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Original Paper

OSMOTIC RESPONSES OF *SEGARA ANAKAN* FINE SHRIMP (*Metapenaeus elegans*) ADULTS IN VARIOUS SALINITY AND MOLTING STAGES

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ABSTRACT

A research on eco-physiological characteristics of fine shrimp (Metapenaeus elegans) with special emphasis on the osmotic responses and isosmotic medium requirement for adult spawners in various molting stages was conducted. Adult stocks of M. elegans origin from the Segara Anakan lagunas of the South West of Central Java region were collected and used as experimental shrimps. The shrimps were hold in three 500 l-acclimation tanks and treated according to Anggoro and Nakamura's method. The seawater salinity level in the tank 1, 2, and 3 was 25, 28, and 22 ppt, respectively. Osmotic response of the shrimps was examined during 3 molting stages, i.e. pre-molt/ post-molt, molt, and inter-molt phases by using an automatic microosmometer Roebling. The results showed that osmotic responses were closely related to the salinity of water medium and molting stages. It was also found that the minimum osmotic works of fine shrimp occurred in isosmotic medium, i.e. 16 to 20 ppt for post-molt, 28 to 30 ppt for molt, and 22 to 25 ppt for inter-molt stages. It was concluded that the range of isosmotic media for the adult of fine shrimp was 22 to 28 ppt or equals to 642.06 to 817.31 mOsm/l H_2O.

Keywords : Fine shrimp; *Metapenaeus*, Elegans; Molting; Osmotic; Salinity.

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INTRODUCTION

Attempts to optimize shrimp culture production have faced with several problems related to its biotechnical management, which was suspectedly unable to meet the needs of the cultured species. This is primarily due to eco-physiological characteristics of shrimp, particularly those related to the osmoregulation mechanisms in connection with their growth, which have not been sufficiently understood. In principle, any failure occurring in shrimp culture could be attributed to the inappropriateness between biotechnical management used and the ecophysiological needs of the cultivated shrimp.

Previous studies (Kumlu, *et. al*., 2001; Anggoro and Muryati, 2006;) stated that there were indicators that could be used to determine the success of a shrimp culture operation, i.e.: 1) the ability of brood stocks to survive and spawn in the right time; 2) sufficiently high hatching rate with adequate number and high quality of larvae; 3) the large number of biomass production, characterized by high survival rate and rapid growth rate; and 4) the efficiently used feed while maintaining the quality of water medium. However, some profound problems still exist in the attempt to achieve the above success. Issues predominantly occurred in shrimp culture operation in Indonesia which contribute largely to the failure of shrimp culture are: 1) low hatching rate and high mortality of brood stocks due to molt death syndrome (MDS) and osmotic stress (OS), especially those which were spawned using ablation technique; 2) poor quality and quantity of shrimp larvae; 3) frequent failure in harvesting due to high mortality rate or retarded growth of shrimp cultured; and 4) degradation of water quality owing to the accumulation and putrefaction of feed residue and fecal matters

which in turn lowering the carrying capacity of pond environment (Tim Satgas Tambak Udang, 2005).

In principal, it was considered that there are 2 major factors that contribute to the above condition, i.e.: 1) internally, the eco-physiological characteristics of shrimp, particularly those related to the need of molting and osmoregulation process are not fully understood; and 2) externally, the optimum level of water quality and feeding suitable for molting and osmoregulation have not been determined. It is, therefore, highly crucial to address the issues in order to improve shrimp production technique, and thus improving shrimp production at large.

The general objectives of this experiment were to examine the eco-physiological aspects of *M. elegans*, especially the relationship of molting stages and osmoregulation pattern, as a basis information for the improvement of biotechnical management of shrimp culture. More specifically, this experiment was aimed at investigating more deeply the eco-physiological characteristics of *M. elegans* that relate to biotechnical management of on-growing and spawning of adult shrimp brood stocks, with special emphasis on the osmotic responses and the need for isosmotic medium by the adult brood stocks in various molting stages.

MATERIALS AND METHODS

The examination of osmoregulation was conducted on the adult stocks of *M. elegans*. In addition, osmotic responses of the brood stock's haemolymph were also observed during molting stage. Adult stocks were collected from the Segara Anakan lagunas of the South West of Central Java region. They were kept in the acclimation tanks and treated according to Anggoro and Nakamura's method (2005). In order to prevent thermal shock during the transportation of the adult shrimp, temperature of water medium was set below 25^{o}C (i.e. by using ice flakes scattered around the container). As for osmoregulation and molting examination adult stocks were acclimatized in a medium similar to their natural environment (with a salinity of 20 to 25 ppt) prior to the main treatment.

Osmotic response of *M. elegans* was examined during 3 molting stages, i.e. pre-molt/ post-molt, molt, and inter-molt stages. This experiment was arranged using completely randomized design. Three 500 l-concrete tanks were used as experimental units. The treatment was designed as follows: a) Tank 1; containing seawater with salinity of 25ppt or equal to 726.2 mOsm/ l H_2O. This condition is close to intermolt isosmotic level; b) Tank 2; containing seawater with salinity of 28 ppt or equal to 875.46 mOsm/l H_2O. This is close to molt isosmotic level; and c) Tank 3; containing seawater with salinity of 22 ppt or equal to 626.31 Osm/l H_2O. This is close to post-molt and pre-molt isosmotic level. Each tank was partitioned into 3 similar compartments with nylon netting and covered with plastics on the top. Each compartment was then filled with 1 adult spawner with a body length ranged between 10.50 and 11.00 cm and weighing about 189.45 to 106.55 gr. Aerators were used in each tank to supply oxygen, and temperature regulator and biofilter recirculation unit were also placed in the tank to maintain water quality in the desired condition. The treatment was done in 29 $\pm 1^{o}$ C.

Tested shrimps were fed on minced fresh squids as much as 17.3 g/day which was given in every afternoon and dawn. Osmotic response (osmolarity) examination on adult spawner was conducted following the method previously applied by Anggoro and Nakamura (2005). Based on results from preliminary molting experiments, only haemolymph samples from 40-hr post-molt animals were taken 1, 2, 3, 5, 7 and 9 days after transfer to the new salinity. Shrimps were sacrificed by severing the ventral nerve cord after wiping the animal dry. Haemolymph was drawn from the pericardiac cavity with a #23 gauge needle and 1 ml tuberculine syringe. Osmotic pressure was measured with an Automatic Microosmometer Roebling (for 0.10 ml sample). The data were then analyzed using variance and descriptive analysis.

RESULTS AND DISCUSSION

Osmotic responses of brood stock shrimp were evaluated based on the osmolarity of their haemolymph and the osmotic work level in various molting stages. This was done in the three treatments with different salinity of water medium.

The results could be seen, in **Fig. 1 and 2**, and revealed that the osmolarity of haemolymph and the osmotic work level (TKO) of brood stock shrimp were changing according to their

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molting stages. Such changes were necessary to occur from the viewpoint of their ecophysiological mechanism in accordance with the need of molting and osmoregulation processes (Ferraris, *et al*., 1987; Gilles, 2004; Venkitraman, *et al., 2010).*

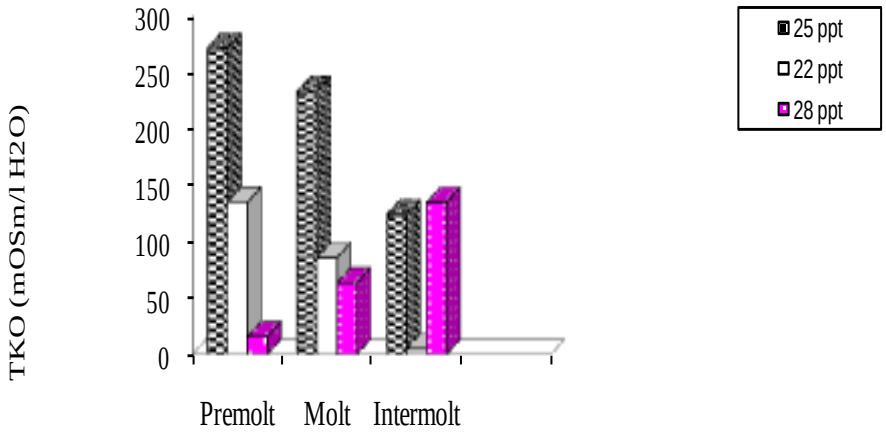

Fig.1. Osmotic Work (TKO) of Adult Spawners Shrimp in Various Molting Stages and in Different Salinity Media (Molting Cycle I)

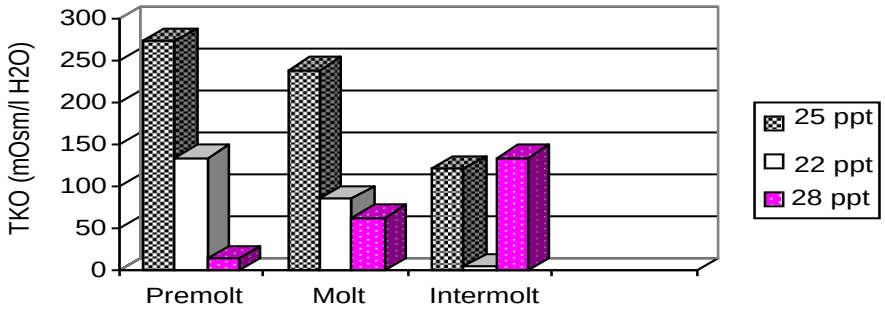

Fig.2. Osmotic Work (TKO) of Adult Spawners Shrimp in Various Molting Stages and in Different Salinity Media (Molting Cycle II)

In the pre-molt stage, the osmolarity of haemolymph was considerably high, i.e.: a) in 25 ppt (intermolt isosmotic) medium: 726.20 to 729.35 mOsm/l H_2O or equal to salinity of 25 to 26 ppt; b) in 28 ppt (molt isosmotic) medium: 817.15 to 848.57 mOsm/l H_2O or equal to salinity of 28 to 29.7 ppt; and c) in 22 ppt (postmolt isosmotic) medium: 640.70 to 642.72 mOSm/l H_2O or equal to a salinity of 20 to 22 ppt.

In this phase the minimum level of osmotic work of the shrimp was 15.59 to 15.60 mOsm/l H_2O, which was achieved in 28 ppt medium. Such very high level of haemolymph osmolarity in pre-molt and molt stage was also found in previous study by Anggoro and Muryati (2006). There were two factors considered responsible for the above result. First, during pre-molt stage, mobility and accumulation of osmoefector substance reserve, especially calcium, phosphorus and organic nutrient, occur in haemolymph and hepatopancreas as an initial step of molting process. Second, the preparation of new integument formation is accompanied by the absorption of organic nutrient and calcium from the old integument to haemolymph (Yamaoka and Scheer, 1990; Mantel and Parmer, 1983; Venkitraman, *et. al*., 2010). The regulation and transport of calcium into haemolymph are sustained by ion pumping system (Na-K-ATP-ase and Ca-Mg-ATP-ase) and calmoduline (Ferraris, *et al.*, 1987; Che Mat, 1987). In this case, calmodulin is responsible for modulating Ca

concentration in the cells (CIS). The modulation is stimulated by Ca transfer from CIS (in the epidermis) into the haemolymph using the energy supplied from ATP. It is through this process that Ca is pumped out from epidermis cells (old integument) into haemolymph and finally accumulates in hepatopancreas (as a raw material for the formation of new integument). Consequently, the increase of osmotic concentration occurs in the extra cellular fluids, which result in a considerable increase in haemolymph osmolarity.

During molt or early post-molt stages, haemolymph osmolarity level is slightly lower than that in pre-molt phase as described below: a) in 25 ppt medium: 726.20 to 729.35 mOsm/l H_2O or equal to salinity of 25 to 26 ppt; b) in 28 ppt medium: 817.15 to 848.57 mOsm/l H_2O or equal to salinity of 28 to 29.7; and c) in 22 ppt medium: 640.70 to 642.72 mOSm/l H_2O or equal to a salinity of 20 to 22 ppt. The decrease of haemolymph osmolarity in the early molt and post-molt stage was suspectedly caused by: 1) the increase in water absorption during molt phase, and 2) the increase in the utilization of osmoefector (organic and inorganic nutrient) in the haemolymph as raw materials of somatic tissue formation (Gilles and Pequeux, 1983; Dalla Via, 1986; Gilles, 2004; Roy, 2006)

Intermolt stage is the longest period in the entire molting cycle of shrimp, that is around 70% of the whole cycle (Anggoro and Nakamura, 2005; Venkitraman *et al*., 2010). In this phase, haemolymph osmolarity was relatively low and steady within the ranges described below: a) in 25 ppt medium: 726.10 to 729.30 mOsm/l H_2O or equal to salinity of 25 to 26 ppt; b) in 28 ppt medium: 817.10 to 848.50 mOsm/l H_2O or equal to salinity of 28 to 29.7; and c) in 22 ppt medium: 640.52 to 642.65 mOSm/l H_2O or equal to a salinity of 20 to 22 ppt.

The minimum value of osmotic work of the shrimp occurred at a salinity of 25 ppt, that was 4.80 to 5.31 mOsm/l H_2O. Osmolarity and osmotic work level in intermolt phase is considered ideal as a standard reference for shrimp culture (Brito *et al*., 2000; Lemaire *et. al*., 2002; Saoud and David *et al*., 2005; Anggoro and Muryati, 2006). The length of intermolt cycle is related to several contributing factors mentioned below: a) the process of somatic cells and tissues growth and the hardening of new integument that stimulate X- organ to continuously producing molt inhibiting hormone (MIH); b) the production of MIH will inhibit the activity of Y-organ in such a way that the secretion of molt alternating hormone is restrained; and c) the accumulation of materials and energy for the successive molting process is highly dependent upon the availability of foods and suitable environment, thus it needs a longer time to happen (Anggoro and Nakamura, 2005; Roy, 2006; Hesni, *et al.*, 2008).

From the above discussion, it could be predicted that the salinity of water medium that ensure the minimum osmotic work load in compliance with the molting process in shrimp could be described as follows: a) inter-molt stage: 25 to 26 ppt or equals to osmolarity of 726.18 to 729.30 mOsm/l H_2O; b) molt- stage: 28 to 30 ppt or equals to osmolarity of 817.10 to 906.08 mOsm/l H_2O; and c) post-premolt stage: 20.5 to 23 ppt or equals to osmolarity of 640.04 to 653.84 mOsm/l H_2O.

CONCLUSION

1. Osmotic response of *M. Elegans* adult shrimp was closely related to the osmolarity/salinity of water medium and molting stages of shrimp;
2. The minimum osmotic work of the shrimp occurred in isosmotic media, i.e. 20 to 22 ppt for post-molting stage, 28 to 30 ppt for molting stage, and 25 to 26 ppt for intermolting stage; and
3. The range of isosmotic media for the adult of *M. Elegans* shrimp was: 22 to 28 ppt or equal to 642.06 to 817.31 mOsm/l H_2O.

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