Aquaculture International
https://doi.org/10.1007/s10499-024-01557-6

RESEARCH

Dietary supplements of β-1,3/1,6-glucan derived from baker's yeast results in enhanced seed production and robustness in larvae of the freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879)

Riyad Taguemount[1] · Jarunan Pratoomyot[2] · Andrew P. Shinn[3,4] · Khor Waiho[1] · Rasina Rasid[1]

Received: 9 November 2023 / Accepted: 27 May 2024

Abstract
This study explored the effects of β-1,3/1,6-glucan derived from *Saccharomyces cerevisiae* cell walls on the growth, survival, and physiological responses of post-larvae (PL) of *M. rosenbergii*. Over a 3-week period, larvae were fed a formulated egg custard diet containing varying amounts of β-glucan. The findings revealed that incorporating β-glucan into the diet had a substantial positive impact. The inclusion of β-glucans significantly enhanced survival (59.14% for the PL fed 0.2% β-glucan versus 46.58% for the control; $p < 0.05$), promoted growth (13.58 mg wet weight for the PL fed 0.2% β-glucan versus 9.53 mg for the PL fed the control diet; $p < 0.05$), and accelerated the time to metamorphosis (26.67 days for the PL fed 0.2% β-glucan versus 28.0 days for the PL fed the control diet). As the amount of β-glucan in the diet increased, larval growth performance consistently improved. The group receiving 0.2% β-glucan exhibited the highest performance in terms of wet and dry weight, total length, and mean production of PL. Furthermore, the study assessed the influence of β-glucan supplementation on larval tolerance to hypersaline stress. Although the differences were not statistically significant, the addition of β-glucan resulted in incremental improvements in the ability of larvae to withstand hypersaline conditions. In conclusion, the dietary supplement containing 0.2% β-glucan exhibited the highest performance among the inclusion levels tested. Further investigation is recommended to determine the nutritional and physiological effects of β-glucan supplementation under salinity stress conditions.

Keywords B-glucan · Larval quality · Growth performance · Survival · Metamorphosis rate · Stress test

Handling Editor: Daniel Merrifield

Extended author information available on the last page of the article

Published online: 14 June 2024

Introduction

Macrobrachium rosenbergii, also known as the giant freshwater prawn, is the world's fifth most farmed crustacean species, representing 2.62% of global crustacean production and 3.96% of worldwide shrimp and prawn aquaculture. In 2020, the global production of *M. rosenbergii* was 294,019 tons with China, Bangladesh, and Thailand as the top producers of this species. Malaysia, the country focus of this study, ranked 10th; national production had decreased from 205.58 tons in 2019 to 192.69 tons in 2020 against a backdrop of rising global production of 7.95% year-on-year over the period 2016–2020 (FAO FishStatJ 2023). Well-established hatchery technologies have contributed to the widespread success of freshwater prawn farming, particularly the cultivation of *M. rosenbergii*, in various regions across the globe (Wilder et al. 1999; Vici et al. 2000). The success of freshwater prawn farming, like any other aquaculture sector, is dependent on the availability of high-quality seed for stocking grow-out ponds. Hatchery-raised post-larvae (PL) prawns are favoured due to their selection from breeding programs and fed dietary regimes, which together can contribute to improved disease resistance (Khasani et al. 2022; Zarantoniello et al. 2023). Using aquaculture stocks means a reduced reliance on wild-caught seed and broodstock. Nevertheless, the low survival rate of prawn seed in commercial production remains an ongoing challenge (Hongtuo and Jin 2018).

In commercial hatcheries of giant freshwater prawns in Southeast Asia, it is common to use egg custard as an artificial diet that includes seafood, milk powder, and chicken egg yolk (Harun et al. 2021; Liew et al. 2022; Xu et al. 2023). In a study by Nik Sin and Shapawi (2017), it was found that by supplementing a common egg custard diet with poultry byproduct meal and fish oil resulted in successful improvement in larval survival (i.e. 46% survival rate) when provided alongside *Artemia* nauplii. However, they found that when a mixture of egg custard containing shrimp, squid, and bivalve meal was used as a complete replacement for live feed, the survival rate dropped significantly to only 9%. More recently, several studies have focused on exploring the utilisation of additional ingredients/microorganisms in small quantities to enhance the egg custard base diet, operating at both biochemical and physical levels (Kangpanich et al. 2017; Mohmad et al. 2020; Xu et al. 2023). Similarly, functional feeds containing beneficial agents such as bacterial preparations, complex carbohydrates nutritional factors, plant and animal products, hormones, cytokines, and products derived from synthetic sources are extensively used to enhance seed production in a wide range of shrimp species (Vici et al. 2000; Apines-Amar et al. 2015; Wang et al. 2020). These feed supplements have been recognised for their ability to enhance growth, disease resistance, and various other qualities in fish and shrimp (Deshimaru and Yone 1987; Burrells et al. 2001; Dash et al. 2016; Dawood et al. 2018; Lim et al. 2018). Out of the numerous prebiotics employed in aquaculture, β-glucan stands out as a well-documented immunomodulator. Extracted from various organisms such as yeast and plants, this glucose polymer has been extensively studied in the aquaculture industry. Its immunomodulatory effects have been thoroughly evaluated (Soltanian et al. 2009; Meena et al. 2013). Among the various types of β-glucans, it is suggested that the β-1,3/1,6-glucans derived from baker's yeast exhibits high potency as an enhancer of the immune system (Ringø et al. 2011). Studies such as Miest et al. (2016) and Khanjani et al. (2022) have demonstrated the immunostimulatory effects of β-glucan. These effects include improved stress tolerance and disease resistance in various aquatic species, which are crucial for the survival and growth of shrimp larvae. Additionally, β-glucan acts as a prebiotic, fostering the growth

of beneficial bacteria within the digestive system of crustaceans. This promotes nutrient uptake, overall health and subsequent improvements in survival rates (Song et al. 2014; Kühlwein et al. 2014; Shah et al. 2016; Nieves-Rodríguez et al. 2018; Shoukat and Sorrentino 2021).

Numerous studies have highlighted the benefits of incorporating β-glucan into shrimp diets. Oral administration of glucans has been shown to protect against opportunistic pathogens and environmental stress in various cultured species. Dietary supplementation of 0.05–0.20% β-glucan has been found to enhance growth, survival, and low-salt stress tolerance in Pacific whiteleg shrimp, *Penaeus vannamei* (see Qiao et al. 2022). In *P. vannamei*, inclusion of 0.2% β-glucan positively impacted growth performance, particularly under low salinity conditions (Murthy et al. 2009). Moreover, feeding *P. vannamei* with 0.05–0.15% β-glucan improved growth, antioxidant activities, non-specific immunity, and disease resistance against *Fusarium solani* infection (Eissa et al. 2023). Low dietary β-glucan supplements at concentrations of 0.02–0.04% improved digestibility, antioxidant capacity, immunity, growth performance, and survival in *P. vannamei* under low salinity conditions (Li et al. 2019). In banana prawns, *Penaeus merguiensis*, treatment with 0.2% β-glucans from *Chaetoceros muelleri* and *Thalassiosira weissflogii* increased survival after *Vibrio parahaemolyticus* infection (Pooljun et al. 2022). The findings indicate that β-glucan has the potential to mitigate the effects of low-salinity stress in penaeid shrimp. Moreover, the results highlight that β-glucans derived from the same species but from different sources and obtained through diverse biotechnological processes can demonstrate distinct biological characteristics after extraction and purification.

The physiological processes of shrimp are directly influenced by the surrounding water, and various environmental factors have specific impacts on their innate immunity (Chen and He 2019). It has been observed that water pH, temperature, and other physical and chemical factors can weaken the shrimp's immune system (Cheng and Chen 2000). In crustacean hatcheries, stress tests, including variations in salinity, temperature, pH, ammonia, or exposure to formalin, are conducted to evaluate the quality of post-larvae (PL), along with assessing their survival capacity. Salinity stress testing, commonly employed by shrimp farmers, assesses the ability of shrimp to cope with stressful environmental conditions but does not necessarily indicate disease resistance. Activity tests have been utilised to evaluate the quality of *Macrobrachium* sp. seed through salinity shock, enabling a quick assessment of larval quality by subjecting test organisms to short-term salinity shock and evaluating their survival potential through osmoregulation (Devresse et al. 1990; De Caluwe et al. 1995; Merchie et al. 1995; Cavalli et al. 2000; Kangpanich et al. 2017; Rodrigues et al. 2018; Wei et al. 2021). Similar tests have been conducted in marine shrimp and fish (e.g. Tackaert et al. 1989; Dhert et al. 1990; Zacarias et al. 2021). This study aims to assess the potential benefits of supplementing the diet of larval/post-larval *M. rosenbergii* with β-glucan on their survival and tolerance to environmental stressors, such as sudden changes in salinity levels. Such information can contribute to the optimisation of shrimp aquaculture production and support conservation efforts.

Limited published evidence exists regarding the effects of β-glucan on *M. rosenbergii* larvae. To the best of the authors' knowledge, no studies have specifically examined the impact of dietary β-glucan supplementation on larval development and physical stress resistance, such as exposure to hypersaline water. Therefore, the present study aimed to investigate the effect of the selected doses of β-glucan supplementation on the growth, survival, and robustness of offspring under high salinity conditions (65 ppt).

Materials and methods

Origin of the experimental animals and their maintenance

Three gravid *M. rosenbergii* females, with an average length of 15.5 cm (measured from rostrum to telson) and an average weight of 44.05 g, were captured by a licensed fisherman from the Manir River (05°, 17′ 39.2″ N and 103°05′ 23.1″ E) near Kampung Teluk Menara, Kuala Terengganu, Terengganu. The three female individuals were carefully selected from a larger pool to guarantee uniformity in size, and the eggs within their brood chambers were in comparable stages of development. They were then transported to a research aquarium facility at the Faculty of Fisheries and Food Sciences (FPSM), Universiti Malaysia Terengganu (UMT), Malaysia, for the purposes of the current study. Each female was placed in a separate 80-L black plastic tank, containing freshwater (0 ppt) with a temperature range of 26.9–29.2 °C. The tanks were equipped with supplementary aeration and subjected to a 12-h light and 12-h dark photoperiod. Sections of plastic pipe measuring 30 cm in length and 5 cm in diameter were provided as shelter. Brackish water (11 ppt) was prepared by filtering untreated seawater collected from the Pantai coast, passing it through an 80 µm sand filter and a 3 µm filter bag, and then mixing it with dechlorinated freshwater to the required salinity. The prawns were allowed to acclimate to the aquarium conditions; then the salinity of the water was gradually increased to 11 ppt over a period of 4 days. The females were fed a commercial pelleted diet with 40% protein content from Gold Coin, twice a day at a rate of 5% of their body weight per day. The eggs carried by the females took approximately 15 to 20 days to hatch and to be released from their brood chambers. The counting of hatched larvae was conducted in the absence of light when the larvae have a uniform distribution; this practice results in greater count accuracy. In the presence of light, larvae exhibit positive phototaxis and tend to gather at the tank wall, which can make counts more challenging.

Experimental procedures and test diets

This study set out to assess the impact of four distinct diets on the performance of *M. rosenbergii* larvae. The control diet, serving as the reference point, was formulated to meet the nutritional requirements for optimal prawn growth. It was developed by modifying the egg custard recipe specifically designed for *M. rosenbergii* larvae, with chicken egg and powdered milk being the primary protein sources (Barros and Valenti 2003; Nik Sin and Shapawi 2017). To assess the effect of different inclusions of β-glucan, three experimental diets were created by supplementing the basal diet with β-1,3/1-6 D-glucan derived from the cell wall of baker's yeast (*S. cerevisiae*) obtained from Horbäach (USA) and incorporated at three different concentrations: 0.05% (βG1), 0.1% (βG2), and 0.2% (βG3) (see Table 1) based on the content of active ingredient in the commercial product (i.e. 70%; see https://horbaach.com/products/beta-glucan-1-3-1-6-d-500 mg-180-capsules). The dry ingredients were measured, and eggs and fish oil were included in the mixture. The mixture was then blended using a kitchen mixer. Subsequently, the mixture was steamed in a water bath (75–100 °C) for 15 min and allowed to cool. It was then stored in a freezer at a temperature of −18 °C until it was time for feeding. The daily amount of feed required was kept refrigerated at 4 °C. Prior to feeding, the feeds were weighed and sieved using a stainless-steel mesh with a size range of 425–1000 µm.

Table 1 The composition of the three experimental diets containing the different levels of β-glucan supplementation and their respective nutrient content*

Ingredients	Control (0% βG)	βG1 (0.05% βG)	βG2 (0.1% βG)	βG3 (0.2% βG)
Chicken egg	47	47	47	47
Milk powder[1]	20	20	20	20
Rolled oats[2]	10	10	10	10
Fishmeal[3]	8	8	8	8
Fish oil[4]	5	5	5	5
Diatomaceous earth[5]	2	2	2	2
Starch / (CMC)[6]	2	1.95	1.9	1.8
Mixed minerals[7]	1.5	1.5	1.5	1.5
Mixed vitamins[8]	1.5	1.5	1.5	1.5
β-1,3/1,6-glucan[9]	--	0.05	0.1	0.2
Catalyst[10]	3	3	3	3
Proximate analysis (DW)				
Moisture	53.00 ± 0.80	53.12 ± 0.42	54.03 ± 0.38	53.67 ± 0.80
Ash	10.41 ± 0.27	10.69 ± 034	10.44 ± 0.13	10.18 ± 0.58
Protein	52.70 ± 1.34	53.45 ± 0.68	53.63 ± 2.50	53.23 ± 1.59
Lipid	33.75 ± 1.41	33.43 ± 0.90	33.90 ± 0.62	33.99 ± 0.40
Fibre	1.37 ± 0.01	1.37 ± 0.01	1.37 ± 0.01	1.37 ± 0.01
NFE	1.74 ± 2.73	1.03 ± 1.48	1.13 ± 0.85	1.59 ± 0.53

*The values are shown as the mean ± 1 standard deviation derived from three replicates. Abbreviations: *CMC*, carboxymethylcellulose; [1]Nestle products Sdn. Bhd., Petaling Jaya, Kuala Lumpur, Malaysia; [2]Scc Marketing (M) Sdn Bhd., Puchong, Selangor, Malaysia; [3]TripleNine, 700 g kg^{-1} crude protein; [4]TripleNine, 100% marine oil;[5]Global Agora Resources., Bandar Pinggiran Suban 40150 Shah Alam, Malaysia; [6]D Chemie Chemical Supplie, Skudai, Johor, Malaysia ; [7]Vitamin pre-mix contained (as g/kg) vitamin A, 50; vitamin D3, 10; vitamin E, 130; vitamin K3, 10; vitamin B1, 10; vitamin B2, 25; vitamin B6, 16; vitamin B12, 0.1; niacin, 20; pantothenic acid, 50; folic acid, 8; biotin, 0.5; anti-caking agent, 20. DSM Nutritional Products (Thailand) Ltd 700/437 Chonburi, Thailand; [8]Mineral pre-mix contained (as g/kg) copper, 7.5; iron, 125; manganese, 25; zinc, 125; cobalt, 0.5; iodine, 0.175; selenium, 0.3; anti-caking agent, 10; [9]Horbäach (USA); [10]Barkath Foods Sdn. Bhd, Seberang Perai Tengah, Malaysia

Proximate analysis

The nutrient content of the experimental diets was determined through proximate analysis using techniques detailed in the Association of Official Analytical Chemists (AOAC) (1995). All analyses were carried out in triplicate. The moisture content was measured by drying 2 g samples of each diet at 105 °C for 24 h. The crude ash level was determined by subjecting the samples to a temperature of 600 °C for 6 h using a muffle furnace (Gallenkamp). The Kjeldahl method determined the crude protein content, involving acid digestion and distillation with 0.01 HCL using a Kjeltec Autoanalyser (FOSS KT200 Kjeltec™, Höganäs, Sweden). Crude lipids were extracted using petroleum ether (boiling point 40–60 °C) via the Soxtec™ system and the 2043 Extraction Unit (FOSS Tecator, Höganäs, Sweden). Fibre was analysed using a Fibretherm FT12 automatic system (C. Gerhardt GmbH & Co. KG, Germany). Nitrogen-free extract (NFE) was determined by subtracting protein, lipid, fibre and ash from 100.

Larviculture system

The larvae were collected and placed into the experimental tanks 24 h after hatching. The larvae of *M. rosenbergii* were randomly assigned to the larviculture tanks at an initial density of 60 larvae per litre (i.e. 7800 larvae per experimental tank). The experiment involved raising four groups of larvae in a recirculation system consisting of twelve rectangular fibreglass tanks (i.e. three replicates per dietary group). Each tank had a capacity of 150 L and was filled with 130 L of brackish water. The system was connected to a 400-L submerged biological filter divided into four chambers. To maintain water flow, gravity was used to force water from the larviculture tanks to the biological filter. An outlet screen with a mesh size of 250 µm was placed at the top of the drain pipe (2.5 mm) to allow water to flow back into the biological filter while retaining the larvae and *Artemia* within the rearing tank. The water in the system had a high turnover rate of 24 times per day. An air-lift pump was used to pump the filtered water back into the rearing tank. To prevent water overflow, the filter screen was cleaned on a daily basis. If there was any water loss due to evaporation, it was compensated by adding more brackish water. Brackish water (av. 11.16 ± 0.60 ppt; range 10.9–12.2) was prepared by mixing natural, 3 µm filtered seawater and dechlorinated freshwater.

Feeding trials and analytical procedures

Twenty-four hours post-hatch, the *M. rosenbergii* larvae were fed exclusively on *Artemia* nauplii, *Artemia franciscana* (Great Salt Lake strain, Aquatic Artemia Cysts™, USA) until the larvae reached stage VI. The preparation of the *Artemia* was in accordance with the supplier's instructions. During this first phase of feeding, *Artemia* nauplii were given four times a day (i.e. 10:00 AM, 15:00 PM, 20:00 PM, 00:00 AM).

From stage VI to post-larvae (PL), the larvae were fed live *Artemia* nauplii supplemented with the egg custard diets containing the different inclusion levels of β-glucan, namely 0.0% (Control), 0.05% (βG1), 0.1% (βG2), and 0.2% (βG3). The control group (βG1) was fed the basal diet without any functional supplement, while the other groups were fed the basal diet with a supplement of β-glucan. The prepared feeds were offered during daylight three times a day, while *Artemia* nauplii were provided to each tank of prawns once a day as an overnight feed to ensure a continuous food supply. The feeding regimen involved 25 *Artemia* nauplii per prawn larva for days 1–7 (i.e. 1500 *Artemia* nauplii/L), 58 *Artemia* nauplii per larva plus 0.2–0.3 g of the relevant egg custard diet for days 8–15 (i.e. 3,480 *Artemia* nauplii/L), and 83 *Artemia* nauplii per larva supplemented with 0.3–0.5 g of the relevant egg custard diet for days 16–28 (i.e. 4980 *Artemia* nauplii/L). Feed was slowly added to each tank using a Pasteur pipette, ensuring larvae were able to catch and carry feed particles. To ensure larvae were fed to satiation but not overfed, the quantity of formulated feed provided to the larvae was closely monitored and carefully evaluated on the amount of leftover feed. Diets were assigned to the experimental tanks using a complete random design. Larval prawn feeding requirements were determined through subjective evaluation of their growth stage, feeding response, and other influencing factors. Any larvae dying during the experimental trial were recorded but not replaced. The average total amount of feed added to each tank in each experimental group throughout the entire feed trial as given in Table 2 was used to provide an indication of diet utilisation.

Table 2 Performance of the *Macrobrachium rosenbergii* larvae fed on the experimental diets containing different β-glucans supplementation

Parameters	Treatments*			
	Control (0% βG)	βG1 (0.05% βG)	βG2 (0.1% βG)	βG3 (0.2% βG)
Final mean PL production/L[A]	27.95 ± 3.23[a]	31.09 ± 3.00[b]	32.69 ± 1.58[b]	35.49 ± 4.49[b]
Post-larvae/L	26.55 ± 3.07[a]	29.54 ± 2.85[ab]	31.06 ± 2.50[ab]	33.71 ± 4.27[b]
Final mean wet wt PL (mg)	9.53 ± 0.93[a]	12.73 ± 0.78[b]	13.04 ± 1.25[b]	13.58 ± 1.19[b]
Final mean dry wt PL (mg)	3.31 ± 0.27[a]	6.80 ± 0.90[b]	6.80 ± 0.04[b]	7.23 ± 0.31[b]
Total length PL (mm)	10.01 ± 0.51[a]	10.9 ± 0.20[ab]	10.77 ± 0.87[ab]	11.16 ± 0.45[b]
First PL (days)	22.33 ± 0.58	23.67 ± 0.58	22.33 ± 0.58	23.33 ± 1.16
95% PL (days)	28.00 ± 0.00[a]	29.00 ± 0.00[ab]	29.00 ± 1.00[ab]	26.67 ± 1.16[b]
Mean survival (%)	46.58 ± 5.39[a]	51.82 ± 5.00[ab]	54.45 ± 2.63[ab]	59.14 ± 7.49[b]
Metamorphosis rate (%)[B]	44.2 ± 5.11[a]	49.23 ± 4.75[ab]	51.76 ± 2.50[ab]	56.12 ± 7.11[b]
Total feed added per tank (g)	58.11	61.23	61.23	53.96
Ratio of PL biomass to diet	1:4.832	1:2.228	1:2.228	1:1.618

*The values are shown as the mean ± standard deviation derived from three replicates. Different letters in the same line indicate a significant difference between the treatments according to Duncan test

[A]Final number of larvae (L) and post-larvae (PL) produced per litre

[B]Metamorphosis rate = (final number of PL/number of stocked larvae) × 100

Routine husbandry and water quality

Starting from the third day of the experimental trial and before the feeding with the egg custard commenced, the residual feed particles, dead larvae, and other wastes were removed from the bottom of each rearing tank, and then daily thereafter. To maintain water quality, 7–10% of the total water volume was exchanged daily. The water temperature, pH, salinity, and dissolved oxygen levels were closely monitored and recorded twice daily using a YSI multiparameter metre (5908 Cap Membrane Kit, USA). Ammonia and nitrite concentrations were measured weekly using a nitrogen and ammonia test kit (Model NI-SA, Loveland, CO, USA) to assess water conditions.

Throughout the experimental period (26.67–29.00 days; Table 2), the tanks and larvae were exposed to natural photoperiods for at least 12 h daily. The recorded water quality results showed that the temperature ranged from 26.48 to 28.30 °C (average 27.33 ± 0.64 °C). Adequate aeration in the rearing tanks with a biological filter maintained oxygen levels above 5 mg/L (av. 6.19 ± 0.52 mg/L; range 6.19–6.51). Nitrogenous compounds such as ammonia, nitrite, and nitrate were measured at average concentrations of 0.19 ± 0.1 mg/L NH_3 (range 0.12–0.23), 0.23 ± 0.95 mg/L NO_2^- (range 0.134–0.33), and 20 mg/L NO_3^-, respectively. The pH values averaged 7.53 ± 0.32 (ranging from 7.04 to 8.11).

Evaluation of growth parameters

The progress in larval development was evaluated every alternate day using an Olympus STM7 dissecting stereo microscope interfacing with Olympus Stream Essential 24 image analysis software by randomly selecting five larvae from each treatment group within the larval rearing tank. The average stage of larval development was determined utilising the approach outlined by Uno and Kwon (1969). The larval stage index (LSI) was calculated using the following formula: $\mathrm{LSI} = (\sum S_i x n_i)/N$ proposed by Maddox and Manzi (1976) where S is the stage of the larvae (i = 1–12), n_i is the number of larvae in each stage, and N is the number of larvae examined. The time taken for 95% of the larvae to complete their metamorphosis into post-larvae was determined for each rearing tank.

At the conclusion of the experiment, the survival rate of each feeding group was assessed by conducting a comprehensive count of post-larvae originating from each tank. Furthermore, a sample of 50 juvenile prawns, comprising a mix of larvae and PL, was randomly selected from each treatment group, and their total length determined (TL; measured form the tip of the rostrum to the end of the telson) using digital callipers (± 0.1 cm) The wet weight of post-larvae were determined using a Mettler Toledo AT21 balance with a resolution of 1 µg (Mettler Toledo, Inc., Shanghai, China). Thereafter the post-larvae were oven dried at 60 °C for 24 h. After drying, the samples were then transferred to a desiccator to cool for 2 h before the dry weight was determined.

Survival to salinity stress test

The robustness of each larvae tank was assessed following the completion of the 30-day experimental feeding trial. To determine their resilience to changes in salinity, a salinity stress test was conducted using the method applied by Devresse et al. (1990) and Cavalli et al. (2000). To gauge the larvae's ability to survive drastic environmental conditions, i.e. their capacity to withstand osmotic shock for 1 h at 65 ppt, the mortality of the

post-larvae was determined every 3 min. The tests were carried out on post-larvae placed in plastic beakers filled with water at a temperature of 28 ± 1 °C. Each treatment involved 30 PL, with 10 PL per replicate, which were exposed to a salinity of 65 ppt compared to a corresponding control group for each experimental diet held for the same time duration in 8 ppt brackish water (i.e. 10 PL per replicate, 3 replicates per experimental group). The test solution was prepared by combining natural seawater (33 ppt) with artificial salts (provided by Qingdao Sea-Salt Aquarium Technology Co., Ltd., China). Mortality observations were recorded at 3-min intervals for a maximum duration of 60 min. Larvae were considered dead if they exhibited no response or immobility of the pleopods when touched with a pipette. The strength and resilience of the *M. rosenbergii* post-larvae were evaluated through a salinity test. Pairwise comparisons between different experimental groups of prawns were performed using a Mantel–Cox log-rank test in Windows Excel. The pooled mortality data (consisting of three replicates with 10 PL per group) were analysed as the time-to-event variable. To determine the overall survival probabilities across all time points, the time-stratified Cochran–Mantel–Haenszel test was employed, considering the number of observed and expected events at each time point. All statistical comparisons were conducted at a significance level of 0.05.

Statistical analysis

The data was subjected to analysis using SPSS version 26.0 software. The experimental data underwent statistical analysis using a one-way analysis of variance (ANOVA). In instances where statistically significant differences ($p < 0.05$) were observed, pairwise comparisons were conducted using the least significant difference (LSD) test, also referred to as Duncan's test. Statistical analysis using ANOVA was not conducted for either the total feed quantity added or for the ratio of PL biomass (wet weight) to biomass of the experimental diet added to each tank.

Results

Composition of the experimental diets

The proximate analyses of the four diets in Table 1 showed consistent levels of moisture, crude protein, crude lipid, NFE, and ash content. The moisture content ranged from 53.00 to 54.03%, crude protein from 52.70 to 53.63%, crude lipid from 33.43 to 33.99%, NFE from 1.03 to 1.60%, and ash from 10.18 to 10.69%. The selected combination of ingredients, including fish meal, chicken eggs, milk powder, rolled oats, fish oil, and diatomaceous earth, ensured a well-balanced and nutrient-rich diet. These ingredients are known for their nutritional value, palatability, and positive impact on larval survival and growth. Additionally, the diet formulation aimed to maintain essential physical characteristics such as buoyancy and water stability; as a result of the inclusion of a catalyst and carboxymethylcellulose in the diet which contributed to the buoyancy, stability, and binding properties of the diets as demonstrated by early experimental trials (Taguemount et al., unpublished).

Larval survival

The findings indicated a progressive enhancement in survival as the amount of β-glucan supplement in the diet increased. Table 2 displays the average survival rates of *M. rosenbergii* larvae (with 3 replicates per treatment group) that were fed experimental diets containing varying β-glucan supplements. At the conclusion of the experiment, the survival rates (mean ± SD) for the four experimental groups were as follows: 46.58 ± 5.39% (control), 51.82 ± 5.0% (βG1 = 0.05% β-glucan), 54.45 ± 2.63% (βG2 = 0.1% β-glucan), and 59.14 ± 7.49% (βG3 = 0.2% β-glucan). Significantly lower survival rates ($p < 0.05$) were observed in the control treatment compared to the βG3 treatment, where a supplement of 0.2% β-glucan was administered. No significant differences ($p > 0.05$), however, were found between the other treatment groups (see Table 2).

Larval growth

All groups exhibited incremental performance improvements as the amount of βG added to the diet increased (see Table 2). On completion of the trial, the group of prawn larvae that were reared on a diet supplemented with 0.2% βG consistently demonstrated significantly better performance compared to the other groups. Figure 1, however, shows no significant difference in larval development (LSI) was observed between the control group (0% βG) and the groups fed the experimental diets containing 0.05%, 0.1%, and 0.2% βG.

All three experimental groups that were fed diets supplemented with βG exhibited significantly higher wet and dry weight rates than the control group ($p < 0.05$).

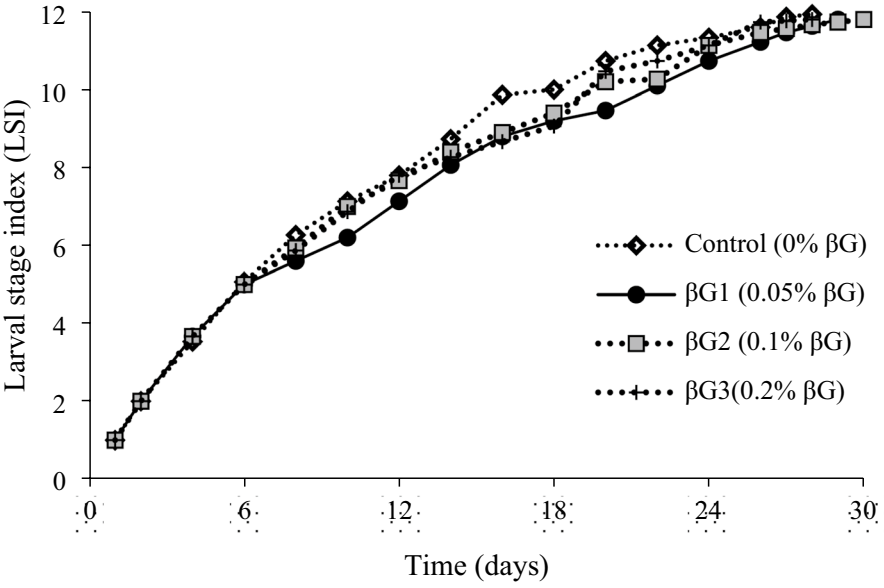

Fig. 1 Larval stage index (LSI) of the *Macrobrachium rosenbergii* larvae fed diets containing different inclusion rates of β-glucan. Each curve is based on data from three replicates. No error bars are shown for clarity. Abbreviations: Control = no inclusion of β-glucan; βG1 = 0.05%; βG2 = 0.1% β-glucan; and βG3 = 0.2% β-glucan

Specifically, the average wet weight for the control group was 9.53 mg, while the dietary groups βG1-3 had average wet weights ranging from 12.73 to 13.53 mg. The average dry weight for the control group was 3.31 mg, while the dietary groups βG1-3 had average dry weights ranging from 6.8 to 7.23 mg. The final wet and dry weights of the larvae in the βG treatments did not exhibit any significant differences ($p \geq 0.05$) among themselves, although the highest values were observed in the βG3 treatment (i.e. 13.58 mg and 7.23 mg, respectively).

The average total length (TL) ranged from 10.01 to 11.16 mm, with significantly higher values ($p < 0.05$) recorded in the βG3 treatments (Table 2). The larvae in the βG3 group exhibited a significantly greater TL compared to the control group ($p < 0.05$), while no significant differences were observed among the βG fed larvae groups ($p > 0.05$). The βG1-3 groups displayed significantly higher final mean productions per litre (ranging from 31.09 to 35.49 PL/L; $p \geq 0.05$) compared to the control group (i.e. 27.95 PL/L) (Table 2). The βG3 group had significantly higher post-larval production (averaging 33.71 PL/L; $p < 0.05$) compared to the control group (i.e. 26.55 PL/L) and was similar to the βG1 and βG2 groups (averaging 29.45–29.54 PL/L; $p > 0.05$). However, the groups of prawns fed βG1 and βG2 did not exhibit any significant differences in PL/L when compared to the control group (see Table 2). The feed treatments did not significantly affect the appearance of the first post-larvae, and the control diet resulted in a similar behaviour to the other diets. From day 16 of the culture, the control group displayed a slightly faster development (Fig. 1). The first PL emerged between 22 and 24 days after rearing, and approximately 95% of the larvae underwent metamorphosis to PL within 26.6 to 29.0 days (Table 2). The βG3 diet notably reduced the larval rearing period to 26.67 days, which was significantly shorter ($p < 0.05$) when compared to the βG1 and βG2 diets with rearing periods of 29 days ($p < 0.05$). However, when compared to the control diet with a rearing period of 28 days, the larval rearing period for the βG3 diet did not exhibit a statistically significant difference ($p > 0.05$). Additionally, the rearing period of the control diet was similar to the βG1 and βG2 diets. Likewise, the metamorphosis rate for the βG3 diet was significantly higher (56.12%; $p < 0.05$) and significantly different only from the control group (44.2%). There was no significant difference ($p > 0.05$) in rates between the control group and those determined for the prawns in groups βG1 and βG2 (49.23–51.76%, respectively).

The biomass of the experimental diet (excluding *Artemia*) provided to each experimental group was calculated as follows. During days 1–6 of the trial, the larvae were exclusively fed *Artemia*. From days 7 to 15, each tank received a daily allocation of 1.95 g of the respective experimental diet (calculated based on a feeding rate of 0.00025 g per larva per day). This phase of the trial operated under the assumption of no larval loss, with 7800 larvae per tank. Each larval tank adhered to this feeding regimen for a total of 9 days, resulting in a cumulative provision of 17.55 g of experimental diet per tank during this period. Starting from day 16 onwards, each tank was provided with a daily ration of 3.12 g of the relevant experimental diet (calculated based on a feeding rate of 0.0004 g per larva per day), again under the assumption of no larval loss. Since the endpoint of this final phase varied among the experimental groups (specifically, when 95% of the larvae reached the post-larval stage), the total feed amount provided during this period also varied accordingly. By combining the feed quantities administered from days 7 to 15 with those from day 16 until the trial's conclusion, the total experimental diet allotment for each experimental group was determined as follows: 58.11 g (control), 61.23 g (for βG1 and βG2), and 53.96 g (for βG3) (refer to Table 2 for details). Evaluating the mean larval survival and mean wet weight of larvae within each experimental group in relation to the feed provided yields the following amounts:

12.025 g (control), 27.486 g (βG1 and βG2), and 33.352 g (βG3). Consequently, this translates to ratios of post-larvae biomass (wet weight) to experimental diet of 1:4.832 for the control group, 1:2.228 for βG1 and βG2, and 1:1.618 for βG3.

Salinity stress testing

The 65 ppt stress test resulted in the complete mortality of all prawns within the 60-min duration (see Fig. 2); there were no losses observed in any of the corresponding 8 ppt control groups (data not shown).

Mantel–Cox log-rank tests were performed to analyse the pattern of prawn mortality during the first 30 and 45 min, as well as at the end of the 60-min salinity stress test. The tests revealed no significant differences between any of the experimental groups. However, when the data from each replicate within an experimental group were combined, and the time points at which 50%, 75%, 90%, and 95% of the population had died were determined, the data presented in Table 3 shows that prawns fed diets supplemented with β-glucan exhibited increasing levels of resilience as the inclusion rate of β-glucan increased, up to the first 70% population loss. Diets βG2 (0.1% βG) and βG3 (0.2% βG) resulted in longer durations until 90% of the population was lost compared to the control group, while prawns fed the βG3 diet displayed greater resilience than the control group for 95% of the population. Since the observations were derived from aggregated data, it was not possible to conduct any statistical tests on the time-to-event observations.

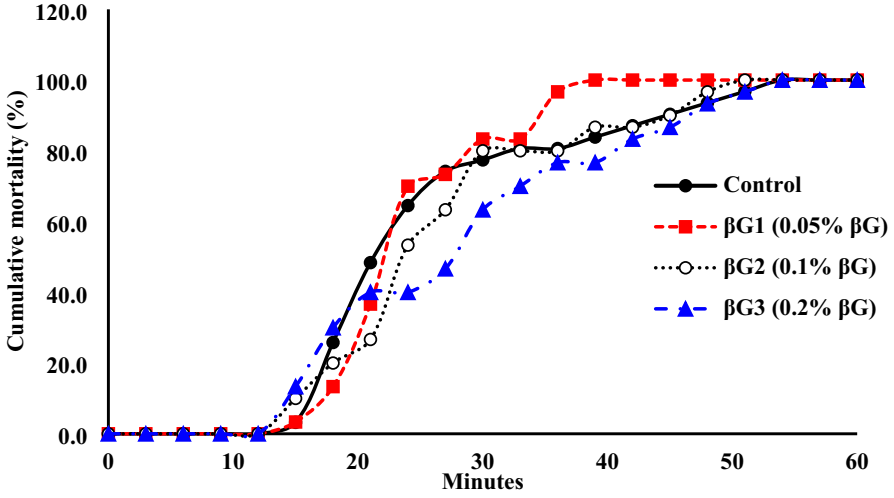

Fig. 2 Cumulative mortality of *Macrobrachium rosenbergii* post-larvae (PL) assessed in a 65 ppt stress test conducted at 3-min intervals over a total duration of 60 min. The graph illustrates the mortality rates of four experimental groups that were fed diets with varying inclusion rates of β-1,3/1,6-glucan. The curves on the graph represent pooled data obtained from three replicates, each consisting of 10 PL. To account for background mortality, each experimental diet was compared against a corresponding control group in an 8 ppt test, which did not exhibit any mortality (data not shown). The experimental groups included the following: Control (no β-glucan inclusion), βG1 (0.05% β-glucan), βG2 (0.1% β-glucan), and βG3 (0.2% β-glucan)

Table 3 Time-to-event observations are given as minutes·seconds (mean ± SD) by which a proportion of the *Macrobrachium rosenbergii* post-larvae (PL) population was lost under a 65 ppt stress test. The data shows that there are increasing levels of resilience with a rising inclusion rate of β-glucan in the diet. Each test group was run against a corresponding control held in 8 ppt brackish water over the same time; there was no mortality of PL in these controls and the data is not shown

	Dietary group			
% Mortality	Control (min)	0.05% βG (min)	0.1% βG (min)	0.2% βG (min)
50%	21.18 ± 2.29	21.49 ± 3.62	23.38 ± 1.73	27.36 ± 1.61
75%	27.33 ± 6.58	27.33 ± 4.58	29.06 ± 4.54	35.15 ± 3.12
90%	44.42 ± 10.52	34.30 ± 7.23	45.00 ± 7.94	46.30 ± 7.55
95%	49.21 ± 10.53	35.38 ± 7.01	47.15 ± 7.94	49.30 ± 6.87

The figures are based on pooled data from three replicates and 10 PL per replicate

Discussion

This trial demonstrates that β-glucan supplementation (0.05–0.2%) of diets presented to *M. rosenbergii* larvae led to enhanced survival, growth, and production, with 0.2% being the most effective inclusion level of those assessed.

Growth performance

Recent studies in aquaculture have highlighted the potential benefits of incorporating probiotics, prebiotics, and β-glucans to enhance the health and growth performance of fish and crustaceans (Kühlwein et al. 2014; Dawood et al. 2015a, b; Miest et al. 2016; Khanjani et al. 2022). β-glucan, recognised as a prebiotic, supports the growth of beneficial gut bacteria, aiding in nutrient absorption and overall health (Shoukat and Sorrentino 2021). Studies have indicated that β-glucan supplementation can improve the digestive system of crustaceans, potentially leading to enhanced nutrient uptake and higher survival rates (Song et al. 2014; Shah et al. 2016; Nieves-Rodríguez et al. 2018).

In this study, the addition of β-1,3/1,6-glucan derived from baker's yeast, in combination with egg custard, had a positive impact on the growth of *M. rosenbergii* larvae. Among the various treatments, the inclusion of 0.2% β-glucan in the diet resulted in the highest overall production, survival, growth performance, and metamorphosis of the larvae. However, supplementation with lower levels of β-glucan (i.e. 0.05–0.1%) only improved the final mean production and final mean weight compared to the control group. These findings align with a previous study on juvenile white Pacific shrimp, *P. vannamei*, which showed that 0.1% β-glucan resulted in the highest final mean weight but not survival (Murthy et al. 2009).

Supplementation of 0.2% β-glucan in the diets emerged as the most effective among those evaluated, exhibiting beneficial effects on growth performance, survival, and the production of *M. rosenbergii* larvae. It is possible that the 0.2% β-glucan supplement enhanced the digestive system, leading to increased energy production (López et al. 2003) and alterations in the microbial community composition, favouring beneficial bacteria in the intestinal microbiota (Yang et al. 2015; Li et al. 2019). These improvements in growth performance and production of *M. rosenbergii* larvae can be attributed to enhanced digestion and nutrient absorption when β-glucan is included in the diet. These findings are consistent

with previous studies that highlight the positive effects of β-glucan on growth in various species (Dawood et al. 2017; Li et al. 2019).

Previous research on *P. vannamei* has shown that feeding with low dosages of β-1,3-glucan (i.e. 0.01–0.15%) improved growth but not survival (Boonanuntanasarn et al. 2016; Li et al. 2019; Eissa et al. 2023). Qiao et al. (2022) conducted a study feeding *P. vannamei* diets supplemented with 0.05–0.4% β-glucan for 35 days, revealing that optimal growth was achieved with a 0.2% inclusion level. Although there were no statistical differences in growth between 0.1 and 0.2% inclusions, the higher dosage of 0.2% was recommended. However, increasing the supplementation to 0.4% in the diet did not promote the growth of *P. vannamei* larvae and instead resulted in lower growth performance (Qiao et al. 2022).

Survival

At the end of the trial, *M. rosenbergii* larvae fed on 0.2% β-glucan exhibited a significantly higher metamorphosis rate, leading to a higher number of post-larvae per litre, final mean production, mean weight, and total length. However, there were no significant differences in the larval stage index (LSI) at each stage between the larvae fed on different dietary treatments and the control group. The number of days to obtain the first post-larva and to reach 95% post-larvae in the population was not significantly different from the control group, resulting in variations in the number of larvae or post-larvae per litre.

The survival rate of *M. rosenbergii* larvae fed a diet containing 0.2% β-1,3/1,6-glucan was approximately 12% higher compared to the group fed the control diet. This finding is consistent with previous research showing enhanced survival in tiger shrimp, *Penaeus monodon*, after being fed a diet supplemented with 0.1% β-1,3-glucan derived from *Schizophyllum commune* for 40 days (Chang et al. 2003). Similarly, juvenile white Pacific shrimp, *P. vannamei*, exhibited the highest survival when fed a diet supplemented with 0.2% β-glucans for 30 days (Murthy et al. 2009). Kuruma shrimp, *Marsupenaeus japonicus*, also showed improved survival when fed peptidoglycan at a dosage of 0.2 mg/kg body weight/day for a total of 95 days, using an alternating 7-day regime of supplemented diet followed by 7 days without supplementation (Itami et al. 1998). In contrast, feeding *P. vannamei* with 0.1% β-1,3-glucan derived from *Saccharomyces cerevisiae* continuously for 7 weeks did not result in improved survival or weight gain (Scholz et al. 1999).

The study offers insights into the ratio of PL biomass (wet weight) to the biomass of experimental diet added to each tank, but cautious interpretation is advised. While larvae were fed to satiation, efforts were made to avoid overfeeding, assessed by visually monitoring leftover feed. Feeding was tailored to the larval count (7800 larvae/tank) and tank volume (130 L). To enhance precision, recovering, drying, and weighing leftover feed daily is recommended. Moreover, monitoring survival rates throughout the trial would enhance the precision of estimations for each dietary regimen.

Robustness

The incorporation of β-glucans in aquaculture feeds aims at enhancing the immune system, improving stress tolerance, and disease resistance (Miest et al. 2016; Khanjani et al. 2022). This study tested the robustness of prawn post-larvae (PL) to salinity shock as an indicator of their physiological condition and quality, utilising a 65 ppt salinity stress test. *Macrobrachium rosenbergii* can tolerate salinities up to 25 ppt, with rapid mortality beyond 30 ppt (Sandifer et al. 1975; New 1995). Total mortality was observed in the experimental

groups derived from wild-sourced broodstock under the 65 ppt stress test in this study, unlike in previous studies where mortality rates were lower.

While the time-stratified Cochran–Mantel–Haenszel and Mantel–Cox log-rank tests did not reveal significant differences in mortality patterns among the experimental groups, the time to reach specific population loss percentages (i.e. 50%, 75%, 90%, and 95%) indicated that higher β-glucan inclusion levels (up to 0.2% in the diets) led to increased tolerance during salinity testing. These findings suggest that conducting a larger-scale trial, increasing the number of post-larvae per replicate (i.e. 30–50), and testing at a lower salinity level (e.g. 45–50 ppt) could yield stronger data supporting the inclusion of up to 0.2% β-glucan in the diet. This could result in significant improvements in the robustness of prawn post-larvae compared to those fed a standard egg custard diet.

The use of a 65 ppt stress test for freshwater juvenile crustaceans, is considered severe. It is recommended to adopt a gradual salinity stress test involving a slow increase in salinity over time to better observe how prawns adapt to changing conditions. The extreme conditions utilised in the study may not accurately replicate real-world scenarios, limiting the practical applicability of the findings.

Conclusion

In conclusion, this study demonstrates that supplementing an egg custard with β-1,3/1,6-glucan at an inclusion rate of 0.2% can lead to significant improvements in growth performance, increased survival rates, and potentially enhance the robustness of prawn larvae. Therefore, it is recommended to be used in further studies as an ideal amount for prawn larvae diet. However, while the study has extensively delved into the effects of 0.2% β-glucan inclusion, it briefly acknowledges that lower levels (0.05–0.1%) only improved final mean production and final mean weight. A more in-depth exploration and detailed analysis of both lower and higher β-glucan levels on various parameters would, therefore, contribute to a nuanced understanding of the dosage-response dynamics.

To better understand the effects of β-glucan on prawn larvae, we recommend investigating the nutritional and physiological parameters such as hemolymph composition, digestive enzyme activities, energy reserves, cholesterol levels, and digestive gland histology. Further research could also explore the impact of β-glucan on gene expression, stress resistance, and long-term growth performance. Assessing larval performance and survival throughout the trial, rather than a single evaluation at the end, would offer a more dynamic perspective on the impact of β-glucan over time. Comprehensively understanding the physiological effects of β-glucan will aid in optimising its application as a nutritional supplement in aquaculture. Using a 65 ppt stress test for freshwater juvenile crustaceans is severe and requires modifying. It is recommended a gradual salinity stress test is conducted, where the salinity is slowly increased over time, to observe how the prawns adapt to the changing conditions. This could provide valuable insights into their resilience and survival capabilities under salinity stress.

Author contribution All authors approve of the submitted version of the manuscript. RT and RR conceived the study. RT conducted feed trials and stress tests. RT, JP, and RR worked on the dietary formulations. All authors contributed to the interpretation of the results. RT led in writing the manuscript in consultation with JP, APS, WHK, and RR.

Funding This research was funded by Universiti Malaysia Terengganu (UMT) under the Talent and Publication Enhancement Research Grant (TAPE-RG) (Vot No. 55332) (UMT/TAPE-RG/2022/55332).

Data availability The datasets generated during and/or analysed during the current study are available on request.

Declarations

Ethics approval The use of *M. rosenbergii* larvae in these nutrition-based trials adheres to the ethical principles in the handling of animals for scientific research and complies with animal welfare regulations in Malaysia. The research was conducted with due consideration minimising harm to the larvae and maximising their welfare throughout the trials. The larvae were treated humanely; their culture environment was tested and monitored closely; were given a feed optimised for growth and robustness; their health and condition were monitored on a daily basis. The use of the larvae in nutrition trials is justified by the potential benefits gained through improved culture practices in the larval phase. The experimental design was checked by a statistician to ensure a robust experimental design and to reduce the overall number of larvae required and any potential impacts. Additional senior oversight of the trial was provided by experienced personnel trained in animal handling and crustacean welfare.

Competing interests The authors declare no competing interests.

References

Apines-Amar MJ, Mary JS, Edgar CA (2015) Chap. 3. Use of immunostimulants in shrimp culture: an update. In: Caipang CMA, Bacano-Maningas MBI, Fagutao FF (Eds.) Biotechnological Advances in Shrimp Health Management in the Philippines, Research Signpost. pp. 45–71

Barros HP, Valenti WC (2003) Food intake of *Macrobrachium rosenbergii* during larval development. Aquaculture 216:165–176. https://doi.org/10.1016/S0044-8486(02)00505-7

Boonanuntanasarn S, Wongsasak U, Pitaksong T, Chaijamrus S (2016) Effects of dietary supplementation with β-glucan and synbiotics on growth, haemolymph chemistry, and intestinal microbiota and morphology in the Pacific white shrimp. Aquac Nutr 22:837–845. https://doi.org/10.1111/anu.12302

Burrells C, Williams PD, Forno PF (2001) Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to disease in salmonids. Aquaculture 199:159–169. https://doi.org/10.1016/S0044-8486(01)00577-4

Cavalli RO, Menschaert G, Lavens P, Sorgeloos P (2000) Maturation performance, offspring quality and lipid composition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. Aquac Int 8:41–58. https://doi.org/10.1023/A:1009220932268

Chang CF, Su MS, Chen HY, Liao IC (2003) Dietary β-1,3-glucan effectively improves immunity and survival of Penaeus monodon challenged with white spot syndrome virus. Fish Shellfish Immunol 15:297–310. https://doi.org/10.1016/S1050-4648(02)00167-5

Chen YH, He JG (2019) Effects of environmental stress on shrimp innate immunity and white spot syndrome virus infection. Fish Shellfish Immunol 84:744–755. https://doi.org/10.1016/j.fsi.2018.10.069

Cheng W, Chen JC (2000) Effects of pH, temperature and salinity on immune parameters of the freshwater prawn *Macrobrachium rosenbergii*. Fish Shellfish Immunol 10:387–391. https://doi.org/10.1006/fsim.2000.0264

Dash G, Raman RP, Prasad KP et al (2016) Evaluation of *Lactobacillus plantarum* as a water additive on host associated microflora, growth, feed efficiency and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquac Res 47:804–818. https://doi.org/10.1111/are.12539

Dawood MAO, Koshio S, Ishikawa M, Yokoyama S (2015a) Effects of heat killed *lactobacillus plantarum* (LP20) supplemental diets on growth performance, stress resistance and immune response of red sea bream, *pagrus major*. Aquaculture 442:29–36. https://doi.org/10.1016/j.aquaculture.2015.02.005

Dawood MAO, Koshio S, Ishikawa M, Yokoyama S (2015b) Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. Fish Shellfish Immunol 45:33–42. https://doi.org/10.1016/j.fsi.2015.01.033

Dawood MAO, Koshio S, El-Sabagh M et al (2017) Changes in the growth, humoral and mucosal immune responses following β-glucan and vitamin C administration in red sea bream, *Pagrus major*. Aquaculture 470:214–222. https://doi.org/10.1016/j.aquaculture.2016.12.036

Dawood MAO, Koshio S, Esteban MÁ (2018) Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev Aquac 10:950–974. https://doi.org/10.1111/raq.12209

De Caluwe J, Lavens P, Sorgeloos P (1995) The influence of *Macrobrachium rosenbergii* broodstock diet on egg and larval characteristics. Larvi 95:97–82

Deshimaru O, Yone Y (1987) Effect of dietary supplements on the feeding behaviour of prawn. Bull Jpn Soc Sci Fish 44:903–905

Devresse B, Romdhane MS, Buzzi M et al (1990) Improved larviculture outputs in the giant freshwater prawn *Macrobrachium rosenbergii* fed a diet of *Artemia* enriched with n-3 HUFA and phospholipids. World Aquaculture 21:123–125

Dhert P, Lavens P, Duray M, Sorgeloos P (1990) Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using ω3-HUFA-enriched live food. Aquaculture 90:63–74

Eissa EH, Ahmed RA, Abd Elghany NA et al (2023) Potential symbiotic effects of β-1,3 glucan, and fructooligosaccharides on the growth performance, immune response, redox status, and resistance of Pacific white shrimp, *Litopenaeus vannamei* to *Fusarium solani* infection. Fishes 8(2):105. https://doi.org/10.3390/fishes8020105

FAO FishStatJ (2023) A tool for fishery statistics analysis. Berger, T., Sibeni, F. & Calderini, F. (Eds.) Food and Agriculture Organization of the United Nations. FAO Fisheries Division (NFI), Release 4.02.08. https://www.fao.org/fishery/en/statistics/software/fishstatj

Harun HC, Muhammad Amiruddin W, Leng OS et al (2021) Optimisation of treated black soldier fly larvae (BSFL) using acidic salt to improve protein digestibility of giant freshwater prawn (*Macrobrachium rosenbergii*) larvae. IOP Conf Ser Earth Environ 842:012067. https://doi.org/10.1088/1755-1315/842/1/012067

Hongtuo F, Jin S (2018) Chap. 3.3. Culture of the oriental river prawn (*Macrobrachium nipponense*). In: Gui JF, Tang Q, Li Z, Liu J, De Silva SS (eds) Aquaculture in China: success stories and modern trends. John Wiley & Sons Ltd, pp 218–225. https://doi.org/10.1002/9781119120759.ch3_3

Itami T, Asano M, Tokushige K et al (1998) Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. Aquaculture 164:277–288. https://doi.org/10.1016/S0044-8486(98)00193-8

Kangpanich C, Pratoomyot J, Siranonthana N, Senanan W (2017) Effects of arachidonic acid supplementation in maternal diet on low-salinity tolerance of newly hatched larvae of giant freshwater prawn (*Macrobrachium rosenbergii* De Man). J Agric Sci Technol 13:59–72

Khanjani MH, Sharifinia M, Ghaedi G (2022) β-glucan as a promising food additive and immunostimulant in aquaculture industry. Ann Anim Sci 22:817–827

Khasani I, Sopian A, Pamungkas W (2022) Growth, resistance to vibriosis, and tolerance to stressors of the selected giant freshwater prawn, *Macrobrachium rosenbergii* seeds. IOP Conf Ser Earth Environ Sci 1119:012058. https://doi.org/10.1088/1755-1315/1119/1/012058

Kühlwein H, Merrifield DL, Rawling MD et al (2014) Effects of dietary β-(1,3)(1,6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L). J Anim Physiol Anim Nutr (Berl) 98:279–289. https://doi.org/10.1111/jpn.12078

Li H, Xu C, Zhou L et al (2019) Beneficial effects of dietary β-glucan on growth and health status of Pacific white shrimp *Litopenaeus vannamei* at low salinity. Fish Shellfish Immunol 91:315–324. https://doi.org/10.1016/j.fsi.2019.05.052

Liew HJ, Rahmah S, Tang PW et al (2022) Low water pH depressed growth and early development of giant freshwater prawn *Macrobrachium rosenbergii* larvae. Heliyon 8(7):e09989. https://doi.org/10.1016/j.heliyon.2022.e09989

Lim KC, Yusoff FM, Shariff M, Kamarudin MS (2018) Astaxanthin as feed supplement in aquatic animals. Rev Aquac 10:738–773. https://doi.org/10.1111/raq.12200

López N, Cuzon G, Gaxiola G et al (2003) Physiological, nutritional, and immunological role of dietary β 1–3 glucan and ascorbic acid 2-monophosphate in *Litopenaeus vannamei* juveniles. Aquaculture 224:223–243. https://doi.org/10.1016/S0044-8486(03)00214-X

Maddox MB, Manzi JJ (1976) The effects of algal supplements on static system culture of *Macrobrachium rosenbergii* (De Man) larvae. Proceedings of the 7 th Annual World Mariculture Society Meeting 677–698

Meena DK, Das P, Kumar S et al (2013) Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol Biochem 39:431–457. https://doi.org/10.1007/s10695-012-9710-5

Merchie G, Lavens P, Radull J et al (1995) Evaluation of vitamin C-enriched *Artemia* Nauplii for larvae of the giant freshwater prawn. Aquac Int 3:355–363. https://doi.org/10.1007/BF00121623

Miest JJ, Arndt C, Adamek M et al (2016) Dietary β-glucan (MacroGard®) enhances survival of first feeding turbot (*Scophthalmus maximus*) larvae by altering immunity, metabolism and microbiota. Fish Shellfish Immunol 48:94–104. https://doi.org/10.1016/j.fsi.2015.11.013

Mohmad BNARB, Rojab NNBA, Wahab MAB et al (2020) Feed formulation of improved egg custard formulation using response surface methodology (RSM). IOP Conf Ser Earth Environ Sci 596. https://doi.org/10.1088/1755-1315/596/1/012077

Murthy HS, Li P, Lawrence AL, Gatlin DM (2009) Dietary β-glucan and nucleotide effects on growth, survival and immune responses of Pacific white shrimp, *Litopenaeus vannamei*. J Appl Aquac 21:160–168. https://doi.org/10.1080/10454430903113644

New MB (1995) Status of freshwater prawn farming: a review. Aquac Res 26:1–54. https://doi.org/10.1111/j.1365-2109.1995.tb00859.x

Nieves-Rodríguez KN, Álvarez-González CA, Peña-Marín ES et al (2018) Effect of β-glucans in diets on growth, survival, digestive enzyme activity, and immune system and intestinal barrier gene expression for tropical gar (*Atractosteus tropicus*) juveniles. Fishes 3(27):1–13. https://doi.org/10.3390/fishes3030027

Nik Sin NN, Shapawi R (2017) Innovative egg custard formulation reduced rearing period and improved survival of giant freshwater prawn, *Macrobrachium rosenbergii*, larvae. J World Aquac Soc 48:751–759. https://doi.org/10.1111/jwas.12391

Pooljun C, Jariyapong P, Wongtawan T et al (2022) Effect of feeding different types of β-glucans derived from two marine diatoms (*Chaetoceros muelleri* and *Thalassiosira weissflogii*) on growth performance and immunity of banana shrimp (*Penaeus merguiensis*). Fish Shellfish Immunol 130:512–519. https://doi.org/10.1016/j.fsi.2022.09.047

Qiao Y, Zhou L, Qu Y et al (2022) Effects of different dietary β-glucan levels on antioxidant capacity and immunity, gut microbiota and transcriptome responses of white shrimp (*Litopenaeus vannamei*) under low salinity. Antioxidants 11. https://doi.org/10.3390/antiox11112282

Ringø E, Erik Olsen R, Gonzalez Vecino JL, Wadsworth S (2011) Use of immunostimulants and nucleotides in aquaculture: a review. J. Mar Sci Res Dev 2012,2:1. https://doi.org/10.4172/2155-9910.1000104

Rodrigues MM, De Almeida LCF, Bertini G (2018) Survival rate of *Macrobrachium acanthurus* (Caridea: Palemonidae) larvae in laboratory conditions under different salinities and diets. Panam J Aquat Sci 13:121–130

Sandifer PA, Hopkins JS, Smith TIJ (1975) Observations on salinity tolerance and osmoregulation in laboratory-reared *Macrobrachium rosenbergii* post-larvae (Crustacea: Caridea). Aquaculture 6:103–114. https://doi.org/10.1016/0044-8486(75)90063-0

Scholz U, Garcia Diaz G, Ricque D et al (1999) Enhancement of vibriosis resistance in juvenile *Penaeus vannamei* by supplementation of diets with different yeast products. Aquaculture 176:271–283. https://doi.org/10.1016/S0044-8486(99)00030-7

Shah A, Gani A, Ahmad M et al (2016) β-glucan as an encapsulating agent: Effect on probiotic survival in simulated gastrointestinal tract. Int J Biol Macromol 82:217–222. https://doi.org/10.1016/j.ijbiomac.2015.11.017

Shoukat M, Sorrentino A (2021) Cereal β-glucan: a promising prebiotic polysaccharide and its impact on the gut health. Int J Food Sci Technol 56:2088–2097. https://doi.org/10.1111/ijfs.14971

Soltanian S, Stuyven E, Cox E et al (2009) Beta-glucans as immunostimulant in vertebrates and invertebrates. Crit Rev Microbiol 35:109–138

Song SK, Beck BR, Kim D et al (2014) Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol 40:40–48. https://doi.org/10.1016/j.fsi.2014.06.016

Tackaert W, Abelin P, Leger PH, Sorgeloos P (1989) Stress resistance as a criterium to evaluate quality of post-larval shrimp reared under different feeding procedures. III Simposio Brasileiro Sobre Cultivo de Camarao MCR Aquacultura, pp 393–403

Uno Y, Kwon CS (1969) Larval development of *Macrobrachium rosenbergi* (De Man) reared in the laboratory. J Tokyo Univ Fish 55:179–190

Vici V, Singh ISB, Bhat SG (2000) Application of bacterins and yeast *acremonium dyosporii* to protect the larvae of *Macrobrachium rosenbergii* from vibriosis. Fish Shellfish Immunol 10:559–563. https://doi.org/10.1006/fsim.2000.0278

Wang H, Chen G, Li X et al (2020) Yeast β-glucan, a potential prebiotic, showed a similar probiotic activity to inulin. Food Funct 11:10386–10396. https://doi.org/10.1039/d0fo02224a

Wei J, Tian L, Wang Y et al (2021) Effects of salinity, photoperiod, and light spectrum on larval survival, growth, and related enzyme activities in the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture 530:735794. https://doi.org/10.1016/j.aquaculture.2020.735794

Wilder MN, Wei-Jun Y, Do Thi Thanh H, Masachika M (1999) Reproductive mechanisms in the giant freshwater prawn, *Macrobrachium rosenbergii* and co-operative research to improve seed production technology in the Mekong Delta Region of Vietnam. United States-Japan Natural Resources (UJNR) Technical Report 149–156

Xu J, Zhang S, Zhang Z et al (2023) Effects of supplementary astaxanthin in egg custard on antioxidant capacity and non-specific immunity during larvae metamorphosis of giant freshwater prawn (*Macrobrachium rosenbergii*). Aquacult Int 1–14. https://doi.org/10.1007/S10499-023-01199

Yang G, Xu Z, Tian X et al (2015) Intestinal microbiota and immune related genes in sea cucumber (*Apostichopus japonicus*) response to dietary β-glucan supplementation. Biochem Biophys Res Commun 458:98–103. https://doi.org/10.1016/J.BBRC.2015.01.074

Zacarias S, Fegan D, Wangsoontorn S et al (2021) Increased robustness of postlarvae and juveniles from non-ablated Pacific whiteleg shrimp, *Penaeus vannamei*, broodstock post-challenged with pathogenic isolates of *Vibrio parahaemolyticus* (*Vp*AHPND) and white spot disease (WSD). Aquaculture 532:736033. https://doi.org/10.1016/j.aquaculture.2020.736033

Zarantoniello M, Chemello G, Ratti S et al (2023) Growth and welfare status of giant freshwater prawn (*Macrobrachium rosenbergii*) post-larvae reared in aquaponic systems and fed diets including enriched black soldier fly (*Hermetia illucens*) prepupae meal. Animals 13(4):715. https://doi.org/10.3390/ani13040715

Authors and Affiliations

Riyad Taguemount[1] · Jarunan Pratoomyot[2] · Andrew P. Shinn[3,4] · Khor Waiho[1] · Rasina Rasid[1]

✉ Riyad Taguemount
taguemount.ryad@gmail.com

✉ Rasina Rasid
rasinarasid@gmail.com

[1] Faculty of Fisheries & Food Science, Universiti Malaysia Terengganu (UMT), Kuala Terengganu 21300 , Terengganu, Malaysia

[2] Institute of Marine Sciences, Burapha University, Chonburi 20131, Thailand

[3] INVE, Thailand, 471 Bond Street, Bangpood, Pakkred, Nonthaburi 11120, Thailand

[4] Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville QLD4811, Australia