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Alteration of Phagocytic Activity of White Shrimp *Litopenaeus vannamei* (Boone, 1934) Reared in Isosmotic Salinity and Different Density

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Abstract

Effect of stocking density to assess phagocytic activity changes of white shrimp has been carried out. White shrimp reared at a salinity of 25 ‰ with five levels of density that is 150, 250, 350, 450, and 550 shrimps/m^3, with three replications. Phagocytic activity of hemocytes cells was analyzed at time 0, 24, 48, 96, and 144 hours after exposure to different density. The phagocytic activity data were analyzed by repeated measurement analysis of variance at the 5% significance level. Phagocytic of the density of 150, 250, and 350 shrimps/m^3 showed significant difference with a density of 450 and 550 shrimps/m^3. Phagocytic activity decreased fastest occur at the level densities of 450 and 550 shrimps/m^3 which is at the 24 hours after the treatment and recovery of the 144 hours, while the level of density of 350 shrimps/m^3 decreased activity at 96 hours after the treatment, and the level of density of 150 and 250 shrimps/m^3 relatively stable. These findings indicate that increasing stocking density increased phagocytosis activity disorder and disturbance density against phagocytic activity occurs faster in high population density.

Keywords: White shrimp; *Litopenaeus. vannamei*; phagocytic activity; stress; density.

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1. Introduction

White shrimp, *Litopenaeus vanamei,* is one of the major aquaculture commodities in the world, including Indonesia, which is an alternative to the cultivation of organisms after the decline of the production of tiger shrimp *Penaeus monodon.* These organisms have a better resistance to disease and environment than the tiger shrimp and can be maintained at a high density. Cultivation of the shrimp is still encountering many problems in the form of infectious diseases, particularly viral infections Taura syndrome virus [1] and the bacteria *Vibrio alginolyticus* and *Vibrio harveyi* [2,3]. White shrimp can live on a wide range of salinity of 1-2 ‰ to 40 ‰ [4,5] that is hyper-osmotic in low salinity and hypo-osmotic in high salinity with isosmotic point 718 mOsm / kg is equivalent to 25 ‰ [5].

Increasing stocking density is one of the strategies to optimize productivity in shrimp farming. Shrimp stocking density is one of the technical factors that directly affect the survival, growth, behavioral, health, production and profit [6]. In semi-intensive cultivation of *L.vannamei* for three months can be produced 1,825 kg/ha of shrimp 10 g with a stocking density of 25 shrimps/ m^2 [7], within 85 days had obtained 2,363 kg/ha of shrimp measuring 18.5 g with a stocking density of 18 tail -22 / m^2 [8]. White shrimp were maintained at a density of 300 shrimps/m^2 showed fast growth and high survival rate [9]. Different things reported that increased stocking density of 5,720 to 11,430 shrimp/m^3 does not significantly increase biomass production [10], indicating a decline in the growth of the individual. High stocking density affects the biochemical composition, physiological condition, immune status, and hematological [11], and inhibit the metabolic activity of antioxidant enzymes and leading stress expressed by mRNA expression levels of Hsp 70 which significantly increased [12].

Aquatic organism growth performance is largely determined by the stability of the physiological condition and ability of immune response to face fluctuating environmental conditions. The immunity aquatic invertebrates, including shrimps, which has no antibodies rely heavily on natural defense system (innate immunity) against pathogens. The defense system carried out by hemocytes circulating in hemolymph very important for cellular and humoral immunity of shrimp [13,14]. Hemocytes cellular immune responses such as phagocytosis, nodule formation, and encapsulation, and humoral response i.e. prophenoloxidase and release of antimicrobial peptides and lysozyme [15,16]. In the natural defense system, macrophages are phagocytic cells play a key role in responding to incoming pathogens without waiting time adaptation. Phagocytosis to form reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radicals [17].

Phagocytic activity sensitive to naturally environmental changes and the decreased phagocytic activity can be caused by an anthropogenically-induced stressor, and immune systems also showed seasonal variation due to physiological changes in the organism [18]. One of the stressors which can affect the immune system is the stocking density level. High density can stimulate the hypothalamus-pituitary axis-interrenal (HPI) on neuroendocrine system and therefore increase plasma cortisol fish [19,20,21]. Increased plasma cortisol inhibits the making of food and feed conversion efficiency [22] and led to an increased need for energy to fight stress. Increasing the number of corticosteroids released during stress affects the non-specific immune system that causes disruption to the phagocytic activity [23]. However, very little scientific data revealing the relationship between the stocking density and phagocytic activity changes of white shrimp, *L. vannamei* hemocytes.

Consequently, the purpose of the present work was to evaluate the phagocytic activity changes of *L. vannamei* hemocyte cells induced by rearing stocking density in isosmotic salinity.

2. Materials and Methods

2.1. Experimental animals and rearing conditions

Shrimp used in this experiment is the white shrimp, *L.vannamei*, measuring 11 ± 1.2 g were obtained from shrimp farming centers in Mandale Pangkep, South Suawesi, Indonesia. The shrimp was transported from farms with a salinity of 15 ‰. Prior to using the shrimp acclimatized on rounded fiberglass tanks with a capacity of 0.3 tons. Salinity levels increased daily to reach the salinity of 25 ‰ (isosmotic). Acclimatization was continued for a week after reach the isosmotic level. To maintain the water quality parameters remain optimal, the acclimatization process is done by passing the water recirculation system with biological filter media. During the acclimatization and the research process, tested shrimp fed with a commercial diett containing 35% protein twice daily at 5% of their body weight in the wet laboratory. After one week of acclimatization at a salinity of 25 ‰, a group of shrimp test taken at random from each tank acclimatization to measure phagocytic activities as the baseline data (n = 10).

The experiment was conducted using a rectangular container with a capacity of 30 L and shrimp test maintained for a week at 25‰ salinity. Water was continuously aerated, changed at 30% level and siphoned of food remains unutilized daily.

2.2. Experimental Design

Tested shrimp is distributed into a rectangle container and containing 30 L of seawater at salinity 25 ‰. This experiment was designed with five levels of density that is 150, 250, 350, 450, and 550 shrimp/m^3 with three replications. Sampling hemocytes and phagocytic activity measurements performed at 0 (baseline), 24, 48, 96, and 144 hours after exposure to different density.

2.3. Hemolymph Sampling

Hemolymph was extracted from the ventral sinus of sampled shrimp from the base of the first swimming leg. 0.1 ml of hemolymph taken with 1 ml tuberculin syringe *(needle* 26G x ½ ") which contains 0.3 ml anticoagulant (30 mM tri sodium citrate, 12:34 M sodium chloride, 10 mM EDTA at pH 7:55, 0.115 M glucose [24]) were homogenized by shaking hands forming a figure eight for 5 minutes.

2.4. Measurement of phagocytic activity

Shrimp hemolymph pipetted of 0.1 ml into microplate and mixed thoroughly with 25 mL of Staphylococcus sp. and incubated for 20 minutes. As many as 5 mL of the incubated hemolymph was dropped on the object glass and a thin monolayer slide was made by smear with another object glass. Furthermore, fixed with 100% methanol for 5 minutes and stained with Giemsa (10%) for 15 minutes. Phagocytic activity measured by the percentage of phagocytic cells that show phagocytic activity [25]. The phagocytic activity was calculated using

the formula:

$$\textbf{Phagocytic activity} = \frac{\textbf{Number of phagocytic cells that perform phagocytosis}}{\textbf{Number of phagocytic cells}} \textbf{ x 100\%}$$

2.5. Data Analysis

To determine the effect of stocking density of white shrimp on the phagocytic activity dynamic, the phagocytic activity data were analyzed using repeated measures analysis of variance [26]. The difference among treatment was analyzed with Duncan's multiple range test. Before the data were statistically analyzed, normality and homogeneity of data were analyzed to anticipate the data outliers that can affect the results and statistical inferences. The statistical difference each data comparisons was setting up at 5% significance level. Data analysis was performed using STATISTICA software release 5 (StatSoft, Inc.).

3. Result

Phagocytic activity of tested shrimp at pre-stocking density treatment was 83.59 ± 1.19% (78.10 to 88.00%) and decreased after 24 hours of treatment densities by 8.22%, 19:17%, 18.71 %, 20.93% and 29.18% for the treatment of 150, 250, 350, 450, and 550 shrimps/m^3, respectively (Table1, Figure 1). The declined phagocytic activity of 150, 250 and 350 shrimps/m^3 not continue until the 48-hour post-treatment and is likely to increase up to the 144 hours post-treatment, except for the treatment of 350 shrimps/m^3 was reduced in 96 hours post-treatment and increased again up to 144 hours post-treatment.

The densities of 450 and 550 shrimps/m^3 showed phagocytic activity continued to decline up to 96 hours and subsequently increase up to 144 hours' post-treatment. Such changes indicate that the phagocytic activity in the treatment immediately returned to the original value at the 48 hours post-treatment after a significant decline in the 24-hour post-treatment.

The pattern of changes in treatment phagocytic activity densities of 150 and 250 shrimps/m^3 tend to be the same as the level of activity was higher in the treatment of 150 shrimps/m^3. The changes of phagocytic activity of stocking density of 350 shrimps/m^3 which is initially equal up to the 48 hours post-treatment with the pattern that of the density of 150 and 250 shrimps/m^3 was changed within 96 hours and follow the pattern of the density of 450 and 550 shrimps/m^3.

Both treatments of the latter show equal pattern change in phagocytic activity. Densities of 450 and 550 shrimps/m^3 showed phagocytic activity continued to decline until 96 hours and increased to 144 hours post-treatment indicate that the phagocytic activity both treatment levels tend experiencing recovery density at 144 hours post-treatment.

Phagocytic activity values of 150, 250, 350 shrimps/m^3 showed were higher and significantly different with 450 and 550 shrimps/m^3 ($P < 0.05$) which both of the treatment of the latter did not show significant differences ($P > 0.05$). The difference among treatments indicated that increasing density $\geq$ 350 shrimp/m^3 can cause a decrease in

phagocytic activity.

Phagocytic activity of tested shrimp before exposure to density treatment showed the highest activity and significantly different with the value of activities at the exposure time of 144 hours ($P < 0.05$), and both of them show a difference also with an exposure time of 24, 48, and 96 hours that have lower phagocytic activity ($P < 0.05$). This finding indicates that the exposure time of density for 24 to 96 hours have a negative influence on the phagocytic activity leading a significant drop. The findings also indicate that the phagocytic activity increased back toward the original value of 144 hours post-treatment.

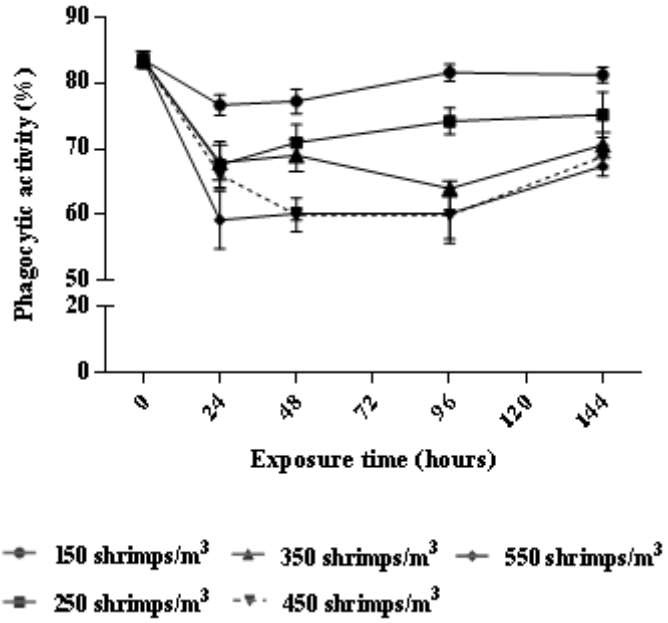

Figure1: The Phagocytic activity of white shrimp, *L. vannamei* (Boone, 1934), reared at a constant salinity of 25 ‰ and differentt density. Vertical bars show mean ± SE

Table 1: Changes in phagocytic activity of white shrimp, L. vannamei, hemosit which is reared at a constant 25 ‰ salinity and different density

Exposure Time (hour)	Changes of phagocytic activity (%)									
	150 shrimps/m^3		250 shrimps/m^3		350 shrimps/m^3		450 shrimps/m^3		550 shrimps/m^3	
	Per time	Kumulatif	Per time	Kumulatif	Per time	Kumulatif	Per time	Kumulatif	Per time	Kumulatif
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	-8.22	-8.22	-19.17	-19.17	-18.71	-18.71	-20.93	-20.93	-29.18	-29.18
48	0.71	-7.57	5.78	-15.07	1.62	-17.39	-9.27	-28.27	1.58	-28.05
96	5.66	-2.34	5.37	-11.21	-7.34	-23.45	-0.06	-28.31	0.13	-27.96
144	-0.44	-2.76	1.62	-10.04	10.38	-15.51	14.89	-17.64	11.98	-19.33

Effect of interaction density of 150 shrimps/m^3 with a time 24, 96, and 144 hours showed no difference in phagocytic activity before treatment density (P> 0.05). Three first interaction showed no difference in the value of activity also with interaction density of 150 shrimps/m^3 with a time 48 hours and the interaction density of 250 shrimps/m^3 with a time of 24.48, 96 dan144 hours, interaction density of 350 shrimps/m^3 with a time of 24.48, and 144 hours, the interaction density of 450 and 550 shrimps/m3 with a time of 144 hours. The condition indicates that the phagocytic activity of white shrimp was maintained at a density of 150 shrimps/m^3 at that time did not change from the original value and the treatment density of 250 shrimps/m^3 showed activity that is relatively stable though different from the original value. Interaction density of 550 shrimps/ m^3 with a time 24 hours which showed the lowest phagocytic activity was no different from the interaction density of 350 shrimps/m^3 with a time 96 hours, the interaction density of 450 shrimps/m3 to 24, 48 and 96 hours, and the interaction density 550 shrimps/ m^3 at 48 and 96 hours (P> 0.05) but significantly different with all the interaction density and other time (P <0.05). These findings indicate that densities of 350, 450, and 550 shrimps/m3 decreased phagocytic activity significantly sequentially each at 96, 24, and 24 hours after exposure density. The pattern of changes in the phagocytic activity of white shrimp reared at a salinity of 25 ‰ constant and different density levels are likely to follow the pattern of cubic polynomials (Table 2). The pattern of these changes indicates that the of phagocytic activity of white shrimp experienced two peak phase changes as a decrease in a certain period of a disturbance density and increased again after being able to adapt to interference received. Phagocytosis is a cellular defense mechanism of the most common [27] which can remove foreign particles in the organism hemocoel crustaceans [28]. Phagocytic activity may change as a result of the stimulation or disorder that triggers phagocytic cells to do the activity. Changes in the environment may put pressure on the immune response of crustaceans that can disrupt the activity of phagocytosis. Phagocytic activity was found to increase with increasing density of the reared of shrimp indicate disturbances that trigger changes in the activity of phagocytic cells.

Table 2: The equation of the regression and coefficient of determination (R^2) phagocytic activity of white shrimp *L. vannamei* (Boone, 1931) reared at a constant salinity of 25 ‰ and different densities

Density (shrimps/ m^3)	**Regression equation**	**R^2**
2150	$y = -0.7119x^3$ 7.3112x+^{2} - 23.206x + 100.19	0.6991
250	y = 3-0.4113x 4.5025x 2^+ - 18.031x +97 446	0.8900
350	$y = -1.6655x^3 + 18.633x^2$ - 63.866x + 129.61	0.9312
450	$y = -1.8258x^3 + 20.253x^2$ - 69.699x +132.84	0.8427
550	$y = 3\text{-}1.6001x + 18.360x^2$ - 66.268x + 131.27	0.8794.

High density causes an increase in plasma cortisol [19] and or hyperglycemia hormone (CHH, hyperglycemic crustacean hormone), which is one indicator of stress. Increased plasma cortisol induces a variety of physiological responses [29], including a decrease in the immune response [30]. When there is stress, allostatic load significantly affects the immune system and *energetic-metabolic machinery.* In this case, requirement energy is an increase during stress occurs because of a significant amount of energy used continuously, causing growth and low activity. In addition, some of the immune response while not active, especially immunocytes synthesis, therefore increasing the susceptibility of the organism against pathogens. Synthesis immunocytes

disturbed affect the number of phagocytic cells that circulate in hemolymph resulting in decreased phagocytic activity. A decrease in phagocytic activity of hemocytes caused by stress due to the density may also be the effect of a decrease in the ability of cells to modify the morphology and formed podosome and pseudopodia needed to move towards and ingest foreign material [31,32].

4. Conclusions

The results of this study indicate that white shrimp were maintained at density levels of ≥ 350 shrimps/m^3 impaired phagocytic activity, as indicated by a significant decrease in phagocytic activity. Impaired phagocytic activity progressively with increasing levels of rearing density where the rate of 150 and 250 shrimps/m^3 were relatively stable and densities of 450 and 550 shrimps/m^3 impaired early in 24 hours up to 96 hours post-treatment, while the density of 350 shrimps/m^3 started to crash at 96 hours post-treatment. Shrimp that maintained at the densities of ≤ 350 shrimps/m^3, though it showed phagocytic activities changes which it was not significant, were immunocompetence. The effect was due to the density stress that can disrupt the immune system of shrimp, therefore it is very important to consider the factors that can lead to stress in the cultivation process to avoid the risks associated with the impaired immunity. This research points show the importance of proper management measure to minimize high-density stress in *L vannamei* culture to avoid loss due to pathogenic infection, thus to maintain comfort and the growth of shrimp have maintained better, is recommended to avoid excessive density level so that energy that converted from feeding is solely used for the growth of such organisms.

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