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The Effect of Dietary Sodium Alginate on the Growth and Survival of Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The giant freshwater prawn, *Macrobrachium rosenbergii*, is a valuable aquaculture species known for its rapid growth, adaptability to environmental variations, and high nutritional value. However, challenges such as high mortality rates, algal blooms, dissolved oxygen depletion, and pathogen

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outbreaks constrain its cultivation. The overuse of antibiotics in aquaculture raises significant environmental and health concerns, including antimicrobial resistance. This study explores the potential of sodium alginate, a natural polysaccharide derived from brown algae, as a prebiotic and immunostimulant for improving growth and feed efficiency in M. rosenbergii. Juvenile prawns (~0.2 g) were cultured for 60 days and fed experimental diets supplemented with sodium alginate at concentrations of 0 g/kg (control, T0), 1.0 g/kg (T1), 2.0 g/kg (T2), and 3.0 g/kg (T3). Growth performance, feed utilization, and survival rates were assessed bi-weekly. Results showed significant improvements (p<0.05) in growth parameters, including mean weight gain (MWG) and specific growth rate (SGR), in prawns fed a 2.0 g/kg sodium alginate diet (T2). At the end of the experiment, T2 recorded the highest mean weight, MWG, SGR, and lowest feed conversion ratio (FCR, 2.395 ± 0.068), demonstrating superior feed utilization efficiency. In contrast, prawns fed a 3.0 g/kg sodium alginate diet (T3) showed reduced growth performance and feed efficiency. This study highlights the efficacy of sodium alginate as a dietary supplement to enhance growth, feed efficiency, and sustainability in freshwater prawn aquaculture, with the optimal dosage identified as 2.0 g/kg. These findings support sodium alginate's potential as an eco-friendly alternative to antibiotics in aquaculture systems.

Keywords: Macrobrachium rosenbergii; sodium alginate; prebiotic; immunostimulant; aquaculture sustainability.

1. INTRODUCTION

The strong demand for giant freshwater prawn, Macrobrachium rosenbergii is due to its high nutritional value, including its high levels of protein, polyunsaturated fatty acids, and low-fat levels (D'Abramo & Sheen, 1994). The giant freshwater prawn, Macrobrachium rosenbergii, is a candidate culturable freshwater prawn species with a high growth rate, euryhaline, wide temperature tolerance (15-35 °C), and larger size. In India, freshwater prawns are mostly cultivated in mono- and polyculture systems in either naturally occurring or controlled bodies of water (Mukhopadhyay et al., 2003). But, apart from these prons, the major constraint in prawn farming is the high rate of mortality due to higher rate of stocking, algal bloom production, DO depletion, man-made food polluted water, and pathogen diseases (Farook et al., 2019). Globally, the giant freshwater prawn contribute 24 thousand tonnes in 2020, accounting for 2.6% of global aquaculture production (FAO, 2022). In India, Among the total shrimp production, the giant freshwater prawn production is 8,303 metric tonnes. In India, for Scampi production, West Bengal remains in the top position (MPEDA, 2021).

The use of antibiotics in aquaculture, especially during larval and growth stages, has raised concerns about environmental and human health impacts, such as bacterial resistance, persistent aquatic diseases, and altered sediment biogeochemistry (Bermúdez-Almada & Espinosa-Plascencia, 2012; Ma et al., 2006).

Excessive antibiotic use promotes antimicrobialresistant bacteria (AMRB) and fosters antibiotic resistance mechanisms (Pruden et al., 2013). To address challenges. environmentally these friendly alternatives probiotics. like immunostimulants, antimicrobial peptides, and quorum sensing interference have developed (Sivakamavalli al., 2021). Probiotics and prebiotics have gained attention as more cost-effective solutions compared to antibiotics or vaccines, with prebiotics offering the advantage of being natural feed ingredients that promote beneficial gut bacteria and enhance health (Gatesoupe, 2005; Yousefian & Amiri, 2009). Additionally, medicinal plants, such as marine algae, show promise as prebiotics due to their bioactive, antibacterial properties that are safe for the environment and beneficial in aquaculture (Alghazeer et al., 2013; Dashtiannasab et al., 2016). Algal extracts can be delivered to prawns through injection, immersion, or feed supplementation (Huang et al., 2006; Yeh et al., 2006).

Sodium alginate, а natural hydrophilic polysaccharide derived from marine brown algae, is composed of 1,4-d-mannuronic (M) and I-guluronic (G) acids and serves as a structural component in algal cell walls, containing 30-60% alginic acid (Maiti & Kumari, 2016; Santos, 2017). Widely used in aquaculture as a feed additive, it functions as a feed binder to improve pellet stability, a dietary fiber, and a prebiotic promoting gut health in herbivorous and omnivorous fish species. Sodium alginate enhances the intestinal absorption of minerals

like calcium and magnesium (Mohapatra et al., 2011) and is recognized as GRAS (generally regarded as safe) by the FDA (Stone et al., 2009). Known for its biocompatibility, nontoxicity. and non-immunogenicity, it also possesses antibacterial, antiviral, antimicrobial, antifungal, immunomodulatory, and antioxidant. inflammatory properties (Sachan et al., 2015; Ngo & Kim, 2013; Guo et al., 2020). Additionally, alginic acid exhibits hypocholesterolemic and antihypertensive effects. Further Yeh et al. (2008) found that dietary sodium alginate at 1.0 and 2.0 g/kg improved growth performance and feeding efficiency in orange-spotted grouper. Its potential for immunostimulatory and resistance studies, such as in Macrobrachium rosenbergii against Aeromonas hydrophila, highlights its versatile applications in aquaculture.

2. MATERIALS AND METHODS

2.1 Sodium Alginate

Sodium alginate, which was purchased from Isochem Laboratories in Kochi, Kerala.

2.2 Experimental Diet

The commercial diet was cooked, sterilized, and powdered. Then, powdered Sodium alginate was added to diets in four doses, including control (T0), 1.00 g/kg (T1), 2.00 g/kg (T2), and 3.00 g/kg (T3). After that, the dough was thoroughly mixed to distribute evenly all the ingredients. Then prepare pellets, and spread them into trays for sun drying. After drying, pellets were packed into airtight plastic containers, labeled, and stored properly.

2.3 Experimental Animal & Husbandry Trial

The experiment was conducted at the Hands-on Training Center, Department of Aquaculture, situated within the College of Fisheries Science, Kamdhenu University, Veraval. *Macrobrachium rosenbergii* juveniles (~0.2 g) were obtained from Dev Fisheries in Valsad, 695 km from the experimental site. Packaged in 15-liter plastic bags with treated freshwater and pure oxygen, sealed bags (1,000 juveniles per bag) were transported in thermocol boxes to minimize stress during transit. Upon arrival, the juveniles underwent prophylactic treatment in a 0.05% potassium permanganate solution following the normal bath method (Cruz-Lacierda et al., 2000)

and were transferred to a 500-liter tank for acclimatization. During the 10-day acclimation period, they were fed *Artemia* (live feed) and commercial feed, with continuous aeration to maintain optimal oxygen levels before being moved to experimental tanks.

The experiment was conducted in 16 plastic tanks (2 x 1 x 1 feet, 40 liters), disinfected with 2 ppm KMnO₄ and rinsed thoroughly. The tanks were divided into four treatment groups with four replicates each. A total of 240 *Macrobrachium rosenbergii* juveniles (~0.2 g initial weight) were acclimatized and randomly distributed (15 prawns per tank) following a completely randomized design (CRD). The prawns were cultured for 60 days, and fed experimental diets twice daily—initially at 10% of body weight for the first month, then reduced to 5% thereafter. Feedings occurred from 9:00–9:30 a.m. and 6:30–7:00 p.m. Weight was monitored biweekly, and feeding rations were adjusted accordingly.

2.4 Growth Parameter Analysis

- Weight Measurement: Prawn juveniles' weight was recorded bi-weekly using an electronic balance with a glass beaker of water to reduce stress.
- Mean Weight (MW): Average weight per prawn in each tank calculated at bi-weekly intervals.
- **Mean Weight Gain (MWG)**: Calculated using the formula:

MWG(g) = Final weight(g) - Initial weight(g)

• Specific Growth Rate (SGR): Calculated using the formula by Manivannan and Saravanan (2012):

SGR =

 $\frac{(Average\ Final\ weight\ -\ Average\ Initial\ weight)}{Number\ of\ days}X\ 100$

2.5 Feed Utilization Parameter Analysis

• Feed Conversion Ratio (FCR): Measures feed efficiency, calculated as:

$$FCR = \frac{Feed\ intake}{Weight\ gain}$$

• Protein Efficiency Ratio (PER): Assesses protein utilization efficiency, calculated as (on a dry matter basis):

$$PER = \frac{Wet \ weight \ gain}{Protein \ intake}$$

2.6 Survival Rate Analysis

- Survival Monitoring: Daily inspection of tanks for live and deceased prawns; dead prawns removed and counted.
- Survival Rate Formula:

 $Survival\ rate\ (\%) =$

 $\frac{No.\,of\,\,prawn\,stocked}{No.\,of\,\,prawns\,\,survived\,\,after\,\,rearing}\times\,\,100$

2.7 Statistical Analysis

After the experiment, data was evaluated using conventional statistical techniques by Snedecor and Cochran (2014). The analysis was carried out using the SPSS program. To assess "Mean ± Standard Error (SE)," one-way analysis of variance (ANOVA) was utilized; to test for significant differences across treatments, the Duncan multiple range test (DMRT) was employed.

3. RESULTS

3.1 Growth Parameters Analysis

3.1.1 Mean weight analysis

During the 60-day trial, *Macrobrachium rosenbergii* juveniles from each tank were sampled bi-weekly to monitor growth. The initial mean weights of treatments T0, T1, T2, and T3

were $0.209 \pm 0.003g$, $0.211 \pm 0.055g$, $0.216 \pm$ 0.006g, and $0.207 \pm 0.003g$, respectively, showing no significant differences initially. However, as the experiment progressed, mean weights increased significantly (p<0.05) across treatments, with T2 consistently showing the highest growth, followed by T1, T0, and T3. By day 30, prawns fed a 2.0 g/kg sodium alginate diet (T2) exhibited significantly higher growth. At day 45, T2 maintained superior growth, while T3 showed the lowest growth. At the end of the experiment, the mean weights were 1.011 ± 0.015g (T0), $1.223 \pm 0.003g$ (T1), $1.497 \pm$ 0.003g (T2), and $0.876 \pm 0.012g$ (T3), confirming that T2 produced the highest growth (p<0.05), whereas T3 resulted in the lowest.

3.1.2 Mean weight gain (MWG) analysis

The fortnightly growth performance Macrobrachium rosenbergii juveniles assessed through mean weight gain, presented in Fig. 2. On day 15, the mean weight gains for T0, T1, T2, and T3 were 0.186 ± 0.008 g, $0.208 \pm$ 0.006g, $0.352 \pm 0.001g$, and $0.163 \pm 0.006g$, respectively, with T2 showing the highest gain (p<0.05). By day 30, T2 continued to outperform with $0.547 \pm 0.012g$, followed by T1 (0.461 ± 0.012g), T0 (0.385 \pm 0.005g), and T3 (0.213 \pm 0.006g). On day 45, T2 maintained the highest gain $(0.788 \pm 0.008g)$, while T0, T1, and T3 showed lower gains. By day 60, mean weight gains were $0.801 \pm 0.012g$ (T0), $1.012 \pm 0.004g$ (T1), 1.281 \pm 0.009g (T2), and 0.669 \pm 0.009g (T3). The results confirm that T2 had significantly higher weight gain throughout the culture period.

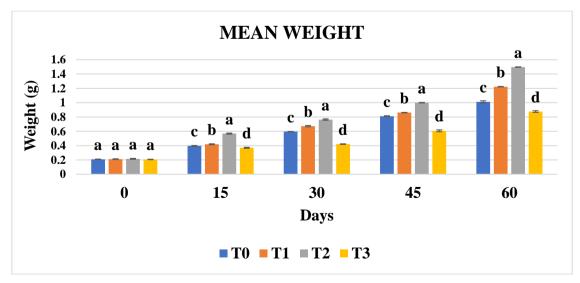


Fig. 1. Impact of sodium alginate diet on *M. rosenbergii* mean weight (MW) after the experiment

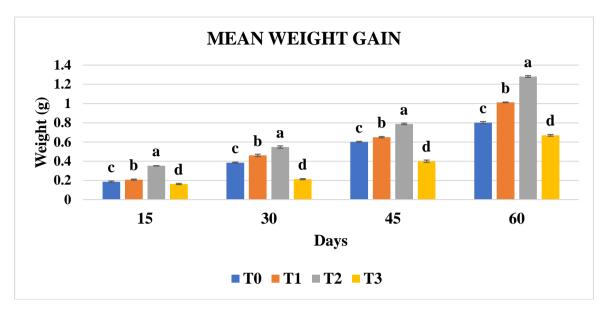


Fig. 2. Impact of sodium alginate diet on *M. rosenbergii* mean weight gain (MWG) after the experiment

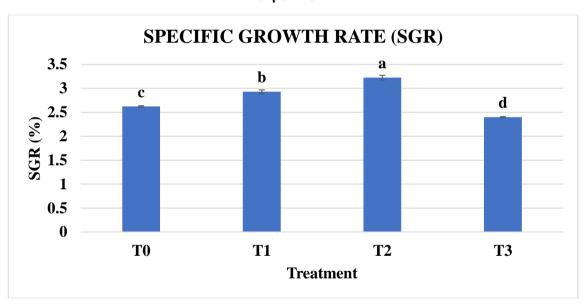


Fig. 3. Impact of sodium alginate diet on *M. rosenbergii* specific growth rate (SGR) after the experiment

3.1.3 Specific growth rate analysis

The specific growth rate (SGR) of *M. rosenbergii* juveniles at the end of the study is shown in Fig. 3. The lowest SGR (2.399 ± 0.009) was observed in T3, where prawns were fed a 3.0 g/kg sodium alginate diet. The highest SGR (3.224 ± 0.049) was recorded in T2, fed a 2.0 g/kg sodium alginate diet, followed by T1 (1.0 ± 0.000 g/kg sodium alginate) and T0 (fish meal-based diet). All treatments showed significant differences (p<0.05), with T2 demonstrating the most favorable growth performance.

3.2 Feed Utilization Parameter Analysis

3.2.1 Feed conversion ratio (FCR) analysis

At the end of the 60-day study, the feed conversion ratio (FCR) of *M. rosenbergii* juveniles was calculated and presented in Fig. 4. The FCR values for treatments T0, T1, T2, and T3 were 2.878 ± 0.081 , 2.612 ± 0.204 , 2.395 ± 0.068 , and 3.106 ± 0.089 , respectively. The lowest FCR was observed in T2 (2.0 g/kg sodium alginate diet), indicating the most efficient feed utilization, while the highest FCR was in T3 (3.0 g/kg sodium alginate diet).

Significant differences (p<0.05) were observed among all treatments.

3.2.2 Protein efficiency ratio (PER) analysis

The protein efficiency ratio (PER) of *M. rosenbergii* juveniles was evaluated after the experiment and is presented in Fig. 5. Prawns were fed a diet containing 35% protein. The highest PER (1.194 \pm 0.034) was observed in T0, followed by T1 (1.099 \pm 0.090), T2 (0.993 \pm 0.028), and T3 (0.920 \pm 0.026). Significant differences (p<0.05) were noted among treatments, with T2 (2.0 g/kg sodium alginate diet) showing the most effective protein utilization.

3.3 Survival Rate Analysis

The survival rate of *M. rosenbergii* juveniles was monitored every two weeks and is summarized in Fig. 6. At the end of the culture period, the highest survival rate (93.33 \pm 2.721%) was observed in T2, where prawns were fed with a 2.0 g/kg sodium alginate diet, followed by T1 and T3 (both 90.00 \pm 1.924%), and T0 (85.00 \pm 1.667%). While no significant differences (p<0.05) were found among treatments, T2 had a notably higher survival rate compared to the others, with no significant difference between T1 and T3.

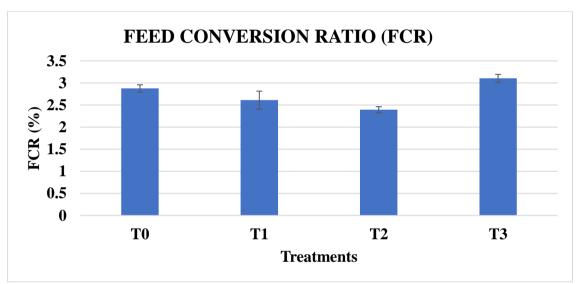


Fig. 4. Impact of sodium alginate diet on *M. rosenbergii* feed conversion ratio (FCR) after the experiment

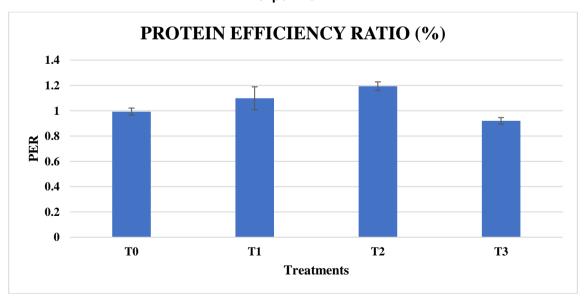


Fig. 5. Impact of sodium alginate diet on *M. rosenbergii* protein efficiency ratio (PER) after the experiment

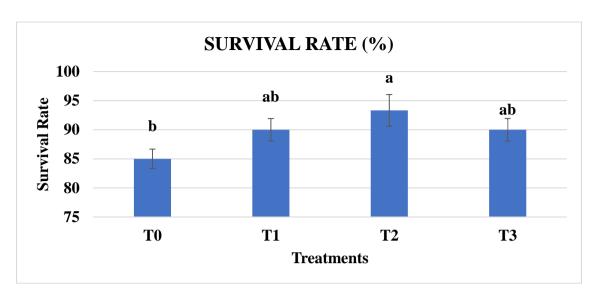


Fig. 6. Impact of sodium alginate diet on M. rosenbergii survival rate after the experiment

4. DISCUSSION

The dietary inclusion of prebiotics such as sodium alginate can enhance immunity as well as growth parameters of M. rosenbergii. The T2 treatment showed higher growth compared to the other treatments, where prawns were fed with a 2.0 g/kg sodium alginate incorporated diet. As the concentration of the sodium alginate increases, the growth of the M. rosenbergii increases proportionally, but the higher growth parameters obtained at 2.0 g/kg of the sodium alginate incorporated diet, and 3.0 g/kg of sodium alginate incorporated diet showed less growth than the other all treatments. The reason behind these results may be due to the theory given by Bagheri et al. (2023) that the prawn's microbiota and digestive system were most likely not enhanced by altering the weight. A similar trend was found by Bagheri et al. (2023), where the L. vannamei fed with a 2.0 g/kg sodium alginate diet showed significantly higher growth parameters compared to other treatments. Previous studies on sodium alginate-based diets also showed significantly higher SGR of Oreochromis niloticus (Van Doan et al., 2016), Atlantic cod (Vollstad et al., 2006), Penaeus monodon (Chung et al., 2011), Epinephelus coioides (Yeh et al., 2008), Sea cucumber (Xia et al., 2012), and Procambarus clarkii (Mona et al., 2015). The reasons for these results could be the prebiotic effects differ according to fish species, water temperature, feeding duration, prebiotic dosages, and solubility (Van Doan et al., 2016). A similar trend was also found by Santos et al. (2019), where the weight gain of L. vannamei was proportional to the concentration

of sodium alginate in the shrimp diet. The present study suggests that the presence of beneficial bacteria and digestive enzymes may be responsible for the notable enhancement in the growing performance of *M. rosenbergii*.

The present investigation indicated that the lowest FCR was found in the T2 treatment, which shows higher growth parameters. Bagheri et al. (2023) also found a similar result where a diet containing 2.0 g/kg sodium alginate showed lower FCR than other treatments. In the case of finfish, Vollstad et al. (2006) and Yeh et al. (2008) also found similar results in Gadus morhua L. and **Epinephelus** coioides. respectively. Due to its high protein content and abundance of additional growth stimulants, algae, and algae extracts have gained a lot of interest when added to prawn diets. As a result, it can enhance immunity, boost growth and appetite, and strengthen resistance to infections (Schiener et al., 2014; Akbary & Aminikhoei, 2018; Akbary et al., 2021). The study conducted by Akbary et al. (2023) found that the extract of the brown seaweeds positively affects the growth parameters and also PER of the shrimp, Litopeneous vannamei.

At the end of the study, a higher survival rate was found in T2 treatment. The T2 treatment was significantly higher than the other treatments. There were no significant differences between T0 and T1 treatment. Control treatment shows a significantly lower survival rate than all treatments. A previous study by Santos et al. (2019) also found a similar trend of survival rate in shrimp as the concentration of sodium

alginate increased. Contradictory, Bagheri et al. (2023) found no significant difference in the survival rate of white leg shrimp. Neamat-Allah et al. (2019) evaluate that The immunological response being triggered by sodium alginate may be the reason for the increased catfish survival rate.

5. CONCLUSION

The study highlights the positive impact of sodium alginate as a dietary prebiotic on the growth performance, feed utilization, and survival rate of Macrobrachium rosenbergii. Among all treatments, the T2 group, fed with a 2.0 g/kg sodium alginate-incorporated diet, exhibited the best overall results, including higher growth performance, improved feed conversion ratio (FCR), higher protein efficiency ratio (PER), and increased survival rate. The findings align with previous research, suggesting that optimal concentrations of sodium alginate enhance the prawn's microbiota and digestive enzyme activity, resulting in superior growth and immunity. However, a higher concentration (3.0 g/kg) of sodium alginate showed reduced growth, possibly due to a saturation effect on the microbiota and digestive system. The study also corroborates earlier reports that algae and their extracts, due to their protein and growthpromoting components, significantly enhance immunity, growth, and disease resistance in aquatic species.

This investigation confirms the potential of sodium alginate at an optimal dose as a sustainable and effective feed additive to enhance the aquaculture performance of *M. rosenbergii*. Future research should explore the long-term impacts and underlying mechanisms of sodium alginate and other prebiotics in aquaculture systems.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative Al technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. Details of the Al usage are given below:

1. Chat GPT Original Manuscript

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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