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Characteristics of Haemolymph's Juvenile Tiger Prawn, *Penaeus monodon* (Fabricius) Reared in Ponds

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Abstract: The mechanism of stress and diseases resistance to extreme environments of tiger prawn (*Penaeus monodon*, Fabricius) is very complex and little understood. Information about the characteristics of natural haemolymph can be a useful guide to assess the performance of physiological responses of juvenile tiger prawn to extreme weather and environmental stresses and changes. The objective of this paper is therefore to examine and characterize the juvenile tiger prawns natural haemolymph. The study was conducted through three field observation times within a 10 days interval period when the juvenile tiger prawns were about two months old. Sampling of juvenile tiger prawns each weighing 5-16 g were carried from four to six random location points at each corner of the pond where two points were taken from the middle of the pond. Growth parameters of the juvenile tiger prawn haemolymph such as the number of hemocytes, total protein, glucose, plasma protein osmotic and the percentage types of different hemocyte cells were monitored. Using a correlation analysis (*á* = 0.01), it was observed that the increased weight of juvenile tiger prawn was positively correlated to the increased number of hemocytes, total protein and haemolymph plasma osmolality. However, it was not correlated with increased haemolymph glucose. Based on the percentage types of hemocytes, the small juvenile tiger prawns had a high body resistance towards diseases and environmental stress but a low percentage of the alleged hyaline cell antibody function which were in contrast to the large size juvenile tiger prawns. This study implies that bigger size tiger prawns need a careful treatment when reared in a pond. More studies need to be done in the future to obtain more information on the response of different sizes of tiger prawns to physiological stresses as a results of extreme environmental conditions.

Key words: Characteristics · Haemolymph · Hemocyte · Glucose · Plasma osmolality · Juvenile tiger prawn

INTRODUCTION

Nowadays, rearing of tiger prawns (*Penaeus monodon*, Fabricius) are very vulnerable to diseases which may lead to death due to the intense and prolonged stress from homoeostasis. Stress to a certain extent is not always harmful; instead it can be a form of adaptive response by the juvenile tiger prawns. Despite such situation, excessive response may threaten the growth of the juvenile tiger prawns due to severe pathological conditions and deadly diseases [1]. In most aquaculture projects, tiger prawn may be exposed to the stressed

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interactions between extreme climatic conditions and pathogens of which the mechanism is still very complex and little understood. In order to determine the characteristics of haemolymph, in-depth information is required to examine the relationship between stress and mechanisms of tiger prawn and resistance to extreme environments and viral diseases.

Haemolymph is a sensitive indicator to characterise and assess the vitality of juvenile tiger prawns to be reared in ponds. In lieu of the above, the objective of this paper is to examine the important features of haemolymp juvenile tiger prawns that were reared in the ponds.

MATERIALS AND METHODS

The research was carried in an aquaculture pond farm owned by Balitbangda Provincial Research Station, which is located in District Kupa, Barru South Sulawesi. The analysis of juvenile prawn samples were conducted in The Maros Brackish Water Aquaculture Research Center, Makassar Bestari Permai Clinical Laboratory and Laboratory of Disease and Agriculture Polytechnic, respectively in Pangkep, South Sulawesi. The samples used in this study were two months juvenile tiger prawns weighing 5-16 g each under artificial feeding. Field sampling and measurements were done three times a day with interval of 10 days.

Sampling was conducted at six points at four corners of the pond. Samples were also taken from two points in the middle of the pond. The parameters observed and measured were total hemocyte count (THC), the percentage of hemocytes differentiation by types, glucose, total haemolymph protein and plasma osmolality as supporting data to monitor water physico-chemical properties. Sampling on haemolymph tiger prawn was obtained from the ventral abdominal using a 1 ml needle syringe and 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 the anti-coagulant with haemolymph volume is 3:1, the number of hemocytes was calculated by using hemocytometer (0.1 mm) under a light microscope with a magnification of 1000x, while the counting of hemocytes was done using a calculator [2].

The glucose level of haemolymph was determined using the Enzymatic/GOD-PAP) standard glucose solution of 100 mg/dl and recommended equations [3]. The total haemolymph protein was determined using the Biuret method with standard protein of 6 g/dl and calculated with a formula by Bradford Protein Essay. The plasma osmolality of the haemolymph was obtained by injecting into the avendoff tubes and centrifuge at speed 3,000 rpm for 5 minutes. Later part of the supernatant (plasma) was taken using a micropipette automatic of 0.1 ml sample tube inserted in the Fiske osmometer tool, Micro-osmometer Model 210 with 1ìl sample volume.

Rearing of the prawns was done traditionally in a pond where the prawns were fed four times a day at 0600H, 1400H, 1800H and 2400H). Feed dose is 5% weight of biomass. The nutrient content of the feed was comprised of 30.88% protein, 12.74% carbohydrate, 17:46% fat, 7.6% moisture and 11% ash. All statistical data obtained were subjected to a correlation test at the 95% confidence level [4].

RESULTS AND DISCUSSION

Number of Hemocytes Counts: Figure 1 indicated that the hemocytes of the juvenile tiger prawn increased with increasing size and weights, but there was no increase with the age. Based on the correlation test is known that the number of hemocytes was positively correlated to the size of the weight of juvenile tiger prawn (*á* = 0:01). This fact supports the results of previous studies that the number of hemocytes varied in nature and is a function of the stage of development, post-larval moulting cycle of *Penaeus japonicas* [1]. Further, it is found that the number of hemocytes also varies due to salinity and temperature. When the temperature increases, the number of circulating hemocytes in the haemolymph also increases due to the energy required to pump the heart. In addition it involves the availability of feed and nutrition, infectious diseases as well their effects. If the nutrient supply in the body is reduced, as the effect of decreased appetite due to stress, it will also decrease the number of hemocytes [5, 6]. Stress can be due to the environment, food and intensive rearing as well as infectious diseases. The reduced number of hemocytes due to infectious diseases specifically proved that the number of hemocytes circulating in the blood of fish around the infected organ is greater than in uninfected organs [7]. The previous research has shown that post-larval shrimps that are immunized were seen to be on an increase in the number of hemocytes cells of 50.99 x 10^6 cells/ml to 69.91 x 10^6 cells/ml. Subsequently, it was reported that an increasing number of haemocyte cells are evidence of immune responses to the post larval shrimp [8, 9].

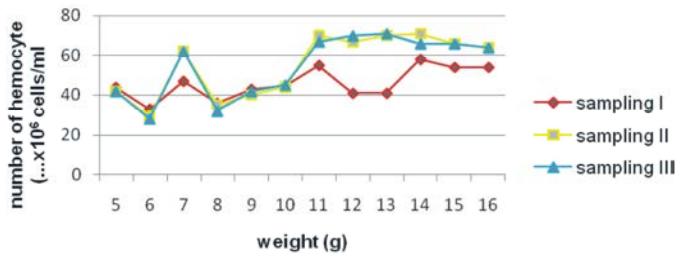

Fig. 1: The relationship between the weight (g) of juvenile tiger prawn against the number of hemocytes

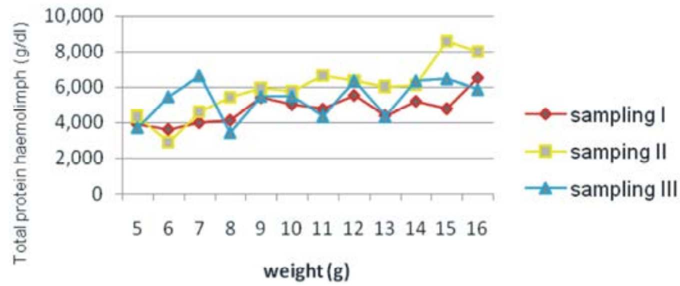

Fig. 2: Graph relationship between weights (g) of juvenile tiger prawn with total protein haemolymph (g/dl)

Total Protein of Haemolymph: Total protein of haemolymph is one of the three types of proteins in the body that are formed from amino acids in the form of colloidal solution in the blood plasma. The usefulness of the blood proteins are to circulate lipid molecules, hormones, vitamins and iron, protease inhibitors and precursors, regulatory activity, non functional in the cellular immune system. In addition, the number of hemocytes based on the results of statistical analysis has shown that the total protein haemolymph juvenile tiger prawn weighing 5-16 g each is also correlated with increased weight (á = 0:01). Tiger prawn haemolymph total protein intake is influenced by nutritional, physiological and intermouth stadia conditions. The need for protein in the growth of young tiger prawn is higher than on the needs of adult tiger prawn. When the tiger prawns are ready to reproduce, their protein requirements decreased, but there is a need to increase the nutritional intake of fat since fat is required for the formation of egg cells while the energy for growth has been declining. Therefore, a protein intake of nutrients needed for growth and the fact that in this study the total protein of haemolymph in juvenile tiger prawn is increasing its weight is a necessity as displayed in Figure 2.

Further statistical analysis showed that the total haemolymph protein was positively correlated to the increase in weight (á = 0:01). Similar results was obtained in total male and female haemolymph protein of *Macrobrachium rosenbergii* weiging 17-48 g, but there was an increase in *Macrobrachium rosenbergii* males weighing 50 g [10, 11]. It was also reported that a decline in total protein, osmolality and solubility of Cl^-ions, Na^+, K^+, Mg^{2+} and Ca^{2+} in the blood of the matured gonads of a pond cultivated tiger prawn [10, 11]. Many other researchers have reported that there was an association between increased weight and total haemolymph protein in the number of juvenile tiger prawn hemocytes [12]. This is due to the fact that the haemoglobin in red blood cells of fish composed of protein. The higher the haemoglobin, the higher will be the total haemolymph protein [12]. Nevertheless, simillar research has not been carried out in the black tiger prawn. Based on this phenomenon, it can be assumed that the higher the number of hemocytes in juvenile tiger prawn, the higher the total haemolymph protein since hemocyte cells are mainly composed of protein as can be found in the haemoglobin of fish cells. Based on the haemolymph low total protein level and high dietary fat, it can be assumed that juvenile tiger prawn weighing 5-16 g was still in the early growth period. More research on salinity, quantity and quality of feed and intensity of prawn rearing should be undertaken by the relevant authorities.

Haemolymph Glucose: Figure 3 showed that the glucose in the haemolymph juvenile tiger prawn was not correlated with the weight increase (á = 0.05 level). Glucose fluctuations occur in tiger prawn haemolymph when there were external and internal disturbances such as extreme environments, high rearing density, infectious diseases that may lead to stresses in the tiger prawn.

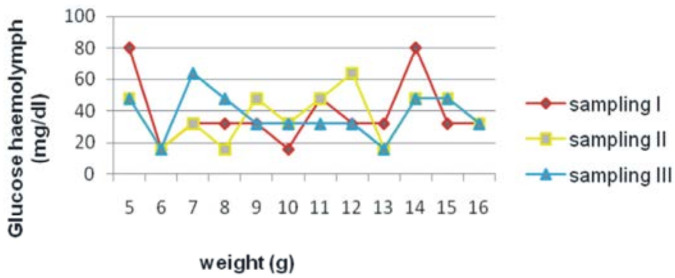

Fig. 3: The relationship between weight graph (g) of total juvenile tiger prawn and haemolymph glucose (mg /dl)

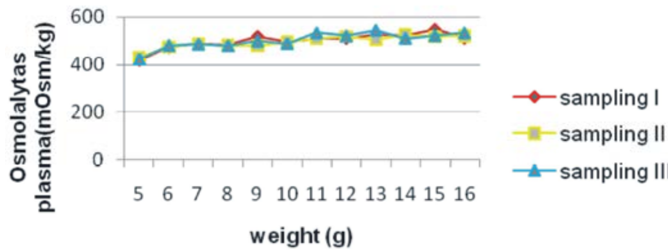

Fig. 4: The relationship between weight (g) of juvenile tiger prawn and plasma osmolality (mOsm / kg)

Sensitive quick fluctuations in glucose haemolymph of juvenile tiger prawn were probably due to the external interferences during sampling. The high glucose content found in the haemolymph is one of the efforts by the stimulus in respond to the stress. This is one of the early indicators of the tiger prawn's physiological responses to increase resistance during the homoeostasis process. The response to these external factors is controlled by the endocrine system through the release of the hormone cortisol and cotecholamine [13-15]. There was an increase in glucose metabolism in the body triggered by the hormone cortisol and catecholamines. Stress causes increased secretion of cortisol (glucocorticoids) which in turn can trigger an increase in blood glucose of fish [16]. Gluconeogenesis, lipolysis, glycogenesis and lipogenesis each play a significant role in maintaining the stability of blood glucose. The conversion of glucose to glycogen or fat is stored as deposits to be converted into glucose which gets into the fish blood. Therefore, the number of mechanisms that play a role in maintaining blood glucose homeostasis, stability of blood glucose is essential to the health and life of the tiger prawns [17].

Plasma Osmolality: Plasma osmolality is another haemolymph parameter which was measured. In fact, the plasma osmolality of juvenile tiger prawn increased with increasing weight size as shown in Figure 4. At the time of the pond salinity sampling, a 32-40 ppt was observed and such range was higher than the optimal value for juvenile tiger prawn. The optimal plasma osmolality for a tiger prawn at the weight range of 11-16 g is 26 ppt while at 5-10 g weight, the salinity should be at 20 ppt. The salinity of the water in the pond was higher as tested using the bivariate correlation test. It is known that an increase in plasma osmolality of juvenile tiger prawn was positively correlated with increased weight (á = 0:01). This fact suggests that there is a correlation between weight size gain and an increase in plasma osmolality which requires an increase in water salinity in line with juvenile tiger prawn weight gained as presented in Figure 4.

Osmotic balance between body fluids and water media is very important for the life of aquatic animals since the ions were actively absorbed by the body through the gills when there is water absorption process [18, 19]. Generally, the energetic requirement for ion regulation was lower in the isoosmotic water environment which is substantial enough to boost growth [20, 21]. Thus, it is preferable for the juvenile tiger prawns be reared and maintained in an isoosmotic pond environment.

Composition of Hemocyte Cells: Hemocyte cells are usually referred to hyaline, granular and semi granular [22] and they can be easily differentiated using Transmission Electron Microscope (TEM) technique. The percentage composition of the different number of cell types is presented in Figure 5. Semi granular cells/semiglobular known as primary cells are involved in phagocytosis of foreign particles in shrimp [23, 24]. Granular or globular cells are also capable of a less active phagocytosis of foreign bodies [25, 26]. However, granular cells are shown to play an important role as a defence system in shrimp

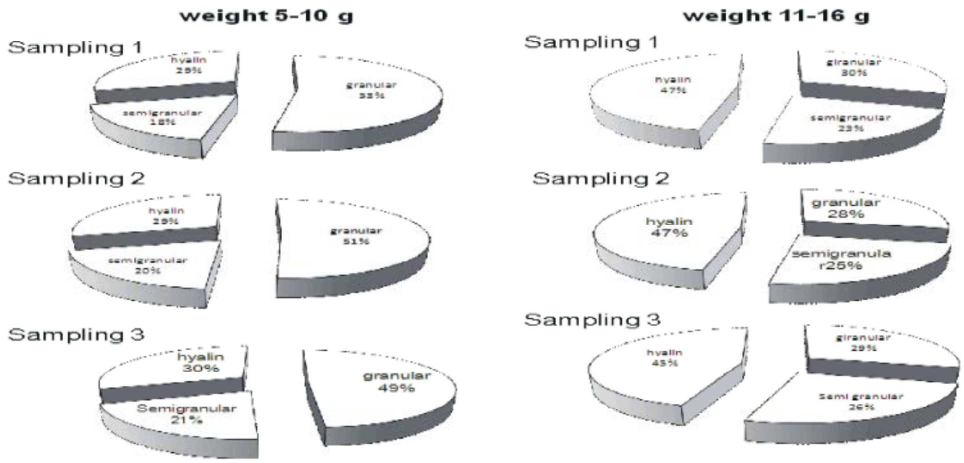

Fig. 5: Comparison of haemolymph cell composition of juvenile tiger prawn at different weights

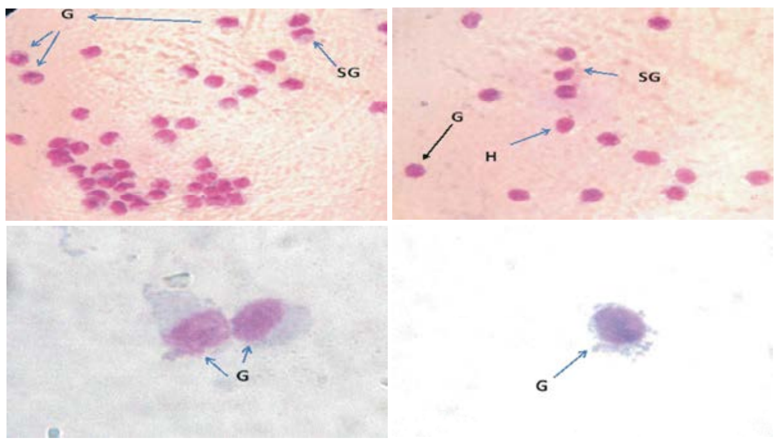

Fig. 6: Micrograph haemocyte cells of juvenile tiger prawn (G = Globular, SG = Semi globular and H = Hyaline) (magnification 1000 X)

because of anti-bacterial activity [27]. Hyaline cells were reported to be the smallest type of hemocyte cell found in tiger prawns [28].

Results indicated that the natural juvenile tiger prawn with smaller weights created a more resistant defence system compared to the larger weight size by deactivating any foreign objects activities into their life cycle system. This is presumably true when a small percentage of hemocyte cells allegedly act as antibodies as found in fish. The role of glycoproteins in the haemolymph lectin or crustaceans has the ability to react with many types of molecules for example, lipoproteins or â-glucan which was able to agglutinate and activate foreign objects. The major constituent of hyaline cell in tiger prawns is lectin. The intake of crude extract LPS dose from *E. coli* for 96 hours showed a trend of decreasing mortality with increasing time immune-stimulant. Tiger prawn can boost the immune cells in particular tiger prawn hyaline and phagocytosis of hemocytes which is expected to be resistant to microbial pathogens in ponds. However, smaller weight size tiger prawns may sometimes be more vulnerable and sensitive to diseases and sudden changes in the environment.

CONCLUSION

Based on hemocyte cell types, the number of natural hemocyte, total haemolymph protein and plasma osmolality were positively correlated with the increased weight of the juvenile tiger prawn. The smaller natural juvenile shrimp with a low percentage of hyaline antibody and low globular and semi-globular cells has a higher immune system compared to the bigger size shrimp. Future studies should be done to monitor the haemolymph cortisols and glycogen in response to physiological stresses such as fluctuations in salinity, temperature and water pH in a large scale pond rearing.

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