Journal of Fish Health
Volume 6, Issue 2, May 2026
https://doi.org/10.29303/jfh.v6i2.10076

Optimization of Probiotic Dosage in Tomato Meal-Enriched Diet to Improve Growth and Feed Utilization Efficiency in Whiteleg Shrimp (*Litopenaeus vannamei*)

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Received:
April 27th, 2026
Accepted:
May 7th, 2026
Published:
May 28th, 2026

Keywords:
Feed Efficiency, Growth Performance, Probiotic, Tomato Meal, Whiteleg Shrimp

ABSTRACT

Probiotics and prebiotics are promising functional feed additives in aquaculture because they can improve gut health, nutrient utilization, growth, and survival of cultured aquatic species. This study aimed to evaluate the effects of different probiotic dosages in a tomato meal enriched diet on growth performance, feed utilization efficiency, and survival of whiteleg shrimp. The experiment was conducted using a completely randomized design consisting of four treatment and three replicates. The treatments included PR-5, PR-10, and PR-15, representing tomato meal-enriched diet supplemented with 5, 10, and 15 mL probiotic/kg feed, respectively, and WPR, representing tomato meal-enriched diet without probiotic supplementation. The shrimp were reared for 30 days and evaluated for weight gain, specific growth rate, feed conversion ratio, feed efficiency, and survival rate. The results showed that probiotic supplementation significantly affected weight gain, feed conversion ratio, feed efficiency, and survival rate ($P < 0.05$), but did not significantly affect specific growth rate on day 15 or day 30 ($P > 0.05$). The PR-15 treatment produced the highest weight gain of 7.53 g, the lowest feed conversion ratio of 15.68, the highest feed efficiency of 6.39%, and the highest survival rate of 80.00%. These findings indicate that supplementation with 15 mL probiotic/kg feed in a tomato-meal enriched diet was the most effective treatment for improving growth performance, feed utilization efficiency, and survival of whiteleg shrimp. Therefore, this dietary strategy may support more efficient and sustainable whiteleg shrimp culture.

INTRODUCTION

Litopenaeus vannamei is widely recognized as one of the most important commodities in global aquaculture, due to its rapid growth, broad environmental tolerance, and consistently strong market demand. Global shrimp production has continued to expand alongside the intensification of farming practices. Nevertheless, this expansion is still

constrained by several major challenges, particularly suboptimal feed utilization, high production costs, and persistent disease outbreaks that can compromise farm performance (Kamaliah *et al*., 2025; Maurus *et al*., 2025). In intensive culture systems, feed accounts for at least half of the variable production costs, highlighting feed efficiency as a critical determinant of both economic viability and long-term sustainability (Ayisi *et al*., 2017; Weldon *et al*., 2021). Accordingly, the development of feed formulations capable of simultaneously promoting growth and improving feed utilization efficiency has become a major focus of contemporary aquaculture research.

Dietary probiotic supplementation has emerged as a promising functional strategy in shrimp aquaculture. Evidence indicates that probiotics can modulate gut microbiota, enhance digestive and immune functions, and thereby improve growth performance, feed efficiency, and survival in *L. vannamei* (Dawood *et al*., 2020; Amoah *et al*., 2019). These benefits also include reduced feed conversion ratio and improved nutrient utilization through better gut physiological and microbial status (Wang *et al*., 2019). Recent meta-analyses further confirm that probiotic application consistently enhances daily growth, feed efficiency, and survival of Pacific white shrimp, particularly under intensive farming conditions (Li *et al*., 2024; Muthu *et al*., 2024).

On the other hand, nutrition-based strategies utilizing natural ingredients, including tomato meal, are increasingly being explored as alternative prebiotic sources in aquafeed formulations. Tomato fruit is known to certain dietary fiber such as pectin, along with diverse antioxidant and bioactive compounds that may facilitate the proliferation of beneficial microorganisms within the gastrointestinal tract (Coelho *et al*., 2023a; Lu *et al*., 2019). Recent studies have further demonstrated that tomato-derived ingredients can modulate gut microbiota composition and stimulate the production of functional metabolites, such as short-chain fatty acids, which are essential for maintaining intestinal health and enhancing nutrient metabolic efficiency (Coelho *et al*., 2023b; Wang *et al*., 2024). From an applied perspective, the inclusion of tomato meal in aquaculture diets has been reported to improve the nutritional quality of feed and contribute to enhanced growth performance and feed utilization efficiency in Pacific white shrimp (Abidin *et al*., 2024). Furthermore, the synergistic combination of probiotics and prebiotic substrates in the form of synbiotic has been shown to exert more comprehensive benefits, particularly in improving growth performance, feed efficiency, and resistance to pathogenic infections in *L. vannamei* (Khanjani *et al*., 2024; Banafsha *et al*., 2025).

The effectiveness of probiotic application in aquaculture is highly influenced by the administered dosage. Numerous studies have demonstrated a dose-response relationship in probiotic supplementation, indicating that the determination of an optimal dosage is a critical factor in enhancing feed efficiency and growth performance in aquatic organisms (Hai, 2015; Dawood *et al*., 2019). Based on that, the present study aimed to evaluate the effects of dietary probiotic supplementation at different dosage in tomato meal-enriched diet on growth performance, feed utilization efficiency, and survival rate of Pacific white shrimp.

METHODS

Preparation of Tomato Meal and Enrichment of the Experimental Diets

Tomato was obtained from the Baruga traditional market, South Konawe Regency, Southeast Sulawesi, Indonesia. The fruits were first washed thoroughly, and the seed portion, which contains a high moisture content, was separated from the pulp. The tomato pulp was

then cut into small pieces and dried in an oven at 50°C for 5 days. The dried tomato pulp was subsequently ground using a chopper and sieved to obtain a fine tomato meal. A total of 81.2 g of tomato meal was produced from 7 kg of fresh tomato fruits.

The experimental diets were prepared using the repelleting method following Abidin *et al*. (2024). Commercial feed was first ground into powder and then supplemented with tomato meal according to the treatment design. Tapioca flour was added as a binder and mixed thoroughly until homogeneous, after which hot water was gradually added until a dough-like consistency was obtained. The dough was then repelleted and sun-dried until completely dry. Dietary probiotic enrichment followed the procedure described by Hasmin *et al*. (2025). The probiotic used was a commercial formulation containing *Saccharomyces cerevisiae* (1×10^8 CFU/mL), *Lactobacillus acidophilus* (1×10^8 CFU/mL), *Bacillus subtilis* (1×10^8 CFU/mL), *Aspergilus orizae* (1×10^8 CFU/mL), *Rhodopseudomonas* (1×10^8 CFU/mL), *Actinomycetes* (1×10^8 CFU/mL), and *Nitrobacter* (1×10^8 CFU/mL). The probiotic was applied to the experimental diets by uniformly spraying it onto the surface of the feed pellets.

Experimental Animals and Rearing Containers

Whiteleg shrimp were obtained from a traditional shrimp farmer in Ranooha Raya Village, Moramo District, South Konawe Regency, Southeast Sulawesi, Indonesia. The shrimp used in the experiment had an initial body weight ranging from 5 to 7 g individual^{-1}. A total of 120 shrimps were used, with 10 individuals stocked in each container.

The rearing system consisted of 12 containers measuring 61 × 43 × 38 cm. Prior to use, all containers were thoroughly washed with detergent, rinsed with clean water, and dried. Seawater with a salinity of 24–25 ppt was then added to each container to a depth of 25 cm, followed by the installation of an aeration system to ensure continuous oxygen supply. After 24 h of water preparation, 10 shrimp were introduced into each rearing unit.

Rearing of Experimental Animals

The experimental animals were reared for 30 days. Feed was provided daily at 5% of the total biomass. This daily ration was divided into two feedings, administered in the morning and in the afternoon. To maintain water quality, siphoning was carried out every morning prior to feeding. Water replacement or replenishment was adjusted according to the volume of water lost during the siphoning process.

Experimental Design

This study employed a completely randomized design (CRD) consisting of four treatments and three replicates. The experimental treatments were as follows:

PR-5 : feed enriched with 12.5 g tomato meal and supplemented with 5 mL probiotic/kg feed

PR-10 : feed enriched with 12.5 g tomato meal and supplemented with 10 mL probiotic/kg feed

PR-15 : feed enriched with 12.5 g tomato meal and supplemented with 15 mL probiotic/kg feed

WPR : feed enriched with 12.5 g tomato meal without probiotic supplementation.

The tomato meal and probiotic dosages and were determined with reference to the findings of Abidin *et al*. (2024) and Karel *et al*. (2019).

Data Processing

The growth and feed utilization efficiency in whiteleg shrimp was evaluated using the following parameters:

- Weight Gain (WG) was calculated according to Kurnia *et al*. (2025a):

$$WG = Wt - W0$$

Where:
Wt = final body weight of shrimp at the end of the experiment (g)
W0 = initial body weight of shrimp at the beginning of the experiment (g)

- Specific Growth Rate (SGR) was calculated according to Kurnia *et al*. (2025a):

$$SGR = [(\ln Wt - \ln W0) / t] \times 100\%$$

Where:
Wt = final body weight of shrimp at the end of the experiment (g)
W0 = initial body weight of shrimp at the beginning of the experiment (g)
t = rearing period (days)

- Feed Conversion Ratio (FCR) was calculated according to Kurnia *et al*. (2025b):

$$FCR = F / WG$$

Where:
WG = weight gain (g)
F = feed intake

- Feed Efficiency (FE) was calculated according to Kurnia *et al*. (2025b):

$$FE = (WG / F) \times 100\%$$

Where:
WG = weight gain (g)
F = feed intake

- Survival Rate (SR) was calculated according to Kurnia *et al*. (2022):

SR = (total number of shrimp at the end / total number of shrimp at the beginning) × 100

Data Analysis

Data on weight gain, specific growth rate, feed conversion ratio, feed efficiency, and survival rate were analyzed using analysis of variance (ANOVA). When significant differences among treatments were detected, the Tukey test was performed as a post hoc analysis.

RESULTS

Dietary supplementation with different probiotic dosages in tomato meal-enriched diet significantly affected the weight gain of whiteleg shrimp ($P < 0.05$). Shrimp fed with PR-15, consisting of 15 mL probiotic/kg feed enriched with 12.5 g tomato meal, exhibited the highest weight gain of 7.53 g. This value was significantly higher than those recorded in PR-5, PR-10, and WPR treatments, which reached 4.85 g, 5.60 g, and 4.92 g, respectively (Figure 1).

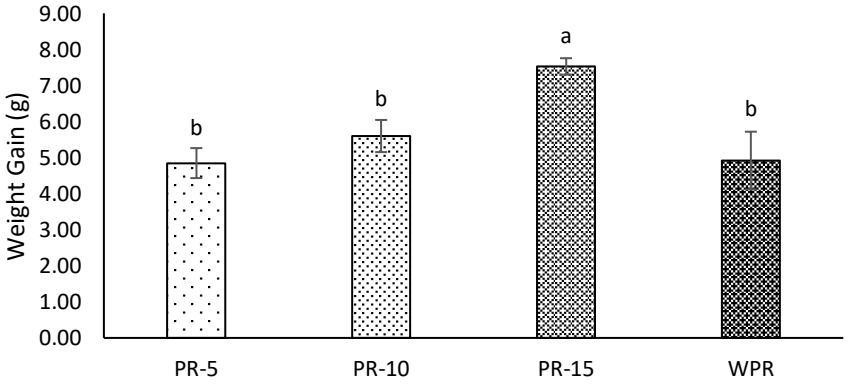

Figure 1. Weight Gain of Whiteleg Shrimp Fed with Tomato Meal-Enriched Diet Supplemented with Different Probiotic Dosages

On day 15, shrimp receiving the PR-15 treatment showed the highest specific growth rate, with a value of 3.54%, followed by WPR, PR-10, and PR-5, which reached 2.97%, 2.70% and 2.62%, respectively. On day 30, specific growth rate decreased in all treatments. The highest specific growth rate was observed in the PR-10 treatment, with a value of 2.04%, followed by PR15, PR-5, and WPR, which reached 2.00%, 1.53%, and 1.49%, respectively. The use of different probiotic dosages in tomato meal-enriched diet did not significantly affect the specific growth rate of whiteleg shrimp on day 15 and day 30 ($P > 0.05$) (Figure 2).

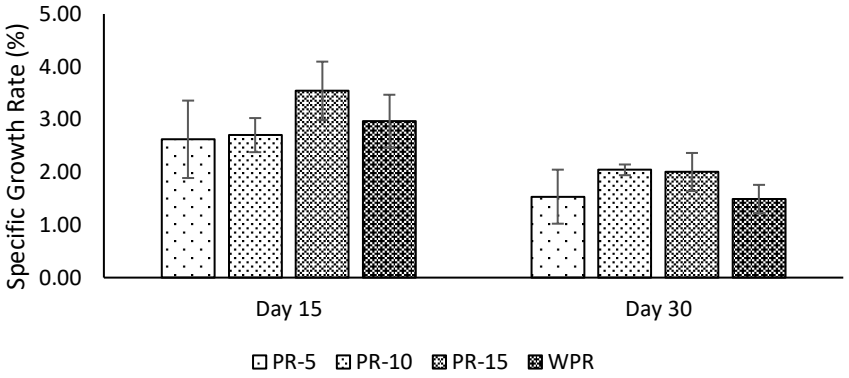

Figure 2. Specific Growth Rate of Whiteleg Shrimp Fed with Tomato Meal-Enriched Diet Supplemented with Different Probiotic Dosages

Dietary supplementation with various dosages of probiotic in tomato meal-enriched diet significantly affected the feed conversion ratio of whiteleg shrimp ($P < 0.05$). Shrimp fed PR-15 showed the lowest feed conversion ratio value of 15.68. This value was significantly lower than those recorded in PR-5 and WPR treatments, which reached 20.85 and 19.81, respectively. Meanwhile, PR-10 showed an intermediate feed conversion ratio value of 18.07 an was not significantly different from PR-15 (Figure 3).

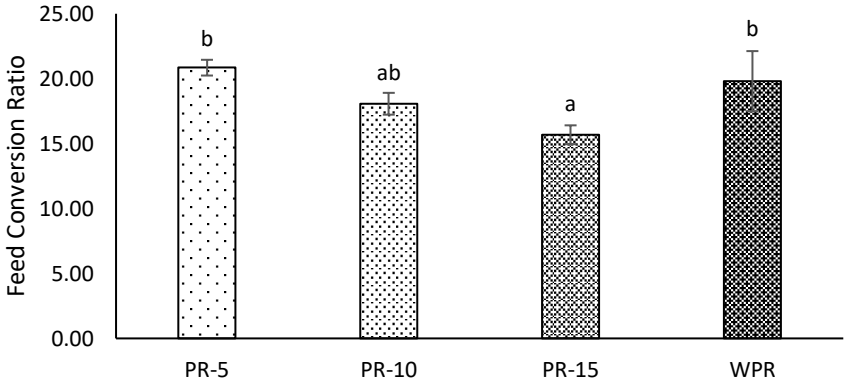

Figure 3. Feed Conversion Ratio of Whiteleg Shrimp Fed with Tomato Meal-Enriched Diet Supplemented with Different Probiotic Dosages

Dietary supplementation with various dosages of probiotic in tomato meal-enriched diet significantly affected the feed efficiency of whiteleg shrimp ($P < 0.05$). The PR-15 treatment showed the highest feed efficiency value, reaching 6.39%. This value was significantly higher than those observed in the PR-5 and WPR treatments, which were 4.80% and 5.09%, respectively. In contrast, PR-10 produced an intermediate feed efficiency value of 5.54% and did not differ significantly from PR-15 (Figure 4).

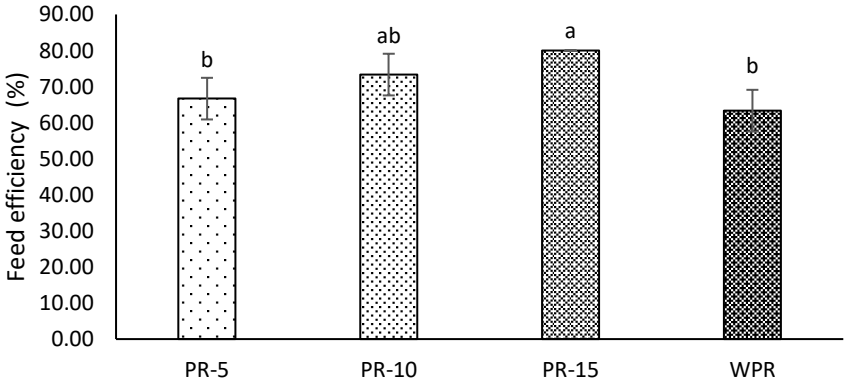

Figure 4. Feed Efficiency of Whiteleg Shrimp Fed with Tomato Meal-Enriched Diet Supplemented with Different Probiotic Dosages

The use of different probiotic dosages in tomato meal-enriched diet had a significant effect on the survival rate of whiteleg shrimp ($P < 0.05$). Shrimp receiving the PR-15 treatment showed the highest survival rate, with a value of 80.00%. This result was significantly greater than the values obtained in PR-5 and WPR, which reached 66.67% and 63.33%, respectively. Meanwhile, the PR-10 treatment resulted in an intermediate survival rate of 73.33% and was not significantly different from PR-15 (Figure 5).

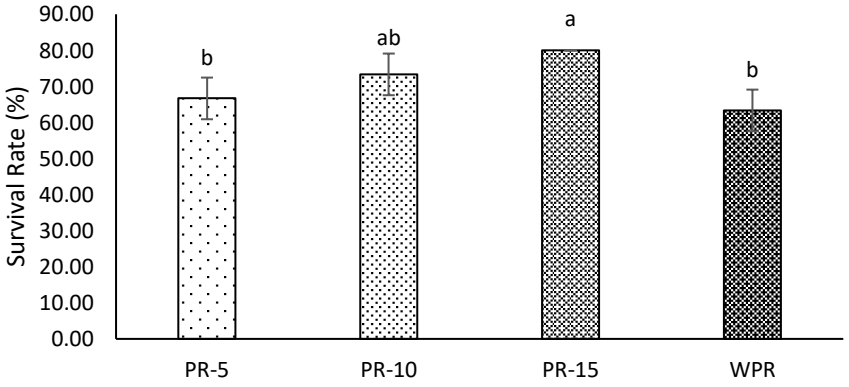

Figure 5. Survival Rate of Whiteleg Shrimp Fed with Tomato Meal-Enriched Diet Supplemented with Different Probiotic Dosages

DISCUSSION

The increased weight gain (WG) or growth observed in whiteleg shrimp receiving probiotic supplementation, particularly at 15 mL/kg feed (PR-15), suggests that the growth response in the present study was most likely driven by improved nutrient utilization and enhanced intestinal environmental stability once the probiotic density reached an effective level. This finding is consistent with previous reports showing that probiotic supplementation in *L. vannamei* diets can improve digestibility, growth performance, and physiological condition through enhanced digestive enzyme activity, improved intestinal morphology, and suppression of opportunistic bacteria in the digestive tract (Du *et al*., 2022; Lee *et al*., 2022; Luo *et al*., 2023). In the context of the present study, the fact that treatment WPR (without probiotic supplementation) still exhibited relatively competitive growth suggest that tomato meal may also have contributed as a source of citric acid. Tomato is known to contain citric acid as one of its major organic acids (Agius *et al*., 2018). In aquatic animal nutrition, citric acid

has been reported to enhance nutrient utilization, improve mineral availability, and promote feed efficiency and growth performance (Zhao *et al*., 2024; Lei *et al*., 2025). Nevertheless, in the absence of probiotic supplementation, these benefits did not appear sufficient to produce growth performance comparable to that achieved in the probiotic-supplemented treatments.

The pattern of specific growth rate (SGR), which showed the superiority of treatment PR-15 on day 15 but then shifted to PR-10 on day 30, suggests that the probiotic effect in whiteleg shrimp was both dose and time dependent, indicating that the highest dose was not necessarily associated with the most stable growth performance throughout the rearing period. During the early phase, the PR-15was presumed to accelerate the colonization of beneficial microorganisms and stimulate digestive function at an earlier stage, thereby resulting in a higher SGR. However, during the later phase, the moderate dose (PR-10) appeared to be more effective in maintaining microbiota homeostasis, thereby sustaining a more efficient and consistent growth trajectory. This interpretation is supported by previous studies in *L. vannamei*, which demonstrated that dietary probiotic supplementation can enhance specific growth rate (SGR) in aquatic animals (Kewcharoen & Srisapoome, 2019). Therefore, the findings of the present study are more appropriately interpreted to suggest that the optimum probiotic dose in tomato meal-enriched diet for maintaining SGR was not the highest dose capable of eliciting the most rapid initial response, but rather the dose that was most effective in preserving the functional balance among digestion, intestinal health, and metabolic efficiency throughout the culture period (Lu *et al*., 2022; Wei *et al*., 2024).

Dietary supplementation with different dosages of probiotic in tomato meal-enriched diet produced a consistent response characterized by a reduction in feed conversion ratio (FCR) and an increase in feed efficiency (FE), with the 15 mL/kg feed treatment (PR-15) yielding the lowest FCR and the highest FE, whereas the 5 mL/kg feed treatment (PR-5) exhibited the highest FCR and the lowest FE. This pattern indicates that, at higher dosage, probiotic acted more effectively in enhancing nutrient utilization, likely through increased digestive enzyme activity, improvement of intestinal microbiota composition, and suppression of opportunistic microorganisms that may interfere with nutrition absorption, thereby allowing a greater proportion of feed to be converted into shrimp biomass. Studies in *L. vannamei* have shown that probiotic supplementation can improve growth performance in parallel with enhanced intestinal condition and modulation of gut microbiota, ultimately leading to more efficient feed utilization (Lee *et al*., 2022). A recent review further emphasized that probiotic application in whiteleg shrimp culture is generally associated with improved FCR, as probiotics facilitate the degradation of complex feed compounds into more readily absorbable forms while also stabilizing the intestinal environment (Amiin *et al*., 2023). The inclusion of tomato meal as a source of functional fiber and bioactive compound may also have served as a substrate for the intestinal microbiota, thereby exerting a prebiotic-like effect and potentially creating a more favorable gut environment for probiotic activity within the digestive system (Coelho *et al*., 2023; Firrman *et al*., 2024). Therefore, the improvement in FCR and FE observed in PR-15 did not merely reflect more productive feed intake, but also indicated that the combination of an adequate probiotic dose and the phytobiotic properties of tomato meal was more effective in directing energy utilization toward growth than either the lower probiotic dose or the treatment without probiotic supplementation.

The increased survival rate observed in the probiotic-treated groups, particularly at the PR-15, further indicates that the benefits of the treatment extended beyond growth performance and also included enhanced physiological resilience of the shrimp during the rearing period. The higher survival was most likely associated with a combination of reduced

microbial pressure in the gut, strengthened immune defense, and a lower oxidative stress burden, thereby reducing the amount of energy that would otherwise have been allocated to maintaining homeostasis. In shrimp, probiotics have been reported to improve intestinal microbiota and enhance immunity, which subsequently contributes to increased survival (Du *et al*., 2022). In addition, other studies in *L. vannamei* have shown that synbiotic supplementation can improve survival in parallel with changes an immune response and gut microbiota, thereby supporting the interpretation that the increase in survival observed in the present study was not merely an indirect consequence of improved growth, but also reflected improved intestinal health and physiological stability (Vega-Carranza *et al*., 2024). In this context, tomato meal was presumed to act as a supportive component through the provision of natural antioxidants, whereas probiotics remained the primary factor stabilizing the biological system of the shrimp.

CONCLUSION

Dietary supplementation with different dosages of probiotic in tomato meal-enriched diet significantly affected the weight gain performance, feed utilization, and survival of whiteleg shrimp. Overall, the treatment with 15 mL probiotic/kg feed produced the best response across most parameters, particularly in terms of weight gain, feed efficiency, and the highest survival rate, while also yielding the lowest feed conversion ratio. Therefore, probiotic supplementation in tomato meal-enriched diet may serve as an effective nutritional strategy to enhance the productivity of whiteleg shrimp culture.

ACKNOWLEDGEMENT

This research was carried out independently using funding provided by the research team, without financial assistance from external parties. The article presented here form part of Khusnul Qarima Firdaus's undergraduate thesis in the Department of Aquaculture. The authors express their sincere appreciation of the laboratory staff of the Faculty of Fisheries and Marine Sciences, University of Halu Oleo, for their valuable support and assistance during the research.

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