

## Research Article

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# Administration of a Combined Probiotic Feed Containing *Streptococcus thermophilus* and *Bacillus coagulans* on Growth Performance and Molting Frequency in Black Tiger Shrimp (*Penaeus monodon* Fab.)

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Received: 17 Apr 2025

Revised: 03 May 2025

Accepted: 18 Jun 2025

<https://doi.org/10.29244/java.v2i1.61537>

## Abstract

The black tiger shrimp (*Penaeus monodon* Fab.) is one of Indonesia's leading aquaculture commodities; however, its production has declined due to various factors affecting growth, such as feed quality and environmental conditions. This study aimed to evaluate the effect of incorporating the probiotics *Streptococcus thermophilus* and *Bacillus coagulans* to shrimp feed on growth performance, feed efficiency, and molting frequency of black tiger shrimp. The study was conducted using a completely randomized design (CRD) with six treatments: one control (no probiotic addition) and five probiotic treatments at a dose of 15 mL/kg of feed, these treatments were as follows: A (control), B (100% *S. thermophilus*), C (100% *B. coagulans*), D (50% *S. thermophilus* and 50% *B. coagulans*), E (75% *S. thermophilus* and 25% *B. coagulans*), and F (25% *S. thermophilus* and 75% *B. coagulans*). Measured parameters included absolute body length, absolute body weight, feed conversion ratio (FCR), specific growth rate (SGR), survival rate (SR), feed efficiency (FE), and molting frequency. The results indicated that treatment with *B. coagulans* (Treatment C) had a significant effect on absolute weight, specific growth rate, and survival rate, but no significant effect on FCR, FE, and molting frequency. In conclusion, the addition of *B. coagulans* to shrimp feed shows potential in enhancing growth performance in *Penaeus monodon*.

**Keywords:** *Bacillus coagulans*, molting, probiotics, *Penaeus monodon*, *Streptococcus thermophilus*

## I. INTRODUCTION

The black tiger shrimp (*Penaeus monodon* Fab.) is one of the leading crustacean commodities and holds significant economic value (Muhammadar *et al.*, 2018; Nurhasanah *et al.*, 2021; Putra, 2021). However, in recent years, the production of black tiger shrimp has declined. According to data from the Central Bureau of Statistics (BPS, 2023), total shrimp production reached 27,493,200 tons, representing a 7% decrease compared to 2021, which recorded 28,696,000 tons. This decline is attributed to various factors affecting the growth and survival of *P. monodon*, including seed quality, feed type and nutritional content, water quality, pest and

disease outbreaks, and the success rate of the molting process (Nur'Aisyah *et al.*, 2017; Safriani *et al.*, 2019; Putra, 2021; Putra *et al.*, 2020, 2021).

Feed is a key factor that plays a crucial role in the growth process of shrimp. Inadequate nutritional content in feed can lead to physiological disorders, including molting syndrome, which is characterized by fungal and parasitic infections, cannibalism, and abnormal growth—all of which ultimately reduce the survival rate of black tiger shrimp (*Penaeus monodon*) (Budi & Aqmal, 2021). One strategy to improve feed

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efficiency is the addition of probiotics, which function to enhance digestion and increase the activity of digestive enzymes (Muhammadar *et al.*, 2018; Dachi *et al.*, 2019). Probiotics contain live microorganisms that can suppress pathogenic microbial populations through the production of antimicrobial compounds, while also improving in feed, making it more easily absorbed by the shrimp (Abdul & Abdul, 2018).

Probiotic bacteria are known to produce various enzymes including amylase, protease, lipase, and cellulase, which function to break down complex compounds into simpler, more digestible forms (Muhammadar *et al.*, 2018). One of the commonly found probiotic bacteria in the digestive tract is *Streptococcus thermophilus*, which can produce lactic acid and lactase enzymes, and plays a role in maintaining intestinal microflora balance (Wulanningsih, 2022). In addition, bacteria from the genus *Bacillus*, particularly *Bacillus coagulans*, are recognized as effective probiotics capable of enhancing shrimp metabolism and inhibiting the growth of pathogenic microorganisms (Amoah *et al.*, 2019; Supano *et al.*, 2020; Priya *et al.*, 2024). The enzymes produced by these bacteria contribute to energy metabolism, cell regeneration, and strengthening the shrimp's immune system against disease (Zongzheng *et al.*, 2009).

The molting process is a critical phase in the growth of black tiger shrimp, during which the exoskeleton is periodically shed to allow for body expansion (Putra, 2021). The inclusion of probiotics in feed is believed to support the molting process by enhancing the efficiency of calcium metabolism within the body. This, in turn, enables calcium from the feed to be optimally absorbed and stored as a reserve necessary during molting (Handayani *et al.*, 2019). Although the use of probiotics has been shown to positively influence the growth of various fish species, studies on the effects of a combined probiotic feed containing *S. thermophilus* and *B. coagulans* on the growth and molting frequency of black tiger shrimp remain limited. Therefore, further research is required to determine the optimal dosage of this probiotic combination to maximize the growth performance and survival rate of *Penaeus monodon*.

## II. MATERIALS AND METHODS

### 2.1 Experimental Design

The experimental design used in this study was a Completely Randomized Design (CRD) with six treat-

ments and three replications. The treatments involved the addition of probiotics, modified from the method of Suri *et al.* (2018), with a total dosage of 15 mL of probiotics per kilogram of feed. Treatment A served as the control and received feed without any probiotic supplementation. Treatment B consisted of feed supplemented with 100% *S. thermophilus* based on the designated dosage. Treatment C involved feed supplemented with 100% *B. coagulans*. Treatment D received a combination of *S. thermophilus* and *B. coagulans*, each comprising 50% of the total dosage. Treatment E included 75% *S. thermophilus* and 25% *B. coagulans*, while Treatment F had the reverse composition—25% *S. thermophilus* and 75% *B. coagulans*.

The probiotics were directly mixed into commercial floating pellet feed using the wet spraying method. The probiotic mixture was first diluted with an appropriate amount of sterile water and then evenly sprayed onto the feed surface using a manual sprayer. After application, the feed was air-dried for approximately 30 min in a shaded area to allow the probiotics to be absorbed into the feed and to prevent the growth of contaminant microbes. To minimize probiotic leaching into the water during rearing, the surface of the probiotic-coated feed was lightly coated with fish oil at a dosage of 3–5 mL per kilogram of feed. Functioned as both an adhesive and a protective layer to keep to maintain probiotic adherence to the pellets during feeding. The feed used was commercial shrimp feed containing 35.62% crude protein, 5–7% crude fat, a maximum of 4% crude fiber, a maximum of 13% ash, and a maximum moisture content of 12%. The feed size ranged from 1.2 to 1.5 mm, appropriate for the mouth size and growth stage of *Penaeus monodon*.

### 2.2 Research Procedures

#### 2.2.1 Probiotic Culture and Feed Preparation

The probiotics used in this study were obtained from the Freshwater Aquaculture Center (Balai Budi-daya Air Tawar/BBAT), Sungai Gelam, Jambi. A total of 70 g of probiotic powder was dissolved in 1.4 liters of distilled water (aquadest), and vigorously shaken until a homogeneous, milky-white solution was formed. The probiotic culture was incubated for five days, with daily agitation to prevent the sedimentation of the probiotic powder at the bottom of the culture container. Culture media were prepared using tryptic soy agar (TSA) to isolate and quantify bacterial colonies. A total

of 12 g of TSA powder was homogenized in 300 mL of distilled water in a Duran bottle using a hot plate magnetic stirrer set at 300°C and a stirring speed of 8. Once the mixture became homogeneous—indicated by a yellowish-white color—it was sterilized in an autoclave at 121°C for 20 min. The sterile media were poured into petri dishes (6–8 mL per dish), leveled, and allowed to solidify into agar. Once solidified, the media were stored in a refrigerator to maintain sterility until use. Additionally, a 0.9% physiological saline solution (NaCl) was prepared by dissolving 4.5 g of NaCl powder in 500 mL of distilled water. The mixture was homogenized using a hot plate magnetic stirrer at a speed of 8 for 4 min, then sterilized in an autoclave at 121°C for 20 min. The sterile saline solution was stored at room temperature for subsequent use in bacterial inoculation and dilution procedures (Pakaya *et al.*, 2022).

### 2.2.2 Maintenance

The test animals used in this study were post-larval black tiger shrimp (*Penaeus monodon*) at the PL-15 stage, which were reared for 60 days using feed enriched with a combination of *Streptococcus thermophilus* and *Bacillus coagulans*. The amount of feed provided was adjusted to meet daily requirements, equivalent to 4% of the total shrimp biomass. Feeding was carried out four times a day, specifically at 08:00, 11:00, 15:00, and 18:00 Western Indonesian Time (WIB) (Basir *et al.*, 2022).

## 2.3 Research Parameters

### 2.3.1 Absolute Weight and Length Growth

The absolute body weight and length growth were calculated using the formula proposed by (Effendie, 2002).

$$Wm \text{ (absolute body weight gain)} = Wt \text{ (final body weight)} - Wo \text{ (initial body weight)}$$

$$Lm \text{ (absolute body length gain)} = Lt \text{ (final body length)} - Lo \text{ (initial body length)}$$

### 2.3.2 Survival Rate (SR)

The survival rate of *Penaeus monodon* was determined by counting the number of surviving shrimp at the final sampling. According to Effendie (2002), the survival rate can be calculated using the following formula:

$$SR (\%) = \frac{\text{Number of shrimp at the end of the rearing period (individuals)}}{\text{Number of shrimp at the beginning of the rearing period (individuals)}} \times 100$$

### 2.3.3 Feed Conversion Ratio (FCR)

FCR represents the amount of feed required to

produce a unit of shrimp biomass. It was calculated using the formula from Effendie (2002):

$$FCR = \frac{F(\text{total feed weight})}{(Wt \text{ (final biomass)} + D \text{ (biomass of dead shrimp)} - Wo \text{ (initial biomass)})}$$

### 2.3.4 Feed Efficiency (FE)

Feed efficiency was calculated according to Effendie (2002) using the formula:

$$FE (\%) = \frac{1}{FCR} \times 100$$

### 2.3.5 Specific Growth Rate (SGR)

SGR was calculated using the formula proposed by (Abbas *et al.*, 2025; Putra *et al.*, 2025; Syafira *et al.*, 2025):

$$SGR (\%) = \frac{(\ln Wt \text{ (final weight)} - \ln Wo \text{ (initial weight)})}{t(\text{duration of the experiment}) \text{ days}} \times 100$$

### 2.3.6 Molting Frequency (MF)

Molting frequency was calculated based on the formula proposed by Saidi *et al.*, (2018) as follows :

$$MF \text{ (times/individual)} = \frac{(\text{Total number of molting event})}{(\text{Total population})}$$

## 2.4 Data Analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) to identify significant differences among treatments, followed by a post-hoc test (Hanafiah, 2010).

## III. RESULT

The results of the study on the effect of probiotic supplementation with *S. thermophilus* and *B. coagulans* on the growth of *Penaeus monodon* are presented in Table 1. Based on the absolute body length parameter, there were no significant differences observed among treatments ( $p > 0.05$ ), with all groups showing relatively similar values, ranging from 2.59 cm to 2.86 cm. Similarly, initial body weights were uniform across all treatments ( $0.02 \pm 0.00$  g), indicating that at the outset and that no initial differences influenced the final outcomes. However, a significant difference was observed in the absolute body weight parameter, where Treatment C (100% *B. coagulans*, 15 mL/kg feed) yielded the highest value and was significantly different ( $p < 0.05$ ) compared to Treatments A, B, D, E, and F.

Furthermore, the survival rate (SR) parameter



Table 1. Results of measured parameters in Black Tiger Shrimp (*Penaeus monodon*) rearing for 60 days

Parameters	Treatment					
	A	B	C	D	E	F
Absolute Length (cm)	2.71±0.13 <sup>a</sup>	2.59±0.16 <sup>a</sup>	2.86±0.11 <sup>a</sup>	2.79±0.09 <sup>a</sup>	2.81±0.17 <sup>a</sup>	2.72±0.08 <sup>a</sup>
Initial Body Weight (g)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Final Body Weight (g)	0.62±0.05	0.70±0.03	0.77±0.05	0.63±0.02	0.69±0.02	0.70±0.02
Absolute Weight Gain (g)	0.6±0.05 <sup>a</sup>	0.68±0.03 <sup>ab</sup>	0.75±0.05 <sup>b</sup>	0.61±0.02 <sup>a</sup>	0.67±0.02 <sup>ab</sup>	0.7±0.02 <sup>ab</sup>
Feed Conversion Ratio (FCR)	1.02±0.03 <sup>a</sup>	1.08±0.04 <sup>a</sup>	1.04±0.02 <sup>a</sup>	1.02±0.78 <sup>a</sup>	1.02±0.05 <sup>a</sup>	1.06±0.02 <sup>a</sup>
Specific Growth Rate (SGR. %/day)	5.73±0.13 <sup>a</sup>	5.92±0.07 <sup>ab</sup>	6.1±0.12 <sup>b</sup>	5.74±0.07 <sup>a</sup>	5.9±0.06 <sup>ab</sup>	5.93±0.04 <sup>ab</sup>
Survival Rate (SR. %)	88.9±3.81 <sup>b</sup>	85.6±3.86 <sup>ab</sup>	90.0± 6.70 <sup>b</sup>	87.8±6.92 <sup>ab</sup>	82.2±5.08 <sup>ab</sup>	77.8±5.08 <sup>a</sup>
Feed Efficiency (FE. %)	98.5±2.89 <sup>a</sup>	92.5±3.29 <sup>a</sup>	95.92±2.24 <sup>a</sup>	98.28±7.20 <sup>a</sup>	98.0±0.70 <sup>a</sup>	94.29 ±2.46 <sup>a</sup>
Molting Frequency (times/individual)	2.18±0.59 <sup>a</sup>	2.21±0.32 <sup>a</sup>	4.3±0.54 <sup>a</sup>	3.42±0.90 <sup>a</sup>	2.4±0.16 <sup>a</sup>	3.1±0.15 <sup>a</sup>

Note: Different superscript letters in the table indicate significant differences among treatments ( $P < 0.05$ ), and the  $\pm$  symbol represents the standard deviation. The presented values are mean values of the measured parameters evaluating the effect of *S. thermophilus* and *B. coagulans* probiotic supplementation on the growth performance of black tiger shrimp (*Penaeus monodon*), based on one-way ANOVA results showing significant differences. (FCR = Feed Conversion Ratio; SGR = Specific Growth Rate; SR = Survival Rate; FE = Feed Efficiency; MF = Molting Frequency). Treatment A = control (no probiotic); treatment B = 100% *S. thermophilus* at 15 mL/kg feed; treatment C = 100% *B. coagulans* at 15 mL/kg feed; treatment D = 50% *S. thermophilus* + 50% *B. coagulans* at 15 mL/kg feed; treatment E = 25% *S. thermophilus* + 75% *B. coagulans* at 15 mL/kg feed; treatment F = 75% *S. thermophilus* + 25% *B. coagulans* at 15 mL/kg feed.

also exhibited significant differences, with the highest values observed in Treatment C (90.0%) and Treatment A (88.9%), both of which were significantly different from Treatments B–F. This finding suggests that certain probiotic compositions had a notable effect on the survival rate of *Penaeus monodon*. The feed conversion ratio (FCR) did not differ significantly among treatments ( $p > 0.05$ ), indicating that feed utilization efficiency was statistically similar across Treatments A–F. Similarly, the feed efficiency (FE) parameter did not differ significantly ( $p > 0.05$ ). In contrast, the specific growth rate (SGR) in Treatment C ( $6.1 \pm 0.12\%$ /day) was significantly higher than in all other treatments, suggesting a distinct enhancement in daily growth rate. The molting frequency (MF) also did not differ significantly among treatments ( $p > 0.05$ ). Although numerical variation was observed, the results indicate that all treatments resulted in statistically comparable feed efficiency and molting frequency.

Overall, treatment C yielded the most favorable results in terms of absolute weight gain, specific growth rate (SGR), and survival rate (SR), indicating that the sole administration of *B. coagulans* at a dosage

of 15 mL/kg feed has the potential to significantly enhance the growth performance and survival of *Penaeus monodon*.

#### IV. DISCUSSION

The administration of feed supplemented with probiotics plays a crucial role in enhancing nutrient absorption efficiency in aquaculture organisms. Probiotics are known to produce enzymes such as lactase and amylase, which function in the hydrolysis of carbohydrates into simpler forms, making them more easily absorbed by the digestive system. The effectiveness of nutrient absorption greatly influences the growth rate of organisms, including *Penaeus monodon*, which depends heavily on the availability of high-quality nutrients and sufficient feed throughout the rearing period (Harnentis & Syahrudin, 2016). In this context, *Bacillus coagulans* has the potential to serve both as a probiotic source and an alternative protein, supporting the availability of essential nutrients required for growth (Suri *et al.*, 2018).

Several previous studies support the potential of

*Bacillus coagulans* in enhancing shrimp growth performance. Supano *et al.* (2020) reported that supplementation of *B. coagulans* at a dose of 10 mL/kg of feed significantly increased the absolute weight of *Metapenaeus ensis* to  $2.11 \pm 0.16$  g. Similar results were observed by Yuhana *et al.* (2022) in *Macrobrachium rosenbergii*, with a final body weight of  $29.67 \pm 1.88$  mg. In *Litopenaeus vannamei*, Suri *et al.* (2018) noted that the administration of *B. coagulans* at a dose of 70 mL/kg resulted in a body weight of 1.920 g. These findings underscore the potential of *B. coagulans* as a promising probiotic across multiple shrimp species.

In this study, the control group (Treatment A), which received no probiotic supplementation, exhibited the lowest absolute weight gain, at  $0.60 \pm 0.05$  g. However, this value is still higher than that reported by Sihombing *et al.* (2023), where the body weight of *Penaeus monodon* only reached  $0.15 \pm 0.03$  g when fed commercial feed without probiotic supplementation. Feed effectiveness is closely linked to the efficiency of the digestive system, which in shrimp consists of three main sections: anterior, median, and posterior (Kilawati *et al.*, 2025). The combination of feed and probiotics has been shown to improve digestive function, particularly in the intestinal region, which plays a critical role in fermentation and nutrient absorption.

Both *Bacillus coagulans* and *Streptococcus thermophilus* play biological roles that support intestinal fermentation and lactic acid production. The combination of these probiotics has been shown to increase lactic acid levels in the digestive tract, which positively influences appetite stimulation and digestive efficiency. Pan *et al.* (2024) stated that elevated lactic acid levels promote the formation of protein hydrolysates, thereby accelerating the growth of beneficial gut bacteria. This process also contributes to improved feed utilization efficiency, as reported by Hou *et al.* (2017) through enhanced feed conversion ratio (FCR) values.

The feed conversion ratio (FCR) is a critical parameter for assessing feed utilization efficiency, where a lower value indicates more effective digestion and absorption by the organism (Shofura *et al.*, 2017). In this study, the feed efficiency values were relatively high, exceeding the findings of Anwar *et al.* (2016), who reported an efficiency of only  $48.80 \pm 1.23\%$ , and approaching the optimal result recorded by Suri *et al.* (2018), with an FCR of  $1.513 \pm 0.029$ . Supano *et al.* (2020) noted that low feed efficiency is often due to

high protein content that is not fully hydrolyzed into simple amino acids. Therefore, good feed efficiency reflects the organism's ability to convert feed into energy and body tissues, and support vital physiological processes such as molting.

Molting, or the shedding of the exoskeleton, is a critical physiological indicator in the shrimp growth cycle. The inclusion of probiotics in feed has been shown to enhance molting frequency by improving nutrient absorption and feed quality (Basir *et al.*, 2022). That study demonstrated supplementation with *Bacillus* spp. increased molting frequency to as many as 9.03 times per individual. Furthermore, probiotics support improved calcium absorption, which plays a vital role in exoskeleton formation during the molting process (Handayani *et al.*, 2019).

The survival rate of black tiger shrimp (*Penaeus monodon*) in this study was relatively high, indicating favorable rearing conditions. According to the classification by Mulyani *et al.* (2014), a survival rate above 50% is considered good. This finding suggests that the shrimp demonstrated strong adaptability to the environmental conditions and water quality throughout the cultivation period. Pratiwi *et al.* (2021) noted that factors such as feed quality, stocking density, and environmental conditions including water quality are critical variables influencing survival rates in shrimp aquaculture.

## V. CONCLUSION

This study concludes that *Bacillus coagulans* (treatment C) significantly improved the absolute weight gain, specific growth rate (SGR), and survival rate (SR) of *Penaeus monodon*, with no notable effects on feed conversion ratio (FCR), feed efficiency (FE), or molting frequency (MF). Despite consistent FE across treatments, *B. coagulans* demonstrated strong potential to enhance shrimp growth by supporting nutrient absorption, gut health, and the molting process. Thus, it is a promising probiotic for improving black tiger shrimp cultivation performance.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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