management strategies are critical to mitigate these challenges (Rimmer and Glamuzina, 2019). One promising solution is the use of probiotics which are live microorganisms that provide health and performance benefits to the host when administered in sufficient quantities (El-Saadony *et al.*, 2021; Yilmaz *et al.*, 2022). In shrimp cultivation, probiotics have been shown to enhance growth, suppress pathogenic bacteria, and improve water quality through organic matter degradation and nitrogen removal (Yun *et al.*, 2021; Wei *et al.*, 2022). These microorganisms can be applied via feed or directly into the culture water and are widely marketed as water conditioners and feed additives (Khanjani *et al.*, 2024).

Bacillus and lactic acid bacteria (LAB) are among the most widely used probiotics due to their proven efficacy in improving growth, resistance to environmental stressors, and pathogen inhibition (Kuebutornye *et al.*, 2019; Soltani *et al.*, 2019; Jlidi *et al.*, 2022). Additionally, purple non-sulfur bacteria such as *Rhodobacter sphaeroides* and *Afifella marina* have demonstrated bioremediation potential (Jamal *et al.*, 2019). Multispecies probiotics are hypothesized to offer greater benefits than single strains, due to synergistic effects such as stronger colonization and higher antimicrobial compound production (Amenyogbe *et al.*, 2022).

Despite increasing commercial use, many studies focus on individual strains or isolated performance metrics, limiting our understanding of how commercial probiotic blends function holistically in shrimp aquaculture. Therefore, this study aimed to evaluate the efficacy of three commercial probiotic formulations administered via feed on Pacific white shrimp, focusing on key performance indicators including: growth, water quality, bacterial population dynamics, and total hemocyte count. The results of this study are expected to support evidence-based probiotic selection and application strategies, promote more sustainable and cost-effective shrimp farming practices, and reduce reliance on antibiotics in intensive aquaculture systems.

Materials and Methods

Experimental design

This study employed a randomized research design consisting of four treatments, each with three replicates. The treatments involved the dietary administration of three commercial probiotic powder products to Pacific white shrimp reared in a clear-water system. The probiotic products included: PB containing multi-species *Bacillus*; PL containing multi-species *Lactobacillus*; and PMB composed of probiotic strains from different genera. A control group receiving a commercial diet without probiotics was also included. Each probiotic product contained a consortium of bacteria with a minimum density of 10^7 CFU·g^{-1} which was manufactured in Karanganyar, Indonesia. All data were compiled using Microsoft Excel 2021 and analyzed with SPSS version 22. Normality and homogeneity of variances were verified using the Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) was performed to detect significant differences among treatments, followed by Duncan's post-hoc test at a 95% confidence level (P<0.05).

Preparation of experimental feed

A commercial shrimp feed containing 40% crude protein served as the basal diet. Probiotic powders were incorporated into the feed at a concentration of 0.2% (w/w). A binder solution was also prepared by dissolving binder (Boster® Progol) at a dose of 5 g.kg^{-1} feed into 125 mL distilled water, following the method of Yudiati *et al.* (2020). The probiotics were mixed with the binder solution and then sprayed evenly onto the feed (Untsa *et al.*, 2024). The coated feed was finally air-dried and stored in sealed plastic bags at room temperature until use.

Feeding trial

Pacific white shrimp (PL12) were obtained from a hatchery in Jembrana, Indonesia. Prior to the trial, shrimp were acclimated in a 5.98 × 2.86 × 1.00 m³ concrete tank for 40 days and fed a commercial diet containing 40% protein four times daily at 08:00, 12:00, 16:00, and 20:00 as described by Noman *et al.* (2024). The feeding trial was conducted using 12 glass aquaria, each measuring 80 × 40 × 40 cm³. All aquaria were thoroughly cleaned, sun-dried, and filled with 90 L disinfected seawater. Aeration was provided by a low-noise air pump (Resun® LP 100, Shenzhen, China). Shrimp with an initial average body weight of 3.0 g were randomly stocked into each aquarium at a density of 40 individuals per tank. Shrimp were fed the prepared diets at a daily feeding rate of 4% of total biomass, divided into three feedings at 08:00, 12:00, and 16:00. Uneaten feed and fecal matter were siphoned daily and a 60% water exchange was carried out every 4 days to maintain water quality. Sampling was conducted at 10-day intervals to adjust feeding rates according to biomass. For each tank, three shrimp were randomly selected, weighed using a digital scale, and measured for total length using a digital calliper. The trial lasted for 40 days.

Observation of growth performance

Growth performance was evaluated by measuring specific growth rate (SGR), feed efficiency, weight gain, and length gain. Calculations were conducted using established formulas as described by Aanyu *et al.* (2018) and Li *et al.* (2020):

$$Weight\ gain\ (g) = Average\ final\ weight\ (g) - Average\ initial\ weight\ (g)$$

$$Length\ gain\ (mm) = Averager\ final\ total\ lenght\ (mm) - Average\ inital\ total\ lenght\ (mm)$$

$$Specific\ growth\ rate\ (\%.day^{-1}) = 100\ \times \frac{\ln Final\ weight\ (g) - \ln Initial\ weight\ (g)}{period\ (day)}$$

$$Feed\ efficiency\ (\%) = 100\ \times \frac{Final\ biomass\ (g) - Initial\ biomass\ (g)}{Feed\ intake\ (g)}$$

Measurement of water quality

Water quality parameters measured in this study included temperature, pH, salinity, dissolved oxygen, total ammonia nitrogen, nitrite, and nitrate. Temperature, pH, salinity, and dissolved oxygen were monitored daily using standard water quality probes. Total ammonia nitrogen, nitrite, and nitrate levels were measured every 10 days using commercial test kits, consisting of ammonia marine test kits (Salifert®, Duive, the Netherlands) and nitrate-nitrite marine test kits (Red Sea®, Wan Chai, Hong Kong).

Quantification of bacteria

Bacterial populations were quantified from the rearing water, hepatopancreas, and intestine of the shrimp using the total plate count method with spread plating. Water samples were collected every 10 days, and 0.1 mL of each sample was spread onto trypticase soy agar (TSA) for total heterotrophic bacteria and thiosulfate citrate bile salt agar (TCBSA) for *Vibrio* spp. (HiMedia Laboratories LLC, Pennsylvania, USA). To assess bacterial loads in the shrimp organs, 0.1 g of hepatopancreas and intestine tissues was collected before and after the trial and spread onto TSA and TCBSA, respectively. At the end of the experiment, total *Bacillus* and LAB in the intestine were quantified using HiCrome™ Bacillus Agar Base M1651 (HiMedia Laboratories LLC, USA) and GranuCult™ de Man, Rogosa and Sharpe agar (MRS; Merck KGaA, Darmstadt, Germany).

Observation of the total hemocyte count

Total hemocyte counts were determined after the feeding trial by extracting hemolymph from the ventral sinus of shrimp using a sterile 1-mL syringe preloaded with anticoagulant solution. The anticoagulant consisted of 3.8% sodium citrate, 30 mM trisodium citrate, 338 mM sodium chloride, 115 mM glucose, and 10 mM EDTA, adjusted to pH 7.0. Hemolymph was mixed with anticoagulant in a 1:3 (v/v) ratio (Huynh *et al.*, 2018). Hemocyte counts were conducted using a hemocytometer under a binocular microscope at 100× magnification (Liu and Chen, 2004).

Results and Discussion

Growth performance

The incorporation of commercial probiotics into the diet of Pacific white shrimp significantly enhanced growth performance (Table 1). Among the three probiotic treatments ($P<0.05$), the PB group showed the most notable improvement, with the highest specific growth rate (2.9866±0.0027%. day^{-1}), feed efficiency (57.48±0.09%), weight gain (6.35±0.01 g), and length gain (54.26±0.34 mm). In contrast, the control group exhibited the lowest values for all parameters, including a specific growth rate of 2.7396±0.0288%.day^{-1}, feed efficiency of 49.73±0.86%, weight gain of 5.50±0.10 g, and length gain of 45.37±1.07 mm.

These results demonstrate that probiotic supplementation, particularly with the PB formulation, enhances growth and feed conversion efficiency which are key determinants of economic viability in shrimp farming. Improved feed efficiency is particularly important as this reduces the amount of feed needed per unit of biomass gained which directly lowers feed-related costs (Ali *et al.*, 2022; Duy *et al.*, 2023). In this study, probiotics were estimated to improve feed efficiency by 3%-8% compared to the control, representing a substantial potential cost-saving. Reduced feed waste also contributes to better water quality and less frequent water exchange which can further decrease operational inputs.

The beneficial effects of probiotics on feed efficiency are attributed to enhanced digestion, modulation of intestinal microbiota, and increased production of digestive enzymes, which improve nutrient absorption and reduce metabolic waste (Li *et al.*, 2019; Rahayu *et al.*, 2024; Zhang *et al.*, 2024). These physiological improvements shorten the culture cycle and increase the frequency or flexibility of harvests, enabling producers to optimize stocking schedules and respond better to market demand.

Based on product composition, PB and PL contained probiotics from a single genus (*Bacillus* and *Lactobacillus*) while PMB combined species from different genera. The PB product included *B. cereus*, *B. megaterium*, *B. polymyxa*, *B. pumilus*, and *B. subtilis*. The PL comprised *L. plantarum*, *L. fermentum*, *L. bulgaricus*, and *L. lactis*. The PMB combined *B. subtilis*, *Bifidobacterium bifidum*, *L. plantarum*, *Nitrobacter winogradsky*, and *Saccharomyces cerevisiae*. The superior performance of PB can be attributed to the synergistic action of multiple *Bacillus* strains, known for their ability to colonize the shrimp gut, secrete extracellular enzymes, and tolerate environmental stress as a result of their spore-forming capability (Kuebutornye *et al.,* 2019; Soltani *et al*., 2019). *Bacillus* also produces bioactive compounds such as biotin, vitamin B12, fatty acids, and essential amino acids, which directly support growth (Amin, 2018). *Lactobacillus* strains, while not as effective in this trial, are known for producing antimicrobial substances that help suppress pathogenic bacteria (Amin *et al*., 2020). *S. cerevisiae* contributes to nutrient uptake and immune stimulation through its cellular components like β-glucan and mannan-oligosaccharides (Devi *et al*., 2019; Akanmu *et al*., 2022).

The observed benefits of probiotics in this study align with prior research reporting growth improvement in shrimp and other species. Shrimp fed *B. velezensis* showed enhanced growth metrics compared to controls (Chen *et al*., 2021) while other studies noted similar effects from *B. licheniformis*, *B. pumilus*, and *B. subtilis* (Omar *et al*., 2024). The inclusion of *S. cerevisiae* and *L. bulgaricus* improved growth in *Mugil capito* (Shehata *et al*., 2024). Likewise, Nile tilapia fed with S-PS (*Rhodobacter* and *Rhodococcus*) and Z (*Streptococcus faecalis*, *B. mesentericus*, and *Clostridium butyricum*) probiotic products also showed enhanced growth and feed efficiency (Tabassum *et al*., 2021; Azad *et al*., 2023).

Water quality

The administration of commercial probiotics did not significantly affect salinity, temperature, dissolved oxygen, or pH in the rearing media ($P>0.05$; Table 2). However, probiotic treatments notably improved nitrogen removal efficiency, as reflected in lower concentrations of total ammonia nitrogen, nitrite, and nitrate (Figure 1A, B, and C). Probiotic groups consistently showed reduced ammonia levels across all observation periods compared to the control ($P<0.05$). The control group recorded its highest nitrite levels on day 40, while PB maintained the lowest nitrate concentrations on days 10, 30, and 40.

Table 1. Growth performance of Pacific white shrimp fed different commercial probiotics products

Parameters	Treatments			
	Control	PB	PL	PMB
Specific growth rate (%.day^{-1})	2.7396±0.0288^{a}	2.9866±0.0027^{d}	2.8455±0.0029^{b}	2.9217±0.0043^{c}
Feed efficiency (%)	49.73±0.86^{a}	57.48±0.09^{d}	52.95±0.09^{b}	55.37±0.14^{c}
Weight gain (g)	5.50±0.10^{a}	6.35±0.01^{d}	5.85±0.01^{b}	6.12±0.02^{c}
Length gain (mm)	45.37±1.07^{a}	54.26±0.34^{b}	45.92±2.03^{a}	45.03±1.26^{a}

Values are expressed as the mean ± standard deviation. Different superscript letters in the same row indicate significantly different results ($P<0.05$).

Table 2. The salinity, temperature, dissolved oxygen, and pH value of the rearing water of Pacific white shrimp fed different commercial probiotic products

Parameters	Treatments			
	Control	PB	PL	PMB
Salinity (ppt)	30.39±0.10^{a}	30.38±0.11^{a}	30.42±0.20^{a}	30.41±0.32^{a}
Temperature (°C)	28.49±0.10^{a}	28.44±0.11^{a}	28.36±0.20^{a}	28.19±0.32^{a}
Dissolved oxygen (mg.L^{-1})	2.01±0.19^{a}	1.86±0.15^{a}	1.87±0.22^{a}	2.05±0.13^{a}
pH	8.15±0.12^{a}	8.09±0.05^{a}	8.11±0.09^{a}	8.16±0.06^{a}

Values are expressed as the mean ± standard deviation. Different superscript letters in the same row indicate significantly different results ($P<0.05$).

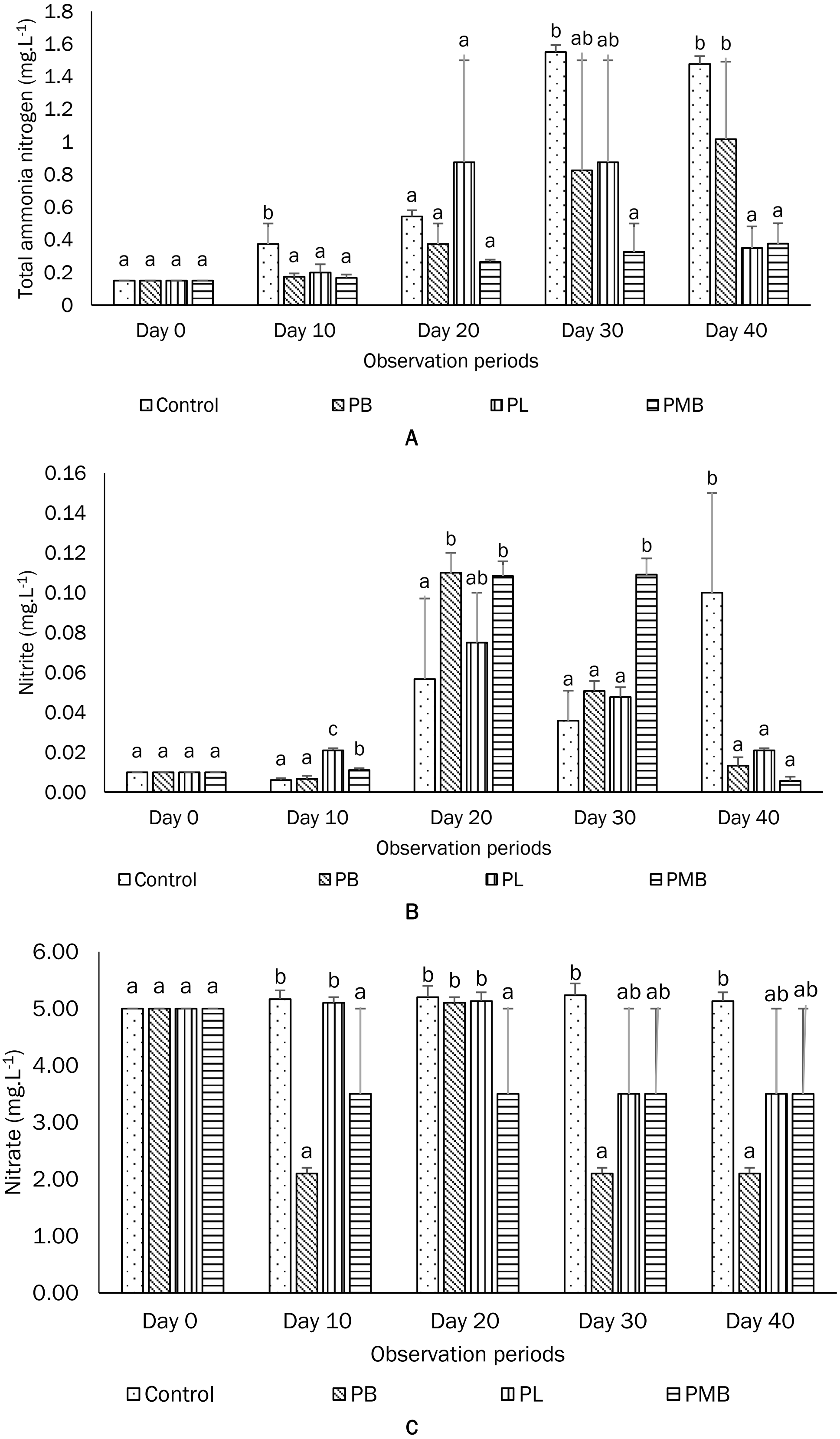


Figure 1. The nitrogenous compounds present in the rearing water of Pacific white shrimp fed different commercial probiotics included (A) total ammonia nitrogen; (B) nitrite; and (C) nitrate. Different letters on bars in the same observation periods indicate significantly different results (*P*<0.05)

The observed reductions in total ammonia nitrogen, nitrite, and nitrate in probiotic groups, especially PB, emphasize the critical role of *Bacillus*-based probiotics in enhancing water quality. These bacteria secrete extracellular enzymes that degrade organic material and facilitate nitrogen transformation, which is essential in minimizing toxic accumulation in intensive systems (Cai *et al.*, 2019). This enzymatic activity supports a more stable environment, reducing the frequency of water exchange and, consequently, lowering energy and water usage costs (Mang *et al.*, 2024).

Bacillus species are particularly effective in this role due to their participation in all stages of the nitrogen cycle, including ammonification, nitrification, denitrification, and nitrogen fixation. For instance, *B. amyloliquefaciens* converts organic nitrogen into ammonium, while *B. cereus* removes nitrite from aquatic systems (Hui *et al.*, 2019). *Lactobacillus*, by contrast, lacks this capability, as noted in previous studies (Flores-Valenzuela *et al.*, 2021). The enhanced microbial activity in the rearing environment, driven by *Bacillus* colonization, also supports better shrimp performance by maintaining a cleaner, more stable system (Amin *et al.*, 2023; Ghosh, 2025).

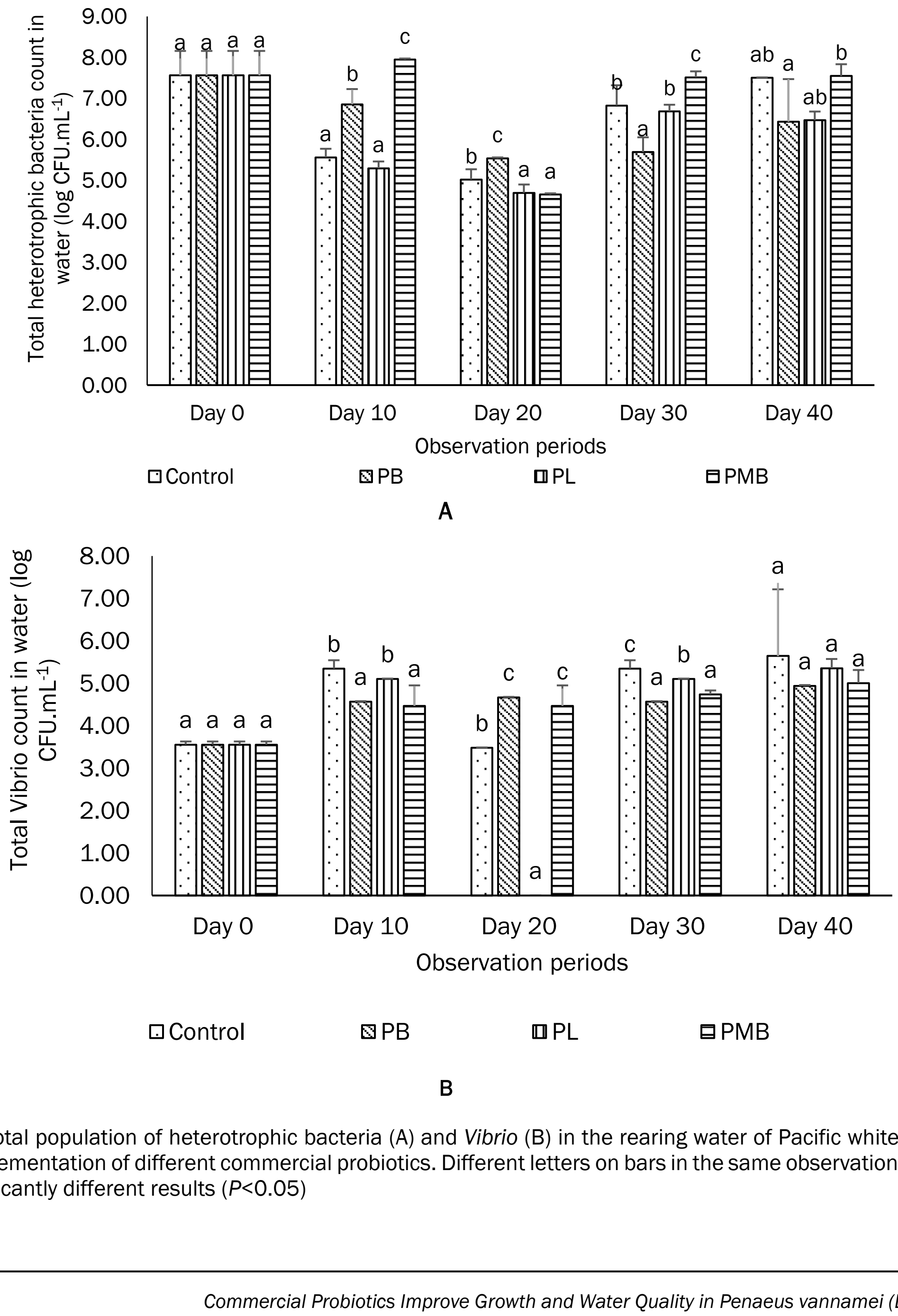
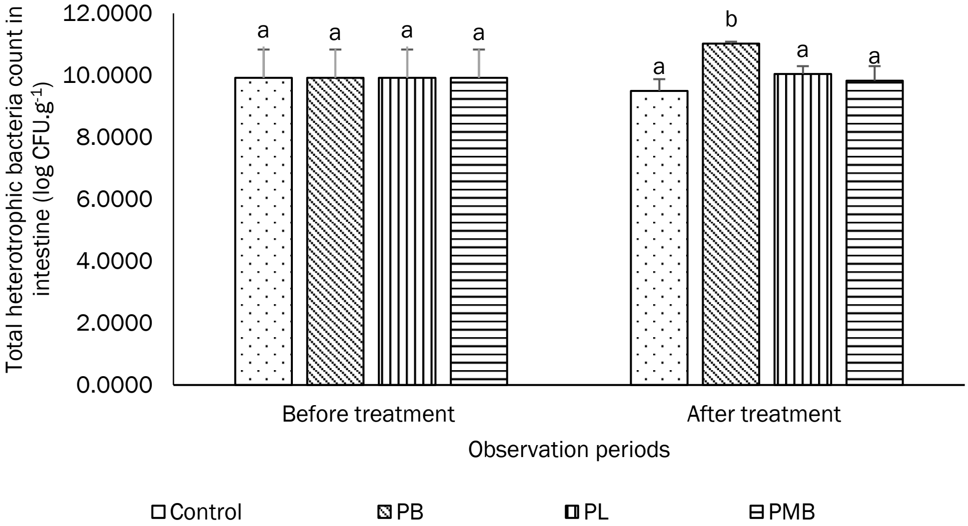
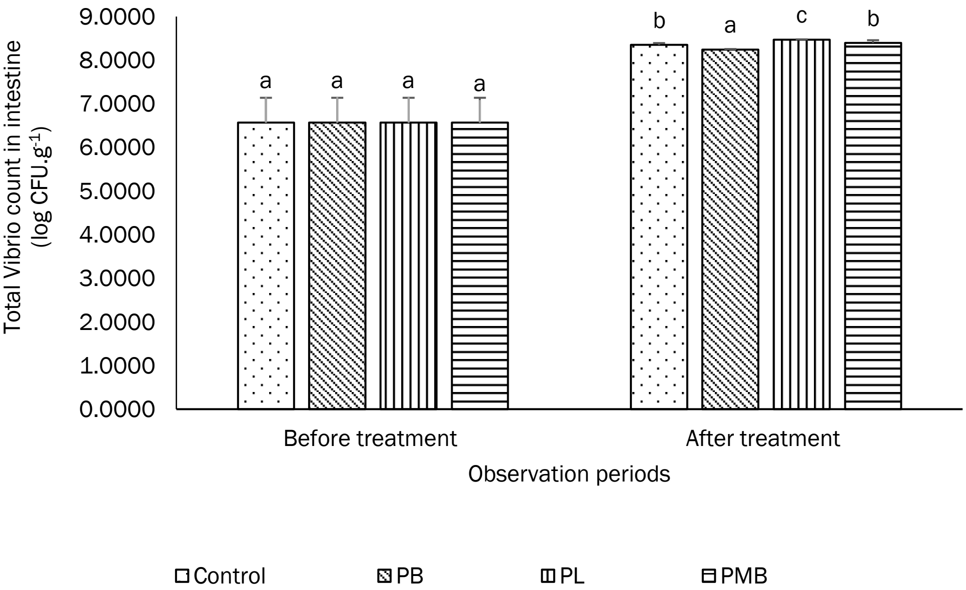


Figure 2. The total population of heterotrophic bacteria (A) and *Vibrio* (B) in the rearing water of Pacific white shrimp fed with supplementation of different commercial probiotics. Different letters on bars in the same observation periods indicate significantly different results (P<0.05)



A



B

Figure 3. The heterotrophic bacteria count (A) and *Vibrio* count (B) in the intestines of Pacific white shrimp fed with supplementation of different commercial probiotic products. Different letters on bars in the same observation periods indicate significantly different results ($P<0.05$)

These findings are especially relevant for biofloc and recirculating aquaculture systems (RAS) which heavily depend on microbial regulation of water quality. Biofloc relies on heterotrophic microbial communities to convert waste into microbial biomass, while RAS systems utilize controlled filtration loops to maintain water quality with minimal water replacement (Akange *et al.*, 2024; Jafari *et al.*, 2024). The ability of probiotics to suppress nitrogenous accumulation and reinforce microbial balance makes them well-suited for integration into both systems. In biofloc, probiotics may enhance floc formation and stability, while in RAS it can support microbial resilience in biofilters that improve overall system efficiency and reliability (Ramiro *et al.*, 2024).

Bacterial population

The PMB treatment yielded the highest total heterotrophic bacterial count in the water on days 10 and 30 (log 7.95±0.02; 7.51±0.15 CFU·mL^{-1}), while both PMB and PB treatments recorded the lowest

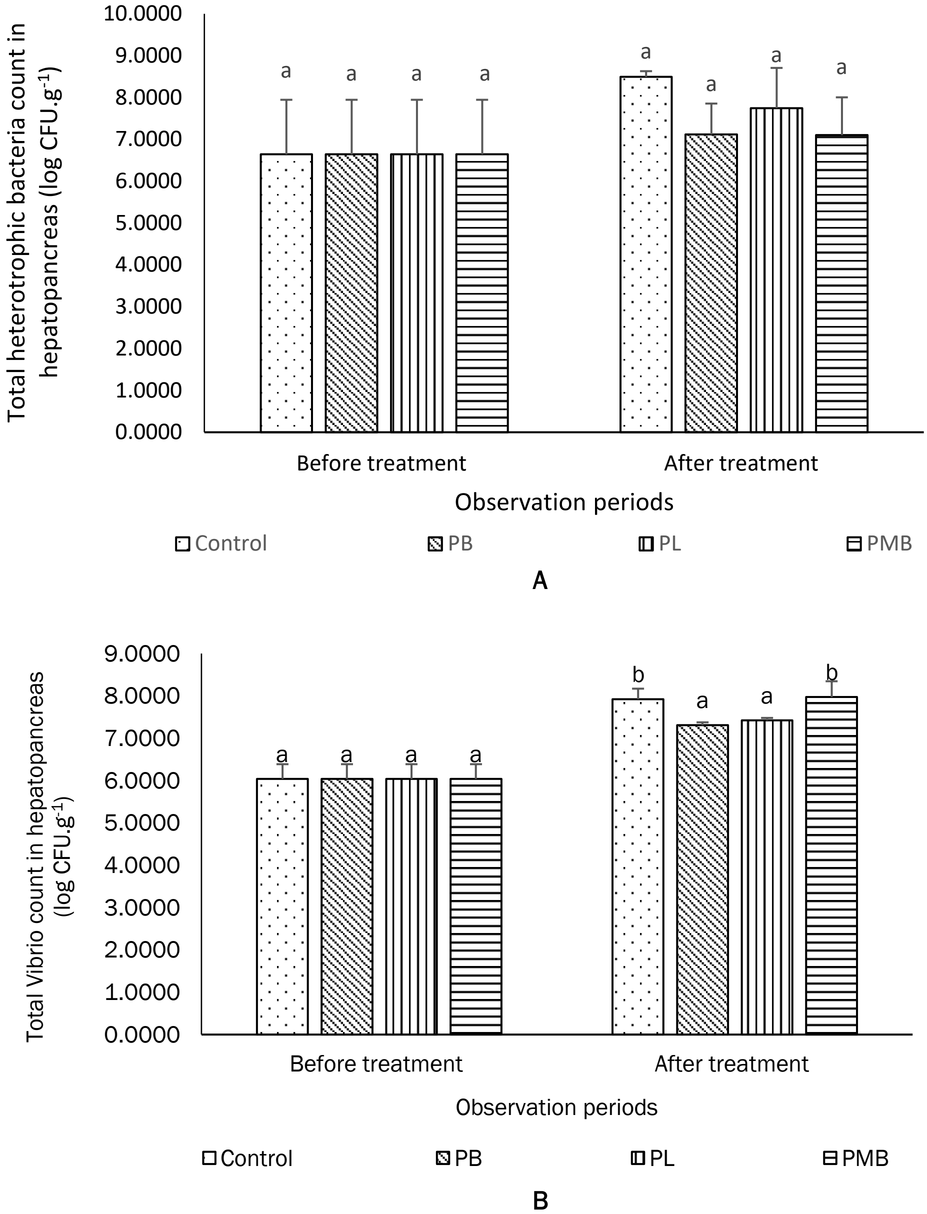


Figure 4. The total (A) heterotrophic bacteria count and (B) *Vibrio* count in hepatopancreas of Pacific white shrimp fed with supplementation of different commercial probiotics. Different letters on bars in the same observation periods indicate significantly different results (P<0.05)

total *Vibrio* levels compared to PL (Figure 2A and B). This indicates that PMB, when administered orally, effectively altered the microbial community by increasing beneficial heterotrophic bacteria and suppressing *Vibrio* populations. These shifts could be the product of competitive exclusion or successful colonization of the introduced strains (Dai *et al.*, 2022). *Vibrio* spp. are opportunistic pathogens common in shrimp farming environments and reducing their abundance is crucial for disease prevention (Huang *et al.*, 2021).

The ecology of *Vibrio* is closely tied to organic load since it is an obligate heterotroph that thrives in environments rich in dissolved organic carbon. This explains the elevated *Vibrio* counts in systems with higher waste accumulation. The reduced *Vibrio* levels in the PB and PMB groups align with previous studies that demonstrated the effectiveness of probiotics, especially *Bacillus*-based forms, in enhancing water quality by degrading organic matter and outcompeting harmful microbes (Hemaiswarya and Doble, 2013; Hassan *et al.*, 2022). *Bacillus* is notably more effective than Gram-negative bacteria in converting organic substrates into carbon dioxide, limiting the carbon available for *Vibrio* proliferation (Rahayu *et al.*, 2024). Moreover, enzymatic activity accelerates waste decomposition which further disrupts *Vibrio* survival niches (Wang *et al.*, 2021; Goh *et al.*, 2023). Improved water conditions in the probiotic groups likely explain the lower *Vibrio* prevalence, as this genus thrives in environments with elevated pH, temperature, and ammonia (Hassan *et al.*, 2022).

In the intestinal tract, the PB treatment produced the highest total heterotrophic bacterial count (log 11.03±0.06 CFU·g^{-1}) and the lowest *Vibrio* count (log 8.24±0.02 CFU·g^{-1}) (Figure 3A and B). These findings support the role of PB in beneficial microbiota modulation, which contributes to improved digestive function and disease resistance. The gut plays a central role in digestion, nutrient absorption and immune regulation (Wang *et al.*, 2020), and a well-balanced microbiota is key to optimal performance.

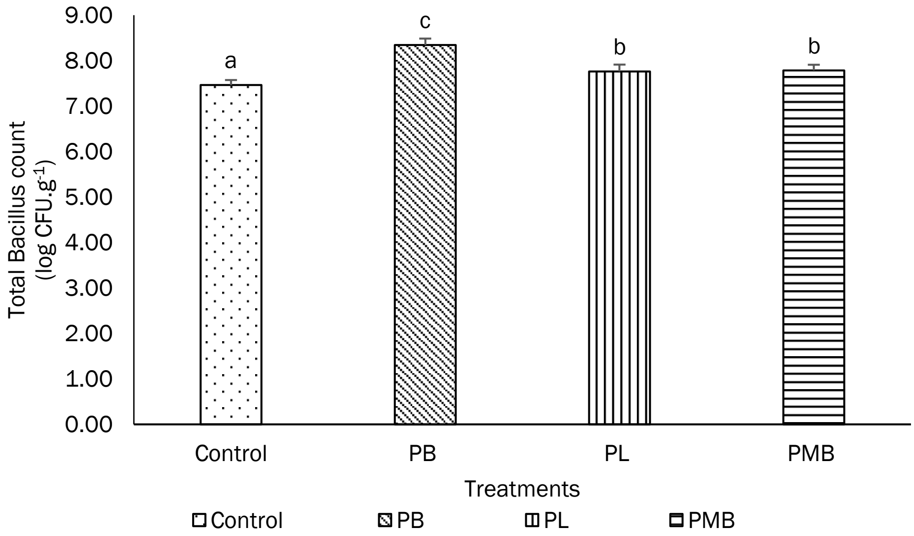


Figure 5. Total *Bacillus* count in the intestine of Pacific white shrimp fed with supplementation of different commercial probiotics. Different letters on bars indicate significantly different results (*P*<0.05)

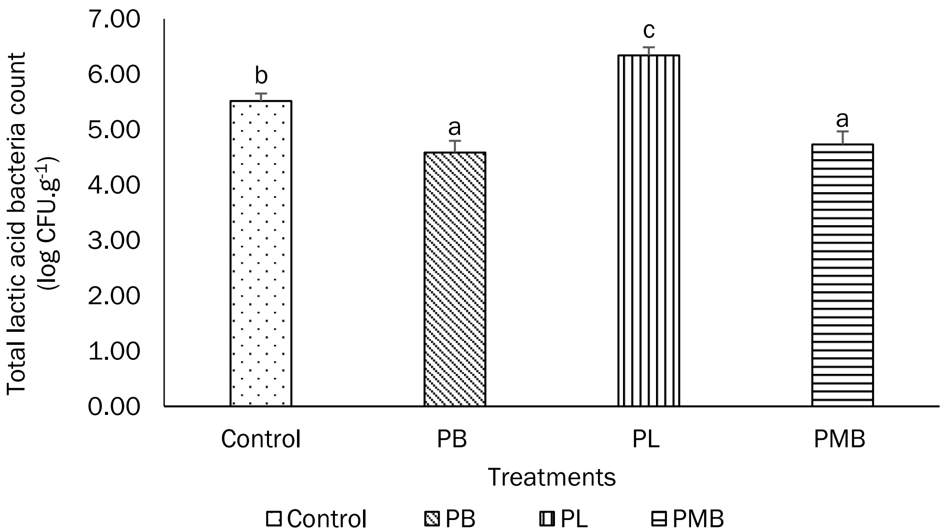


Figure 6. Total lactic acid bacteria count in the intestine of Pacific white shrimp fed with supplementation of different commercial probiotics. Different letters on bars indicate significantly different results (*P*<0.05)

Effective colonization is critical for probiotics to exert sustained benefits. The PB demonstrated this capacity by suppressing *Vibrio* and enhancing the abundance of beneficial microbes. *Bacillus* strains produce various antimicrobial compounds, including bacteriocins, lipopeptides, and quorum-quenching agents that inhibit pathogen adhesion and biofilm formation (Vinoj *et al.*, 2014; Shleeva *et al.*, 2023). For example, *B. licheniformis* disrupts *Vibrio* communication via AHL-lactone interference, reducing pathogenicity. Other studies have similarly shown that commercial probiotics can reshape intestinal microbial communities to favor host health (Aribah *et al.*, 2022; Hien *et al.*, 2022).

Although total heterotrophic bacterial counts in the hepatopancreas did not differ significantly across treatments (P>0.05), both PB and PL resulted in lower *Vibrio* counts compared to the control and PMB (Figure 4A and B). This suggests that while probiotics may not colonize the hepatopancreas, they can exert systemic effects that reduce pathogen load in this metabolically vital organ. The hepatopancreas is often a target for *Vibrio* invasion, and its impairment can lead to compromised growth and survival. LAB strains such as *L. plantarum* and *Pediococcus acidilactici* are known to secrete antimicrobial agents like lactic acid and bacteriocins, which may contribute to *Vibrio* suppression in internal organs (Thompson *et al.*, 2022). Supporting this, Hien *et al.* (2022) reported reduced *Vibrio* in the hepatopancreas of shrimp fed a *Bacillus*-based probiotic blend.

Overall, the ability of PB and PMB to reduce *Vibrio* in both the external (water) and internal (gut and hepatopancreas) environments highlights their potential as functional disease control agents. This microbial suppression reduces the need for antibiotics, aligning with sustainable aquaculture goals and helping mitigate risks of antimicrobial resistance (Choudhary *et al.*, 2025; Gadhiya *et al.*, 2025; Thakur *et al.*, 2025).

PB and PL treatments also demonstrated strong colonization in the intestine with PB producing the highest *Bacillus* count (log 8.34±0.14 CFU·g^{-1}) and PL yielding the highest lactic acid bacteria (LAB) count (log 6.34±0.15 CFU·g^{-1}) (Figures 5 and 6). These colonization profiles are essential for probiotic persistence and long-term effects (Alp and Kuleasan, 2019). Although long-term colonization dynamics remain unclear, *Lactobacillus* is known to adhere well to mucosal surfaces (Altermann *et al.*, 2005). *Bacillus* also competes for nutrients and adhesion sites, securing dominance in the gut microbiota and reinforcing its functional role. Consistent with this, Vidal *et al.* (2018) found that *B. cereus* persisted in the intestines of shrimp post-larvae after probiotic feeding, while Roomiani *et al.* (2018) reported detectable LAB only in treated shrimp. These outcomes confirm that both *Bacillus* and LAB strains used in this study were effective in colonizing and modulating the shrimp gut ecosystem.

Total hemocyte count

The effect of probiotics on shrimp health was also assessed via total hemocyte count (THC) (Figure 7). The PB treatment achieved the highest THC (3.80±0.04 × 10^5 cells·mm^{-3}), significantly higher than other treatments (P<0.05). An elevated THC reflects improved immune competence, enabling crustaceans to respond more effectively to stress and

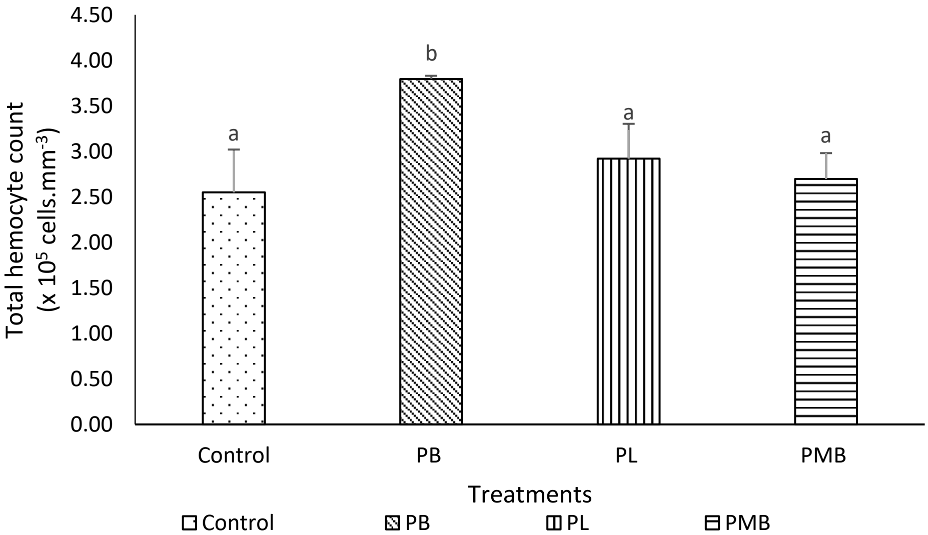


Figure 7. Total hemocyte count of Pacific white shrimp fed with supplementation of different commercial probiotics. Different letters on bars indicate significantly different results (P<0.05)

pathogens (Liu *et al.*, 2020). The observed increase suggests that *Bacillus* strains in PB enhanced hemocyte production.

These results are consistent with Kesselring *et al.* (2019), who reported higher THC in shrimp fed multispecies probiotics (*B. subtilis*, *P. acidilactici*, *Enterococcus faecium*, and *Lactobacillus reuteri*). Similarly, probiotic supplementation increased THC in *Procambarus clarkii* juveniles by accelerating hemocyte precursor maturation and release from hemopoietic tissues (Sequeira *et al.*, 1996; Mona *et al.*, 2015). The modulation of intestinal microbiota which is marked by a higher abundance of beneficial bacteria and reduced *Vibrio*, likely reinforced the immune response, as reflected in the THC data. This immunostimulatory effect helps lower disease incidence and antibiotic use, improving survival and overall productivity, which are critical for profitable and sustainable high-density farming (Bondad-Reantaso *et al.*, 2023).

Conclusion

This study suggests that commercial probiotics, particularly the *Bacillus*-based product, significantly improved growth performance, feed efficiency, immune response, and water quality in Pacific white shrimp culture. Probiotic supplementation also reduced *Vibrio* populations and nitrogenous waste which support better shrimp health and environmental stability. The findings highlight the potential of probiotics as a cost-effective, antibiotic-free strategy suitable for intensive systems such as biofloc and RAS.

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