Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 372-378

Received: 22-03-2018
Accepted: 23-04-2018

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Dietary administration of β-1, 3-Glucan enhances the immune ability of freshwater prawn, *Macrobrachium rosenberg*ii and it's resistance against white muscle disease (De man)

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Abstract
Infectial trial, disease resistance against white muscle disease virus, phenoloxidase activity and respiratory burst were investigated during the year 2008-2009 at college of fisheries, Mangalore, India in *Macrobrachium rosenbergii* juveniles (1.5 ±1.20 g) which were fed diets containing 1.0, 1.5 and 2.0 g/kg ß-1, 3-glucan for three months in growth trial. In eight days of challenge study, prawn fed diet devoid of ß1-3 glucan lead to 60% mortality within five days. The mortality of prawn fed with 1.0, 1.5 and 2.0 g/kg of ß-1,3-glucan containing diet were 46.66, 26.60 and 40% respectively. In the present study, *M. rosenbergii* fed diet containing 1.5 g/ kg ß-1, 3-glucan had enhanced disease resistance against white muscle disease virus, phenoloxidase activity and respiratory burst.

Keywords: ß-1, 3-glucan, *Macrobrachium rosenbergii*, white muscle disease virus, infection trail, phenoloxidase activity and respiratory burst

Introduction
The giant freshwater prawn, *Macrobrachium rosenbergii* is a commercially important Palaeomonid species for aquaculture in Asia and other parts of the world [1]. It is widely cultured in freshwater ponds in India. Till recently, the giant freshwater prawn, *M. rosenbergii* was regarded as relatively disease resistant when compared to farmed *Penaeid* shrimps [2]. Increased stocking densities and poor water quality management in grow out ponds could lead to health problems and out break of diseases in *M. rosenbergii.* Since November 2001 prawn hatcheries situated on the South- East coast of India have been suffering heavy losses due to newly emerged disease called "white muscle disease" (WMD), this disease also referred as "whitish disease" or "white tail disease" in some countries [1]. The occurrence of WMD causes significant damage to the critical life stage of post-larvae. In India more than 18 cases of WMD in freshwater prawn hatcheries with post larvae mortalities ranging from 30% to 100% were reported from November 2001 to December 2002 [3]. The mortalities were up to 60% in 28 day old post larvae cultured under intensive conditions showing signs of a milky diffuse white body described as idiopathic muscle necrosis (IMN) was reported in *M. rosenbergii* from Thailand [4] and Taiwan [5]. The association of gram positive cocci, *Lactococcus garviae* and yeasts were reported to cause the white muscle disease in *M. rosenbergii* [6]. In India Macrobrachium *rosenbergii* Nodavirus (*Mr*NV) and extra small virus (XSV) have been found to be associated with the WMD. However, the role of *Mr*NV and XSV is not yet clearly demonstrated. *Macrobrachium rosenbergii* Nodavirus (*Mr*NV) belongs to family *Nodaviridae*. *Mr*NV is an icosahedral non-enveloped RNA virus with a size of 26-27 nm in diameter. XSV is a satellite virus with a diameter of 14-16 nm, associated with *Mr*NV [2]. Therefore, the health of prawn and enhancement of its immunity are of primary concern. In general Decapods' crustaceans have three types of haemocytes namely, hyaline cells, semi-granular cells and granular cells. Each has distinctive morphological features and physiological functions [7]. In crustaceans these circulating haemocytes are involved in the production of melanin via the prophenoloxidase (proPO) system, which plays an important role in the defense reaction [8, 9]. Based upon the recent classification of *M. rosenbergii* haemocytes [10] large ovoid haemocytes and undifferentiated round haemocytes believed to be carrying out the functions of the proPO

system, like semigranular and granular haemocytes in other crustaceans [9]. The activity of phenoloxidase has already been reported in *M. rosenbergii* [11-4]. Conversion of proPO to PO is through proPO activating enzyme, (ppA) a serine protease [15]. PpA is activated by several microbial polysaccharides, including β-1, 3-glucan from fungal cell walls [13]. Oral administration of β-1, 3-glucan to shrimp was reported to increase the resistance against bacterial infections [16]. An oral administration of schizophyllan, ß-1,3-glucan extracted from the fungus *S. commune* has been reported to increase the resistance of *M. japonicus* against *Vibrio* sp [17] and the resistance of *P. monodon* against *V. damsela* [18]. Positive increase in disease resistance was reported in *P. japonicus*, when fed with different feeding schedules of 0.2 mg ß1-3 glucan per kg diet [19]. The different glucans, ß 1-3-1-6 extracted from the yeast cell wall was reported to enhance resistance of *P. monodon* to *Vibriosis* and white spot syndrome virus (WSSV) infection [14]. The effectiveness of dietary incorporation of beta 1-3 glucan from *Schizophyllum commune* in enhancing the resistance of post larvae and juveniles of *P. monodon* against WSSV was evaluated [20]. The present study was aimed at examining the immune parameters, phenoloxidase activity and respiratory burst of *M. rosenbergii* and its resistance to WMDV after feeding graded level of ß-1, 3-glucan for three months.

2. Material and Methods

2.1 Diet preparation

This study was carried out during the year 2008-2009 in college of fisheries Mangalore, India, where in four test diets containing different levels of ß-1,3-glucan were prepared as tabulated in Table 1, by using the square method [21]. Proximate analysis of basal diet indicated 34.73% crude protein and 5.57% crude lipid. The ß-1, 3-glucan is a poly glucose molecule linked through ß-1, 3 bonds in a long chain with ß-1, 6 branches consisting of single glucose molecule or a chain of glucose molecules. The ß-1, 3-glucan was added to the test diets at levels of 1.0, 1.5 and 2.0 gram per kilogram (g /kg) diet with corresponding decrease in the amount of rice bran. Experimental diets were prepared by mixing the dry ingredients with required quantities of water until stiff dough yielded. The dough obtained was cooked under steam in a pressure cooker at 105 ºC for 30 minutes. The cooked feed was cooled to room temperature rapidly by spreading in an enamel tray and required dose of ß-1, 3-glucan was mixed with pre-weighed vitamin and mineral premix and then blended with wet diet. The dough was thoroughly mixed again and extruded through a pelletizer having 2 millimeter (mm) die. Pellets were dried in a hot air oven at 60 ºC till the moisture content was reduced to less than 10%. The pellets were stored in plastic bins at −4 °C until use.

2.2. Feeding trial

Larvae of prawn, *M. rosenbergii* produced in the Freshwater Prawn Hatchery of College of Fisheries, Mangalore, India, were reared up to post larval stage and used for the study. Prior to start of experiment, the post larvae were acclimatized in the closed aerated recirculatory system which has of 12 circular fiber glass tanks of 120 litre (l) capacity each and fed with control diet for one week. Uniform sized post larvae with an average initial weight of 0.25 gram (g) were stocked at a rate of 30 numbers/ tank in triplicate. Post larvae were fed respective diets at a rate of 10% of their biomass twice daily for a period of 90 days. Prawns were weighed once in two weeks and the daily ration was adjusted accordingly. During the rearing period, water temperature was ranged from (25.0 ºC - 26.8 ºC), pH from (6.96 - 7.90), carbon dioxide from (0.11- 3.73) miligram per litre (mg/l), dissolved oxygen level from 7.06 to 8.56 mg/l and ammonia nitrogen from (0.01 - 6.2) microgram of nitrogen per litre (µg N/l). Growth and survival of prawn was calculated at the end of the feeding trial.

2.3 Experimental infection of white muscle disease virus (WMDV)

2.3.1 Collection of sample

The samples used for the study comprised of a batch of naturally WMDV infected moribund post larvae and juveniles of *M. rosenbergii* having abdomens of milky white appearance were collected from hatchery and farms from Nellore region of Andra Pradesh, India, during January 2006. For virus purification, prawn samples were brought to laboratory on ice and stored at -20^{0}C until it was used for infection and resistance studies.

2.3.2 Preparation of inoculum

The inoculums were prepared by the filtration of 2 g homogenized post larvae of *M. rosenbergii* having abdomens of milky white appearance in 1:10 (w/v) of TNE buffer (0.1-M Tris-HCl, 0.4M NaCl 0.02M EDTA-Na_2 and pH 7.4). In order to prepare the viral extract the homogenate was centrifuged at 20,000 g for 15 minutes at 4^{0}c and resultant supernatant was filtered through 0.25µm sartorius syringe filter. The filtrate obtained was collected in to 2 ml aliquots and stored at -40 ºC until use.

2.3.3 Virus test

Healthy juveniles of freshwater prawn, *M. rosenbergii* of size ranging 1.0-1.5 g were used for infectivity study to determine the lethal concentration 50 (LC_{50}) values against white muscle disease. There were four treatments and one control group. Each group had two aquaria each was stocked with 11 juveniles. The stored virus incoculum was diluted up to 1:100 with 0.9% $NaCl_2$ (sterilized). Each prawn juvenile in control and treatment group was individually injected inter muscularly with viral inoculum at 25, 50, 75 and 100 µl by using 1 ml syringe. The control group was injected with saline. After injection, prawns were kept in aerated aquaria and fed with basal diet and closely monitored for mortality for 6 days. The concentration at which 50% of the experimental prawns died was conceded as $LC_{50.}$ Water was exchanged daily. White muscle disease virus (WMDV) was further confirmed by using RT-PCR [1].

2.4 Challenge study for white muscle disease virus (WMDV)

After three months of feeding trial 45 prawns from each treatment and control group (15 prawns from each replication) were randomly sampled and transferred to 60 litre (l) fiber glass aquaria (15 / aquarium) and fed with control diet at the rate of 10% of their biomass twice daily for a week. Only prawns in the intermoult stage were used. The moult stage was determined by examination of uropoda in which partial retraction of the epidermis could be distinguished [22]. The Infectivity test was conducted in triplicate by the injection of 50 µl of white muscle viral inoculum into the ventral sinus of the cephalothorax. The prawn that received saline (20µl) served as the saline control group. In total there were five treatments. Each treatment was conducted with 45 prawns. Water was replaced daily, and the experiment lasted for 8

days. During Infectivity study clinical signs and mortalities of prawn were observed. The relative percentage of survival (RPS) and mortality of prawn was calculated at the end of the study. The infected sample were tested and confirmed for WMDV by RT-PCR.

2.5 Analysis of immune response in *M. rosenbergii*

At the end of the susceptibility study all the survived prawns from different treatments and control group were sampled for Prophenoloxidase Assay (PPO) and Nitroblue Tetrazolium Assay (NBT) assay to study the immuno response in treated *M. rosenbergii* against the white muscle disease virus.

2.5.1 Prophenoloxidase Assay (PPO)

L-dihydroxyphenylalanine (L-DOPA) used as a substrate as per the procedure [24] to determine the phenoloxidase activity. The haemolymph was drawn from the experimental prawns and a thin layer of haemolymph was made on slide and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 1hour at 4^0C. The smear was washed in phosphate buffer thrice (15 minutes each). It was incubated in 0.1% L-DOPA in phosphate buffer for 16 to 18 hours at room temperature. The slides were observed under a microscope (100X). The black staining of the granules indicated positive reaction and the percentage of positive cells were counted.

2.5.2 Nitroblue Tetrazolium Assay (NBT)

To determine cellular activity, NBT assay was performed as described by *Chang et al*. [20]. At the end of challenge study all the survived prawns from each treatment and control group were bled and haemolymph was drawn. The haemolymph was drawn into pyrogen free eppendroff tube containing a drop of 3.8% sodium citrate. One drop of heamolymph (0.1 ml) was placed on glass slides and incubated for 30 minutes at room temperature (28 ^{O}C) on damp paper before being gently washed with phosphate buffered saline (PBS) at pH 7.5. A drop from 0.1 ml of 0.2% Nitro Blue Tetrazolium in PBS was placed on slides and dried for another 30 minutes. The dried slides were stained by Wright's stain as described by Citarasu *et al*. [13] for 30 seconds and then washed with distilled water. The activated cells contained bluish granules when treated with NBT dye while non activated cells did not contain these bluish granules. The activated granular cells were counted under microscope at 400X. Granular cells were mostly spherical and highly refractive.

2.6 Statistical Analysis

The percentage mean values of NBT, proPO assay and mortality of prawn were transferred to logarithmic transformation. One -way ANOVA and Duncan multiple range test were employed to analyse the data statistically at 0.05 level of significant.

3. Results

3.1. Growth and survival of *M. rosenbergii* fed ß-1, 3-glucan containing diets

Mean weight gain of prawn fed with control diet and diets containing 1.0, 1.5 and 2.0 g/kg of ß-1, 3- glucan were 1.19, 1.17, 1.17 and 1.15 g respectively as tabulated in Table 2. The survival of prawn fed control diet and diets containing 1.0, 1.5 and 2.0 g/kg of ß-1,3-glucan were 74, 78, 76 and 75% respectively as given in Table 2. There were no significant differences in weight attained and survival of prawn among different treatments and control group.

3.2 Experimental infection

Healthy juveniles of freshwater prawn, *M. rosenbergii* as given in (Figure 1) of size ranging 1.0-1.5 g were used for infectivity study to determine the LC_{50} values against WMDV. In the laboratory infectivity study (using the inoculum prepared from WMDV infected post larvae) mortality was recorded from third day of post infection. The cumulative mortality of the *M. rosenbergii* juveniles injected with viral inoculum at 25, 50, 75 and 100 µl reached up to 36.36%, 45.45%, 54.54% and 72.72% respectively on day 5^{th} of post infection (DPI). The concentration at which 50% of the experimental prawn died was considered as LC_{50}. The mortality recorded among control group was 9.0% and it was apparently not because of WMDV, but because of moulting and related cannibalism. The experimental infected prawn juveniles were anorexic, lethargic, freshly developing opaqueness in the abdomen and later turning to a milky white appearance was also observed. LC_{50} value for Juveniles of freshwater prawn against white muscle disease was found to be 50 µl/ juvenile as tabulated in Table 3.

3.3. Challenge study for white muscle disease virus

The clinical signs such as poor feeding, anorexic, lethargy, ataxic swimming behaviour and soft shell of pale whitish coloration (Fig. 2) of the prawns were observed after second and fourth days of post injection (dpi) in control and treatment groups respectively. These clinical signs were identical to those found in prawn naturally infected with white muscle disease. Few juveniles having whitish appearance (Fig. 3) with multifocal or diffuse distribution in the cephalothorax and abdominal muscles were noticed after third and seventh day of post injection resulting mortality in control and treatment groups. Initially the whitish color was apparent only against a dark background. Later this whitish discolouration was gradually diffused both anteriorly and posteriorly from abdominal segments (Fig. 4) followed by high mortality. In eight days of challenge study against WMDV, the control group of prawn fed on diet devoid of ß-1,3-glucan lead to 60% mortality within five days. Highest mortality of (59.99%) was recorded in T_0 followed by T_1 (46.66%), T3, (33.31%) and T_2 (26.66%) respectively as tabulated in Table 4. Highest relative percentage survival of 55.65% was recorded in T_2 and lowest in T_1 22.22% (Table 4). The mortality recorded among saline group was 6.66% and it was apparently not because of WMDV, but due to molting and related cannibalism. Mortality of prawn recorded in different treatment groups was significantly higher (P<0.05) than that of control group.

3.4. Immune response of *M. rosenbergii*

3.4.1 Prophenoloxidase Assay (PPO)

Phenoloxidase activity of prawn fed control diet and diet containing 1.0, 1.5 and 2.0 g/kg of ß-1, 3- glucan were 40.33%, 50.67%, 68.33% and 58.33% respectively as recorded in Table 5. A significant difference (P<0.05) in phenoloxidase activity was observed among the treatment groups and than that of control group.

3.4.2 Nitroblue Tetrazolium Assay (NBT)

Respiratory burst of prawn fed control diet and diet containing 1.0, 1.5 and 2.0 g/kg of ß-1, 3- glucan were 39.33%, 50.0%, 68.0% and 58.67% respectively as recorded in Table 5. A significant difference (P<0.05) in respiratory burst was observed among the treatment groups than that of control group.

Table 1: Composition of the test diets (g/ kg) for *M. rosenbergii*

ß1-3 glucan /kg	Fish meal /kg	Ground nut oil cake /kg	Rice bran /kg	Tapioca flour /kg	Vitamin mineral mix /kg
0	240	550	100	100	10
1.0	240	550	99	100	10
1.5	240	550	98.5	100	10
2.0	240	550	98	100	10

Table 2: Mean weight attained and survival by *M. rosenbergii* fed with graded levels of β-1.3-glucan for three months.

β-1.3-glucan/kg diet	Survival (%)	Mean weight(g)
0	74	1.19
1.0	78	1.17
1.5	76	1.17
2.0	75	1.15

Table 3: LC_{50} of *M. rosenbergii* juvenile against virulent white muscle disease virus (WMDV)

Viral inoculum µl/ juvenile	Challenged (N)	Survived	Mortality (%)	LC_{50}
0	11	10	9.0	
25	11	7	36.36	
50	11	6	45.45	50 µl
75	11	5	54.54	
100	11	3	72.72	

Table 4: Mean mortality of *M. rosenbergii* challenged with white muscle disease virus

β-1.3-glucan/kg diet	Mortality (%)	Relative percentage survival (%)
0	60.0^{d}	
1.0	46.66^{c}	22.22
1.5	26.60^{a}	55.65
2.0	40.0^{b}	44.47

(Values with unlike superscripts in the same column are significantly different $p<0.05$.)

Table 5: Mean phenoloxidase activity (ProPO) and NBT reduction in *M. rosenbergii* challenged with white muscle disease virus

β-1.3-glucan/kg diet	PO positive cells (%)	NBT positive cells (%)
0	40.33^{a}	39.33^{a}
1.0	50.67^{b}	50.0^{b}
1.5	68.33^{d}	68.0^{d}
2.0	58.33^{c}	58.67^{c}

(Values with unlike superscripts in the same column are significantly different $p<0.05$.)

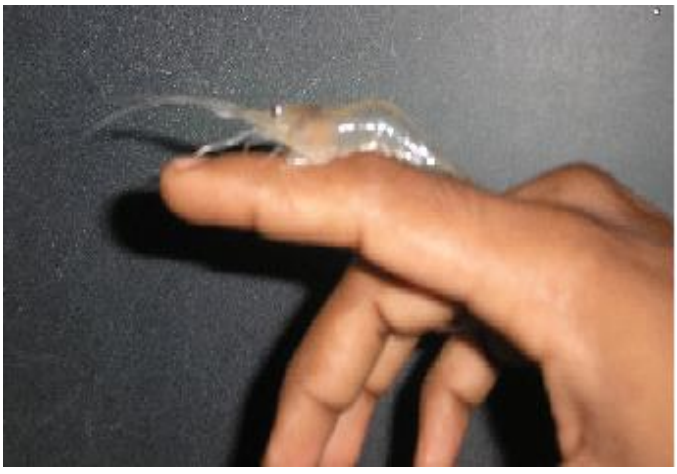

Fig 1: Healthy juvenile of *M. rosenbergii*

Fig 2: Juvenile of *M. rosenbergii* Showing pale whitish coloration

Fig 3: Juvenile of *M. rosenbergii* showing whitish discoloration of abdominal segments

Fig 4: Juvenile of *M. rosenbergii* showing whitish appearance

4. Discussion

Nutritional status is considered as one of the important factors that determine the ability of animals to withstand infections and considered as a good health indicator of shrimp [23, 24]. Circulating haemocytes have been reported to serve a variety of functions including haemolymph coagulation and defense against invading microorganisms or parasites. Feeding of some immunostimulants does not necessarily enhance growth and survival of shrimp. Weight attained of shrimp, *Litopenaeus Vannamei* fed with control diet and diet containing 0.5, 1.0 and 2.0 g/ kg sodium alginate were not significantly different [26]. Similar results of no significant differences in mean weight attained were observed among treatments by earlier workers [26, 27]. In the present growth study, there were no significant difference in weight attained by *M. rosenbergii* fed different level of ß-1, 3-glucan and control diet. The survival of *L. vannamei* following 7 weeks feeding with ß -1, 3-glucan extracted from the yeast *Saccharomyces cerevisiae* at 1g / kg was less than that of shrimp fed control diet [28]. The survival of *P. monodon* brooders fed a diet containing ß-1, 3-glucan extracted from the fungus *Schizophyllum commune* at 2 g/ kg for 40 days was significantly higher than that of brooders fed a diet without ß-1, 3-glucan [6]. Survival of shrimp fed control diet and different levels of sodium alginate were not significantly different [25]. Similar results of no significant differences in survival were observed among treatments by earlier workers [26, 27]. The survival achieved in prawn fed ß-1, 3-glucan based diets and control diet were not significantly different (P<0.05) and reconfirmed that immunostimulants not necessarily enhances growth and survival.

The infected prawn juveniles in the present study were anorexic, lethargic and pocess freshly developing opaqueness in the abdomen and later turning to a milky white appearance. In the experimentally infected adult animals of *M. rosenbergii* showing the above clinical signs and mortality went up to 50% within 5 days [2]. Few researchers have tried to study as to how prawn defense factors, including a possible induction mechanism, influence prawn health and resistance to disease, but the information gained has not yet led to any general conclusions. The laboratory studies have demonstrated that glucan has a short term effect on the immuno system of shrimp by providing non-specific protection against bacterial and viral diseases. Activated haemocytes also produce extra bactericidal substances such as H_2O_2 and superoxide anion (O_2^-) that may increase disease resistance [29]. Oral administrations of β-1, 3-glucan to shrimp were reported to increase the resistance against bacterial infections [16]. An oral administration of schizophyllan, ß-1, 3-glucan extracted from the fungus *S. commune* has been reported to increase the resistance of *M. japonicus* against *Vibrio* sp [17] and the resistance of *P. monodon* against *V. damsela* [18]. Positive increase in disease resistance was reported in *P. japonicus*, when fed with different feeding schedules of 0.2 mg ß-1, 3 glucan/ kg diet [25]. The different glucans, ß 1-3-1-6 extracted from the yeast cell wall is reported to enhance resistance of *P. monodon* to *Vibriosis* and white spot syndrome virus infection [30]. The effectiveness of dietary incorporation of ß 1-3 glucan from *Schizophyllum commune* in enhancing the resistance of post larvae and juveniles of *P. monodon* against WSSV was evaluated [31]. *M. rosenbergii* fed with levamisole showed an increased resistance against *Pseudomonas fluorescens* [32].

The survival of *P. monodon* following 20 days feeding with a diet containing schizophyllan (ß-1, 3-glucan) at 10g/kg and when challenged with WSSV was significantly higher by 9th day than that of the shrimp fed with control diet [14]. *M. rosenbergii* fed a diet containing lactoferrin for a period of 7 to 14 days significantly reduced the percent mortality compared to the control group when challenged with *A. hydrophila* [1]. In the present study, *M. rosenbergii* fed a diet containing ß-1,3- glucans at 1.5g/kg diet showed significantly increased resistance against white muscle disease virus and demonstrated that dietary glucans enhance disease resistance against white muscle disease virus. The prophenoloxidase (proPO) system has been considered to play an important role in the defence system of crustaceans [13]. Activation of the proPO system which is measured in terms of the PO activity has been used by some investigators to measure immunostimulation in shrimp [33-35]. In the present study, the PO activity in hemocytes of *M. rosenbergii* was used as an indicator for measuring immunostimulation induced by ß-1, 3- glucan. Enhanced PO activity in *M. rosenbergii* fed diets containing 125 mg and 250 mg levamisole/ kg for 115 days was reported by Baruah *et al.* [32]. Significant enhancement of in vitro PO-activity in all hemocytes treated with the yeast derived-products against control diet was reported by Chang *et al.* [6]. Administration of sodium alginate by injection (50 µg/g body weight) was reported to increase the PO activity and enhanced resistance against *V. alginolyticus* [36]. Phenoloxidase activity of shrimp that received chitin at 4 and 6 mg/g was significantly higher than the control shrimp after 1, 2 and 4 days challenge with *V. alginolyticus* [37]. Phenoloxidase activity of shrimp fed 1.0 and 2.0 g/kg sodium alginate was significantly higher than that of shrimp fed control diet [25]. Significantly increased PO activity in the prawns fed diets containing graded levels of lactoferrin for 7 days as well as 50 or 100 mg/kg diet groups for 14 days was reported by Chand *et al.* [1]. In the present study, phenoloxidase activity increased significantly in the prawn fed diets containing 1.0, 1.5 and 2.0 g ß-1, 3-glucan /kg diet than control group and demonstrated the effect of ß-1, 3-glucan in enhancing PO activity. Therefore, it was revealed by study that fungus ß-1, 3-glucan added to the diet can trigger the phenoloxidase activity indicating an increase in immune ability.

Being the first product released during the respiratory burst, 0_2^-concentration is widely accepted as an accurate parameter quantifying the intensity of a respiratory burst [29]. The oxygen-dependent defence mechanism of mammalian phagocytic cells is involved in the generation of reactive oxygen intermediates (ROIs) that are powerful microbicidal agents. Nitroblue tetrazolium (NBT) staining has been used for both qualitative and quantitative analyses of superoxide anion generated by haemocytes [38]. In an in-vitro experiment using haemocytes of *P. monodon* it was reported that ß-1, 3 and ß-1, 6-glucan extracted from yeast *S. cerevisiae*, zymosan extracted from yeast *S. cerevisiae* and PMA (phorbol 12-myristate 13-acetate) could increase the release of superoxide anion [30]. *P. monodon* which were immersed in ß-1, 3-1, 6-glucan and zymosan, showed increased release of superoxide anion [39]. *P. monodon* which had been fed a diet containing ß -1, 3-glucan at 2.0 g/kg showed significantly increased production of superoxide anion in 24 days [40]. Single immunostimulation by ß-1-3 glucan and capability of sulphated polysaccharide in enhancing respiratory burst of *L. vannamei* hemocytes was reported [41]. Respiratory burst of shrimp that received chitosan at 2 and 4 mg/ g was significantly higher than the control shrimp after 1 and 2 days challenge with *V. alginolyticus* [37]. *L. vannamei* fed sodium alginate containing diet at 2.0 g/ kg showed increased release

of superoxide anion indicating that sodium alginate enhances the immune ability of *L. vannamei* [25]. Increasing proPO activity and O_2– production in the herbal immunostimulant added diets seem to act as a promoter of shrimp immune system against the WSSV infection [42]. In the present study, the respiratory burst activity increased significantly in the prawn fed diets containing ß-1, 3-glucan than control group and the results are comparably with those of earlier works. It may be concluded that dietary administration of ß-1, 3-glucan at 1.0, 1.5 and 2.0 g /kg levels enhances resistance to white muscle disease virus and immuno response in freshwater prawn, *M. rosenbergii.*

5. Conclusion

This study provides a basic information regarding enhancement of immune response in freshwater prawn, *M. rosenbergii* due to dietary administration of ß-1, 3-glucan at 1.0, 1.5 and 2.0 g /kg levels and further to enhance resistance against white muscle disease virus. This study can be used in recent times as baseline information for biotic stress management of prawns against WMDV.

6. Acknowledgement

The authors are grateful to the Head of Department of Aquaculture, and Dean College of Fisheries, Mangalore for providing all the necessary facilities to carry out this research.

7. References

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