

The Cellular Response of the Juvenile White Shrimp (*Litopenaeus vannamei*) to Rearing with Different Levels of Cultivation Technology

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ABSTRACT

This research aimed to examine the cellular response of the juvenile *Litopenaeus vannamei* when reared with different levels of technology. The research was carried out by taking samples directly to the locations of semi-intensive ponds and intensive ponds. The frequency of sampling was 2 times at an interval of 15 days when the juvenile *Litopenaeus vannamei* were \pm 2 months old. The cellular response was observed by monitoring the expression of physiological parameters, namely the hemocyte count and the percentage of hemocyte cell differentiation based on type. The physiological response expressed from the research results showed that there was an increase in the hemocyte count of juvenile *Litopenaeus vannamei* during monitoring at 16:30, each increasing from 32×10^6 cells/ ml in semi-intensive ponds, and 36.8×10^6 cells/ ml in the intensive ponds. At 12:00 PM monitoring, it was 70.7×10^6 cells/ ml in the semi-intensive ponds and 76.8×10^6 cells /ml in intensive ponds. Then, it decreased toward 3:00 AM to 26.8×10^6 cells/ ml in the intensive ponds and 36.7×10^6 cells/ ml in the semi-intensive ponds. Furthermore, the percentage of hyaline cells was higher (54- 62%) in the semi-intensive ponds compared to globular cells (31- 35%) and semi-globular cells (6- 65%), but in intensive ponds, globular cells were higher than hyaline cells and globular at every 24-hour monitoring. This fact shows that the vitality condition of the *Litopenaeus vannamei* juveniles, based on the cellular response expressed by the animals in the semi-intensive ponds, is better than in the intensive ponds. In intensive ponds, the physiological condition of the *Litopenaeus vannamei* juveniles is thought to be vulnerable to stress, especially before dawn (4:30 AM).

INTRODUCTION

The difference between semi-intensive and intensive technology levels is mainly due to differences in rearing density and application of technology levels and relying on the artificial feed as a nutrient supply.

The application of semi-intensive and intensive levels of technology can influence the cellular response, which is expressed through changes in the hemolymph of juvenile *Litopenaeus vannamei*. One of the hemolymph parameters that is most sensitive and

constant to stress conditions in cultivating *Farfantepenaeus paulensis* shrimp according to **Perazzolo *et al.* (2002)** is the number of hemocytes. Changes in the number of hemocytes to a certain extent are usually followed by changes in the composition of the differentiation of hemocyte cells (**Hartinah, 2012**). Furthermore, **Hartinah *et al.* (2014)** stated that, changes in the number of hemocytes and changes in the composition of cell differentiation can be early indicators of the early vitality condition of *Litopenaeus vannamei* juveniles. It is reasonable to assume that there is a higher shock to the cellular response expressed through the number of hemocytes and cell differentiation at the intensive technology level. High rearing density is a common consequence in intensive ponds since metabolic waste and unutilized artificial feed harm water quality, which can further cause stress for *Litopenaeus vannamei* juveniles. The stressful conditions of *Litopenaeus vannamei* juveniles can trigger significant outbreaks of deadly diseases in pond cultivation. Changes in the composition of blood cells and blood chemistry in several types of fish, and shrimp have been known to be related to stress, but the mechanism of this relationship is very complex and very little understood for *Litopenaeus vannamei*.

For this reason, research has been carried out to provide information on cellular responses through the expression of the number of hemocytes and cell differentiation during 24 hour monitoring so that extreme conditions that require extra attention are known at different levels of cultivation technology, namely semi-intensive and intensive. Monitoring the health of *Litopenaeus vannamei*, which includes observing hemolymph responses, constitutes the primary response. This proactive approach ensures a prompt action in case of any issues, particularly instances of sudden death in intensive ponds, which are often attributable to stress in *L. vannamei*.

This study aimed to examine the cellular response expressed through the number of hemocytes and hemocyte cell differentiation of juvenile white shrimp (*Litopenaeus vannamei*) at different levels of cultivation technology (semi-intensive and intensive). Intensive cultivation according to **Chiang and Liang (1985)** is characterized by high stocking density ($<100 \text{ PL/ m}^2$), ($>100 \text{ PL/ m}^2$).

MATERIALS AND METHODS

This research was carried out using the observation method, namely taking samples directly to intensive pond locations in Mallusetasi District and semi-intensive ponds in Soppeng Riaja District, Barru Regency. The frequency of sampling is 2 (two) times with an interval of 15 days when the juvenile *Litopenaeus vannamei* are ± 2 months old. Sampling was carried out at five sampling points, with each sampling point at the corner of the pond, and one sampling point in the middle of the pond. Hemolymph parameters observed include total haemocyte count (number of hemocytes) and percentage of hemocyte differentiation based on cell type.

Hemolymph was obtained from the ventral hemocoel in the second abdominal segment using a syringe needle. 1ml, 27 gauge hypodermic containing anti-coagulant (0.01 M tris-HCL, 0.25 M sucrose, 0.1 M Sodium citrate; at pH 7.6) was used for this purpose. The volume ratio between anti-coagulant and hemolymph volume was 3:1, then the number of hemocytes were counted using a hemocytometer (0.1mm) under a light microscope with a magnification of 1000 \times . The number of hemocytes was counted using a counter (**Gopinath & George, 2000**). The variables monitored, monitoring methods, and formulas used in data analysis can be seen in Table (1).

Table 1. The variables monitored, monitoring methods, and formulas used in data analysis

No	Variables	Method	Formula
1	Number of hemocytes	Modification of the Blaxhall and Daishley method (1973).	Number of hemocytes = $P \times N \times 10^3 \times 25 \times \text{cell/ml}$ Information : P = Number of small squares inside hemocytometer (16) N = Average number of hemocytes calculated in several small boxes 10^3 = Sample volume in a large box (if using a hemocytometer with a depth of 0.1 mm) 25 = Number of boxes in the hemocytometer
2	Cell differentiation	Martin and Graves (1995).	Calculated based on cell type to reach 100%

RESULTS

Cellular responses can be detected through changes in hemolymph. Hemolymph is one of the parameters of the immune system in shrimp which can be an indicator to determine its phagocytosis activity. It was further stated that hemocyte phagocytosis is directly proportional to the number of hemocytes (**Direkbusarakom & Danayadol, 1998**).

The application of the level of *Litopenaeus vannamei* cultivation technology, namely between intensive and semi-intensive, is very different, especially in the supply of feed and oxygen. To understand how conditions of low dissolved oxygen (1.8–2.0ppm), a challenge test was previously carried out on a type of bioluminescent bacteria, it was reported that this condition showed a significant negative effect on the body's immune system of *Litopenaeus vannamei* and therefore could cause disease outbreaks and death.

The number of hemocytes in juvenile *Lithopenaeus vannamei* tends to increase toward 14:30 PM, respectively, rising from 32×10^6 to 68.7×10^6 cells/ ml in semi-intensive ponds, and 34.8×10^6 to 64.8×10^6 cells/ ml in intensive ponds. Then it decreased toward 4:30 AM to 29.6×10^6 cells/ ml in intensive ponds and 33.6×10^6 cells/ ml in semi-intensive ponds.

Based on the cellular response expressed through the number of hemocytes monitored for 24 hours in semi-intensive and intensive ponds, it tends to increase at 16:30 PM, and there is a lower decrease in intensive ponds compared to semi-intensive ponds at 4:30 AM, but both do not cause stress. Along with changes in the number of hemocytes, the percentage composition of the number of hyaline hemocytes increased at 16:30 PM in semi-intensive ponds, but the percentage of globular cells in intensive ponds was more dominant at each monitoring time for 24 hours. The physiological condition of *Lithopenaeus vannamei* juveniles at 16:30 PM was the best, both in semi-intensive and intensive ponds, and based on the percentage composition of hemolymph cells, it is reasonable to assume that the immunity of *Lithopenaeus vannamei* juveniles in semi-intensive ponds was better than in intensive ponds.

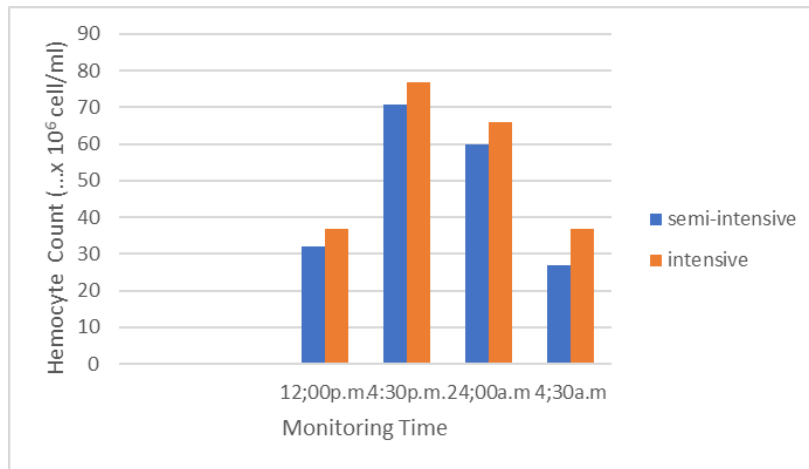


Fig. 1. Characteristics of the hemocyte count in *Lithopenaeus vannamei* juveniles during 24-hour monitoring in semi-intensive and intensive ponds

The percentage of each type of hemocyte in *Lithopenaeus vannamei* juveniles weighing 5– 16g was monitored over a 24-hour period, specifically at 12:00 PM, 4:30 PM, 12:00 AM, and 4:30 AM, in both intensive and semi-intensive ponds. The obtained characteristics are depicted in Figs. (2, 3).

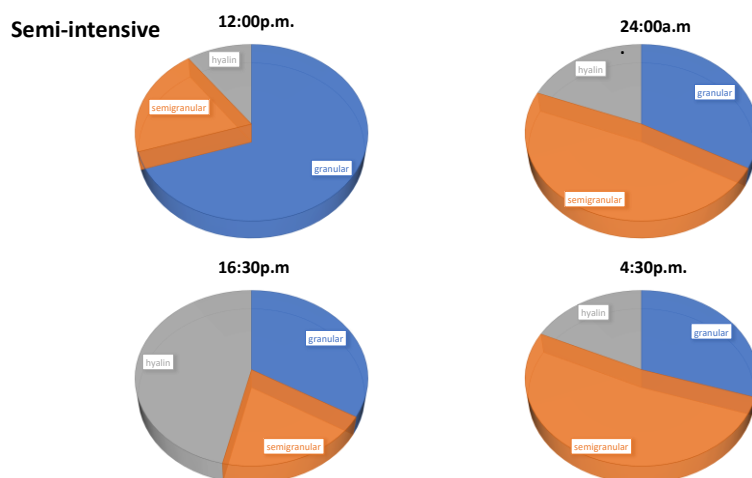


Fig. 2. Cell differentiation characteristics in semi-intensive ponds at different monitoring times

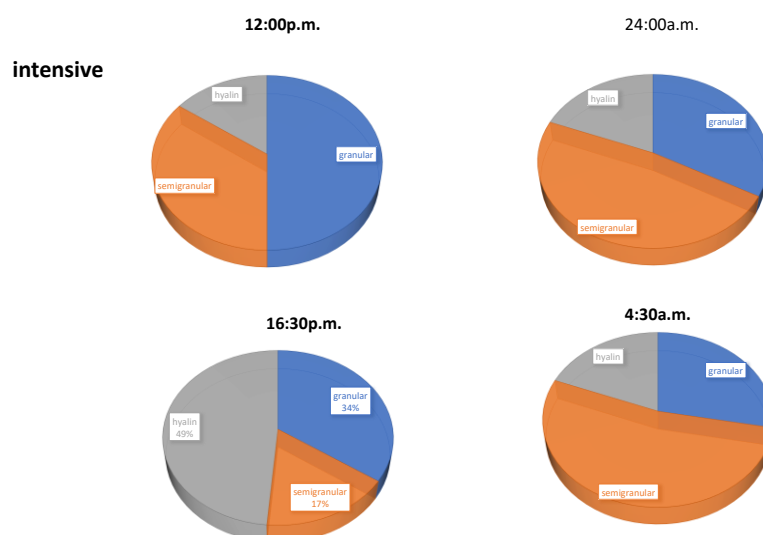


Fig. 3. Cell differentiation characteristics in intensive ponds at different monitoring times

With the observed increase in the number of hemocytes toward 4:30 PM, as previously explained, it is reasonable to assume that the immunity of the juvenile *Lithopenaeus vannamei* reared in both intensive and semi-intensive ponds is at its peak toward the evening (4:30 PM). In semi-intensive ponds, the percentage composition of hyaline cells is higher (54- 61%) compared to globular cells (33- 35%) and semi-globular cells (7- 14%). This indicates an increased ability to destroy macromolecules, such as DNA, carbohydrates, and pathogenic proteins as a form of resistance. Subsequently, hyaline cells decreased until monitoring at 4:30 AM in both semi-intensive and intensive ponds.

DISCUSSION

The number of hemocytes in the juvenile *Litopenaeus vannamei* tends to increase toward 14:30 PM, rising from 32×10^6 to 68.7×10^6 cells/ ml in semi-intensive ponds, and 34.8×10^6 to 64.8×10^6 cells/ ml in intensive ponds, respectively. Then, it decreased towards 4:30 AM to 29.6×10^6 cells/ ml in intensive ponds and 33.6×10^6 cells/ ml in semi-intensive ponds. The seller's response, as indicated by the juvenile white tiger prawns, can be understood to occur before 4:30 PM (Fig. 1). The juvenile *Litopenaeus vannamei* are actively eating, leading to an increase in energy supply, which in turn has a positive effect on increasing the number of hemocytes. An increase in the number of hemocytes is an indicator of increased body resistance (immunity); However, an increase in the number of hemocytes to a non-compensatory limit can cause stress in shrimp. Long before, **Maynard (1960)** had reported that shrimp could increase the number of hemocytes for body defense. The increase in hemocytes in intensive ponds, apart from the increase in consumption activity, before 16:30 PM is also thought to be triggered by environmental conditions due to the high density so that the oxygen content changes, even though the wheel is still installed. This increase is thought not to cause stress to the *Litopenaeus vannamei* juveniles that are reared. This is confirmed by data from dawn sampling, namely that the hemocyte count of the juvenile *Litopenaeus vannamei* decreased drastically compared to monitoring at 24:00 AM.

Increases and decreases in the number of hemocytes during 24-hour monitoring did not cause stress to the *Litopenaeus vannamei* juveniles in either intensive or semi-intensive ponds. According to **Wardoyo (1978)**, the most critical oxygen conditions in closed waters occur at 5:00, as a negative effect of competition from organisms living in the waters to obtain dissolved oxygen including plankton. That's why it is recommended to keep the water wheel installed optimally until 6:00 AM in intensive ponds.

Several previous studies reported that, in nature the number of hemocytes varies and is a function of the development stage, post-larval molt cycle of *Penaeus japonicus* (**Tsing *et al.* 1989; Wolffrom, 2004**). The number of hemocytes also varies due to environmental factors, such as salinity and temperature (**Oliver & Fisher, 1995; dan Rustam *et al.*, 2013**). If the temperature increases, then the number of hemocytes circulating in the hemolymph increases due to the need and strength of the heart pump. Additionally, the availability of food and nutrition, as well as disease infection also have an influence. If the supply of nutrients in the body decreases, as an effect of decreased appetite due to stress, the number of hemocytes will also decrease. Stress can be caused by environmental factors, feed, and high maintenance density, as well as disease infection. There is a decrease in the number of hemocytes as an effect of specific disease infections. This is evident from the fact that the number of hemocytes circulating in fish blood around the infected organs is greater than that recorded in the uninfected organs (**Kamiso *et al.*, 2009**). Research conducted by **Mahasri (2008)** showed that the immunized tiger prawn post larvae showed an increase in the number of hemocyte cells

from 50.99×10^6 to 69.91×10^6 cells/ ml. This increase in the number of hemocyte cells serves as an evidence of an immune response to post-larval tiger prawns. Furthermore, **Maynard (1960)** reported that shrimp can increase the number of hemocytes for body defense.

Several researchers previously reported that hemocyte cells have special functions according to their cell type. The function of globular cells of juvenile tiger shrimp in the presence of foreign objects in their body is to act as an anti-bacterial protein and increase the activity of enzymes that play a role in the body's defense system and can degrade if there are microbes when phagocytosis occurs. Meanwhile, hyaline cells have the function of destroying macromolecules, such as DNA, carbohydrates, and pathogenic proteins, as a form of resistance. **Hartinah et al. (2013)** reported that under stress conditions, juvenile tiger prawns measuring 6- 16g were able to survive, but their ability to fight against foreign objects in the body decreased. This was indicated by a decrease in the percentage of hyaline cells and a different behavioral pattern expressed.

The percentage of each type of hemocyte in juvenile tiger prawns measuring 5– 16g was monitored over a 24-hour period, specifically at 12:00 PM, 4:30 PM, 12:00 AM, and 4:30 AM, in both intensive and semi-intensive ponds. The obtained characteristics are depicted in Figs. (2, 3). Along with the increase in the number of hemocytes toward 16:30 PM, as previously explained, it is reasonable to assume that the immunity of the juvenile *Litopenaeus vannamei* reared in intensive and semi-intensive ponds is at its peak toward the evening (16:30 PM). In semi-intensive ponds, the percentage composition of hyaline cells is higher (54- 61%) compared to globular cells (33- 35%) and semi-globular cells (7- 14%). This indicates an increased ability to destroy macromolecules, such as DNA, carbohydrates, and pathogenic proteins, as a form of resistance. Subsequently, hyaline cells decreased until monitoring at 4:30 AM in both semi-intensive and intensive ponds. In intensive ponds, at 4:30 AM monitoring, there was a decrease in the number of the juvenile *Litopenaeus vannamei* hemocytes, which was lower than that recorded in semi-intensive ponds. During 24-hour monitoring, the percentage of globular cell hemocytes was higher, meaning that the immunity of the white tiger prawn juveniles, reared with intensive technology levels, was only able to survive while their ability to fight was lower compared to the *Litopenaeus vannamei* juveniles reared in semi-intensive ponds. This fact provides an information regarding that the *Litopenaeus vannamei* juveniles reared in intensive ponds are susceptible to stress. However, the observed decrease in the number of hemocytes and the percentage composition of hemolymph cells does not cause the reared *Litopenaeus vannamei* juveniles to experience stress. The results of this research support the farmer's opinion that *Litopenaeus vannamei* is more resistant to extreme environments compared to *Penaeus monodon*.

This phenomenon answers the problem of the intensive shrimp cultivation in ponds where crop failure often occurs, the solution of which has not yet been definitively

resolved. The stress experienced by the juveniles of *Lithopenaeus vannamei* causes them to solely survive, but their ability to fight or destroy foreign objects in their body decreases.

CONCLUSION

Based on the cellular response expressed through the number of hemocytes monitored for 24 hours in semi-intensive and intensive ponds, there is a tendency for an increase at 4:30 PM, and there is a lower decrease in the intensive ponds compared to the semi-intensive ponds at 4:30 AM. However, neither condition causes stress. Along with changes in the number of hemocytes, the percentage composition of the number of hyaline hemocytes increased at 16:30 PM in the semi-intensive ponds, but the percentage of globular cells in the intensive ponds was more dominant at each monitoring time for 24 hours. The physiological condition of the *Lithopenaeus vannamei* juveniles at 16:30 PM was at its peak, both in the semi-intensive and intensive ponds, and based on the percentage composition of hemolymph cells, it is reasonable to assume that the immunity of the *Lithopenaeus vannamei* juveniles in the semi-intensive ponds was better than in the intensive ponds.

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