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Original Article

Effects of pine pollen as a dietary feed additive on growth performance, survival, and stress response of postlarval giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract: The rising costs and environmental concerns associated with fishmeal-based feeds have spurred efforts to identify sustainable, viable alternatives in freshwater prawn farming. The present study was performed to investigate the effects of supplementation of pine pollen meal (PPM) on growth performance, length development, survival, microbial characteristics, stress tolerance, and feed cost in postlarval *Macrobrachium rosenbergii*. A 45-day feeding experiment was conducted in a completely randomized design with three dietary treatments: a control diet without PPM and diets supplemented with 25% and 50% PPM. Growth parameters, including weight gain, specific growth rate, final length, and length increment, were evaluated alongside survival rate, water quality, microbial counts, and stress response. The 25% PPM diet provided the maximum growth and length increment for post-larval prawns, whereas the 50% PPM diet showed the highest survival and stress resistance. Water quality conditions were within acceptable limits during the experiment. Pine-pollen supplemented treatments resulted in enhanced lactic acid bacterial populations in the culture water. These findings suggested that pine pollen meal could be a promising functional dietary additive to improve growth performance, length development, survival, and stress tolerance of juvenile *M. rosenbergii* as it supports its potential application in sustainable freshwater prawn production.

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Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important freshwater crustaceans in aquaculture, especially in Asia, because of its fast growth, big size, and high market demand. However, the profitability and long-term sustainability of prawn farming are severely limited by high feed costs, which often account for 50-70% of total production costs. Fishmeal (FM) is the primary protein source in prawn diets, and its production is costly and ecologically unsustainable due to overfishing and competition with other livestock and aquaculture industries. These issues have led to a search for alternative feed ingredients that are both nutritionally sufficient and environmentally sustainable.

Recently, pine pollen meal (PPM) has been introduced as a promising natural feed ingredient and nutritional supplement in animal nutrition. Pine pollen is a nutrient-dense food source, with significant quantities of protein, essential amino acids, vitamins, minerals, lipids, and a variety of bioactive substances, including polysaccharides, phenolics, phytohormones, and antioxidants. These components have been reported to boost growth performance, feed efficiency, immune stimulation, and stress tolerance in aquatic species (Cheng et al., 2023).

Dietary supplementation with pine pollen has been reported to significantly improve growth performance, feed utilization, and immune response in several finfish species, such as Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). Moreover, there are reports of pine pollen affecting reproductive performance and sex ratios through its

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phytoandrogenic activity, suggesting potential anabolic and endocrine-modulating effects (Abaho et al., 2022; Cheng et al., 2023). These findings suggest that pine pollen has functional potential beyond basic nutrition and propose it as a desirable feed supplement rather than simply a protein source. Although there is growing scientific evidence for the benefits of pine pollen in finfish nutrition, its use in crustacean aquaculture remains largely unexplored. Alternative feed additives for *M. rosenbergii* have been limited, especially during the postlarval (PL) stage, a period of rapid development, high metabolic demands, and vulnerability to environmental and nutritional stress. Nutritionally balanced diets are a prerequisite for optimal growth, efficient feed utilization, and high survival rates in postlarval prawns. Thus, evaluating pine pollen meal as a feed additive for postlarval *M. rosenbergii* could provide new insights into its potential to enhance growth performance, survival, and overall culture efficiency.

Materials and Methods

Experimental design and set-up: The study was conducted using a completely randomized design (CRD) to evaluate the effects of pine pollen meal (PPM) as a functional dietary ingredient in postlarval *M. rosenbergii*. Three dietary treatments were prepared: a control diet containing 100% fishmeal (FM) as the primary protein source (T1), and two experimental diets in which fishmeal was maintained as the primary protein source, while pine pollen meal was included as a dietary additive at different inclusion levels (T2 and T3). Each treatment was replicated three times, resulting in a total of nine experimental units.

The experiment was conducted in a controlled laboratory setting, using blue plastic drums as culture units. Each drum had a total capacity of 100 L and was cut in half, providing an effective working volume of approximately 50 L per experimental unit. A total of nine half-drums were used and arranged according to the experimental design. Prior to stocking, all experimental units were thoroughly washed, disinfected, and rinsed to prevent contamination. Each unit was filled with dechlorinated freshwater and supplied with continuous aeration using air stones to maintain adequate dissolved oxygen levels. The system was maintained under ambient laboratory conditions with a natural photoperiod. All experimental units were managed uniformly throughout the study to ensure that observed differences among treatments were attributable solely to dietary effects.

Experimental organism and acclimation: Postlarval *M. rosenbergii* (PL35) were obtained from the Mindanao State University at Naawan Ulang Hatchery and transported to the experimental setup. The post-larvae had a mean body weight of 0.10±0.01g and a mean total length of approximately 16.0 mm at stocking. Upon arrival, the post-larvae were acclimated for a week before the experiment began. During the acclimation period, post-larvae were fed a commercial prawn diet and maintained under continuous aeration. Healthy, active post-larvae of relatively uniform size were selected for the experiment. After acclimation, post-larvae were randomly stocked into the experimental units at a density of 30 individuals per unit.

Diet formulation, preparation, and proximate analysis: All experimental diets were formulated to be isonitrogenous and isoenergetic, ensuring comparable crude protein and energy levels across treatments. The control diet was formulated using 100% fishmeal as the primary protein source. Two experimental diets were prepared by incorporating pine pollen meal (PPM) as a feed additive into the control formulation at inclusion levels of 25 and 50% (w/w), respectively.

For diet preparation, all dry ingredients were accurately weighed and thoroughly mixed to ensure uniform distribution of pine pollen meal in the experimental diets. The dough was pelletized using a laboratory pelletizer and dried using a food dehydrator at 60°C to remove excess moisture. The dried pellets were then stored in airtight containers until use. Proximate composition of the experimental diets was analyzed according to standard methods of the Association of Official Analytical Chemists (AOAC, 2019).

Feeding management: Postlarval *M. rosenbergii* were fed as a percentage of total biomass, following standard freshwater prawn culture practices. Feeding rates were adjusted based on the average body weight (ABW) of the postlarvae, which was determined through biweekly sampling (SEAFDEC/AQD, 2025). The daily feed ration was calculated based on total biomass using the formula: Daily feed ration (g day^{-1}) = Total biomass (g) × Feeding rate (%)

Total biomass was calculated by multiplying the average body weight (ABW) of the post-larvae by the total number of individuals in each experimental unit. Post-larvae weighing less than 1 g were fed at 30% of the total biomass per day. As growth progressed, feeding rates were gradually reduced in accordance with recommended feeding guidelines and the changing nutritional requirements of the cultured animals. The total daily feed ration was divided into four equal feedings and administered at regular intervals throughout the day to improve feed utilization efficiency and minimize feed wastage (Jia et al., 2021). Uneaten feed and fecal matter were siphoned daily (Valente et al., 2024).

Experimental units and water quality management: The experiment was conducted using blue plastic drums (approximately 56 cm in diameter) as culture units. Each drum had a total capacity of 100 L and was cut in half, providing an effective working volume of approximately 50 L per experimental unit. All units were filled with dechlorinated freshwater and provided with continuous aeration throughout the 45-day feeding trial. Shelters were provided in each experimental unit using half-cut PVC pipes (1-inch diameter) to reduce stress and minimize aggressive interactions among post-larval *M. rosenbergii*. Uneaten feed and fecal matter were siphoned daily, and a 50% water exchange was performed twice per week to maintain adequate water quality.

Water quality parameters, including temperature, dissolved oxygen (DO), and pH, were monitored throughout the 45-day feeding trial. Temperature was measured with a digital thermometer, and dissolved oxygen and pH were measured with a D.O. meter and a pH meter, respectively. All water quality parameters remained within acceptable ranges for post-larval *M. rosenbergii* culture as reported in previous studies (Camarin et al., 2023; Kasan et al., 2023).

Microbial analysis of culture water: Water samples were aseptically collected from each experimental unit to assess the microbial load. Samples were analyzed using the standard plate count method, and microbial counts were expressed as colony-forming units per milliliter (CFU mL^{-1}). Initial microbial samples were collected during the culture period prior to the implementation of water management practices, when no water exchange was conducted. Final microbial samples were collected after water management was applied at the end of the experimental period.

Stress response: A stress response was conducted at the end of the 45-day feeding trial to evaluate stress-related responses of juveniles. Individuals from each dietary treatment were first exposed to seawater for 30 minutes to induce osmotic stress, then transferred back to freshwater for 30 minutes to allow recovery. Subsequently, juveniles were subjected to air exposure for 5 minutes to simulate handling stress. After the stress challenge, prawns were returned to freshwater, and survival was recorded (Wang et al., 2020; Wang et al., 2022; Bir et al., 2024).

Growth performance and survival assessment: The initial body weight of the post-larvae was recorded at the start of the feeding trial. Growth performance was monitored through biweekly sampling. At the end of the 45-day experimental period, final body weight was recorded. Growth performance and feed utilization parameters, including weight gain (WG), specific growth rate (SGR), average daily gain (ADG), and survival rate (SR), were calculated using standard formulas that are commonly applied in studies on *M. rosenbergii* (Thawinwan et al., 2022; Habib et al., 2014), as follows:

Weight gain WG (g) = Final weight - Initial weight

Specific growth rate (SGR) (%day-1) = (ln (Final weight) − ln (Initial weight) /Culture Days) × 100

Average daily gain (ADG) (gday−1) = Final Weight−Initial weight / Number of Days

Survival rate (SR%) = Number of total surviving PL / Total number of PL stocked x 100

Table 1. Proximate composition (% as fed) of control and pine pollen-supplemented experimental diets fed to *Macrobrachium rosenbergii* post larvae.

Treatment	Protein (%)	Fiber (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrates
Control	35.8±0.30^{a}	6.50±0.10^{a}	1.18±0.08^{c}	10.6±0.10^{a}	10.4±0.10^{a}	35.52±0.09^{b}
25% Pine Pollen	35.9±0.21^{a}	6.20±0.10^{b}	2.60±0.10^{a}	10.3±0.10^{b}	10.0±0.10^{b}	35.03±0.35^{c}
50% Pine Pollen	35.8±0.46^{a}	6.00±0.10^{c}	1.70±0.10^{b}	10.2±0.06^{b}	9.8±0.10^{c}	36.50±0.52^{a}

Values are mean±SD. Means within the same column sharing the same superscript letter are not significantly different (P>0.05), whereas different letters indicate significant differences (P<0.05).

Table 2. Growth performance, feed utilization, and survival of juvenile *Macrobrachium rosenbergii* fed partial addition of pine pollen meal diets over a 45-day culture period.

Treatment	Pine pollen meal level (%)	Final mean weight (g)	Weight gain (g)	SGR (%$^{-1}$)	Survival (%)
Control	0	0.32±0.03^{b}	0.22±0.03^{b}	2.58±0.03^{c}	34.44±5.09^{c}
25% Pine Pollen	20	0.47±0.03^{a}	0.37±0.03^{a}	3.44±0.03^{a}	55.55±8.38^{b}
50% Pine Pollen	50	0.34±0.04^{b}	0.24±0.04^{b}	2.72±0.04^{b}	62.22±6.94^{a}

Values are mean±SD. Means within the same column sharing the same superscript letter are not significantly different (P>0.05), whereas different letters indicate significant differences (P<0.05).

Statistical analysis: Statistical analyses were performed using Jamovi Software (version 2.7.12). All data were expressed as mean ± standard deviation (SD). Differences among dietary treatments were analyzed using one-way analysis of variance (ANOVA), and when significant differences were detected, post hoc tests were conducted to compare treatment means. Statistical significance was set at P<0.05.

Results

The prawns were stocked as post larvae and developed into juveniles during the 45-day culture period; thus, the growth, survival, microbial, and stress-response results discussed in this section refer to juvenile *M. rosenbergii*. The proximate composition of the control and pine pollen–supplemented diets is presented in Table 1. Protein content did not differ significantly among treatments (P>0.05). However, significant differences (P<0.05) were observed in fiber, fat, ash, moisture, and carbohydrate contents across diets. The 25% pine pollen diet (T2) exhibited the highest fat content, while fiber and moisture contents decreased as pine pollen inclusion increased.

Growth performance and survival of juvenile *M. rosenbergii* fed diets containing different levels of pine pollen meal are shown in Table 2. After 45 days of culture, prawns fed the diet with 25% pine pollen meal (T2) achieved the highest final mean body weight (0.47±0.03 g), weight gain (0.37±0.03 g), and specific growth rate (3.44±0.03% day^{-1}). In comparison, the control group (T1) showed lower growth performance, with a final mean weight of 0.32±0.03 g and an SGR of 2.58±0.03% day^{-1}. Prawns fed the 50% pine pollen meal diet (T3) exhibited moderate growth performance compared with the other treatments. Survival also varied among treatments. The highest survival rate was observed in T3 (62.22±6.94%), followed by T2 (55.56±8.39%), whereas the control group exhibited the lowest survival rate (34.44±5.09%). These results suggest that the dietary inclusion of pine pollen meal influenced both the growth performance and the survival of juvenile *M. rosenbergii*.

Length performance of juvenile *M. rosenbergii* is presented in Table 3. After 45 days of culture, prawns fed the diet containing 25% pine pollen meal (T2) attained the highest final length (35.6±1.01 mm) and length gain (19.2±2.21 mm). The treatment containing 50% pine pollen meal (T3) also showed a substantial increase in length, while the control group (T1) exhibited the lowest final length and length gain. These results indicate that the dietary inclusion of pine pollen meal affected the growth in length of juvenile prawns (Fig. 1).

Water quality parameters recorded during the 45-day culture period are summarized in Table 4. Temperature, pH, and dissolved oxygen values were

Table 3. Initial length, final length, and length increment (mm ± SD) of juveniles *Macrobrachium rosenbergii* fed diets containing different levels of pine pollen meal.

Treatment	Initial Length (mm)	Final Length (mm)	Length Increment (mm)
Control	16.4 ± 1.38^{a}	22.0 ± 0.73^{c}	5.60 ± 2.09^{c}
25% Pine Pollen	16.4 ± 1.86^{a}	35.6 ± 1.01^{a}	19.2 ± 2.21^{a}
50% Pine Pollen	15.0 ± 0.15^{a}	33.1 ± 1.00^{b}	18.1 ± 1.07^{b}

Values are mean±SD. Means within the same column sharing the same superscript letter are not significantly different (P>0.05), whereas different letters indicate significant differences (P<0.05).

Table 4. Water quality parameters during the 45-day culture period of juvenile *Macrobrachium rosenbergii* fed diets containing different inclusion levels of pine pollen meal.

Treatment	Temperature (°C)	pH	DO (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)
Control	27.6 ± 1.44^{a}	9.21 ± 0.04^{a}	5.93 ± 0.45^{a}	0.35 ± 0.39^{a}	1.84 ± 0.47^{a}	1.93 ± 0.27^{a}
25% Pine Pollen	27.5 ± 1.39^{a}	9.22 ± 0.09^{a}	5.96 ± 0.47^{a}	0.41 ± 0.34^{a}	1.93 ± 0.60^{a}	2.12 ± 1.62^{a}
50% Pine Pollen	27.6 ± 1.46^{a}	9.18 ± 0.08^{a}	5.92 ± 0.45^{a}	0.35 ± 0.36^{a}	1.47 ± 0.62^{a}	1.25 ± 0.39^{a}

Values are mean±SD. Means within the same column sharing the same superscript letter are not significantly different (P>0.05), whereas different letters indicate significant differences (P<0.05).

Table 5. Initial and final microbial counts (CFU mL^{-1}) in the culture water of juvenile *Macrobrachium rosenbergii* fed diets with different inclusion levels of pine pollen meal.

Treatment	Initial TPC (TSA, CFU mL^{-1})	Final TPC (TSA, CFUmL^{-1})	Initial LAB (MRS, CFUmL^{-1})	Final LAB (MRS, CFU mL^{-1})
Control	$(2.93\pm0.40)\times10^{8a}$	$(2.17\pm0.21)\times10^{8a}$	$(0.40\pm0.27)\times10^{8a}$	$(0.40\pm0.10)\times10^{8b}$
25% Pine Pollen	$(2.43\pm0.64)\times10^{8a}$	$(1.77\pm0.15)\times10^{8b}$	$(0.50\pm0.10)\times10^{8a}$	$(0.57\pm0.15)\times10^{8a}$
50% Pine Pollen	$(2.23\pm1.10)\times10^{8a}$	$(2.23\pm0.25)\times10^{8a}$	$(0.30\pm0.20)\times10^{8a}$	$(0.50\pm0.10)\times10^{8a}$

Values are mean±SD. Means within the same column sharing the same superscript letter are not significantly different (P>0.05), whereas different letters indicate significant differences (P<0.05).

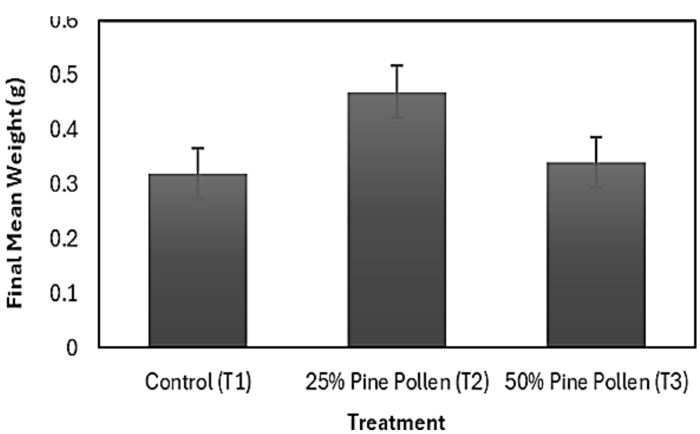

Figure 1. Final mean body weight (g) of juvenile *Macrobrachium rosenbergii* fed diets containing different inclusion levels of pine pollen meal over 45 days. Values are expressed as the mean ± SD.

similar across treatments. Mean water temperature ranged from 27.5 to 27.6°C, while pH values ranged from 9.18 to 9.22. Dissolved oxygen levels were maintained between 5.92 and 5.96 mg/L. Ammonia, nitrate, and nitrite concentrations showed minor variations among treatments. Ammonia levels ranged from 0.35 to 0.41 mg/L, nitrate from 1.47 to 1.93 mg/L, and nitrite from 1.25 to 2.12 mg/L across treatments.

Initial and final microbial counts of the culture water are presented in Table 5. Initial microbial samples collected before water management practices showed variation in total heterotrophic and lactic acid bacterial counts among treatments. Final microbial counts measured after water management also differed across treatments. Total heterotrophic bacteria were quantified on tryptone soy agar, and lactic acid bacteria on MRS agar after 72 h of incubation.

Juveniles fed diets supplemented with 25% and 50% pine pollen meal exhibited 100% survival following stress exposure, whereas the control group showed a lower stress survival rate of 90% (Table 6). The results showed that dietary inclusion of pine pollen meal was associated with differences in growth performance, length increment, survival, and microbial characteristics of *M. rosenbergii* juveniles under the experimental conditions (Fig. 2).

Discussions

The proximate composition of the diets (control and pine pollen addition) showed no significant differences in protein content among treatments. This

Table 6. Stress response (%) survival of juvenile *Macrobrachium rosenbergii* subjected to salinity stress after 45 days of feeding with experimental diets.

Treatment	Stress Survival (%)
Control	90.0±0.0[b]
25% Pine Pollen	100.0±0.0[a]
50% Pine Pollen	100.0±0.0[a]

Means with different superscripts indicate significant differences (P<0.05).

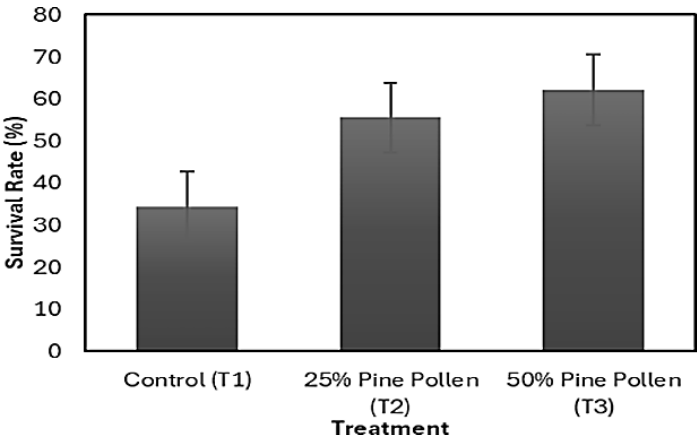

Figure 2. Survival rate (%) of juvenile *Macrobrachium rosenbergii* fed diets containing different levels of pine pollen over 45 days. Values are expressed as mean ± SD.

suggests that the overall protein level of the experimental diets was not compromised by the partial inclusion of pine pollen meal as a supplement. This result is consistent with earlier research that found no effect of pine pollen inclusion in Nile tilapia diets on dietary protein content or consumption (NRC, 2011; Nian et al., 2017). Protein is an important nutrient for freshwater prawns, especially during the early developmental phases, as it is required for tissue development and molting (Nesara et al., 2018).

In contrast, significant differences were observed in lipid, fiber, ash, moisture, and carbohydrate contents among treatments. The maximum lipid content was seen in the diet containing 25% pine pollen meal. Lipids are a significant energy source for crustaceans and can increase growth by saving protein for somatic development rather than energy metabolism (Guillaume et al., 2001; Chen et al., 2024). Thus, the higher lipid content of the diet containing 25% pine pollen may have contributed to the improved growth performance observed in this treatment.

The growth performance results showed a significant difference among dietary treatments, indicating that supplementation with pine pollen meal affected the growth of juvenile *M. rosenbergii*. Juvenile prawns fed 25% pine pollen addition showed the highest final mean weight, weight gain, and specific growth rate compared to control and 50% pine pollen treatments. This indicates that moderate dietary addition of pine pollen meal can provide nutritional and functional benefits that increase growth performance. The enhanced growth observed in the 25% pine pollen treatment may be attributed to the improved dietary energy profile, particularly the increased fat content of this diet. Lipids promote growth in crustaceans at adequate levels by providing readily available energy, thereby reducing the need for dietary protein for metabolic activities and allowing more effective utilization of protein for tissue development (Guillaume et al., 2001; Chen et al., 2024). Protein levels were similar across all diets, so the improved growth performance is likely related to variations in non-protein energy components and functional features of pine pollen meal.

Although the juvenile prawns fed the 25% pine pollen diet showed better growth performance, their survival was lower than that shown in the 50% pine pollen treatment. This may point to a trade-off between growth and survival, where conditions favoring rapid development may not necessarily maximize survival. Those with a faster rate of growth tend to have greater metabolic demands and hence may be more sensitive to environmental or physiological stress. In contrast, higher survival in the 50% pine pollen treatment suggests that increasing supplementation may have improved stress tolerance or general robustness, even though growth performance was moderate.

The length growth of juvenile *M. rosenbergii* was influenced by supplementation with pine pollen meal in the diet. Juvenile prawns fed a diet containing 25% pine pollen meal recorded the highest final length and length increment after a 45-day culture period. This implies that a moderate addition of pine pollen meal increased linear growth and total body development. The greater weight gain observed with this therapy was matched by the higher length gain, suggesting that growth was proportionate and not confined to body mass alone. The improved length growth in the 25% pine pollen treatment could be associated with improved nutrient utilization and increased dietary energy availability. Adequate dietary energy is beneficial for growth and molting, which are necessary for crustaceans to increase body length. In particular, adequate dietary lipid levels provide readily available energy that may improve growth efficiency and support molting and tissue development (Chen et al., 2024). Since protein levels were similar between treatments, the differences seen in length development are likely related to differences in non-protein energy components and the functional features of pine pollen supplementation. Previous research has demonstrated that adequate dietary energy intake can improve linear growth and growth efficiency in freshwater prawns and other crustaceans (Frías-Gómez et al., 2023; Chen et al., 2024).

Based on the results, water quality measures remained steady throughout the 45-day culture period, with little difference among treatments. Temperature, dissolved oxygen, and pH values were maintained within the permitted range for the culture of juvenile *M. rosenbergii*. These characteristics were important for maintaining normal metabolic activity, molting, and growth in freshwater prawns, and their stability indicated that the culture conditions were adequate and carefully managed during the experiment (SEAFDEC/AQD, 2020).

Ammonia, nitrite, and nitrate contents were relatively low and remained within tolerable limits for juvenile prawns. High concentrations of nitrogenous waste, especially ammonia and nitrite, have been found to induce physiological stress, slow growth, and raise mortality in freshwater prawns (Romano and Zeng, 2013). However, the values in this study did not exceed the suggested levels, indicating that the feeding practices and water management were effective in preventing the buildup of toxic metabolites. Ammonia, nitrite, and nitrate concentrations showed modest variation between treatments, but these variations were not expected to have a major impact on growth performance or survival. Similar results have been reported in feeding studies in which water quality was maintained within acceptable standards, allowing examination of the effects of food on growth irrespective of environmental stress (Romano and Zeng, 2013). Adequate water quality during the culture phase often enhances the reliability of experimental outcomes. Stable environmental conditions ensured that the observed differences in growth performance, length increment, microbiological features, and stress response of juvenile *M. rosenbergii* were mostly attributable to nutritional supplements, not complicated by variations in water quality.

The microbial analysis of the culture water demonstrated changes in overall heterotrophic bacterial counts and lactic acid bacterial (LAB) populations between treatments. Microbial counts were relatively high in all treatments prior to the start of water management, which is typical in aquaculture systems where organic matter from feed and metabolic waste accumulates. Total heterotrophic bacterial counts were generally reduced after water management, demonstrating that water exchange could lower the total bacterial load. The lactic acid bacterial counts in pine pollen-supplemented treatments were significantly higher than in the control, especially after water management. Lactic acid bacteria are usually beneficial microorganisms, as they can help establish a microbial balance and inhibit the growth of potentially pathogenic bacteria through competitive exclusion and the production of organic acids (Nayak, 2010). The increase in LAB counts in the pine pollen treatments suggests that dietary supplementation might have influenced microbial dynamics in the culture water.

The increased populations of LAB in treatments with pine pollen as a dietary addition may be linked to increased survival and stress tolerance in these groups. Beneficial microbial communities in the culture environment have been reported to improve water quality and reduce pathogen pressure, thereby promoting host health (Nayak, 2010; Arun and Midhun, 2022). Although the specific processes were not explored in this study, the microbiological data suggest that supplementation with pine pollen may promote a more favorable microbial environment for juvenile *M. rosenbergii*. Microbial data indicate that water management measures were successful in controlling bacterial load (SERD Personnel Editor, 2022; Qui et al., 2023) and suggest that pine pollen supplementation may help build beneficial microbial communities in the culture system.

Juvenile *M. rosenbergii* fed diets containing pine pollen meal as a food additive exhibited greater survival after stress exposure than the control. Both 20% and 50% pine pollen treatments resulted in 100% survival after the stress test. The control group had lower stress survival. The results showed that supplementing the diet with pine pollen meal improved the stress resistance of juvenile prawns. Improved stress resistance may be associated with bioactive compounds in pine pollen, which are known to have antioxidant and immunomodulatory activities. Antioxidants play a crucial role in mitigating oxidative stress and protecting organisms from cellular damage under severe environmental or handling conditions (Muscolo et al., 2024). Although the present study did not explicitly assess antioxidant activity, prior studies have demonstrated that functional feed additives can enhance stress tolerance and survival in aquatic organisms by improving physiological resilience (Citarasu, 2010).

The high-stress survival observed in the 50% pine pollen treatment is consistent with its higher survival during the feeding trial and suggests that increased inclusion of pine pollen meal may lead to greater robustness and stress resistance. On the other hand, the lower stress survival of the control group may represent a reduced physiological capacity to cope with stress in the absence of functional dietary additives. Comparable gains in stress resistance have been documented in crustaceans and fish fed diets containing functional plant-based additives that promote immune function and stress-response mechanisms. Thus, the findings on stress responses add to those on growth, survival, and microbial activity in this study, suggesting that pine pollen meal, as a dietary addition, may contribute to growth performance and improved stress tolerance in juvenile *M. rosenbergii*. Functional feed ingredients have also been demonstrated to enhance nutrient utilization, gut health, and stress tolerance of crustaceans, which may indirectly improve growth performance and survival (Gatlin et al., 2007; Dawood et al., 2018). The results indicated that using dry supplements at up to 50% is economically viable in *M. rosenbergii* diets. The slight increase in formulation cost is a realistic trade-off when considering potential gains in feed efficiency and overall production performance. The results encourage future studies on the utility of the addition as an economical dietary ingredient for freshwater prawn production, particularly under resource-limited farming conditions.

Conclusion

The results of this study revealed that a pine pollen meal nutritional supplement can significantly improve growth performance, length development, survival, and stress tolerance in post-larval *M. rosenbergii*. The prawns fed diets containing 25% pine pollen meal showed the greatest growth and length increases, implying that moderate supplementation is optimal for enhancing somatic and linear growth. In contrast, the inclusion of 50% pine pollen meal increased survival and improved stress tolerance, suggesting greater physiological resilience. Water quality was consistent throughout the experimental period, indicating that the observed treatment effects were primarily due to nutritional supplementation rather than environmental variation. Furthermore, the increased abundance of lactic acid bacteria in treatments supplemented with pine pollen suggests that the pine pollen meal may help maintain a favorable microbial environment. Pine pollen meal can be a promising natural, functional, and low-cost feed element for freshwater prawn culture. Moderate inclusion levels are recommended for optimal growth and length performance, but higher inclusion levels may improve survival and stress resistance. Future studies should include long-term feeding experiments, optimization of inclusion levels under commercial conditions, and evaluation of physiological pathways responsible for the observed benefits.

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