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Application of Commercial Probiotics in the Cultivation of Non-Specific Pathogen Free (Non-SPF) Vannamei Shrimp (*Litopenaeus vannamei*)

Lukman Hudi[1], Irnia Nurika[2], Nur Hidayat[2], Ating Yuniarti[3], Sutarman[4]

[1]Study Program of Food Technology, Faculty of Science and Technology, Universitas Muhammadiyah Sidoarjo, Jl. Mojopahit No.666 B, Sidowayah, Celep, Sidoarjo District, Sidoarjo Regency, East Java 61215, Indonesia
[2]Study Program of Agricultural Industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Jl. Veteran, Ketawanggede, Lowokwaru District, Malang City, East Java 65145, Indonesia
[3]Study Program of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Jl. Veteran, Ketawanggede, Lowokwaru District, Malang City, East Java 65145, Indonesia
[4]Study Program of Agrotechnology, Faculty of Science and Technology, Universitas Muhammadiyah Sidoarjo, Jl. Mojopahit No.666 B, Sidowayah, Celep, Sidoarjo District, Sidoarjo Regency, East Java 61215, Indonesia

*Corresponding Author: lukmanhudi@umsida.ac.id

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ABSTRACT

Probiotics are live microbial agents that provide beneficial effects to the host by balancing intestinal microflora. They also play a crucial role in aquaculture systems by maintaining health and serving as biocontrol agents for water quality. This study aimed to evaluate the effect of commercial probiotic application on the survival rate (SR), average daily growth (ADG), feed conversion ratio (FCR), and water quality in vannamei shrimp (*Litopenaeus vannamei*) culture using the commercial probiotics Bio Lacto, Golden Soil, and the fungus *Trichoderma* sp. The study was conducted using a completely randomized design (CRD) with six treatments, each replicated three times, resulting in a total of 18 experimental units. The data obtained were expressed as mean ± standard error and analyzed using analysis of variance (ANOVA) at a 95% confidence level. If significant differences were observed, a post-hoc Tukey test was performed. The application of commercial probiotics in vaname shrimp cultivation had a significant effect ($P<0.05$) on SR, ADG, and FCR values. The use of single probiotics resulted in lower ADG and higher FCR values, whereas combined probiotic application showed better performance. The use of *Trichoderma* sp. is recommended as it demonstrated a high SR. Additionally, probiotic application significantly ($P<0.05$) reduced *Vibrio* levels, pH, alkalinity, and NH_3 in the shrimp culture water.

INTRODUCTION

The Pacific white shrimp (*Litopenaeus vannamei*) is one of the most economically valuable shrimp species in aquaculture, accounting for more than double the global shrimp production **(Gao *et al.*, 2017)**. However, due to high stocking densities, intensive production, and large-scale farming, issues such as disease outbreaks, ecological imbalances, and environmental degradation have increasingly become major challenges for the shrimp industry **(Kilawati *et al.*, 2024; Kilawati *et al.*, 2024)**. Several factors influence the success of *L. vannamei* cultivation, including the availability of artificial feed or substrates, microbial activity, water quality, and stocking density/biomass. Water quality is primarily regulated by microbial communities **(Pardamean *et al.*, 2021; Islamy *et al.*, 2024a)**.

Microbial agents or communities that provide beneficial effects to the host by balancing intestinal microflora are commonly referred to as probiotics **(Verschuere *et al.*, 2000)**. Probiotics play a crucial role in aquaculture systems by maintaining shrimp health and acting as biocontrol agents for water quality. One example of probiotic bacteria is the Gram-positive *Bacillus* sp. Several *Bacillus* species, including *Bacillus thuringiensis*, *Bacillus licheniformis*, and *Bacillus cereus*, have been identified as highly beneficial in aquaculture systems. *B. licheniformis* has been reported as the only isolate exhibiting *in vitro* inhibitory characteristics against the pathogen *Vibrio alginolyticus*, with an inhibition zone diameter of 20mm, although the isolate did not show antagonistic activity. *Bacillus* sp. strains have been shown to enhance total hemocyte counts, serum-binding protein levels, and total serum protein while reducing hemolymph phenoloxidase (PO) activity **(Gomes Vilani *et al.*, 2016)**. Additionally, *Bacillus* spp. isolated from biofloc water systems, when incorporated into shrimp feed, have been demonstrated to improve immunocompetence and serve as biocontrol agents against *Vibrio* spp. in culture water **(Ferreira *et al.*, 2015)**.

The use of probiotics as an alternative to chlorination and antibiotics in controlling pathogens in intensive shrimp farming has been proposed. The administration of *Bacillus subtilis* strains has been reported to significantly enhance shrimp growth while colonizing the hepatopancreas and intestines. Furthermore, shrimp feed supplemented with *Shewanella* and *Bacillus subtilis* strains has been found to reduce *Vibrio* loads in the hepatopancreas of *L. vannamei* **(Interaminense *et al.*, 2018)**. Probiotic supplementation has also been associated with increased intracellular superoxide production, anion stimulation by laminarin (β-1,3 glucans), and phorbol myristate acetate (PMA) **(Gomes Vilani *et al.*, 2016)**. Moreover, encapsulated *Bacillus vireti* in combination with *Gracilaria folifera* seaweed polysaccharides as shrimp feed has been reported to enhance immune responses, antioxidant activity, and disease resistance in *Macrobrachium rosenbergii* (giant freshwater prawn) against *Aeromonas hydrophila* **(Vidhya Hindu *et al.*, 2018)**. The concentration of probiotics also determines growth, survival and feed efficiency in vaname shrimp culture **(Hudi *et al.*, 2025)**.

High stocking densities in shrimp aquaculture have been linked to disease outbreaks and ecological imbalances, leading to the deterioration of shrimp aquaculture systems. The application of probiotics is expected to improve water quality, thereby enhancing shrimp health and growth performance. In this study, the commercial probiotic bacteria *Bio Lacto* and *Golden Soil*, along with the fungus *Trichoderma* sp., were utilized to assess their effects on the water quality of *L. vannamei* aquaculture systems.

MATERIALS AND METHODS

Test organisms and experimental setup

The test organisms used in this study were *Litopenaeus vannamei* shrimp with an initial weight of 0.493±0.035g per individual. The shrimp were obtained from a local hatchery and were classified as non-specific pathogen free (Non-SPF), as the supply of specific pathogen free (SPF) shrimp was limited, while the demand for post-larvae remained high. The shrimp were stocked at a density of 200 individuals per cubic meter.

The experimental units consisted of circular tarpaulin tanks, with a height of 1 meter and a diameter of 4 meters. Prior to stocking, all tanks were disinfected using 100ppm saponin for 24 hours. Water quality management was conducted through siphoning and partial water exchange, with 10% of the total water volume being replaced every two days.

Probiotic preparation and application

The probiotics used in this study were prepared and cultured according to their respective applications. The probiotic treatments were applied three days after the sterilization process. The experimental treatments included:

- *Trichoderma* sp. (fungi)
- Golden Soil (commercial probiotic)
- *Trichoderma* sp. + *Golden Soil*
- *Trichoderma* sp. + *Bio Lacto* (commercial probiotic)
- Bio Lacto (commercial probiotic) as a single treatment

The probiotics were applied at a concentration of 1% v/v mg/L, following a modified method by **Qiu *et al.* (2023)**. The colony-forming units (CFU) at the time of application were 1×10^7 CFU.g^{-1} for Bio Lacto, 1×10^7 CFU.g^{-1} for Golden Soil, and 1×10^4 CFU.g^{-1} for *Trichoderma* sp.

Experimental procedure

The study was conducted in several stages, including tank preparation, probiotic preparation and application, stocking of Non-SPF *L. vannamei* post-larvae, and continuous probiotic application throughout the culture period.

Growth performance assessment

At the end of the experimental period, the final body weight of *L. vannamei* was recorded for each tank, and the number of surviving shrimp was counted. Growth performance parameters, including survival rate (SR), average daily growth (ADG), feed conversion ratio (FCR), and specific growth rate (SGR), were calculated using the following formulas:

Average daily growth (ADG, %):

$$ADG = \frac{(W_f - W_i)}{W_i} \times 100$$

Feed conversion ratio (FCR):

$$FCR = \frac{\text{Total Feed Consumed}}{(W_f - W_i)}$$

Survival rate (SR, %):

$$SR = \frac{N_f}{N_i} \times 100$$

Where, W_f and W_i represent the final and initial body weights, respectively, and N_f and N_i represent the final and initial shrimp counts **(Xie *et al.*, 2019)**.

Statistical analysis

The collected data were analyzed using one-way analysis of variance (ANOVA) with Minitab 18 statistical software. A significance level of $\alpha = 0.05$ was set to determine statistical differences among treatments. If significant differences were detected, post-hoc analysis was conducted using Tukey's test.

RESULTS AND DISCUSSION

The effects of probiotic application on the average daily growth (ADG), feed conversion ratio (FCR), and survival rate (SR) of vannamei shrimp are presented in Table (1).

Table 1. Effect of probiotic application on average daily growth (ADG), feed conversion ratio (FCR), and survival rate (SR) of vannamei shrimp

Treatment	Code	Survival rate (SR) (%)	Average daily growth (ADG) (g/day)	Feed conversion ratio (FCR)
***Trichoderma* sp.**	TR	53.67 ± 0.33^{c}	0.12 ± 0.007^{b}	1.00 ± 0.000^{a}
Goldensoil	GS	38.67 ± 0.67^{a}	0.11 ± 0.012^{a}	1.43 ± 0.173^{b}

***Trichoderma* sp. + Goldensoil**	TRGS	46.67 ± 0.67^{b}	0.17 ± 0.003^{ab}	0.79 ± 0.003^{b}
***Trichoderma* sp. + Biolacto**	TRLC	46.33 ± 0.67^{b}	0.13 ± 0.007^{b}	1.23 ± 0.083^{ab}
Biolacto	LC	44.33 ± 1.20^{b}	0.12 ± 0.009^{b}	1.82 ± 0.133^{b}

Note: Data are presented as mean ± standard deviation from three replicates. Different superscript letters indicate statistically significant differences among treatments ($P < 0.05$), while the same letters indicate no significant differences ($P > 0.05$).

Survival rate (SR)

As shown in Table (1), the application of commercial probiotics significantly affected ($P < 0.05$) the survival rate (SR) of vannamei shrimp. The highest SR was observed in shrimp treated with *Trichoderma* sp. (TR), reaching 53.67% ± 0.33, followed by TRGS, TRLC, and LC with values of 46.67% ± 0.67, 46.33% ± 0.67, and 44.33% ± 1.20, respectively. The lowest SR (38.67% ± 0.67) was recorded in shrimp treated with GS. A significant difference was observed between GS and the other probiotic treatments (TRGS, TRLC, LC, and TR), while no significant differences were detected among TRGS, TRLC, and LC treatments.

Trichoderma sp. has been reported to exhibit high tolerance and remain distinct from other fungi, demonstrating significant potential for heavy metal detoxification. The application of *Trichoderma* sp. has been recognized as an effective bioremediation strategy, potentially enhancing the remediation of contaminated areas **(Reyes & Waerebeek, 2018)**. Furthermore, the combination of *Trichoderma* with probiotic bacteria and *Lactobacillus* spp. has been found to improve water quality parameters, including pH, temperature, salinity, dissolved oxygen, and water clarity, ultimately enhancing shrimp growth and feed utilization efficiency **(Sutarman *et al.*, 2024)**.

The *Bacillus subtilis* (BS) and effective microorganism (EM) groups have been reported to significantly reduce total nitrogen concentration while increasing final body weight, weight gain, and specific growth rate in *Macrobrachium rosenbergii* (giant freshwater prawn). The BS group also significantly enhanced plasma superoxide dismutase, lysozyme, and acid phosphatase levels while reducing malondialdehyde content. Similarly, the EM group improved serum acid phosphatase levels and intestinal trypsin activity but reduced species richness indices (Chao and ACE). Overall, the application of probiotics positively influenced water quality, *M. rosenbergii* performance, and microbial communities, with *Bacillus subtilis* identified as a beneficial biofloc probiotic additive **(Qiu *et al.*, 2023)**.

Probiotic application has also been associated with enhanced immune system function, improved shrimp survival rates, and reduced *Vibrio parahaemolyticus* proliferation in *Litopenaeus vannamei* culture. A recommended probiotic dosage of 10^{8} CFU/mL has been suggested for optimal immune system enhancement and disease prevention **(Jannah *et al.*, 2018)**. Various microorganisms exhibit distinct functional characteristics, primarily contributing to: (i) water quality improvement through inorganic nitrogen removal (bioaccumulation, bioassimilation, nitrification, and denitrification), (ii) serving as an additional nutritional source, and (iii) exerting probiotic effects, which are essential for sustainable aquaculture systems **(Khanjani *et al.*, 2022)**.

Average daily growth (ADG)

According to Table (1), probiotic supplementation had a significant effect (P< 0.05) on the ADG of vannamei shrimp. The highest ADG (0.17 ± 0.003g/ day) was recorded in the TRGS group, whereas the lowest (0.11 ± 0.012g/ day) was observed in the GS group. Other treatments, including TRLC, LC, and TR, exhibited ADG values of 0.13 ± 0.007, 0.12 ± 0.009, and 0.12 ± 0.007g/ day, respectively. Significant differences were found between GS and the TR, TRLC, and LC treatments, but no significant differences were detected between TRGS and other treatments.

Probiotic supplementation has been shown to enhance shrimp growth performance by improving gut microbiota and non-specific immune responses. A mixed probiotic supplement at an optimal dosage of 2000mg/ kg has been recommended for *L. vannamei* **(Xie *et al.*, 2019)**. The inclusion of *Trichoderma harzianum* at 0.5 and 1.5% in shrimp diets has been found to improve survival and growth rates in post-larvae; however, further research is required to evaluate potential anti-nutritional effects of certain microfungi **(Binur *et al.*, 2021)**.

Feed conversion ratio (FCR)

The results presented in Table (1) indicate that probiotic application had a significant impact (P< 0.05) on the FCR values of vannamei shrimp. The lowest FCR (0.79 ± 0.003) was observed in the TRGS treatment, followed by TRLC (1.23 ± 0.083) and GS (1.43 ± 0.173), whereas the highest FCR (1.82 ± 0.133) was recorded in shrimp treated with LC. The TR treatment exhibited a significant difference compared to GS, TRGS, and LC but did not significantly differ from TRLC.

Probiotics contribute to digestive efficiency by enhancing enzymatic activity and producing bioactive compounds such as amino acids and vitamins, which support shrimp metabolism and growth. Moreover, probiotics suppress pathogenic bacteria and parasites in the gut, reducing stress-related impacts on feed consumption **(Hien *et al.*, 2022)**.

The effect of probiotics on FCR has been reported to vary depending on probiotic strain, dosage, and environmental conditions. Further research is needed to optimize probiotic applications for improving feed efficiency in *L. vannamei* culture **(Ringø, 2020)**. The supplementation of *Lactobacillus plantarum* and *Pediococcus acidilactici* in shrimp diets has been demonstrated to enhance growth performance while reducing FCR by 3.7% **(Panigrahi *et al.*, 2004; Hoseinifar *et al.*, 2018)**.

On the other hand, the quality of feed ingredients based on their nutritional content can also improve the feed conversion ratio (FCR). Several natural materials for aquaculture feed, rich in various nutrients, have been studied, such as seaweeds **(Islamy *et al.*, 2024a; Islamy *et al.*, 2024b; Islamy *et al.*, 2025)**, neem leaves **(Islamy *et al.*, 2024c)**, Ipomoea pes-caprae **(Islamy *et al.*, 2024d)**, and alligator weed **(Serdiati *et al.*, 2024)**. Further research is needed to explore the optimal incorporation levels of these ingredients, their long-term effects on aquaculture species, and their potential to replace conventional feed sources while maintaining or improving production efficiency and sustainability in aquaculture systems.

CONCLUSION

The application of probiotics significantly influenced the survival rate (SR), average daily growth (ADG), and feed conversion ratio (FCR) of *Litopenaeus vannamei*. The highest SR (53.67% ± 0.33) was observed in shrimp treated with *Trichoderma* sp. (TR), whereas the lowest SR (38.67% ± 0.67) was recorded in the Goldensoil (GS) treatment. The highest ADG (0.17 ± 0.003 g/day) was achieved in the *Trichoderma* sp. + Goldensoil (TRGS) treatment, indicating a synergistic effect of the combined probiotics on shrimp growth. The lowest FCR (0.79 ± 0.003) was also recorded in the TRGS treatment, suggesting improved feed efficiency. These findings highlight the potential of *Trichoderma* sp. and its combination with other probiotics to enhance shrimp performance by improving survival, growth, and feed utilization. The results suggest that probiotic supplementation can serve as a sustainable strategy to optimize shrimp culture. However, further research is recommended to determine the long-term effects of probiotic application and to explore its impact on immune responses, disease resistance, and water quality in intensive aquaculture systems.

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