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# Immune Response of White Shrimp (*Litopenaeus vannamei*) to Different Density and IMNV Challenge

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## Abstract

Increasing in stocking density of shrimp affects the physiology and behaviour of their moving space. The health condition of shrimp is influenced by feeding, growth, and its susceptibility on disease. The aim of this study was to determine the development of immune response in relation to density and the presence of IMNV infection. This study used a completely randomized design (CRD) at density of 100 shrimp.m$^{-2}$, 200 shrimp.m$^{-2}$, and 400 shrimp.m$^{-2}$, with three replications in each treatment. The shrimp used was 5.02±0.26 g and the virus infection was exposed orally. This research was facilitated at the Disease Research Centre Laboratory of Central Proteina Prima Company, Pasar Kemis, Tangerang for 30 days. The results showed that the Total Hemocyte Count (THC) in hemolymph of shrimp had different values between negative controls and challenged IMNV. The lowest THC value was found at a density of 400 shrimp m$^{-2}$ (3.00x10$^{6}$ml$^{-1}$). While the highest THC value was at a density of 100 shrimp.m$^{-2}$ (4.75x10$^{6}$ml$^{-1}$). This result is supported by the increasing value of water quality parameters along with the increasing density of shrimp. Histopathology changes on skeletal muscle and lymphoid organs confirmed that the development of IMNV infection was faster at high shrimp densities.

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## 1. Introduction

*Litopenaeus vannamei* farming is one of the priority commodities aquaculture production (Tang *et al*., 2019). On the world market it is estimated that the value from marine shrimp production reaches USD 40 billion (FAO, 2016). Shrimp farming around the world is currently being affected by outbreaks of infectious disease (Apún-molina *et al*., 2017). One of the diseases that attack white shrimp culture is Infectious Myonecrosis Virus (IMNV) in aquaculture ponds (Sarah *et al*., 2018). Several factors that influence the incidence of IMNV are poor water quality, stocking density, shrimp stress, and the impact of climate change Kusumaningrum *et al*. (2012) and Tang *et al*. (2019). In addition, stressful environmental conditions increases susceptibility to pathogens and decreases shrimp immunity (Song *et al*., 2003). Shrimp infected with IMNV disease will reduce the shrimp's immune system (Yudiati, 2016). Stressful environmental conditions increase infectivity of pathogen, because of a reduced capacity of immune response (Tang *et al*., 2005). The immune system in shrimp does not have memory cells, unlike vertebrates, which have specific antibodies and complements. The shrimp immune system does not have immunoglobulins that play important role in the immune mechanism, shrimp only have a natural immune system (Kurniawan *et al*., 2018). The first defence against disease in shrimp is carried out by haemocytes. Haemocytes are a non-specific factor in the cellular defence system (Ridlo and Pramesti, 2009).

In principle, increasing the number of shrimp stocking density increases the risk of disease spreading (Aguilar *et al*., 2011). At very high stocking density, the shrimp are more aggressive and attack each other, resulting in increasing cannibalism and mortality (Miranti, 2016). The increase in density also affects the physiological processes and their movement behavior. This will reduce their health and physiological conditions that affect feed consumption, growth, and survival decreases (Purnamasari *et al*., 2017).

Shrimp farming at high densities provides advantages, although it presents slower growth and even lower survival are observed (Sookying *et al*., 2011). Although high density induces a condition of water quality stress, the effect on shrimp immune with IMNV challenge are not well established (Apún-molina *et al*., 2017). In one experiment, no clear influence of high density of 50, 200, and 600 shrimp $m^{-2}$ on several metabolic and immunological indicators was observed (Li *et al*., 2006). However, other studies have shown that high density affects several immune parameters (Lin *et al*., 2015). On a research by Molina *et al*., (2017), the measured response of shrimp immune with immune parameters did not change at high density.

However, all previous studies were focused on the effect of stocking density on shrimp immune level. To our knowledge, very few studies have been conducted concerning the stocking density (high and low) and IMNV challenge on the immune response of vannamei. Determining stocking densities is a basic procedure in shrimp culture, and the IMNV is the cause of outbreaks of infectious disease.

The purpose of this present study is to assess the effect of the vannamei shrimp immune response observed for fifteen days on immunity (total haemocyte count), conditions of water quality parameters, and the level of viral infection (histopathology) after different density treatments and IMNV challenge.

## 2. Materials and Methods

### *2.1 Materials*

This research was conducted from November 2020 to January 2021 at the Disease Research Center (DRC) laboratory of PT. Central Proteina Prima, Pasar Kemis, Tangerang. The shrimp used was 488 juvenile *Litopenaeus vannamei*, with average mean body weight of 5,02±0,26 g. Myonecrosis virus inoculum was obtained from DRC with a virus copy number of $5,97x10^3$. The aquarium was measured at 60 x 40 x 50 cm and filled with approximately 80L of clean seawater. Water quality measuring instruments consist of thermometer, pH, DO, heater, test kit of TAN, TOM, and TOC.

### *2.2 Methods*

#### *2.2.1 Experimental design*

The experimental design in this study was a completely randomized design (CRD) with 6 treatments with 3 replications. The treatment carried out consisted of 3 treatments with IMNV challenge test and 3 treatments were negative controls. The treatment was different stocking densities; 100 shrimp $m^{-2}$, 200 shrimp $m^{-2}$, and 400 shrimp $m^{-2}$. During the research activity, both IMNV challenged and control without IMNV challenged treatments were carried out.

#### *2.2.2 Viral challenge*

Stock of IMNV virus has been prepared by re-infection of some isolates directly into the shrimp to increase their efficacy. The IMNV inoculum which obtained from the DRC archives of PT. CP Prima, Tan-

gerang was stored at -80$^0$C. A total of 50 shrimps with average mean body weight from 6-8 g were stocked in the aquarium with size of 80L. The inoculum that has been prepared was injected with intra muscularly as much as 0.1 ml shrimp$^{-1}$ into the body of shrimp (Yudiati, 2016). Then the shrimp was cultured and observed. The dead shrimp will be stored in the freezer. Observation were conducted for 14 days, at the end of the observation, both live and dead shrimp in the freezer were mashed. Infected shrimps were dismantled from its carapace, head, and tail; leaving the muscle part of the shrimp and then crushed and homogenized (Tang *et al*., 2005). To confirm the number of copies of the IMNV virus, Real Time-PCR was performed.

The IMNV infected tissue then was fed orally into the shrimp. The number of virus copies of infected tissue was 5.97 x 10$^3$. Shrimp that have been mashed and then weighed was then fed to the tested shrimp for 10% of its biomass. Infected tissue was spread in the aquarium with a frequency of 3 times at 07.00 am, 01.00 pm, and 05.00 pm for 3 days (Umiliana *et al*., 2016).

*2.2.3 Water quality*

Water quality parameters of temperature and pH were observed every day at 8 a.m. and 4 p.m. Weekly measurements of Total Amonia Nitrogen (TAN), Total Organic Meter (TOM), and Total Organic Carbon (TOC) were tested by taking samples of 2 water samples in each treatment and would be duplicated when testing in the laboratory. Water samples are taken using a sample bottle and will be directly tested in the laboratory. During observations, 25% of the water was changed every day by siphoning to remove the rest of the feed and dirt that had settled on the bottom.

*2.2.4 Haemocyte analysis*

Sampling haemocyte analysis was taken at day post infection (dpi) 0, 5, 10, and 15. Approximately 50 µL of haemolymph were taken in each test shrimp, 3 pieces per treatment. It was performed on the ventral sinus of shrimp using a 1 ml syringe and then inserted into a microtube which was already filled with 50 µL of 10% formalin as anticoagulant. Then let it stand for 10 minutes and add 100 µL of rose Bengal for cell colouring. THC was carried out as described by Wang and Chen (2005). The solution mixture was then dripped as much as 10 µL on a haemocytometer and then covered with a cover glass. Total haemocytes were observed and the number of cells was counted under a microscope. Count were made on 5 of the 25 small squares in the centre of the haemocytometer.

*2.2.5 Histopathology*

For confirmation of IMNV virus infection, histopathology has carried out conventionally (Lightner, 1996). The number of shrimp samples were 2 from each density. Observation of histopathological parameters was carried out on skeletal muscles and lymphoid organs of the tested shrimp. Histopathological sampling was carried out on 5$^{th}$, 10$^{th,}$ and 15$^{th}$ day post-infection (dpi). The process of tissue preparations included: fixation, trimming (preparation), processing, embedding, rough, and fine sectioning, staining, respectively.

*2.3 Data Analysis*

Microsoft Excel 2013 was used for analysing water quality data descriptively after comparing it with water quality standards and other relevant research. THC data was analysed through two-way analysis of variance (ANOVA) with 95% confidence level. Then proceeded with Duncan's test to determine the effect of various treatments. The results of histopathology parameters were descriptively analysed by describing the existing results.

## 3. Results and Discussion

*3.1 Water Quality*

The results of this study showed that daily water quality parameter such as temperature that ranging from 29.7$^0$C to 30.5$^0$C and Dissolved oxygen (DO) > 4.0 mg/L were not significantly different (Table 1). The heater installation in the aquarium was set at 30$^0$C during the observation. At this temperature level, the spread of the IMNV virus for challenge test can be evenly distributed. This level would also trigger the development of the IMNV (Disease Research Centre, CP Prima-Company, Unpublished data). According to Tobing (2019), the optimum temperature for rearing shrimp ranges from 22-32$^o$C. While the temperature triggers the development of IMNV is >28$^o$C (Silva *et al*., 2015). As explained by Sulmartiwi *et al*. (2013), the water temperature that accelerates the spread of IMNV is around 30$^o$C.

Observation of the pH value carried out in the morning (8 a.m.) and afternoon (4 p.m.) did not affect the pH value. Changes in the pH value in observations were influenced by the amount of stocking density and IMNV. As the number of stockings increases, the pH value will decrease. In IMNV challenge, the density of 400 shrimp m$^{-2}$ decreased to pH value <7 at dpi 8 (Table 1). While in other treatments, the pH value was >7. This is due to the deteriorating condition of water

 

quality during the observation. As the density increases, the amount of feed remains and metabolism in the water increases. High organic matter causes acidification in the waters, so the pH becomes low. The decreasing pH value was caused by the decomposition of organic matter by microorganisms (Supriatna *et al*., 2020). Reaction of decreasing pH can increase the total organic matter content in the water (Hendrawati *et al*., 2008). The optimal pH value range is 6.8-7.8 (Tobing, 2019). However in other studies, Supriatna *et al*. (2020) conveyed that a good pH for ponds is in the range of 7.6-8.4.

**Table 1.** Average observations of water quality variables according to different densities

| Water Quality variabels | | Density (shrimp $m^{-2}$) | | | Reference |
|---|---|---|---|---|---|
| | | 100 | 200 | 400 | |
| Temperature ($^0$C) | | | | | |
| | A | 29.98±0.37 | 30.01±0.48 | 30.06±0.33 | 26.00 – 32.00 |
| | B | 30.01±0.41 | 30.25±0.59 | 30.04±0.42 | (Tobing, 2019) |
| pH | | | | | |
| | A | 7.31±0.19 | 7.03±0.32 | 6.52±0.15 | 7.60-8.45 |
| | B | 7.54±0.20 | 7.15±0.22 | 7.15±0.22 | (Supriatna *et al*., 2020) |
| Dissolved oxygen (mg $L^{-1}$) | | | | | |
| | A | 5.25±0.09 | 5.19±0.08 | 5.14±0.12 | 4.00 – 6.00 |
| | B | 5.23±0.11 | 5.28±0.09 | 5.19±0.05 | (Fendjalang, 2016) |
| TAN (mg $L^{-1}$) | | | | | |
| | A | 0.98±0.09 | 1.03±0.04 | 2.08±0.59 | 1:50 |
| | B | 0.84±0.02 | 0.92±0.05 | 1.21±0.25 | (Ariadi *et al*., 2020) |
| TOC (mg $L^{-1}$) | | | | | |
| | A | 0.71±0.04 | 1.43±0.04 | 3.71±0.91 | 2:00 |
| | B | 0.57±0.17 | 1.26±0.21 | 1.86±0.34 | (Sansanayuth *et al*., 1996) |
| TOM (mg $L^{-1}$) | | | | | |
| | A | 107.21±0.51 | 118.59±0.72 | 124.91±1.03 | <105.60 |
| | B | 85.72±0.54 | 98.36±0.83 | 116.06±0.52 | (Wafi *et al*., 2020) |

Description : A (IMNV chellenge test), B (control). TAN (Total Ammonia-N, TOM (Total Organic Meter), TOC (Total Organic Carbon).

**Table 2.** The results of observation of the THC value in each treatment

| Trearments | Total Haemocyte Count (x $10^6$ $mL^{-1}$) | | | |
|---|---|---|---|---|
| (shrimp $m^{-2}$) | dpi 0 | dpi 5 | dpi 10 | dpi 15 |
| IMNV+100 | 10.25±0.25$^b$ | 9.33±1.25$^b$ | 4.83±0.29$^b$ | 5.75±0.66$^b$ |
| IMNV+200 | 10.17±0.76$^b$ | 9.50±1.32$^b$ | 5.83±0.29$^b$ | 3.67±0.29$^b$ |
| IMNV+400 | 8.83±1.04$^b$ | 8.00±0.50$^a$ | 4.33±0.76$^a$ | 3.00±0.50$^a$ |
| 100 | 11.50±0.50$^b$ | 13.83±0.29$^b$ | 7.83±0.29$^b$ | 10.83±0.76$^b$ |
| 200 | 11.67±0.29$^b$ | 15.83±0.76$^b$ | 8.33±0.28$^b$ | 10.33±0.76$^b$ |
| 400 | 10.33±0.76$^b$ | 10.17±2.08$^b$ | 8.00±0.87$^a$ | 11.83±0.73$^b$ |

Description : dpi (day post infection), IMNV+density (treatment IMNV challenged test). Different superscripts in the same colomn shows that there are significant differents ($p<0.05$(Total Organic Carbon).

Total Ammonia Nitrogen (TAN) is toxic ammonia and can harm the condition of shrimp. The results of TAN level in this study showed that there were differences between each density in both IMNV challenge test and the control. The amount of TAN content between the IMNV challenge was higher with an average of 0.96-1.66 mg $L^{-1}$ compared to the control 0.698-0.89 mg $L^{-1}$ (Table 1). In the other observations of IMNV challenge, the number of TAN content almost reached a potentially toxic concentration at week-2 (dpi 10), which was in the range of 0.61-2.08 mg $L^{-1}$. Fatal TAN level that can kill shrimp is 1.5 mg $L^{-1}$ (Ariadi *et al*., 2020; Syafaat *et al*., 2013). Research by Aguilar *et al.* (2011) reported the maximum value of TAN is 2,4 mg $L^{-1}$. The highest TAN value was obtained from density of 400 shrimp $m^{-2}$. This was directly proportional to the increasing amount of feed input and waste produced because of shrimp stress from IMNV infection which affect their appetite. The increase in stocking density affects the physiological condition of shrimp due to infection, the shrimp begins to decrease their appetite which results in increasing the remaining feed and the amount of nitrogen released into the water as studied by Syafaat *et al.* (2010).

In addition to TAN, the lowest TOM content was obtained in the treatment of 100 shrimp $m^{-2}$ (A) with an average of 78.14 mg $L^{-1}$, while the highest TOM content was found at 400 shrimp $m^{-2}$ which was 123.64 mg $L^{-1}$ (Table 1). TOM shows the content of organic matter, in this study the TOM content increased according to the increase in the amount of density. High density causes the feeding to be increased, but the presence of IMNV infection causes the shrimp to become weaker. High density also disrupts the physiological process of shrimp due to stress. This increases the concentration of dissolved organic matter along with feed and metabolic waste. Increasing the amount of feed and shrimp metabolism increases the amount of decomposition carried out by microorganisms. Research by Supriatna *et al.* (2020) stated that TOM is a description of the concentration of total organic matter in waters consisting of dissolved, suspended, and colloidal organic matter. On research by Wafi *et al.* (2020), a good content for TOM is <90 mg $L^{-1}$. It was clarified in other studies that the optimum range of TOM values in ponds was <105.6 mg $L^{-1}$ (Supriatna *et al*., 2020).

TOC is the total organic carbon consisting of dissolved organic matter. TOC levels during the same study with other parameters increased at week 2 (dpi 10). In the observation, the highest TOC was found in the IMNV challenge test treatment with high stocking density, at density of 400 shrimp $m^{-2}$ which reached 4.143 mg $L^{-1}$. Poersch *et al.* (2020) said that the factor of shrimp density contributed to the TOC value content of the feed and manure of the reared biota. With increasing density, it increases the amount of feed and metabolic waste increasing organic carbon content (Fast and Lester, 1992).

**Table 3.** Histopathology development in each treatments

| Treatment | Dpi 5 | | Dpi 10 | | Dpi 15 | |
|---|---|---|---|---|---|---|
| (shrimp $m^{-2}$) | Limfoid Organ | Muscle | Limfoid Organ | Muscle | Limfoid Organ | Muscle |
| 100 | - | - | - | - | - | - |
| 200 | - | - | - | - | - | - |
| 400 | - | - | - | - | - | - |
| IMNV+100 | - | - | - | - | LOS -25% | Nec (25%) |
| IMNV+200 | - | - | - | Nec -25% | LOS -60% | Nec -60% |
| IMNV+400 | - | - | LOS -40% | Nec -40% | LOS -80% | Nec -80% |

Description:
- dpi (*day post infection*), Los (*Limfoid Organ Spehroid*), Nec (Necrosis), IMNV+density (treatment IMNV challenged test)
- The percent value indicates the infection rate in each treatment
- (-) IMNV symptoms have not appeared

 

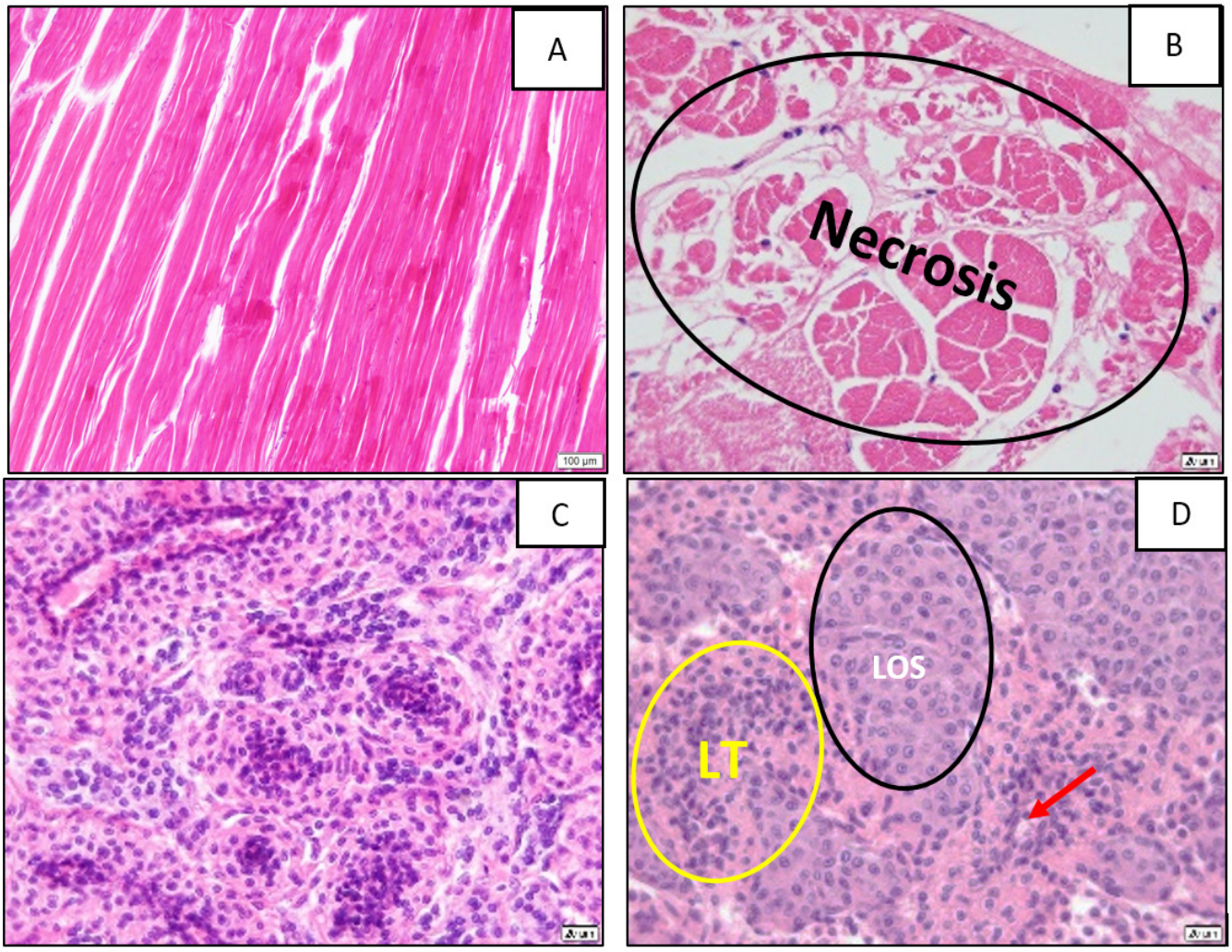


**Figure 1.** Histopathology changes of muscles ad lymphoid organs in the IMNV challenge test.
[A] Normal muscle tissue; [B] Necrotic muscle tissue; [C] Normal lymphoid tubules; [D] Lymphoid organs that form spheroids (LOS) and their comparaison with normal lymphoid tubules (LT). The arrows indicate the formation of vacoula (treatment 400 shrimp m-2 dpi 10).

### *3.2 Haemocyte Analysis*

Based on the results of the study, THC value was different between each treatment. THC values in each treatment generally decreased at the 10$^{th}$ dpi or the 2nd week. Besides this is due to deteriorating water quality conditions (Table 1), it also increasing the development of the IMNV infection in the body of shrimp. This was apparrently different in IMNV challenge and control.

In IMNV challenge, the lowest THC value was 3x10$^{6}$ ml$^{-1}$, while in control was 7,75x10$^{6}$ mL$^{-1}$ (Table 2). Therefore, it can be seen that THC value in all IMNV challenge was lower than control. In other words, it has been showed that in this study, the THC value was significantly influenced by viral factors. Even though the study by Molina *et al.* (2017) showed that high density did not affect THC values and did not confess susceptibility to WSSV virus. However, another study showed that the THC value of vanname shrimp infected with TSV has decreased (Song *et al.*, 2003). The decrease in the value of THC migh be due to foreign objects that enter the shrimp body will be recognized by hemocyte cells and then responded through several stages of mechanisms and various immune responses to pathogen (Muharrama, 2020). In the presence of foreign objects, it causes hemocyte cells to migrate from the shrimp body's circulation system to tissues where many cells are infected (Widanarni *et al.*, 2020). It is indicated that the THC value for every different virus will showed different number.

Observation of THC value in control treatment gave the same effect on increasing stocking den-

sity. The lowest THC value was observed at a density of 400 shrimp $m^{-2}$, which reached $3.00x10^6$cell/ml. Meanwhile, the density of 100 shrimp $m^{-2}$ and 200 shrimp $m^{-2}$ varied. At dpi 0 and dpi 15 THC at a density of 100 shrimp $m^{-2}$ ($10.25x10^6$ $mL^{-1}$ and $4.50x10^6$ $mL^{-1}$) was slightly lower than the density of 200 shrimp $m^{-2}$ ($10.50x10^6$ $mL^{-1}$ and $4.75x10^6$ $mL^{-1}$). This indicated that THC was slightly affected by high density in this study even more to the IMNV infection. On research by Apún-molina *et al.* (2017) mentioned that although high density can cause stress and suppress the immune system in shrimp, there was no significant change in THC at a high density. The normal THC value for shrimp is a minimum of $20x10^6 – 40x10^6$ $mL^{-1}$ (Chang *et al.*, 1999) or minimum is $16,4x10^6$ $mL^{-1}$ (Song *et al.*, 2003), meanwhile in this study appointed that THC value was lower and corresponded to the high density.

### *3.3 Hystopathology*

IMNV infection was confirmed by examining the histopathology of shrimp. Tissue observation was performed in skeletal muscle and lymphoid organs as described by Andrade *et al.* (2008) and Poulos *et al.* (2006). Based on histopathological results, IMNV challenge shrimp were showed abnormalities compared to normal. In IMNV challenge as well as in high density of shrimp, percentage of necrosis muscle tissue and spheroid formation in lymphoid organs are high than normal (Figure 1).

In density of 400 shrimp $m^{-2}$, the fastest clinical symptoms appeared at dpi 10 and the percentage of shrimp exposed to IMNV exceeded 80% of the observed shrimp (Table 3). The last clinical symptom of IMNV that appeared was at a density of 100 shrimp $m^{-2}$ which showed necrosis of muscle tissue and spheroid at the last observation (dpi-15) and the percentage of shrimp exposed was 25% of the observed shrimp. In control, no shrimp were confirmed to be infected with IMNV. Shrimp is declared infected with IMNV if muscle necrosis is found accompanied by the formation of spheroids in lymphoid organs. Necrosis in the muscle tissue could cause loss of transparency in muscle tissue and at advanced stage, it will turn red as a sign of IMNV infection as observed by Poulos *et al.* (2006); Senapin *et al.* (2007); and Sarah *et al.* (2018). Similar results from a study by Sukenda *et al.* (2010) in IMNV infection tissue have shown the formatting of tissue degeneration, necrosis, and infiltration of haemocytes in muscle tissue. Besides the muscle necrosis, the presence of lymphoid organs are used as confirmation of IMNV disease (Andrade *et al.*, 2008). Histopathology of abnormal lymphoid organs was commonly found in cases of shrimp infected with RNA viruses. Therefore, it is generally believed that the formation of spheroids is a non-specific reaction of the shrimp immune system to viral infections (Rusaini and Owens, 2010). The abnormality of the lymphoid organs are when it cannot maintain their normal shape, formation of spheroids, is known as hypertrophy of lymphoid cells, viral inclusions, and degradation of granulocyte haemocyte (Hasan, 2011).

## 4. Conclusion

Based on results of the study, the change in the shrimp immune response which were observed from THC value and histopathology after IMNV challenged has significant difference for each different densities. In high stocking density, the speed and the percentage of appearance of clinical symptoms such as degeneration of muscle necrosis and spheroid in lymphoid organs had increased. The speed of water quality degradation as pH, TAN, TOC, and TOM were also high. From this study, we recommend 100 shrimp $m^{-2}$ for the best stocking density.

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## Authors' Contributions

All authors have contributed to the final manuscript. The contributed of each author are as follows HBU; as a determinant of topic ideas, funding and critical revision of articles. NKB; collecting data, compiling manuscripts, and analyzing data. MN; provide conceptual ideas and critical revision of articles.

## Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

 

Aguilar, V., Racotta, I. S., Goytortua, E., WIlle, M., Sorgeloos, P., Civera, E., & Palacios, E. (2011). The influence of dietary arachidonic acid on the immune response and perfomance of pacific whiteleg shrimp, *Litopenaeus vannamei*, at high stocking density. *Aquaculture Nutrition*, 18(3):258-271

Andrade, T. P. D., Redman, R. M., & Lightner, D. V. (2008). Evaluation of the preservation of shrimp samples with Davidson's AFA fixative for Infectious Myonecrosis Virus (IMNV) in situ hybridization. *Aquaculture*, 278(1-4):179-183.

Apún-molina, J. P., Robles-romo, A., Alvarez-ruiz, P., Santamaria-miranda, A., Arjona, O., & Racotta, I. S. (2017). Influence of stocking density and exposure to White Spot Syndrome Virus in biological performance, metabolic, immune, and bioenergetics response of whiteleg shrimp *Litopenaeus vannamei*. *Aquaculture*, 479:528-537.

Ariadi, H., Wafi, A., Magister, P., Brawijaya, U., Perikanan, P. B., Ibrahimy, U., & Brawijaya, U. (2020). Hubungan kualitas air dengan nilai FCR pada budidaya intensif udang vanname (*Litopenaeus vannamei*). *Samakia: Jurnal Ilmu Perikanan*, 11(1):44-50.

Chang, C. F., Su, M. S., & Chen, H. Y. (1999). A rapid method to quantify total haemocyte count of *Penaeus monodon* using ATP analysis *Fish Pathology*, 34(4):211-212.

FAO. (2016). The state of world fisheries and aquaculture. Rome: FAO.

Fast, A. W., & Lester, L. J. (1992). Marine shrimp culture: Principles and practices. Amsterdam: Elsevier.

Fendjalang, S. N. M., Budiardi, T., Supriyono, E. (2016). Kinerja produksi dan fisiologis udang vaname *Litopenaeus vannamei* pada karamba jaring apung dengan padat tebar berbeda di Selat Kepulauan Seribu. Thesis. Bogor: IPB University.

Hasan, A. S. W. (2011). Ko-infeksi Infectious Myonecrosis Virus (IMNV) dan *Vibrio harveyi* pada udang vaname. Institut Pertanian Bogor. Thesis. Bogor: IPB University.

Hendrawati, Prihadi, T. H., & Rohmah, N. N. (2008). Analisis kadar phosfat dan N-nitrogen (amonia, nitrat, nitrit) pada tambak air payau akibat rembesan lumpur lapindo di Sidoarjo, Jawa Timur. *Jurnal Kimia Valensi*, 1(3):135-143.

Kurniawan, M. H., Putri, B., & Elisdiana, Y. (2018). Efektivitas pemberian bakteri *Bacillus polymyxa* melalui pakan terhadap imunitas non spesifik udang vannamei (*Litopenaeus vannamei*). *E-Jurnal Rekayasa dan Teknologi Budidaya Perairan*, VII (1):739-750.

Kusumaningrum, E. D., Wardiyanto, & Tusihadi, T. (2012). Insidensi Infectious Myonecrosis Virus (IMNV) pada udang putih (*Litopenaeus vannamei*) di Teluk Lampung. *E-Jurnal Rekayasa Dan Teknologi Budidaya Perairan*, I(1):65-70.

Li, Y., Li, J., & Wang, Q. (2006). The effects of dissolved oxygen concentration and stocking density on growth and non-specific immunity factors in Chinese shrimp, *Fenneropenaeus chinensis*. *Aquaculture*, 256(1-4):608-616.

Lightner, D. V. (1996). Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. *Scientific and Technical Review*, 15(2):579-601.

Lin, Y. C., Chen, J. C., Chen, Y. Y., Yeh, S. T., Chen, L. L., Huang, C. L., Hsieh, J. F., & Li, C. C. (2015). Crowding of white shrimp *Litopenaeus vananmei* depresses their immunity to and resistance against *Vibrio alginolyticus* and White Spot Syndrome Virus. *Fish and Shellfish Immunology*, 45(1):104-111.

Miranti, S. (2016). Pengendalian infeksi *Vibrio harveyi* pada udang vaname dengan ekstrak kunyit-sambiloto dalam pakan di karamba jaring apung Kepulauan Seribu. Thesis. Bogor: IPB University.

Molina, J. P. A., Romo, A. R., Ruiz, P. A., Miranda, A. S., Arjona, O., & Racotta, I. S. (2017). Influence of stocking density and exposure to White Spot Syndrome Virus in biological performance, metabolic, immune, and bioenergetics response of whiteleg shrimp *Litopenaeus vannamei*. *Aquaculture*, 479:528-537.

Muharrama, A. R. W. (2020). Pertumbuhan, ekspresi gen dan respons imunitas udang vaname diberi probiotik *Bacillus* NP5 dan prebiotik madu serta diinfeksi *Vibrio parahaemolyticus*. Thesis. Bogor: IPB University.

Poersch, L. H., Bauer, W., Wallner, M., & Wasielesky, W. (2020). Assessment of trace metals , total organic carbon and total nitrogen of a shrimp farm system in Southern Brazil. *Regional Studies in Marine Science*, 39:101452.

Poulos, B. T., Tang, K. F. J., Pantoja, C. R., Bonami, J. R., & Lightner, D. V. (2006). Purification and characterization of Infectious Myonecrosis Virus of penaeid shrimp. *Journal of General Virology*, 87(4):987-996.

Purnamasari, I., Purnama, D., & Utami, M. A. F. (2017). Pertumbuhan udang vaname (*Litopenaus vannamei*). *Jurnal Enggano*, 2(1):58-67.

Ridlo, A., & Pramesti, R. (2009). Aplikasi ekstrak rumput laut sebagai agen imunostimulan sistem pertahanan non spesifik pada udang (*Litopennaeus vannamei*). *Jurnal Ilmu Kelautan*, 14:133-137.

Rusaini, & Owens, L. (2010). Insight into the lymphoid organ of penaeid prawns: A review. *Fish and Shellfish Immunology*, 29(3):367-377.

Sansanayuth, P., Phadungchep, A., Ngammontha, S., Ngdngam, S., Sukasem, P., Hoshino, H., & Ttabucanon, M. S. (1996). Shrimp pond effluent: Pollution problems and treatment by construted Wetlands. *Water Science and Technology*, 34(11):93-98.

Sarah, H., Prayitno, S. B., & Haditomo, A. H. C. (2018). Studi kasus keberadaan penyakit IMNV (Infectious Myo Necrosis Virus) pada udang vaname (*Litopenaeus vannamei*) di pertambakan Pekalongan Jawa Tengah. *Jurnal Sains Akuakultur Tropis*, 2(1):66-72.

Senapin, S., Phewsaiya, K., Briggs, M., & Flegel, T. W. (2007). Outbreaks of Infectious Myonecrosis Virus (IMNV) in Indonesia confirmed by genome sequencing and use of an alternative RT-PCR detection method. *Aquaculture*, 266(1-4):32-38.

Silva, S. M. B. C. D., Rocha, J. L., Martins, P. C. C., Gálvez, A. O., Santos, F. L. D., Andrade, H. A., & Coimbra, M. R. M. (2015). Experimental infection of Infectious Myonecrosis Virus (IMNV) in the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). *Aquaculture International*, 23:563-576.

Song, Y. L., Yu, C. I., Lien, T. W., Huang, C. C., & Lin, M. N. (2003). Haemolymph parameters of Pacific white shrimp (*Litopenaeus vannamei*) infected with Taura Syndrome Virus. *Fish & Shellfish Immunology*, 14(4):317-331.

Sookying, D., Silva, F. S. D., Davis, D. A., & Hanson, T. R. (2011). Effects of stocking density on the perfromance of pacific white shrimp *Litopenaeus vannamei* cultured under pond and outdoor tank conditions using a high soybean meal diet. *Aquaculture*, 319(1-2):232-239.

Sulmartiwi, L., Rekasana, A., & Sudarno, S. (2013). Distribusi penyakit Infectious Myo Necrosis Virus (IMNV) pada udang vaname (*Litopenaeus vannamei*) di pantai Utara Jawa Timur. *Jurnal Ilmiah Perikanan Dan Kelautan*, 5(1):49-54.

Sukenda, Nuryati, S., & Sari, I. R. (2010). Pemberian meniran *Phyllanthus niruri* untuk pencegahan infeksi IMNV (Infectious Myonecrosis Virus) pada udang vaname (*Litopenaus vannamei*). Undergraduate Thesis. Bogor: IPB University.

Supriatna, Mahmudi, M., Musa, M., & Kusriani. (2020). Hubungan pH dengan parameter kualitas air pada tambak intensif udang vanamei (*Litopenaus vannamei*). *Journal of Fisheries and Marine Research*, 4(3):368-374.

Syafaat, M. N., Gunarto, & Mansyur, A. (2013). Evaluasi kualitas air pada udang vaname (*Litopenaeus vannamei*) semi intensif dan intensif dengan aplikasi probiotik. *Prosiding Forum Inovasi Teknologi Akuakultur*:813-823.

Syafaat, M. N., Mansyur, A., & Tonnek, S. (2010). Dinamika kualitas air pada budidaya udang vaname (*Litopenaeus vannamei*) semi-intensif dengan teknik pergiliran pakan. *Prosiding Indoaqua-Forum Inovasi Teknologi Akuakultur*:487–494.

Tang, K. F. J., Melba, & Arthur, J. R. (2019). Shrimp Infectious Myonecrosis strategy manual. Rome: FAO.

Tang, K. F. J., Pantoja, C. R., Poulos, B. T., Redman, R. M., & Lightnere, D. V. (2005). In situ hybridization demonstrates that *Litopenaeus vannamei*, *L. stylirostris* and *Penaeus monodon* are susceptible to experimental infection with Infectious Myonecrosis Virus (IMNV). *Diseases of Aquatic Organisms*, 63(2-3):261-265.

Tobing, S. W. L. (2019). Pertumbuhan dan kelulushidupan udang vaname *Litopenaeus vannamei* pada salinitas 5 ppt dengan kepadatan berbeda. Thesis. Bandar Lampung: Lampung University.

Umiliana, M., Sarjito, & Desrina. (2016). Pengaruh salinitas terhadap infeksi Infectious Myonecrosis Virus (IMNV) pada udang vaname *Litopenaeus vannamei*. *Journal of Aquaculture Management*

*And Technolo*, 5(1):73-81.

Wafi, A., Ariadi, H., Fadjar, M., Mahmudi, M., & Supriatna, S. (2020). Model simulasi panen parsial pada pengelolaan budidaya intensif udang vannamei (*Litopenaeus vannamei*). *Jurnal Ilmu Perikanan*, 11(2):118-126.

Wang, L-U., & Chen, J-C. (2005). The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. *Fish and Shellfish Immunology*, 18: 269-278.

Widanarni, W., Rahmi, D., Gustilatov, M., Sukenda, S., & Utami, D. A. S. (2020). Immune responses and resistance of white shrimp (*Litopenaeus vannamei*) fed probiotic *Bacillus* sp NP5 and prebiotic honey against White Spot Syndrome Virus infection. *Jurnal Akuakultur Indonesia*, 19(2):118-130.

Yudiati, E. (2016). Ekspresi gen dan laju sintasan udang vaname (*Litopenaeus vannamei*) yang tersuplementasi dengan alginat secara oral untuk resistensi penyakit White Spot Syndrome Virus. *Buletin Oseanografi Marina*, 5(2):135-142.