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Research Article

The effects of yeast β-glucan on the immune response and susceptibility of *Macrobrachium rosenbergii* to *Vibrio parahaemolyticus* infection

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Abstract

β-glucans (BG) are widely used as an immunostimulant in aquatic animals through injection, feed, and immersion. The present study aimed to examine the consequences of dietary administration of yeast β-glucan on the immune response and susceptibility of *Macrobrachium rosenbergii* to the *Vibrio parahaemolyticus* infection. Experimental prawns (4.55±0.08 g) were fed a formulated commercial diet supplemented with commercial yeast BG at 0.0% (control), 0.1% (T1), 0.2% (T2), and 0.3% (T3). After the feeding trial of 75 days, a challenge test with *V. parahaemolyticus* (10^4 cfu/mL) was performed for seven days. No significant differences ($p>0.05$) were observed in terms of final weight, specific growth rate, and weight gain, but the prawns fed with 0.1% (T1) BG had the highest survival rates (100%). However, immune parameters, *e.g.* level of total hemolymph protein, albumin, globulin and hemocyte number, were found to be significantly higher ($p<0.05$) in prawns fed with 0.2% (T2) BG-supplemented diet. The total hemocyte count and total hemolymph protein, albumin, and globulin were reduced after the *V. parahaemolyticus* infection. The study's findings suggested that yeast BG can be used at 0.1% to 0.2% to enhance the immune response of juvenile *M. rosenbergii* and increase their resistance to *V. parahemolyticus* infection without upsetting the usual growth and survival.

Introduction

The agro-climatic condition of Bangladesh is congenial for the farming of the giant freshwater prawn, *Macrobrachium rosenbergii*, which is locally known as 'golda'—because of its rapid growth, high fecundity, a wide range of salinity and temperature tolerance (Cheng and Chen, 2000), suitability of polyculture with other fish species as well as integrated farming with paddy (Kunda, *et al.*, 2009), delicious test, increasing demand in the local and international market the farming of *M. rosenbergii* had expanded rapidly in Bangladesh. Every year, on average, nearly 46660.98 Metric Ton (MT) of golda (the local name of *M. rosenbergii*) have been produced, and 6354 MT of golda have been exported from Bangladesh since 2012 (FRSS, 2020). However, Bangladesh's annual production and export volume of golda shrimp did not increase as per demand because of the early mass mortality of larvae, post-larvae and juveniles of *M. rosenbergii*. Invasion of various pathogens is thought to be one of the major causes of early mass mortality of *M. rosenbergii* (Alam *et al.*, 2019; FRSS, 2020).

The bacteria under the *Vibrio* genus are reported to be pathogenic to farmed crustaceans (Alam *et al.*, 2019), responsible for vibriosis, which causes high mortality in *P. monodon* (Sung *et al*., 1994, Rahman *et al.*, 2010) and *M. rosenbergii* (Khuntia *et al.*, 2008) and thus results in severe economic loss to the aquaculture industry. Among different species of Vibrio, *V. parahaemolyticus* was found to be an important pathogen of prawns (Khuntia *et al*., 2008) and shrimp (Sudheesh and Xu, 2001) which causes acute hepatopancreatic necrosis disease (AHPND) in *Litopenaeus vannamei* (Wang *et al.*, 2019), early mortality syndrome in *M. rosenbergii* (Jayaprakash *et al.*, 2006; Khuntia *et al.*, 2008; Tiruvayipati and Bhassu, 2016) and *P. monodon* (Sudheesh and Xu, 2001; Haldar *et al*., 2007)

β-glucan, an essential structural component of bacteria, mycelial fungi, and yeasts cell wall, is a well-recognized immunostimulant that has been widely used in aqua-farm to enhance growth, general performance, immunity and disease resistance of fish, e.g. rainbow trout (Ji *et al.*, 2017), cod (Skjermo *et al*., 2006), Indian major carp (Misra *et al*., 2006); mollusc *e.g.* sea cucumber (Gu *et al*., 2011); shellfish *e.g.* marron (Sang and Fotedar, 2010) against bacterial and viral infections (Raa, 2000; Chang *et al*., 2000, 2003; Sajeevan *et al.*, 2009). Among the immunostimulants used in the aquaculture sector, BG is regarded as promising and effective because of its structure as a homopolysaccharide of glucose molecules connected by a glycoside bond (Meena *et al.*, 2013) and its binding capacity to different receptors on leukocytes, bactericidal activity, cytokine productivity at cellular levels (Hadiuzzaman *et al.*, 2022). In fish and other vertebrates, BG can bind directly with macrophages and other white blood cells, *e.g.* neutrophils and natural killer cells, and thus activate them against any invading microorganisms and ensure optimum resistance against the pathogen (Gantner *et al*., 2003; Herre *et al*., 2004; Meena *et al.*, 2013). On the other hand, shrimp and many other crustaceans have BG binding proteins (BGBP) in their serum as recognition proteins that activate the cellular functions associated with defense mechanisms, *e.g.* encapsulation, coagulation, melanization, and phagocytosis (Vargas-Albores and Yepiz-Plascencia, 2000; Meena *et al.*, 2013). When β-glucan binding proteins (BGBP) interact with the BG molecule, it forms a complex of BGBP-BG that reacts with the hemocyte surface and releases the hemocyte granules, which in turn leads to the activation of prophenoloxidase (proPOs) enzyme (Vargas-Albores and Yepiz-Plascencia, 2000). The enzyme proPOs promotes the oxidation of phenol to semi-quinines and quinines, which helps to kill the pathogens (Raa, 2000; Cerenius and Söderhäll, 2004).

The BG, found in the cell walls of yeast (YBG) differs from bacterial BG in chemical structure and mode of action (Raa, 2000). Yeast BG contains a 1, 3 glucose backbone with elongated 1,6 glucose branches, whereas bacterial BG has linear β-1,3 glucan with no branching (Volman *et al*., 2008; Seo *et al*., 2019). Due to the structural difference, yeast BG generally has less soluble properties than bacterial BG (Choi and Kim, 2023). Among the BG derived from different sources, Yeast BG had a more comprehensive application than bacterial BG in fisheries and aquaculture and is commercially more available in the global market. Moreover, it is readily biodegradable, eco-friendly, and completely secure for human consumption (Luan *et al*., 2021). Hence, we have chosen the yeast BG for the present study.

Previously, many commercially available yeast BG were extensively used to enhance the non-specific defense mechanisms in a wide range of animals, including crustaceans (Sahoo *et al.*, 2008; Murthy *et al.*, 2009). Several feeding trials using dietary BG have been done in different parts of the world to boost the growth, immunity and disease resistance of *P monodon* against *V. vulnificus* (Sung *et al.*, 1994), *V. alginolyticus* (Felix *et al.*, 2008) and white spot syndrome virus (WSSV) (Chang *et al*., 1999, 2003); *M. rosenbergii* against *Aeromonus hydrophila* (Sahoo *et al.*, 2008; Meshram *et al.*, 2015); *Litopenaeus vannamei*, against *V. alginolyticus* (Chang *et al.*, 2011) and Infectious myonecrosis virus (Neto and Nunes, 2015). However, the effects of the dietary BG supplements on *M. rosenbergii* population of Bangladesh have not been evaluated yet. Hence, the study was carried out to assess the effects of BG on the growth, immunological response, and resistance of *M. rosenbergii* against *V. parahaemolyticus* infection as a first step toward resolving the mortality issues that seriously hampers the prawn culture of Bangladesh.

Materials and methods

Collection of the experimental prawns and experimental design

Two hundred (200) juveniles of *M. rosenbergii* were collected from a nursery pond maintained in the Field Laboratory complex of the Faculty of Fisheries, Bangladesh Agricultural University in March 2022. The prawns were acclimatized for seven days before being released into the experimental tank. The 12 experimental tanks with dimensions of 50 x 30 x 30 cm (filled with 30 liters of fresh water) were divided into four groups for the three treatments (T1, T2, and T3) and control (C), each with three replications. At the end of the seven-day adaptation, 108 healthy and robust prawns (4.55±0.08 g) were stocked randomly in 12 experimental aquaria.

Preparation of experimental diet

The commercial prawn feed, Mega Prawn/Shrimp nursery feed, Spectra Hexa Feeds Ltd, Bangladesh (https://www.megafeedbd.com) (ingredients label on the package of the feed: fish meal, soybean meal, maize, wheat flour, De-oiled rice bran, rice polish, oil cake, fish oil, Mono-Calcium Phosphate, di-calcium phosphate, vitamins-minerals premix) was used as control feed. The proximate composition of the feed was analyzed in the Nutrition Lab of the Department of Aquaculture, BAU, Mymensingh (Table 1) and found to be nearly similar to the value label on the package of the feed. A total of 300 g feed was taken into three groups, and each group contained 100 g feed measured by an electronic balance (A and D Korea Ltd, Model: FRH-600, Capacity 620 g, Division: 0.01 g). A total of 600 mg commercial Yeast BG powder was mixed thoroughly into 12 mL distilled water (DW) and used as a stock solution (1mL solution is equivalent to 0.05 g BG). To mix the BG homogenously, 2 mL BG stock solution + 6 mL DW (total 8 mL) was sprayed over the feed by a hand sprayer tube (10 mL) for T_1 (0.1%). Similarly, 4 mL BG stock solution+4mL DW (total 8 mL) for T_2 (0.2% BG) and 6 mL BG stock solution+2 mL DW for T3 (0.3% BG) was sprayed over the feed. A control feed was taken where no BG was added, but only 8 mL DW was sprayed over the feed. A commercial gel (Growth gel, Advanced Chemical Industries, Dhaka, Bangladesh) was used to bind the BG perfectly with the feed. Finally, the prepared feed was airdried for about two hours and then stored at 4^0C in four tightly sealed plastic containers.

Table 1: Proximate composition of the commercial feed used as the control diet.

Parameters	Content (%)
Moisture	12.0
Protein	35.0
Lipid	6.0
Carbohydrate	27.0
Fibre	6.0
Ash	14.0

Feeding and water quality monitoring

The prawns were reared for 75 days and fed with the experimental diets. The feed was supplied at 5% of the body weight twice a day at 8:00 h and 18:00 h. The tanks were siphoned every morning to remove unused food and metabolic wastes. New water from a reserve tank (source: underground water) was added to keep the level constant. An air pump (Resun, ACO-004, China) was used to facilitate continuous aeration, and PVC pipe (6 inches long and 3/4 inches in diameter) was used as an artificial shelter to minimize cannibalism during molting. The top of each tank was covered with a net to prevent the escape of the prawn. Water quality parameters were checked and recorded routinely. pH, temperature, salinity and total dissolved solids (TDS) were checked by a portable multifunctional water quality meter (EZ9909), China; Dissolved oxygen (DO) by a DO meter (Lutron, DO-5509, Taiwan); Ammonia by ammonia test kit, Mars fishcare North America,Inc, USA; Alkalinity by alkalinity test kit. Water parameters were found suitable (Haslawati *et al*., 2022) for prawn culture (Table 2) during the experimental period.

Basic maintenance, including feeding and regular inspection of the health and rearing system, was maintained following rigorous scientific procedures throughout the research and was approved by the animal welfare and ethics committee of the Faculty of Fisheries, Bangladesh Agricultural University.

Table 2: Water quality parameters recorded in the aquarium water during the experimental period.

Parameter	Value
Temperature	28-31ºC
pH	7.6-8.3
DO	5-6.5 ppm
TDS	180-200 ppm
Alkalinity	130
Ammonia	0.25-0.75 ppm

Observation of growth parameters and survival rates

After the 75-day feeding trial, all prawns in the various experimental groups were weighed to determine growth metrics. The weight gain, survival rate, and specific growth rate (SGR) of each of the experimental groups were estimated using the following equations (Mameloco and Traifalgar, 2020):

$$SGR = 100 \times [\ln \text{final weight} - \ln \text{initial weight}] \div \text{total duration of the experiment in days}]$$

$$\text{Weight gain (\%)} = [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100$$

$$\text{Survival (\%)} = (\text{Total number harvested} / \text{Total number stocked}) \times 100$$

Extraction of hemolymph

After completion of the feeding trial, six juvenile prawns from each experimental group were sampled for hemolymph collection. Similarly, after the completion of the bacterial challenge, another six juvenile prawns from each experimental group were sampled for hemolymph collection. Hemolymph was extracted following the method described by Murthy *et al.* (2009) with some modifications. Hemolymph (100 µL) was drawn from the pleopod base of the second abdominal segment using a sterile 1 cc syringe (25 G×13 mm needle). Before hemolymph extraction, the syringe was loaded with 300µL pre-cooled (4°C) 10mM EDTA as an anticoagulant. The hemolymph with an anticoagulant solution was mixed homogeneously and stored on ice. This hemolymph was used to count total hemocytes and estimate total hemolymph protein, albumin, and globulin.

Total hemocyte count

Total hemocyte count was performed following the method described by Murthy *et al.* (2009) with some modifications. For total hemocyte counting, 100 µL aliquot of the hemolymph-anticoagulant mixture was taken into a separate microcentrifuge tube, and 50 µL of 1% Trypan Blue solution was added and mixed thoroughly and then stored on ice for 20 min to allow staining. Then, 5 µl of stained hemolymph was placed on a hemocytometer (Neubauer), and hemocytes were counted using an optical microscope (Olympus-CX21, Japan) connected to a personal desktop computer, and the values were expressed as million hemocyte/mL.

Determination of total hemolymph protein, Albumin, and Globulin

Total protein was measured using a commercial kit (Total protein, Chemelex, S.A, Pol. Ind. Can Castells-C/ Industria 113 Nave J, 08420 Canovelles, Barcelona, Spain) following the manufacturer's instructions. A Semi-Automatic Clinical Chemistry Analyzer (SA-20 Clindiag) was used to measure the absorbance at a wavelength of 540nm. Similarly, Albumin concentration was measured using a commercial kit (Albumin, Linear Chemicals S.L.U, Barcelona, Spain) following the manufacturer's instructions. A Semi-Automatic Clinical Chemistry Analyzer (SA-20 Clindiag) was used to measure the absorbance at a wavelength of 630 nm. Globulin was calculated after subtracting the albumin content from the total protein (Javed and Usmani, 2015).

Bacterial challenge test

After the feeding trial, a bacterial challenge test was done using a pathogenic *Vibrio parahaemolyticus*, which was collected from the microbiology lab of the Fisheries and Marine Resource Technology Discipline of Khulna University, Bangladesh, as a stock bacteria broth. The bacteria were further cultured and a stock solution for the susceptibility study was prepared following the method described by Solidum *et al.* (2016). A total of 18 prawns from each of the treatments were collected and were infected by injecting 100 µL of sterile saline (0.85% NaCl) containing 10^4 *V. parahaemolyticus* cells mL^{-1} (Solidum *et al.*, 2016) between the second and third abdominal segments. During the challenge period, the prawns were fed twice per day at 5% of the body weight with a control diet, and the mortality from each of the experimental groups was checked three times per day. At the end of the challenge test, total hemocyte count, total hemolymph protein, albumin, and globulin were checked again.

Statistical analysis

The statistical significance of the differences was calculated among the control and the treated groups via one-way ANOVA and Tukey’s test using SPSS (ver. 22). Before performing the parametric one-way ANOVA, the data were subjected to the normality test by Shapiro-Wilk test. Data on the initial weight and weight gain (%) were not found to be normal. So the data of initial weight was transformed by “ln” before performing one-way ANOVA. On the other hand, two outliers were resolved from the weight gain (%) and SGR (%) data before performing one-way ANOVA. We also performed a paired sample t-test to assess the effects of bacterial challenge on experimental prawns fed a BG-supplemented diet.

Results

Effects of β-glucan on growth and survival of M. rosenbergii

After the feeding trial of 75 days, maximum growth indices, *e.g.* mean final weight, weight gain (%) and SGR (%), were found in T2 (0.2% BG) and minimum in control (0.0% BG). On the other hand, the highest (100%) survival rates were observed in T1 (0.1% BG) and the lowest (92.6%) in T2 (0.2% BG) during the study period. However, no significant differences (p>0.05) were observed in growth parameters and survival rate among the experimental group (Table 3).

Table 3: Growth and survival of prawns fed with β-glucans supplemented diets.

Parameters	Mean Initial Weight (g)	Mean Final Weight (g)	Weight Gain (%)	SGR. (%)	Survivability (%)
Control	4.53 ± 0.23	6.67 ± 0.28	46.72 ± 5.44	0.53 ± 0.06	96.3 ± 3.70
T1 (0.1% BG)	4.71 ± 0.24	7.00 ± 0.42	52.14 ± 8.00	0.54 ± 0.07	100 ± 0.00
T2 (0.2% BG)	4.60 ± 0.26	7.27 ± 0.38	60.52 ± 7.00	0.60 ± 0.06	92.6 ± 3.70
T3 (0.3% BG)	4.36 ± 0.18	6.96 ± 0.37	61.70 ± 7.03	0.60 ± 0.06	96.3 ± 3.70
F	0.418	0.435	1.025	0.630	0.889
P	0.740	0.728	0.385	0.597	0.487

Values represent the means ± SE of three replicates.

Effects of β-glucan on hemolymph protein, albumin, and globulin level of M. rosenbergii

The total hemolymph protein, albumin, and globulin were measured in two phases, before and after the bacterial challenge test. Before the bacterial infection, we found the maximum level of hemolymph protein, albumin, and globulin in T2 (0.2% BG) and the minimum in control (0.0% BG) with a significant difference (p<0.05). After the bacterial challenge, we also observed significantly higher (p<0.05) levels of hemolymph protein, albumin, and globulin in T2 (0.2% BG) than those of the control (0.0% BG) group. Upon exposure to the bacterial challenge, we observed slightly reduced (paired sample t-test, p>0.05) levels of hemolymph protein, albumin, and globulin as a response to the *V. parahaemolyticus* infection. However, a significant reduction (paired sample t-test, p<0.05) occurred in albumin level in the prawns of the control group (Table 4).

Table 4: Concentrations of hemolymph protein, albumin, and globulin among the experimental groups before and after the challenge with *Vibrio parahemolyticus*.

parameter	Experimental group	Before challenge	After challenge	t	P
Hemolymph protein	Control	6.97 ± 0.27^{c}	6.66 ± 0.34^{b}	0.522	**0.654**
	T1 (0.1% BG)	8.30 ± 0.19^{ab}	7.70 ± 0.32^{ab}	3.980	**0.058**
	T2 (0.2% BG)	8.83 ± 0.09^{a}	8.21 ± 0.24^{a}	2.755	**0.110**
	T3 (0.3% BG)	7.64 ± 0.25^{bc}	6.91 ± 0.25^{ab}	2.68	**0.115**
Hemolymph Albumin	Control	0.76 ± 0.02	0.44 ± 0.06^{b}	8.498	**0.014***
	T1 (0.1% BG)	0.77 ± 0.06	0.67 ± 0.04^{a}	3.875	**0.061**
	T2 (0.2% BG)	0.83 ± 0.01	0.69 ± 0.02^{a}	4.040	**0.056**
	T3 (0.3% BG)	0.75 ± 0.03	0.74 ± 0.03^{a}	0.152	**0.893**
Hemolymph Globulin	Control	6.21 ± 0.27^{c}	6.22 ± 0.30^{b}	0.017	**0.988**
	T1 (0.1% BG)	7.53 ± 0.17^{ab}	7.03 ± 0.30^{ab}	3.768	**0.064**
	T2 (0.2% BG)	8.00 ± 0.08^{a}	7.52 ± 0.26^{a}	2.030	**0.179**
	T3 (0.3% BG)	6.89 ± 0.23^{bc}	6.16 ± 0.24^{b}	2.917	**0.100**

Values represent the means ± SE. of three replicates. Values in the same column with different superscript letters are significantly different (p<0.05, one-way ANOVA). * indicates p-value with significant difference (p<0.05) in the case of paired sample t-test.

Effects of β-glucan on hemocyte counts

We examined the hemocyte count in two phases- before and after the bacterial challenge test. The first phase was done just after the completion of the 75-day feeding trial (before the challenge test), and the second phase was done after the bacterial challenge test. During both phases of the hemocyte count, the maximum hemocyte count was found in prawns fed a 0.2% BG-supplemented diet (T2) and the minimum in the control group. The Total Hemocyte Count (THC) of the β glucan-supplemented prawns was found to be significantly higher (one-way ANOVA, p<0.05) than the control (C) group. (Table 5). Besides, we observed a reduced number of hemocytes in all the experimental groups because of the bacterial infection, and we found a statistically significant reduction in the hemocyte count of the prawns of all the experimental groups except T_2 (0.2% BG) (Paired sample t-test, p<0.05) (Table 5).

Table 5: Total hemocyte count (THC) in *Macrobrachium rosenbergii* of the four experimental groups before and after challenge with *Vibrio parahemolyticus*

Experimental Group	THC		t	p
	Before Challenge (million cells/mL)	After Challenge (million cells/mL)		
Control	2.50 ± 0.12^{c}	1.76 ± 0.09^{d}	9.434	0.000*
T1(0.1% BG)	3.41 ± 0.05^{b}	3.26 ± 0.03^{b}	4.749	0.005*
T2(0.2% BG)	3.75 ± 0.04^{a}	3.63 ± 0.06^{a}	1.606	0.169
T3 (0.3% BG)	3.19 ± 0.02^{b}	3.01 ± 0.03^{c}	4.843	0.005*

Values represent the means ± S.E. of three replicates. Values in the same column with different superscript letters are significantly different (p< 0.05, one-way ANOVA). * Indicates a p-value with a significant difference (p<0.05) in the paired sample t-test.

Effects of β-glucan on the survival rates of M. rosenbergii against Vibrio parahaemolyticus

At the end of the bacterial infection with *Vibrio parahaemolyticus* (10^4 cfu/mL), maximum survival was found in T_2 (0.2% BG) which was significantly higher (one-way ANOVA, F=5.833, p=0.021) than that of the control group (Fig. 1). Prawns of the BG supplemented group were found to be more resistant against *V. parahaemolyticus* than the prawns of the control group. Mass mortality in the prawns of the control group started one day after the infection of *V. parahaemolyticus,* whereas the mortality in T_1 (0.1% BG) and T_2 (0.2% BG) started from 3 and 4 days, respectively. At the end of the challenge test, maximum mortality (66.67±9.62) was found in the prawns of the control group and minimum (22.22±5.56) in T_2 (0.2 % BG) which was significantly lower (p<0.05) than the control group (Fig. 2).

Discussion

Somatic growth characteristics (final weight, weight gain, and SGR) and viability were not affected by the addition of the supplemental BG to the balanced meals developed for *M. rosenbergii* under the current research circumstances. Solidum *et al.* (2016) also observed similar findings in the case of growth and survival of *P. vannamei* fed a diet containing immunostimulant (mixer of mannan oligosaccharide and β-glucan). In contrast, López *et al*. (2003) reported significant effects of BG on the growth of *L. vannamei*. The amount of BG in the diet, the length of culture, the rearing temperature, the kind of BG, and the type of aquatic species being grown are all factors that may influence the effectiveness of these investigations (Wang *et al.*, 2017). The animal having glucanase enzyme in the digestive gland can digest BGββ to produce energy, which can later be used for protein synthesis, thus may boost the somatic growth of those animals (López *et al.*, 2003; Wang *et al.*, 2017).

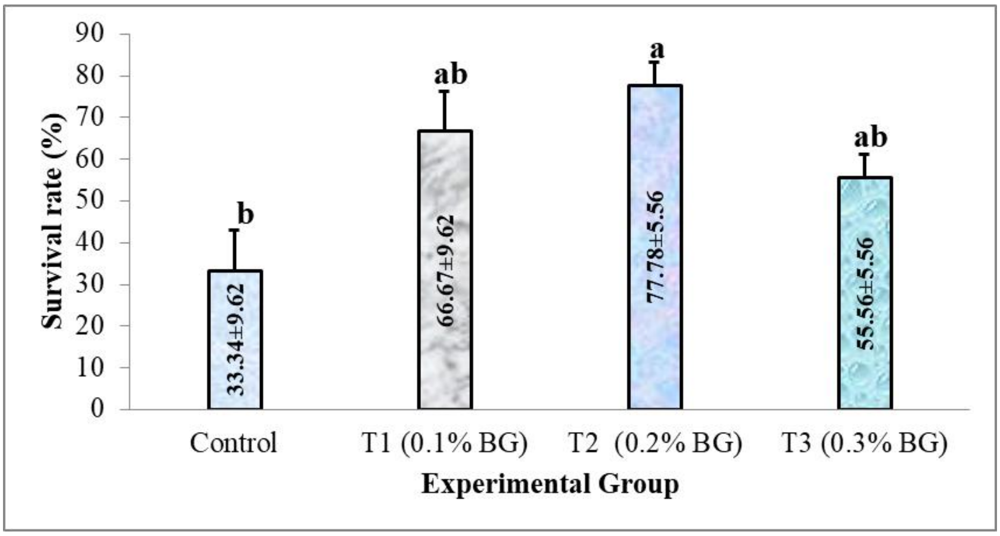

Figure 1: Survival rate (%) of *Macrobrachium rosenbergii* after being infected with *Vibrio parahaemolyticus* (10^4 cfu/mL). Values represent the means±S.E. of three replicates. Different superscripts (a,b,c) indicate significant differences (p<0.05) among control and treatment groups.

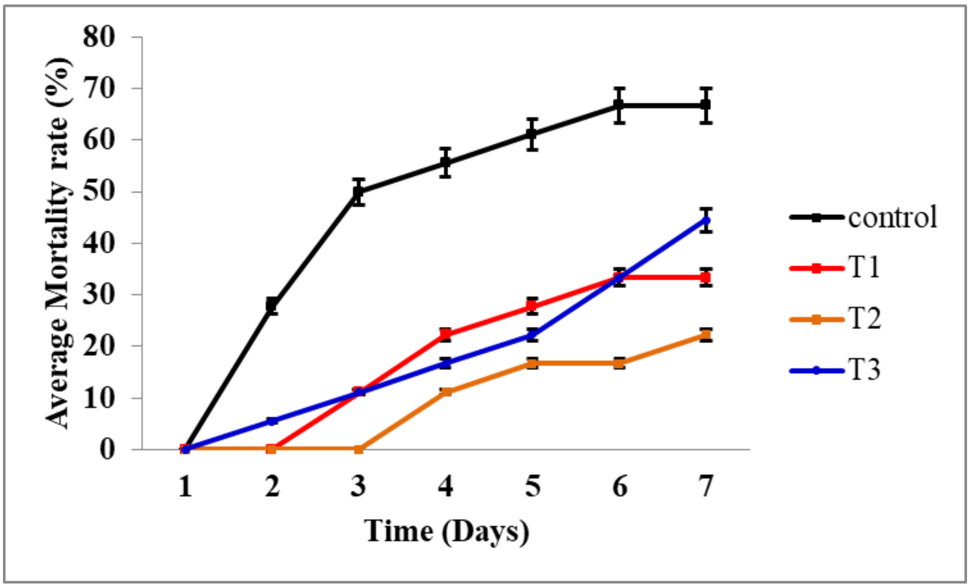

Figure 2: Cumulative mortality rate (%) of *Macrobrachium rosenbergii* after being infected with *Vibrio parahaemolyticus* (10^4 cfu/mL). Error bars represent the means±S.D. of three replicates. Different color and markers represent different experimental groups. Significantly higher (p<0.05) mortality was found in the control group at the end of the 7th day.

Haemocytes are crucial components of the crustacean immune system that play vital roles in processes such as phagocytosis, encapsulation, and nodule aggregation when dealing with foreign particles (Söderhäll and Cerenius, 1992;

Vargas-Albores *et al.*, 2005; Sahoo *et al.*, 2008). These specialized cells contribute significantly to the defence mechanisms of crustaceans, ensuring protection against pathogens and maintaining overall health. The cellular immune response can be measured by counting hemocytes, which serve as a valuable indicator of shrimp health (Sritunyalucksana *et al.,* 2005). Under the current investigation, the THC of *M. rosenbergii* was counted in two phases. During both phases of cell count, we detected significantly higher (p<0.05) cell count in prawns fed with 0.2% BG-enriched feed, which was similar to that, Andrino *et al.* (2014) reported for juvenile, *Penaeus monodon,* Murthy *et al.*, (2009) reported for *L. vannamei*, Meshram *et al.* (2015) reported for *M. rosenbergii* and Sajeevan *et al.* (2009) reported for *Fenneropenaeus indicus.* We detected reduced THC in all the experimental groups after *V. parahemolyticus* challenge, which was similar to the findings of Chang *et al.* (2011) reported for *L. vannamei* challenged with *V. alginolyticus* and Persson *et al.* (1987) reported in crayfish infected with a parasitic fungus (*Aphanomyces astaci*).

Proteins in the hemolymph provide crucial functions throughout a crustacean's life cycle, from facilitating oxygen delivery and reproduction to modulating the animal's reactions to stressors (Lorenzon *et al*., 2011). Hemolymph protein ratios and abundances are significantly influenced by various environmental and physiological conditions, including molting, reproduction, feeding, infection, and a lack of oxygen and salinity (Perazzolo *et al.*, 2002; Arcos *et al.*, 2003). The amounts of albumin, globulin, and total protein indirectly reflect the particular humeral immunological status (Stosik *et al.*, 2001; Maqsood *et al.*, 2009). Several illnesses that affect fish and shellfish could change the total protein, albumin, and globulin concentrations. Andrews *et al*. (2011) reported significantly higher levels of total protein, globulin, and albumin in *L. rohita* fingerlings fed a 1% brewer's yeast extract-supplemented diet. We found significantly higher (p<0.05) levels of hemolymph protein, albumin, and globulin in prawns fed 0.2% BG supplemented diet. Meshram *et al.* (2015) also observed similar findings in the case of hemolymph protein. After being subjected to a bacterial challenge, the levels of total protein, albumin, and globulin were reduced as a response to *V. parahemolyticus*. The changes in total hemolymph protein, albumin, and globulin due to bacterial infection observed in the present study agree with Maqsood *et al.* (2009) and Aydin *et al.* (2001), who observed a significant drop in total serum protein in fish.

During the challenge test, the BG-supplemented prawns were found to be more resistant against *Vibrio parahaemolyticus* challenge. Mass mortality in the prawns of the control group started one day after the bacterial infection, whereas the prawns of T_1 (0.1% BG) and T_2 (0.2% BG) began to die from 3 and 4 days, respectively. Besides, significantly higher survival (Fig. 1) in the BG-supplemented prawns were also a good indicator of enhanced immunity against the pathogenic *V. parahaemolyticus*. We also found decreased levels of THC, hemolymph protein, albumin, and globulin in all experimental groups (Tables 4 and 5) as a result of the response against *V. parahemolyticus* but, the control group's prawns showed a significantly more reduction (p<0.05) in THC and albumin than BG supplemented prawn, suggesting that the BG supplemented prawns have improved immunity against the pathogenic load. The role of β-glucan in improving immunity was also previously reported in *M. rosenbergii* against *A. hydrophylla* (Sahoo *et al.*, 2008; Meshram *et al.*, 2015); *Labeo rohita* against *A. hydrophylla* (Misra *et al.*, 2006); *L. vannamei* against *V. alginolyticus* (Chang *et al.*, 2011)

Each immunostimulant has an optimum dose depending on the species, size, age, physiological conditions of the experimental animal, culture conditions, and water parameters of the experimental unit (Felix *et al*., 2008; Andrino *et al*., 2012; Meshram *et al*., 2015). Below or above the optimum dose, the immunostimulant may not exert its effects properly. For example, 0.2% nucleotide supplementation was found to be optimum for enhancing the growth and immunity of *L. vannamie* (Andrino *et al*., 2012), 0.7% BG supplementation was found to be optimum for improving weight gain and disease resistance of *P. monodon* (Felix *et al*., 2008). Supplementation of 1 g BG per kg feed was found to be optimum for enhancing the immune response and resistance of *M. rosenbergii* against *A. hydrophila* (Meshram *et al.*, 2015). The present study found 0.2 % BG supplementation to be optimum for the juvenile *M. rosenbergii*.

In conclusion, the findings of the present study showed that dietery yeast BG can be supplemented at the dose of 0.1% to 0.2% to enhance the immune response of juvenile *M. rosenbergii* and increase their resistance to *V. parahemolyticus* infection without disturbing the normal growth and survival. Hence, BG supplementation might be helpful to minimize the early mortality problem of *M. rosenbergii* in Bangladesh.

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Conflicts of interest

The author declares no conflict of interest. All the co-authors have seen and approved the final version of the article and have agreed to submit the article to the Journal for publication.

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