

# Non-Specific Immune Response of Vanname Shrimp (*Litopenaeus vannamei*) Induced by Chitosan as an Immunomodulator against IMNV (Infectious Myonecrosis Virus) Exposure

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## Abstract

Chitosan is one of polysaccharide derivatives commonly known as poly D-glucosamin-2 amino-2 dexoy-D-glucose, and glucosamine has a nearly similar chemical structure with  $\beta$ -glucan. It is capable of activating Prophenoloxidase (ProPO) to enhance immune system of shrimp. Chitosan is added to the food with treatment dose of 3 ml, 6 ml, 9 ml, and 12 ml. Then one negative control (not infected with IMNV) and one positive control (infected with IMNV). Shrimps are infected with IMNV through injection and THC, DHC, phagocytosis activity, super anionoxide and phenoloxidase (PO) are carefully observed. The most optimum result was obtained in the treatment of 3 ml Chitosan/kg food and the lowest result was found in the positive control.

**Keywords:** Chitosan, Immune system of shrimp, Immunostimulant, Phenoloxidase.

## 1. Introduction

Disease is considered as the most common factor to cause failure in the culture of vanname shrimp (*Litopenaeus vanname*) in Indonesia. One of the causing diseases known is a disease caused by IMNV (*Infectious Myonecrosis Virus*). Development of IMNV is a very rapid process in the cultural pond and it is potential to cause up to 80% of mortality. Various efforts were performed to overcome IMNV infection, and the most effective method is currently believed to enhance immune system through immunostimulant administration.

Chitosan is a polysaccharide produced through chitin deacetylation. Based on the opinion of Bashir *et al.* (2001), chitosan is one of polysaccharide derivatives and it mainly consists of glucosamine, 2-amino-2-deoxy- $\beta$ -D-glucose or more commonly known as Poly D-glucosamine ( $\beta$  (1, 4) 2-amino-2-deoxy-D-glucose). Presscot *et al.*, (1996) stated that glucosamine is a polymer derivative of microorganism's cellular membrane which is nearly similar with lipopolysaccharide, one type of  $\beta$ -glucan. Therefore, in this case, it is expected that plasma in hemolim can recognize glucosamine and further increase number of hemocytes and activate PPA (Active Serine Protease) to stimulate production of phenoloxidase enzyme. This study is aiming to observe non-specific immune response of vanname shrimp (*L. vanname*) induced with chitosan added into food against IMNV (*Infectious Myonecrosis Virus*) infection through the measurement of THC, DHC, phagocytosis activity, value of super anionoxide and phenoloxidase (PO).

## 2. Materials and Methods

### 2.1 Materials and Tools

Vanname shrimps used in this study were of F1 quality, and SPF (*Specific Pathogen Free*) certified, obtained from PT Suma Benur Situbondo-Jawa Timur, sized 4 grams. Chitosan is a commercial liquid chitosan with composition of 1 gram chitosan dissolved in 100 ml acetic acid 2 % in the ratio of 1:100 (b/v), commercial food from PT Centra Proteina Prima of Irawan brand. Materials used in the hemolim test are: gymsa, anticoagulant KC-199, L-cystein and glutaraldehyde, buffer CAC, L-Dopa and trypsin and tyrosine.

Equipments used: tarpaulin tub 1x1x1 m, aeration equipment, digital scale, syringe 0.1 ml 27.5 G, PCR tool, light microscope, hand tally counter, digital camera, microtube, pastel pellets, centrifuge, vortex, haemocytometer, object glass, cover glass, freezer with temperature  $-80^{\circ}\text{C}$  and micro filter 0.45  $\mu\text{L}$ .

### 2.2 Methods

Vanname shrimp (*L. vanname*) of  $\pm 4$  grams weight acclimatized in the tarpaulin tub, each tub filled with 50 shrimps. Liquid chitosan was added into food with 6 different treatments A: 3 ml; B: 6 ml; C: 9 ml; D: 12 ml/kg food, E: as a negative control (without IMNV infection) and F: as positive control (infected with IMNV). Chitosan was given for 3 weeks (21 days). Food administration was done 4 times in a day. IMNV challenge test was given through injection and data before and after challenge test were observed.

### 3. Result and Discussion

Results were obtained after IMNV challenge. The levels of immune response were measured through recording THC (*Total Hemocyte Count*), DHC (*Differential Hemocyte Count*), phagocytosis activity, and absorbance value of anion superoxide and phenoloxidase (PO).

#### 3.1 Total Haemocyte Count (THC)

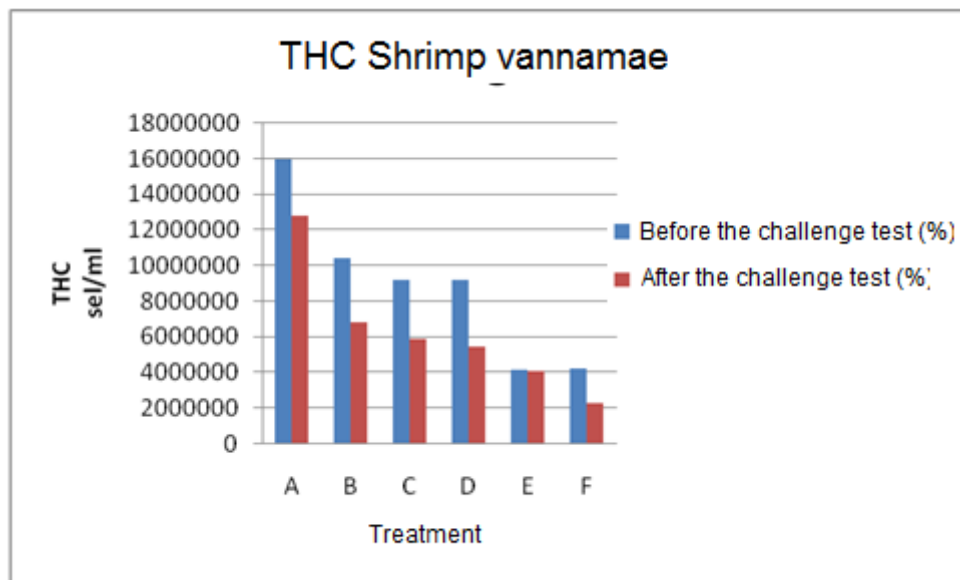


Figure 1. Graphic number of THC of vannamee shrimp before and after IMNV challenge test. Treatment A (3 ml); B (6 ml); C (9 ml); D (12 ml); E (0 ml/negative control); F (0 ml/positive control) per kg food.

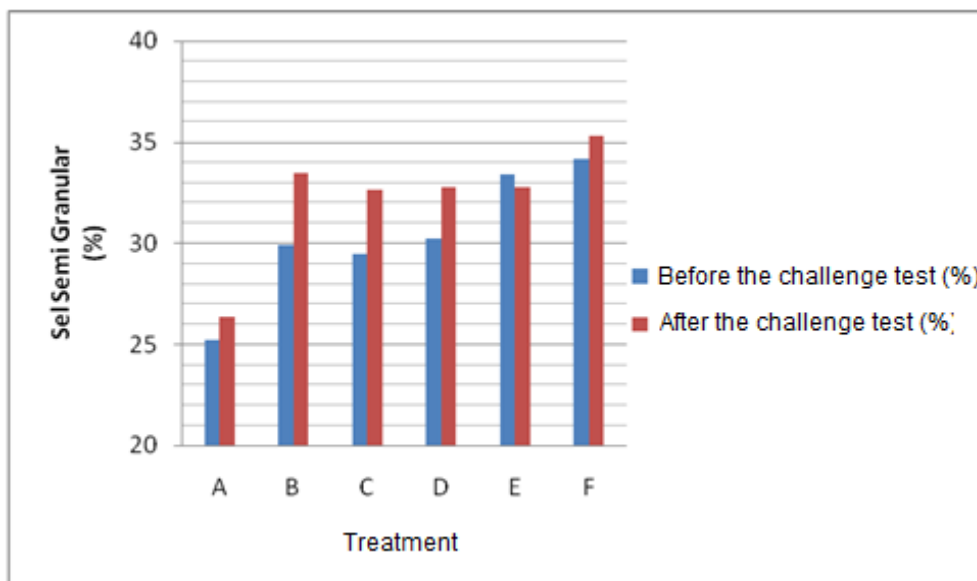
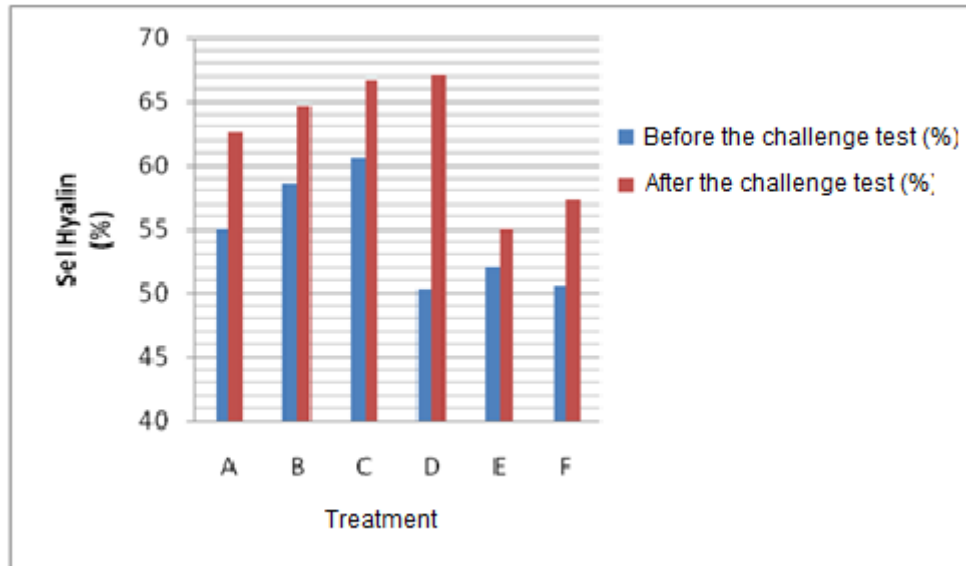
Figure 1 explained that the highest number of THC before challenge test is found in treatment A 3 ml/kg food and the lowest THC number is in control treatment (without chitosan). The high number of THC in shrimp in the treatments showed that chitosan is capable of enhancing immune response, this is in line with the opinion of Van de Braak (2000), Felix *et al.*, (2004), Wang and Cheng (2004) and Yin *et al.*, (2006) stating that the increasing number of THC indicated an increase of both cellular and humoral immune response.

The total number of hemocytes before IMNV challenge test was higher than control. Hemocytes in shrimp hemolymph increased due to the existence of lectin molecules functioning in the recognition of foreign material entering body (Rodriguez and Le Moullac, 2000). Chitosan is a polysaccharide derivative consisting of glucosamine, and lectin molecules recognize it. Lectin will react with glucosamine which then produce hemocytes and increase pro phenoloxidase system.

Total number of hemocytes after challenge test is decreasing, it indicates pathogenic infection caused by IMNV infecting hemocytes. It leads to the loss of hemocytes in hemolymph. Van de Braak (2002) stated that a decrease of hemocytes is caused by pathogenic infection, this is because hemocytes are target for pathogens.

3.2 Differential Hemocyte Count (DHC)

Observation on DHC aimed at identifying pattern of change of hyalin cells, semi-granular cells and granular cells percentage in hemolimf before and after IMNV challenge test



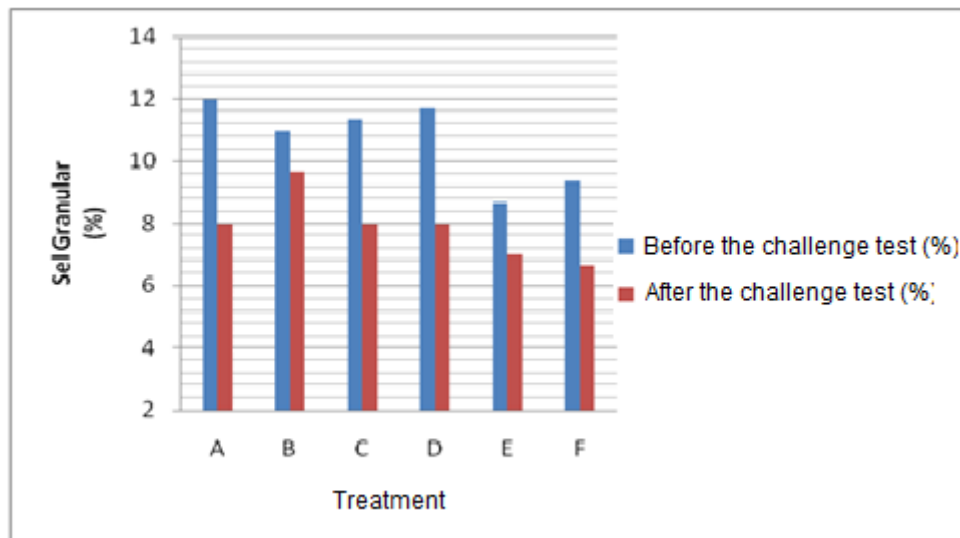


Figure 2. Means of (A) Hyalin Cells, (B) Semi Granular Cells, (C) Granular Cells Vanname Shrimp Before and After IMNV Challenge Test. Treatment A (3 ml); B (6 ml); C (9 ml); D (12 ml); E (0 ml/control -); F (0 ml/control +) per kg food.

It is seen in Figure 2 that different percentage of hemocytes type, and the best result in treatment A. Figure 2A showed high percentage increase in hyalin cells after IMNV challenge test. Hyalin cell is cellular component spread from the whole hemocytes compared with semi-granular cells and granules, as to easily formed and evolved (Sukoso *et al.*, 2012). Hyalin cells have a significant role in microbial phagocytosis process in the body during infection. Based on the greater number of hyalin cells than the other type of hemocyte cells then known that immune response mainly depends on the phagocytosis process taking an important role in cellular defense.

Figure 2B showed a slight percentage increase of semi-granular cells after IMNV challenge test. Semi-granular cells are capable of recognizing and responding to foreign materials or recognized as active cells in encapsulation process (Johanson *et al.*, 2000). Semi-granular cells are capable of recognizing and responding glucosamine in the existence chitosan. In this study, the increase of semi-granular cells have more effective function in the production of phenoloxidase enzyme which is important for humoral defense.

Figure 2C showed that granular cells are decreasing after IMNV challenge test, but they are higher than that of control. Decrease of granular cells was due to its main role in producing, saving, and secreting antimicrobes (Pratiwi, 2008). At the time of infection, granular cells will encounter degeneration and lysis. If infection does not happen, the granular cells will continually increase and multiply cells.

### 3.3 Phagocytosis Activity and Content of Anion Superoxide

Phagocytosis activity and anion superoxide content are proportional with the number of hemocytes, meaning that increase in hemocytes number will be followed by increase of phagocytosis activity and anion superoxide content.

Table 1. Data on Absorbance Value Of Anion Superoxide and Activity Observation Phagocytosis After IMNV Challenge Test

Treatment	Anion Superoxide Absorbance	Phagocytosis activity (%)
3 ml/kg food	0.084	58.08
6 ml/kg food	0.069	42.86
9 ml/kg food	0.042	24.24
12 ml/kg food	0.034	22.14
0 (negative control)	0.029	12.24
0 (positive control)	0.012	7.82

Table 1 shows the highest phagocytosis activity in treatment A(3 ml/kg food). Hemocyte in crustacean is the main component of immune system playing its role in the mechanism of phagocytosis, encapsulation, nodulation and cytotoxicity medium against foreign materials. Smith (1995) specified that total of hemocytes indicate host capability to respond to foreign materials in the body. The higher number of hemocytes and the higher phagocytosis activity given by the host in controlling foreign organisms. In this study, phagocytosis percentage increases due to the increasing ability of hemocytes when shrimp obtain materials for antigen of chitosan.

Anion superoxide content is directly proportional with phagocytosis process performed by hemocyte cells. In Table 2, it was obtained data that phagocytosis activity and absorbance value of anion superoxide added with chitosan is higher than control. When microorganisms are digested by hemocytes then some anti microbial substances are produced, one of them is anion superoxide (O<sub>2</sub><sup>-</sup>). Anion superoxide is the first substance produced during respiratory burst catalyzed by NADPH oxidase.

### 3.4 Phenoloxidase (PO)

Phenoloxidase is the last enzyme produced by hemocytes that function in the body defense of shrimp. Phenoloxidase is produced by prophenoloxidase (ProPO) system. Whereas, ProPO is activated by Prophenoloxidase Activating Enzyme (PPA) and the PPA is activated by a polysaccharide such as beta glucan, polysaccharide and peptidoglycan of microorganisms through protein recognition. Prophenoloxidase Activating Enzyme is a protein located in granulocytes.

Table 2. Absorbance Value of Phenoloxidase (PO) After IMNV Challenge Test

Treatment	Phenoloxidase (PO)
A (3 ml / kg food)	0.070
B (6 ml / kg food)	0.050
C (9 ml / kg food)	0.045
D (12 ml / kg food)	0.030
Negative control	0.018
Positive control	0.010

Table 4 explained that phenoloxidase (PO) is obtained in treatment A with 3 ml/kg food. Findings in this study were quite similar with the findings in the study by Wang and Cheng (2004), chitosan increase PO of vannamee shrimp infected with bacterium *Vibrio* sp. Chitosan is a polysaccharide derivative with glucosamine as the main component. Glucosamine is a

polymer derivative of microorganisms cellular membrane which is nearly similar with lipopolysaccharide or beta glucan. In this study, chitosan in which glucosamine as the main component, is capable of activating PPA which in turn lead to the activation of proPO and PO in shrimp.

#### 4. Conclusion and Suggestion

##### 4.1 Conclusion

Conclusion drawn from the study is that chitosan administration in the food can increase non-specific immune response of vannamee shrimp to the optimum rate, it is shown in treatment of 3 ml/kg food after chitosan administration and IMNV challenge test through the observation of THC, DHC, phagocytosis activity, super anionoxide and phenoloxidase (PO) content.

##### 4.2 Suggestion

Further study should be better to concentrate on non-specific immune response of vannamee shrimp induced with chitosan as immunostimulant against in the presence of different bacteria or viruses infection.

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