Grouper: Jurnal Ilmiah Perikanan
Vol. 17 No. 2 (2026) pp. 540 - 550
pISSN: 2086-8480 | eISSN: 2716-2702
Journal Homepage: https://grouper.unisla.ac.id/index.php/grouper

Impact of *Weissella cibaria* on Growth and Survival Rate in Juvenile Mud Crab, *Scylla paramamosain*

Anggi Alifia Wardani Putri*[1,3], Sukree Hajisamae[1], Pochanart Kanjan[2], Gunanti Mahasri[3]

[1]Department of Agriculture and Fisheries, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000 Thailand
[2]Food Science and Technology Program, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[3]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, MERR (C) Campus Mulyorejo, Surabaya 60115 Indonesia

*Corresponding Author: anggi.alifia.wardani-2024@fpk.unair.ac.id

Submitted: 26 August 2026 Revised: 25 September 2026 Accepted: 30 September 2026

Keywords:
Growth Performance; Probiotic; *Scylla Paramamosain*; Survival Rate; *Weissella Cibaria*

ABSTRACT

Mud crab (*Scylla paramamosain*) is an economically important aquaculture species in Asia. However, low survival and suboptimal growth during the juvenile stage remain major constraints for aquaculture industry due to incomplete development of digestive and immune functions. Dietary probiotics have emerged as a promising strategy to improve nutrient utilization and health. *Weissella cibaria,* a lactic acid bacterium with antimicrobial and probiotic properties, has demonstrated beneficial effects in several aquatic species, but information regarding its application in mud crab culture is still unavailable. Therefore, this study aimed to evaluate the effects of dietary W. *cibaria* supplementation on growth performance and survival rate of juvenile mud crab in captivity. A total of 300 juvenile mud crabs (15 ± 5 g) were reared for 30 days and fed diets supplemented with different concentrations of *W. cibaria* (10^5–10^8 CFU/g feed). Growth performance was assessed based on weight gain (WG), specific growth rate (SGR), carapace width (CW), and carapace length (CL), while survival rate (SR) was also recorded. The results showed that dietary supplementation with *W. cibaria* significantly improved all growth parameters and survival rate ($P < 0.05$). The highest dose (10^8 CFU/g feed) produced the best performance, resulting in WG of 2.76 ± 0.30 g, SGR of 0.63 ± 0.05/day, CW of 0.66 ± 0.13 cm, CL of 0.43 ± 0.10 cm, and SR of 83.33 ± 6.67. These findings indicate that dietary supplementation with W. *cibaria* at 10^8 CFU/g feed effectively enhances growth performance and survival rate in juvenile *S. paramamosain*.

INTRODUCTION

Mud crab, *Scylla paramamosain*, is an economically important aquaculture species in Asian countries due to its high market value and increasing consumer demand (Yang *et al.,* 2020). Mud crab farming has been practiced for more than two decades and has become an important source of seafood in several provinces of Thailand (Maae *et al.,* 2025). Global mud crab aquaculture production has continued to expand, reaching approximately 300,000 tonnes in 2021 (FAO, 2023). In Thailand, mud crab production increased from 1,321 metric tons in 2018 to 3,531 metric tons in 2023, while its economic value increased from 487 million THB to 1,256 million THB (Department of Fisheries, 2023b; Lovatelli *et al.,* 2025). Despite its economic importance, low survival and suboptimal growth, particularly during the juvenile stage, remain major constraints to mud crab aquaculture (Boonyapakdee and Bhujel, 2020). Juvenile mud crabs are particularly vulnerable because their digestive and immune functions are not yet fully developed, which can affect nutrient utilization, growth, and resistance to environmental and

pathogenic stress (Talpur *et al.,* 2011). Therefore, improving growth performance and survival rate is essential for increasing the efficiency of mud crab nursery production.

Feed quality and feeding strategies play important roles in supporting growth and survival of mud crab. Adequate dietary nutrition is required to meet physiological requirements and support tissue development, metabolism, and other biological processes (Basir *et al.,* 2022). However, improving feed utilization through functional dietary supplements has become an important approach in aquaculture. Probiotics have received increasing attention as functional feed additives because they can improve growth performance, survival, digestion, and host health. Several probiotic bacteria, including *Bacillus subtilis, Bacillus licheniformis, Lactobacillus plantarum, Enterococcus faecalis,* and *Pediococcus pentosaceus*, have been reported to improve growth performance, survival, immunity, and disease resistance in cultured crabs (Boonyapakdee and Bhujel, 2020; Wu *et al.,* 2014; Yeh *et al.,* 2014; Yang *et al.,* 2019). These findings indicate that dietary probiotics have potential as an effective tool to improve the production performance of mud crabs.

Among probiotic candidates, the genus Weissella has emerged as a promising group of lactic acid bacteria because of its probiotic and antimicrobial properties (Kahyani *et al.,* 2021). In particular, *Weissella cibaria* can produce antimicrobial metabolites and bacteriocin-like compounds that may inhibit pathogenic microorganisms (Quintanilla-Pineda *et al.,* 2024). The application of *W. cibaria* has shown promising results in aquatic animals. Kanjan *et al.* (2025) reported that dietary supplementation with *W. cibaria* improved growth performance and survival rate of Pacific white shrimp (*Penaeus vannamei*) challenged with *Vibrio parahaemolyticus*. These beneficial effects suggest that W. cibaria may also have potential to improve growth and survival in other economically important crustacean species.

However, information regarding the application of *W. cibaria* in mud crab culture remains limited, particularly its effects on growth performance and survival rate of juvenile S. paramamosain. In addition, the effectiveness of probiotics can vary depending on the supplementation level; therefore, determining an appropriate dietary dose is necessary to obtain optimal benefits. Thus, evaluating different concentrations of *W. cibaria* is important to determine its potential application in juvenile mud crab culture. Therefore, this study aimed to evaluate the effects of dietary supplementation with different concentrations of *W. cibaria* (10^5–10^8 CFU/g feed) on the growth performance and survival rate of juvenile mud crab (*S. paramamosain*) during a 30-day rearing period. The study also aimed to determine the most effective dietary concentration of W. cibaria for improving the growth and survival of juvenile mud crab.

METHOD

Based on the identified research problem, the present study was conducted to address the major constraints in juvenile mud crab (*Scylla paramamosain*) aquaculture, particularly suboptimal growth performance and low survival during the nursery stage. The experimental approach involved evaluating the potential application of the probiotic *Weissella cibaria* as a dietary supplement to improve the growth and survival of juvenile mud crabs under captive rearing conditions. This study implemented different dietary concentrations of *W. cibaria* to determine its effectiveness as a functional feed additive, with growth performance assessed through weight gain, specific growth rate, carapace width, and carapace length, while survival rate was recorded throughout the 30-day feeding trial. The findings of this study are expected to provide scientific evidence regarding the utilization of *W. cibaria* as an environmentally friendly probiotic strategy for supporting sustainable mud crab aquaculture.

Bacterial Cultures and Preparation of Feed

Weissella cibaria, previously isolated from the intestine of Pacific white shrimp, was cultured in De Man, Rogosa, and Sharpe (MRS) broth supplemented with 1.5% NaCl at 30°C for 18 h (Kanjan *et al.,* 2025). The bacterial cells were removed by centrifuged at 8500 rpm for 15 minutes at 4°C and washed twice with 0.1M phosphate buffer saline (pH 7.0), and then

resuspended in 10 % sterile reconstituted skimmed milk. Subsequently, the bacterial cells were immerged in liquid nitrogen at −196 °C for 10–15 min. The sublimation was performed at a capacity of 14 L/24 h using a Dura-Dry MP freeze-dryer (FTS System, USA) for 15–18 h. The freeze-dried W. cibaria (cell viability at 5.54 ×109 CFU/g) was supplemented into the diets at different doses (1×10^5–1×10^8 CFU/g feed). The supplemented feed was coated with 1% squid oil as a carrier, packed in tightly sealed plastic bags, stored at 4°C, and used within 7 days. The control diet was also mixed with the same volume of sterile saline solution, coated with 1% squid oil, and stored under the same conditions as the supplemented diets.

Water Treatment and Experiment Tank Preparation

Water salinity was maintained at 23 ppt, considered suitable for crab nursing. Before use, water in the 500 L reservoir tank was chlorinated using calcium hypochlorite powder (60% available chlorine) at 20 ppm overnight and neutralized with sodium thiosulfate at 10 ppm. After chlorine residue was neutralized, the treated water from the initial tank was transferred into the second reservoir tank for the experiment according to Syafaat *et al.* (2019). Following sterilization, the treated water was distributed into experimental PVC tanks measuring 60 cm × 30 cm × 40 cm, with a working volume of 40 L per tank. Each tank was equipped with net shelters to reduce cannibalism among crabs throughout the experiment. Continuous aeration was supplied using aeration hoses and air pumps to maintain dissolved oxygen levels during the experimental period according to Win *et al.* (2021).

Water quality parameters monitored during the study included temperature, pH, dissolved oxygen (DO), alkalinity, salinity, and ammonia. The measurements were conducted every two days, in the morning, to evaluate fluctuations caused by environmental changes, weather conditions, and biological activities within the tanks.

Maintenance of Mud Crab

The test animals used in this study were crablet stage mud crabs, S. paramamosain, with an initial body weight of 15 ± 5 g and carapace width of 4.55 ± 1.50 cm according to Win *et al.* (2021). A total of 300 mud crabs were used, with 15 individuals stocked in each tank. The crabs were reared for 30 days at a stocking density of one individual per 3 L of water. The experiment consisted of five treatment groups with four replications: a control diet (C) without probiotics; four experimental diets supplemented with W. cibaria at different concentrations: 1 x 105 CFU/g feed (T1); 1 x 106 CFU/g feed (T2); 1 x 107 CFU/g feed (T3); and 1 x 108 CFU/g feed (T4); Throughout the experimental period, the crabs were fed commercial pelleted feed (Lansy Shrimp) once daily at 04:00 pm at a feeding rate of 5% of their total biomass. This Lansy Shrimp PL is specifically formulated for early-stage marine crustaceans (Fu *et al.,* 2017). Feed residues and fecal matter were siphoned every two days, while the crabs were measured every week according to Yang *et al.* (2017).

Observed Parameters

Before feeding, the mud crabs were first weighed before they were weighed again after 30 days of rearing. Furthermore, weight gain (WG), carapace width (CW), carapace length (CL), specific growth rate (SGR), and survival rate (SR) were calculated according to the following formulas.

$$WG = Wt - W0$$
$$CW = CWt - CW0$$
$$CL = CLt - CL0$$
$$SGR = ((LnWt - LnWo))/(t \times 100)$$
$$SR = Nt/No \times 100$$

Where: WG = Weight Gain (gr); CW = Carapace Width (cm); CL = Carapace Length (cm); SGR = Daily Growth Rate (% BW/day) SR = Survival Rate (%).

Data Analysis

The collected data for all parameters were analysed using an SPSS statistics program (IBM SPSS version 20). One-way analysis of variance (ANOVA) with Duncan's post hoc tests were used to determine significant differences between samples at $p < 0.05$. Regression analysis was used to analyse relationship between the growth parameters and different levels of treatment. Raw date was tested for normality before conducting statistical analysis.

RESULT AND DISCUSSION

Growth Performance

One-way ANOVA revealed that dietary supplementation with different doses of *Weissella cibaria* significantly affected the growth performance of juvenile *Scylla paramamosain* during the 30-day nursing period ($P < 0.05$). Duncan's multiple range test showed significant differences among treatments in weight gain (WG), specific growth rate (SGR), carapace width (CW), and carapace length (CL) (Table 1). The highest supplementation level (1×10^8 CFU/g feed; T4) resulted in the highest values for all growth parameters. The WG and SGR values in T4 were 2.76 ± 0.30 g and 0.63 ± 0.05%/day, respectively, which were significantly higher than those of the other treatments ($P < 0.05$). The treatments supplemented with 1×10^7 CFU/g feed (T3) and 1×10^5 CFU/g feed (T1) also showed higher growth values than the control treatment. Meanwhile, the lowest supplementation level of 1×10^6 CFU/g feed (T2) did not significantly increase WG compared with the control, although SGR was significantly higher.

The highest CW and CL were also observed in T4, with values of 0.664 ± 0.13 cm and 0.433 ± 0.10 cm, respectively. Regression analysis indicated a positive relationship between probiotic supplementation level and WG, SGR, CW, and CL ($P < 0.05$) (Fig. 1). The increasing trends observed in these parameters suggest that dietary *W. cibaria* supplementation may enhance nutrient utilization and subsequently promote somatic and morphological growth. The relatively high coefficients of determination for WG ($R^2 = 0.5481$) and SGR ($R^2 = 0.4123$) further indicate that probiotic supplementation contributed to the variation in growth performance among treatments.

The improved growth performance observed in T4 may be associated with the beneficial effects of *W. cibaria* on digestive function and nutrient utilization. Probiotics can contribute to the establishment of beneficial intestinal bacteria and improve feed utilization through the production of extracellular digestive enzymes, including protease, amylase, and lipase (Paramasivan, 2026). Enhanced protein digestion and absorption may be particularly important for juvenile mud crabs because protein provides essential amino acids required for tissue synthesis, muscle development, and exoskeleton formation (Li *et al.,* 2021). Consequently, improved nutrient availability may provide greater resources for tissue growth, resulting in higher WG and SGR in crabs receiving the highest probiotic supplementation.

The increases in CW and CL further indicate improved morphological growth. *W. cibaria* may contribute to better nutrient absorption and metabolic efficiency, providing essential nutrients required for growth and molting. Adequate nutrient availability is important for post-molt recovery and exoskeleton development in crustaceans. Pratoomchat *et al.* (2025) reported that improved nutritional and physiological conditions can support faster post-molt recovery and development of a stronger cuticle. Similarly, Yang *et al.* (2019) reported that probiotic supplementation improved growth performance and physiological responses in *S. paramamosain*, potentially through improved nutrient metabolism and immune function. Therefore, the present findings suggest that dietary *W. cibaria* supplementation, particularly at 1×10^8 CFU/g feed, has potential to enhance growth performance in juvenile mud crabs.

Table 1. Growth performance and survival rate of *S. paramamosain* fed with diets supplemented by different doses of *W. cibaria*. Growth performance parameters included weight gain (WG), specific growth rate (SGR), carapace width (CW), and carapace length (CL), while survival rate (SR) was used to evaluate crab survival. Values are presented as mean ± standard deviation (SD). Different superscript letters within the same row indicate significant differences among

treatments based on one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at $p < 0.05$. Values with the same superscript letter are not significantly different ($p > 0.05$).

Mean values	Feed treatments				
	C	T1	T2	T3	T4
WG (gr)	1.02 ± 0.04^{d}	1.65 ± 0.09^{c}	0.94 ± 0.04^{d}	2.39 ± 0,23^{b}	2.76 ± 0,30^{a}
SGR (%)	0.13 ± 0.03^{d}	0.34 ± 0.15^{bc}	0.19 ± 0.08^{dc}	0.40 ± 0,08^{b}	0.63 ± 0,05^{a}
CW (cm)	0.388 ± 0.01^{b}	0.498 ± 0.28^{ab}	0.486 ± 0.10^{ab}	0.596 ± 0,18^{ab}	0.664 ± 0,13^{a}
CL (cm)	0.240 ± 0.17^{b}	0.330 ± 0.16^{ab}	0.340 ± 0.05^{ab}	0.430 ± 0,15^{a}	0.433 ± 0,10^{a}

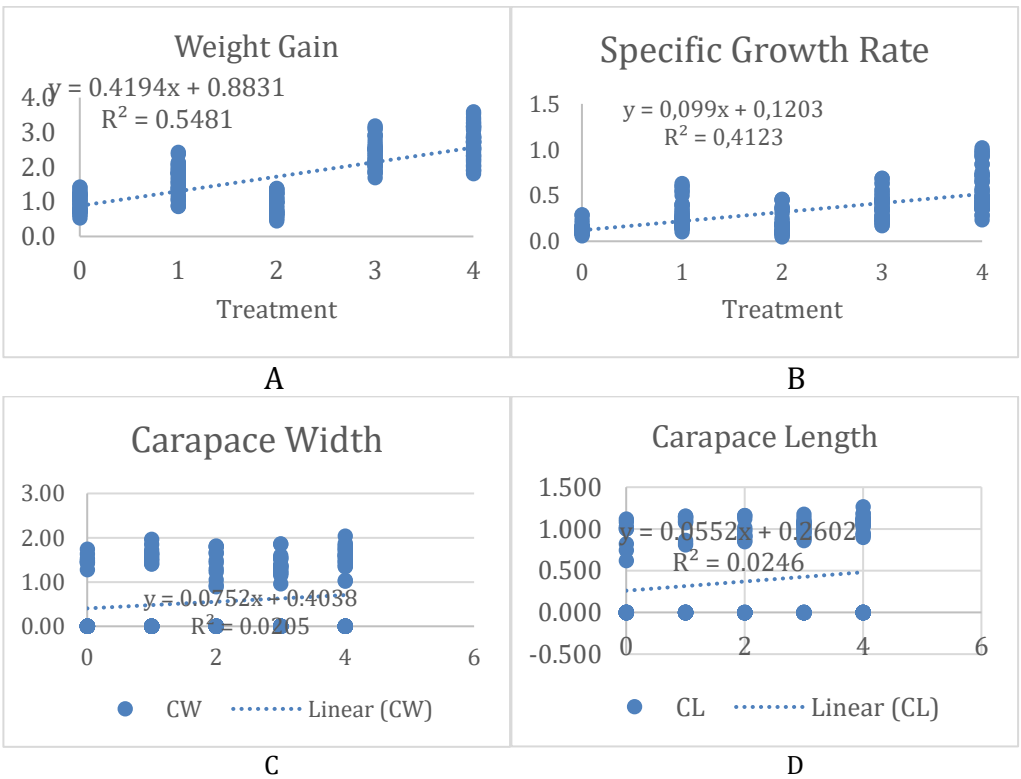


Figure 1. Regression relationships between probiotic dose and growth performance of mud crab (*Scylla paramamosain*) after 30 days of nursing. (A) weight gain (WG), (B) specific growth rate (SGR), (C) carapace width (CW), (D) carapace length (CL). Each panel shows the regression line and the coefficient of determination (R^2). Significant relationships were observed at $P < 0.05$.

Survival Rate

Dietary supplementation with different doses of *W. cibaria* also significantly affected the survival rate (SR) of juvenile *S. paramamosain* during the 30-day nursing period ($P < 0.05$) (Table 1). The highest survival rate was recorded in T4, which received *W. cibaria* at 1×10^8 CFU/g feed, with a value of 83.33 ± 6.67%. This value was significantly higher than those observed in the control and other probiotic treatments ($P < 0.05$). The control treatment showed the lowest survival rate at 52.00 ± 3.33%, while T1, T2, and T3 showed survival rates of 67.00 ± 7.70%, 58.00 ± 6.38%, and 70.00 ± 6.67%, respectively. Regression analysis also demonstrated a positive relationship between probiotic supplementation level and survival rate ($P < 0.05$) (Fig. 1), indicating that increasing the dietary W. cibaria dose was associated with improved survival of juvenile mud crabs.

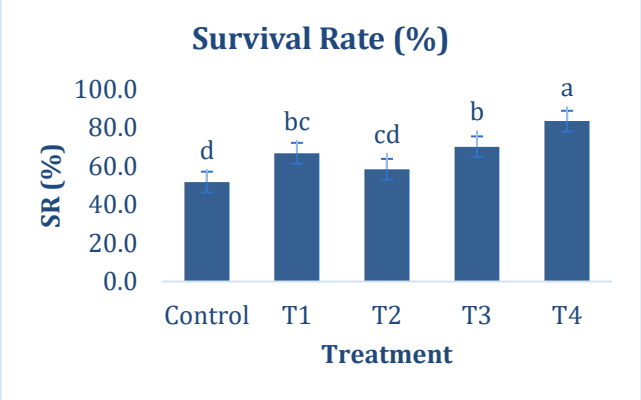


Figure 2. Survival rate of juvenile mud crab (*Scylla paramamosain*) fed diets supplemented with different doses of *Weissella cibaria* after 30 days of nursing. Bars represent mean ± standard deviation (SD). Different superscript letters indicate significant differences among treatments based on one-way ANOVA followed by Duncan's multiple range test (DMRT) at $P < 0.05$.

The higher survival rate observed in T4 may be related to the probiotic properties of *W. cibaria*. Probiotics can improve host health by modulating intestinal microbial communities, stimulating immune responses, and reducing the abundance or activity of pathogenic bacteria (Dawood *et al.,* 2019). In the present study, W. cibaria was originally isolated from the intestine of Pacific white shrimp and demonstrated protein activity and in vitro inhibitory activity against the pathogenic bacterium Vibrio parahaemolyticus (Kanjan *et al.,* 2025). Previous findings showed that dietary W. cibaria supplementation could improve survival and growth performance in Pacific white shrimp through the regulation of intestinal microbiota and inhibition of pathogenic bacteria.

The improved survival observed in juvenile mud crabs may therefore be associated with enhanced gut health and increased resistance to environmental and microbial stress. W. cibaria can produce metabolites such as organic acids and bacteriocins that may inhibit the adhesion and proliferation of pathogenic bacteria in the gastrointestinal tract (Yu *et al.,* 2018). In addition, probiotic supplementation may stimulate immune and antioxidant responses, thereby increasing the ability of cultured animals to withstand physiological stress (Dawood *et al.,* 2019). The present result, in which the highest probiotic dose produced the highest survival rate of 83.33%, suggests that W. cibaria supplementation can provide a beneficial effect on the survival of juvenile S. paramamosain. Therefore, 1×10^8 CFU/g feed can be considered the most effective dose among the tested treatments for improving survival during the 30-day nursing period.

Water Quality Analysis

Water quality was monitored as a supporting parameter to ensure that suitable environmental conditions were maintained throughout the experimental period. The measurements were conducted in each experimental aquarium every other day at 08.00 am in the morning. Results of measurements are in Table 3. It was observed that dietary supplementation with *W. cibaria* had no significant effect on the average water quality parameters among treatments during the 30-day rearing period ($p > 0.05$). Average water temperature ranged from 28.7 to 29.9°C, dissolved oxygen (DO) ranged from 4.42 to 7.3 mg/L, salinity ranged from 23 to 25 ppt, and pH ranged from 7.94 to 8.13. Alkalinity ranged from 90 to 124 mg/L and ammonia concentrations ranged from 0 to 0.423 mg/L.

Table 2. Water quality of *Scylla paramamosain* culture system during the 30-day rearing period

Parameter	Range
Temperature	28,7-29,9°C
DO	4,42-7,3 mg/L

Parameter	Range
PH	7,94 - 8,13
Salinity	23-25 ppt
Alkalinity	90-124 mg/L
Ammonia	0-0,423 mg/L

Overall, the water quality parameters remained within acceptable ranges for juvenile mud crab culture and were relatively stable throughout the experimental period. The temperature range was within the commonly reported suitable range for mud crab culture (27–32°C), supporting normal metabolic activity, feeding, and digestion. Dissolved oxygen concentrations were generally adequate, although the lowest recorded value of 4.42 mg/L approached the lower limit for optimal culture conditions (Pedapoli *et al.,* 2014; Syafaat *et al.,* 2021). The pH values of 7.94–8.13 indicated slightly alkaline conditions suitable for marine and estuarine crab culture. Salinity of 23–25 ppt was also within a suitable range for juvenile mud crab rearing.

Alkalinity values of 90–124 mg/L indicated sufficient buffering capacity to maintain relatively stable pH conditions. Stable alkalinity is important because fluctuations in pH may cause physiological stress in aquatic animals (Syafaat *et al.,* 2021; Vinod *et al.,* 2017). Ammonia concentrations ranged from 0 to 0.423 mg/L and remained relatively low throughout the culture period. Nevertheless, the highest recorded concentration should be monitored because elevated ammonia can negatively affect crab health, growth, and survival (Pedapoli *et al.,* 2014; Syafaat *et al.,* 2021). Overall, the measured water quality parameters remained suitable for juvenile mud crab cultivation and were unlikely to be a major factor contributing to differences in growth performance and survival among treatments.

CONCLUSION

Dietary supplementation with W. cibaria significantly improved the growth performance and survival rate of juvenile *S. paramamosain*. The supplementation resulted in higher weight gain (WG), specific growth rate (SGR), carapace width (CW), carapace length (CL), and survival rate compared with the control treatment. Among the tested doses, 10^8 CFU/g feed was the most effective supplementation level, producing the highest WG (2.76 ± 0.30 g), SGR (0.63 ± 0.05%/day), CW (0.66 ± 0.13 cm), CL (0.43 ± 0.10 cm), and survival rate (83.33 ± 6.67%). Therefore, dietary supplementation with W. cibaria at 10^8 CFU/g feed can be considered the optimum dose for improving growth performance and survival of juvenile S. paramamosain under the conditions of this study.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Prince of Songkla University for providing research facilities and support throughout this study. Special thanks are extended to the PSU-TUYF Charitable Fund for providing the research grant that supported this study. The authors also gratefully acknowledge the assistance, support, and encouragement from the Master's and Ph.D students of Prince of Songkla University during the research activities.

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