Performance of juvenile vaname shrimp (*Litopenaeus vannamei*) hemocyte cells number and differentiation in different cultivation technologies

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Abstract
The Performance from The Number and Differentiation of Haemocyte Cell of udang vaname (*Litopenaeus vannamaei)* . Juvenile on Reared with Different Aquaculture Technology. This study aims to assess the performance of the number of hemocytes and cell differentiation of juvenile Udang vannamei (Litopenaeus vannamei) on reared with different levels of technology. The research was conducted by sampling directly to the location of intensive pond and traditional pond. The sampling frequency is 2 times at intervals of 15 days during the juvenile *Litopenaeus vannamaei* ± 2 months old. Hemolimfe parameters measured were the number of hemocytes, whereas the percentage of hemocytes differentiation by type of cell. The results showed that an increase in the number of juvenile litopenaeus vannamaei hemocytes on monitoring at 16:30, respectively rose from 30×106 cells/ml in traditional pond, and 34.8×106 cells/ml in intensive pond. at 12:00 monitoring, became 68.7×106 cells/ml traditional pond and 64.8×106 cells/ml in intensive pond. Furthermore, cell hyalin is higher percentage (54-61%) on traditional pond than globular cells (33-35%) and cell semiglobular (7-14%), but in intensive pond, globular cells is higher between hyaline cells and globular cells at each monitoring for 24 hours. This fact shows that the condition of vitality juvenile litopenaeus vannamaei reared on traditional pond more vibrant than litopenaeus vannamaei juveniles reared in intensive pond allegedly prone to stress, especially at dawn (4:30 a.m).

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Keywords: vaname shrimp, number of hemocytes, composition of hemocyte cell differentiation, intensive and traditional cultivation technology

Introduction

Increasing the production of vaname shrimp cultivation (*Litopenaeus vannamaei*) is always carried out by increasing stocking density with limited land and water resources, resulting in a decrease in the quality of cultivation water (Ariawan, 2015). The decline in cultivation water quality is caused by cultivation waste which contains organic materials and nutrients in the form of both suspended and dissolved particles. Shrimp cultivation waste in the form of organic material is the main source of ammonia in cultivation media. High levels of ammonia have a negative impact on the life of aquatic organisms and are toxic to organisms. Grow-out activities are an important part of cultivating vannamei shrimp that must be carefully considered. This happens because there are many failures in cultivating vannamei shrimp which are caused by negligence in the feed management process and the water quality of the rearing media so that disease attacks cannot be avoided. The difference in the level of intensive and traditional technology is mainly due to differences in the density of rearing and application of the level of technology and relying on artificial feed. as a nutrient supply.

The level of intensive and traditional technology can influence the performance of the hemolymph response of juvenile white shrimp. One of the hemolymph parameters that is most sensitive and constant to stress conditions in Farfantepenaeus paulensis shrimp farming according to Perazzolo *et al*., (2002) [11] 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 (Rustam, 2013) [12]. Furthermore, Hartinah *et al*., (2014) [6] stated that changes in the number of hemocytes and changes in the composition of cell differentiation can be early indicators of the vitality condition of vaname shrimp juveniles.

In connection with the previous phenomenon, it is reasonable to suspect that there is a higher shock to the performance of hemocyte numbers and cell differentiation at the intensive technology level. High rearing density is a common consequence in intensive ponds because metabolic waste and unutilized artificial feed have a negative impact on water quality, which can further cause stress for vaname shrimp juveniles. The stressful condition of vaname shrimp juveniles can trigger a significant outbreak of deadly disease in shrimp cultivation businesses in ponds. 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 vaname shrimp.

For this reason, research has been carried out to provide information on the performance of hemocyte numbers and cell differentiation during 24 hour monitoring so that extreme conditions that require extra attention are known at different levels of cultivation technology, namely intensive and traditional. Monitoring the health of vaname shrimp which refers to the hemolymph response is monitoring the primary response. So that if a problem occurs, it will be dealt with quickly, especially if sudden death occurs in intensive cultivation which is most likely caused by stress in white shrimp.

This study aims to examine the performance of the number of hemocytes and differentiation of different hemocyte cells of juvenile white shrimp (Litopenaeus vannamaei).

Methods

This research was carried out using the observation method, namely taking samples directly to intensive pond locations in Mallusetasi District and traditional ponds in Soppeng Riaja District, Barru Regency. The frequency of sampling is 2 (two) times with an interval of 15 days when the juvenile white shrimp are ± 2 months old. Sampling was carried out at five sampling points, namely each sampling point at the corner of the pond, 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 is obtained from the ventral haemocoel in the second abdominal segment using a syringe needle. 1 ml, 27 gauge hypodermyc containing anti-coagulant (0.01 M tris-HCL, 0.25 M sucrose, 0.1 M Sodium citrate; at pH 7.6). The volume ratio between anti-coagulant and hemolymph volume is 3:1, then the number of hemocytes is counted using a hemocytometer (0.1 mm) under a light microscope with a magnification of 1000×, the number of hemocytes is counted using a counter (Gopinath and George, 2000) [4].

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

No	Variables	Method	Formula
1	number of hemocytes	Modification of the Blaxhall and Daishley method (1973).	number of hemocytes = PxNx10^3x25xcell/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 haemocytometer with a depth of 0.1 mm 25=Number of boxes in the haemocytometer
2	Cell differentiation	Martin and Graves (1995).	Calculated based on cell type to reach 00 %

Results and Discussion

Hemolymph is one of the parameters of the immune system in white shrimp which can be an indicator to determine its phagosity activity. It was further stated that hemocyte phagocytosis is directly proportional to the number of hemocytes (Direkbusarakom and Danayadol, 1998) [2]. To understand how conditions of low dissolved oxygen (1.8–2.0 ppm), carried out by challenging types of bioluminescent bacteria, it was reported that these conditions showed a significant negative effect on the body's immune system of vaname shrimp and therefore could cause disease outbreaks and death.

The number of hemocytes in juvenile vaname shrimp tends to increase towards 14:30, respectively rising from 30×106 cells/ml to 68.7×106 cells/ml in traditional ponds, and 34.8×106 cells/ to 64.8×106 cells/ml in intensive ponds. Then it decreased towards 4:30 a.m. to 29.6×106 cells/ml in intensive ponds and 33.6×106 cells/ml in traditional ponds.

This can be understood because towards 16:30 the juvenile vaname shrimp are more active in eating, so that the energy supply increases which then 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 white shrimp. Long before, Maynard (1960) [10] had reported that shrimp could increase the number of hemocytes for body defense. The increase in hemocytes in vaname shrimp reared in intensive ponds, apart from increasing consumption activity before 16:30, is also thought to be triggered by environmental conditions due to high density so that the oxygen content changes. Conditions of ponds equipped with waterwheels in intensive ponds. However, this increase is thought to not cause stress to the reared vaname shrimp juveniles. This is confirmed by data from sampling at 4:30 a.m., namely that the number of hemocytes in juvenile vaname shrimp decreased drastically compared to monitoring at 24:00

p.m. Increases and decreases in the number of hemocytes during 24 hour monitoring did not cause stress to white shrimp juveniles in either intensive or traditional ponds. According to Wardoyo (1978) the most critical oxygen conditions in closed waters occur at 5:00 a.m., as a negative effect of competition from organisms living in waters to obtain dissolved oxygen, including plankton.

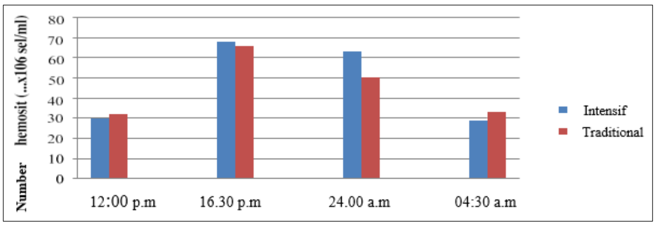

Fig 1: Characteristics of the Hemocyte Number of Vaname Shrimp Juveniles during 24 Hour Monitoring in Intensive and Traditional Ponds

That is why it is recommended to keep the waterwheel installed optimally until 6:00 a.m. 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) [13]. The number of hemocytes also varies due to environmental factors such as salinity and temperature (Oliver and Fisher, 1995), if the temperature increases then the number of hemocytes circulating in the hemolymph increases due to the need and strength of the heart pump, apart from that 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 infected organs is greater than in uninfected organs (Kamiso *et al*., 2009) [8]. Research conducted by Mahasri (2008) [9] showed that in immunized vaname shrimp postlarvae, there was an increase in the number of hemocyte cells from 50.99×106 cells/ml to 69.91×106 cells/ml. This increase in the number of hemocyte cells is evidence of an immune response to the post larvae of vaname shrimp. Maynard (1960) [10] 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 the globular cells of juvenile vaname shrimp is related to the presence of foreign objects in their body, namely acting as an anti-bacterial protein and increasing the activity of enzymes that play a role in the body's defense system and being able to 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) [5] reported that under stressful conditions, juvenile vaname shrimp measuring 6-16 g were only able to survive, but their ability to fight if there was a foreign object in the body decreased, which was indicated by a decrease in the percentage of hyaline cells and a different behavioral pattern. expressed.

The percentage of the number of each type of hemocyte in juvenile vaname shrimp measuring 5 g – 16 g as monitored for 24 hours, namely at 12:00 p.m., 16:30 p.m., 24:00 a.m. and 04:30 a.m. in intensive ponds and Traditionally, the characteristics obtained are as shown in Figure 2 and Figure 3.

Along with the increase in the number of hemocytes towards 16:30, as previously explained, it is reasonable to assume that the immunity of white shrimp juveniles reared in traditional and intensive ponds is best towards evening (16:30). In traditional ponds, the percentage composition of hyaline cells is higher (54-61%) compared to globular cells (33- 35%) and semiglobular cells (7-14%), meaning the ability to destroy macromolecules such as DNA, carbohydrates and pathogenic proteins as a form of resistance. increase. Subsequently, hyaline cells decreased until monitoring at 4:30 in both traditional and intensive ponds.

In intensive ponds, at 4:30 monitoring there was a decrease in the number of white shrimp juvenile hemocytes, which was lower than in traditional ponds. During 24 hour monitoring, the percentage of globular cell hemocytes was higher, meaning that the immunity of vaname shrimp juveniles reared with intensive technology levels was only able to survive while their ability to fight was lower compared to vaname shrimp juveniles reared in traditional ponds. This fact provides information that white vaname shrimp 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 vaname shrimp juveniles to experience stress.

This phenomenon answers the problem of intensive shrimp cultivation in ponds where crop failure often occurs, the solution of which has not yet been definitively resolved. The stress experienced by tiger tiger juveniles means they are only able to survive but their ability to fight or destroy foreign objects in their bodies decreases.

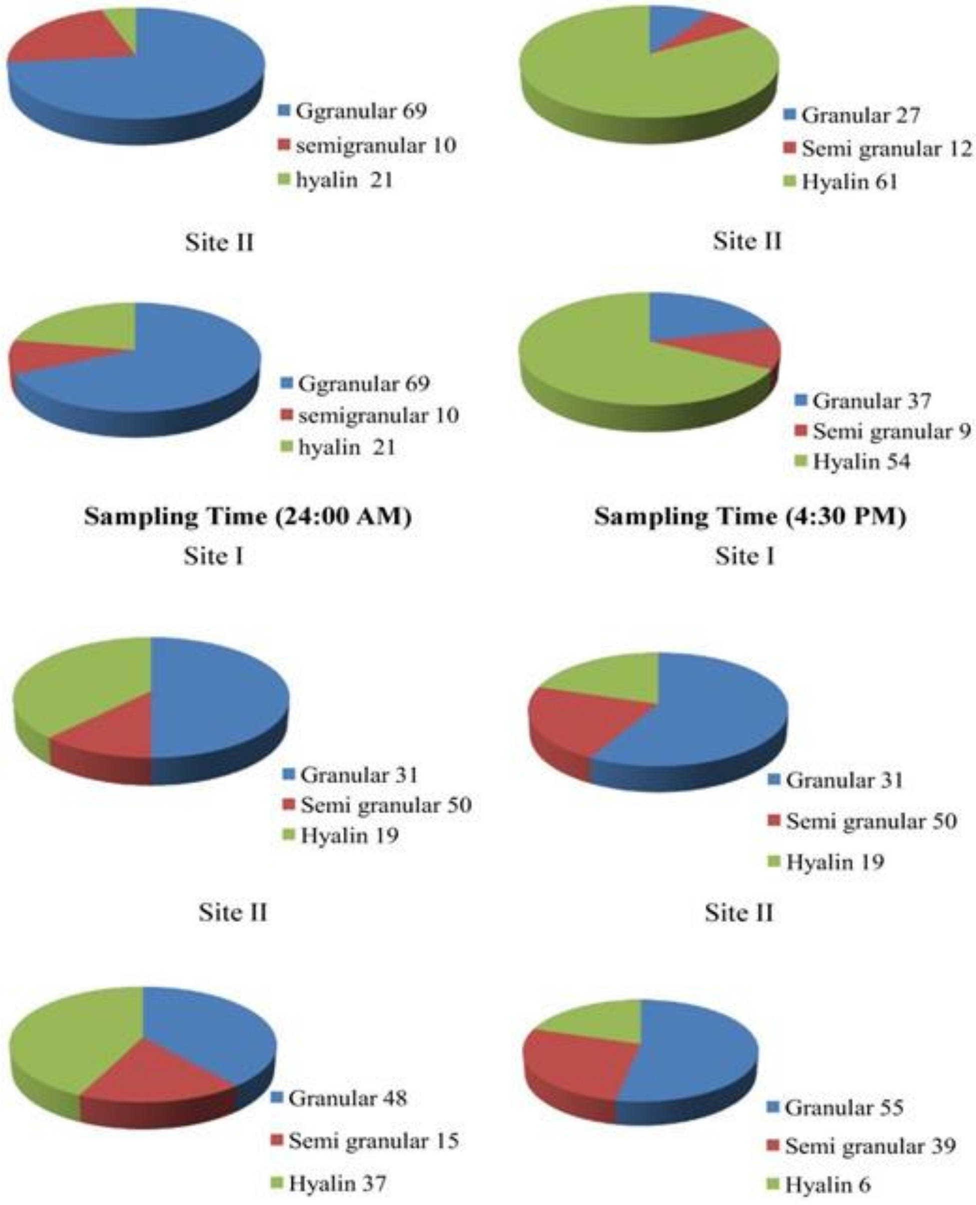

Fig 2: Difernsiasi sel hemosit monitoring pond tradisional

Conclusion

Based on the number of hemocytes monitored for 24 hours in traditional and intensive ponds, it tends to increase at 16:30, and there is a lower decrease in intensive ponds compared to traditional ponds at 4:30, but neither causes stress. Along with changes in the number of hemocytes, the percentage composition of the number of hyaline hemocytes increased at 16:30 in traditional ponds, but the percentage the number of globular cells in intensive ponds was more dominant at each monitoring time for 24 hours. The physiological condition of vaname shrimp juveniles at 16:30 was the best, both in traditional and intensive ponds, and based on the percentage composition of hemolymph cells, it is reasonable to assume that the immunity of vaname shrimp juveniles in traditional ponds was better than in intensive ponds.

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