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ANALYSIS OF WATER SALINITY LEVEL OF FREQUENCY MOLTING IN VANNAMEI SHRIMP (LITOPENAEUS VANNAMEI) ABLATED

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ABSTARCT

Media salinity is a masking factor that plays an important role in controlling the process of shrimp molting and osmoregulation. Both of them are ecophysiological factors for shrimp life, so it is necessary to know the optimum level or range of media salinity for each phase of the molting phase changes in the stage and osmoregulation phase. This study was conducted to examine the molting frequency of adult *L. vannamei* which was ablated and cultivated at various levels of salinity. This research was carried out for 60 days. This study uses experimental laboratory methods with a systematic randomized design (RAS) with 4 treatments and 3 replications in each treatment. The treatments tested were salinity with S1 treatments (10 ppt, 289.20 mOsm / l postmolt H_2O isosmotic), S2 (15 ppt, 432.80 mOsm / l H_2O initial intermolt isosmotic), S3 (25 ± 1 ppt, 725, 15 mOsm / l H_2O isosmotic final intermolt) and S4 (29 ± 1 ppt, 820.10 mOsm / l H_2O isosmotic molt). Data were analyzed with ANOVA. Meanwhile, the difference in effect between treatments was obtained through Duncan's multiple area test. ANOVA results showed that various levels of isoosmotic media salinity at various molting phases had an influence ($p < 0.05$) on molting frequency. The best value of molting frequency was in the S4 treatment (29 ± 1 ppt) (close to isoosmotic molt) 10 times. In the maintenance of *L. vannamei* which is affixed, it should pay attention to the needs of isoosmotic media, namely media with a salinity of 25 ± 1-29 ± 1 ppt (final intermolt isoosmotic range / premolt to molt). the difference in effect between treatments was obtained through Duncan's multiple region test. ANOVA results showed that various levels of isoosmotic media salinity at various molting phases had an influence ($p < 0.05$) on molting frequency. The best value of molting frequency is in the treatment of S4 (29 ± 1 ppt) (close to isoosmotic molt) 10 times. In the maintenance of *L. vannamei* which is affixed, it is better to pay attention to the needs of isoosmotic media, ie media with a salinity of 25 ± 1-29 ± 1 ppt (final intermolt isoosmotic range / premolt to molt). the difference in effect between treatments was obtained through Duncan's multiple region test. ANOVA results showed that various levels of isoosmotic media salinity at various molting phases had an influence ($p < 0.05$) on molting frequency. The best value of molting frequency is in the treatment of S4 (29 ± 1 ppt) (close to isoosmotic molt) 10 times. In the maintenance of *L. vannamei* which is affixed, it is better to pay attention to the needs of isoosmotic media, ie media with a salinity of 25 ± 1-29 ± 1 ppt (final intermolt isoosmotic range / premolt to molt). The best value of molting frequency was in the S4 treatment (29 ± 1 ppt) (close to isoosmotic molt) 10 times. In the maintenance of *L. vannamei* which is affixed, it is better to pay attention to the needs of isoosmotic media, ie media with a salinity of 25 ± 1-29 ± 1 ppt (final intermolt isoosmotic range / premolt to molt). The best value of molting frequency is in the treatment of S4 (29 ± 1 ppt) (close to isoosmotic molt) 10 times. In the maintenance of *L. vannamei* which is affixed, it is better to pay attention to the needs of isoosmotic media, ie media with a salinity of 25 ± 1-29 ± 1 ppt (final intermolt isoosmotic range / premolt to molt).

Keyword : Salinity, Osmoregulation, Molting, *L. vannamei*, Ablation.

I. INTRODUCTION

Shrimp life is controlled by two main ecophysiological factors, osmoregulation and molting. Therefore, the need for optimum media and feed according to the molting and osmoregulation cycle needs to be known. Media salinity, known as the masking factor, plays an important role in controlling the process of shrimp molting and osmoregulation, but the optimum level or range of media salinity for each molting phase changes the stage or size and osmoregulation phase of the shrimp is not yet fully known as the basis for optimum media regulation.

Salinity is a factor that affects osmosis pressure in shrimp, where the higher the salinity, the higher the osmosis pressure. Biota that lives in salt water must be able to adjust to the osmotic pressure emanating from its environment. This adjustment requires a lot of energy derived from the food consumed so that it can reduce the efficiency of the feed (Kordi and Tancung, 2007).

Osmoregulation is a homeostatic system for maintaining interior stability in shrimp by regulating the balance of osmotic concentration between intracellular fluid and extracellular fluid. The activity is carried out by regulating the volume of water in extracellular fluid and regulating ion exchange between intracellular and extracellular fluid (Mantel and Farmer, 1983).

Cutting the eye stalk or better known as ablation is the process of removing x-organs and complex sinus glands that are in the eye stalks (Sainz-Hernandez *et al.,* 2008). This ablation can increase the aquaculture production of larvae from Penaeus spp. and has been known since 1970 (Bray and Lawrence, 1992). The complex sinus gland of the x-organ in the eye stalk is the most important neuroendocrine gland in crustaceans (Beltz, 1988 and Chang, 1992). In this gland the hormone is synthesized, stored and released into the body's fluid (haemolymph) to regulate some of the metabolic processes (Chang, 1992). Another process involved is the molting process (Chang and O'Connor, 1988).

Ablation in *L. vannamei* is expected to accelerate the process of molting so that growth occurs more quickly as well, but it cannot be denied that the presence of ablation can lead to a low survival rate (SR) and osmolarity of haemolymphnya (Meade and Watts, 2001 and Venkitraman *et al.,* 2004). Therefore, knowledge about the osmotic response (needs of isoosomotic media) of *L. vannamei* which is affixed is important to be recognized and mastered to produce high survival and growth.

II. LITERATURE REVIEW

Shrimp molting is influenced by species, life phase, size and environmental conditions. Molting is a physiological process of Shrimp, where the old shell of the shrimp is replaced with new skin after going through several processes and stages. In simple terms the principles and characteristics of molting in Shrimp follow the process flow as follows (Anggoro, 1990):

1 Intermolt, is an active growth phase in muscle tissue and hardening of the new exoskeleton that characterizes the relative calm and storage of material and energy reserves in preparation for the next phase. At this stage isoosmotic intra-cell fluid (CIS) with extracellular fluid (CES).
2 Premolt, is a phase of molting preparation characterized by increased mobility and accumulation of metabolic material into the hepatopancreas and CES, preparation of a new epidermis, formation of a new exoskeleton under the old exoskeleton accompanied by dissolution and resorption of inorganic material (especially Ca) from the old exoskeleton. In this phase, Shrimp are very active in utilizing feed to obtain material and energy for preparation for skin release (molt).
3 Molt, is the release stage of the old exoskeleton and accompanied by absorption of water from outside the body. At this stage the Shrimp does not eat at all, its condition is weak and its osmoregulation power does not play a perfect role, so it is the most vulnerable phase for its life.
4 Postmolt, is the initial phase of muscle tissue growth and new epidermal expansion. During this period the osmotic pressure of the intracellular fluid begins to approach extracellular fluid and will reach isoosmotic pressure at the time of the intermolt.

The central nervous system in shrimp receives specific stimuli both from internal factors originating from the body (molting) and from external factors (environment, such as salinity). The central nervous system then orders the pericardiac cavity to secrete the osmoregulatory hormone and mobilize electrolytes or ions to be

transported into the extracellular fluid when the external media (salinity) changes. The osmoregulation hormone functions to facilitate osmoregulation. In addition, the central nervous system during the intermolt phase instructs the x-organs to work, where in the x-organs there are neurosecretory cells that function for the secretion of the osmoregulatory hormone and secretion of Molt Inhibitory Hormone (MIH) which inhibits molting. Meanwhile, at the time of molting preparation, the inorganic components of the old exoskeleton are absorbed into the blood vessels and stored in the hepatopancreas. At the time of molting there is an increase in size / growth. If osmoregulation goes well, growth will be good too, and vice versa. This happens because, if osmoregulation is disrupted, the energy for growth is widely used for osmoregulation, so that growth does not run optimally (Anggoro and Nakamura, 1996).

III. METHODOLOGY

This research was carried out for 60 days in the Jepara Coastal Development Laboratory (LPWP). This research was conducted in 2 stages, namely preliminary research and core research. The preliminary research is aimed at establishing the treatment media according to isoosmotic needs in the molt, postmolt, initial intermolt and final intermolt phases as the cornerstone of management of cultivation of *L. vannamei* which is layered. *L. vannamei* was acclimatized in seawater media (33 ppt) for 30 days for examination of media osmolarity at each molting phase for this purpose.

The preliminary experimental results of *L. vannamei* isoosmotic level according to the molting phase which will then be used as a treatment media in this study are as follows:

- S1: 10 ppt (289.20 mOsm / l H_2O) is equivalent to the postmolt phase isosmotic;
- S2: 15 ppt (432.80 mOsm/ l H_2O) is equivalent to the initial intermolt isosmotic phase;
- S3: 25 ± 1ppt (725.15 mOsm/ l H_2O) is equivalent to the final intermolt isosmotic phase; and
- S4: 29 ± 1ppt (820.10 mOsm/ l H_2O) is equivalent to the isosmotic molt phase.

Core research was conducted by observing molting to determine the frequency of molting during the study. These observations were made directly on the test container, which is carried out every day during the study (at 08.00, 12.00 17.00 and 22.00 WIB).

The research method used is an experimental laboratory method with a regular observation and planning system for the phenomenon under study. Experiments are planned investigations to obtain new facts or strengthen or refute pre-existing facts (Dwiloka and Srigandono, 2006). The experimental design used was a Systematic Randomized Design (RAS) by applying 4 treatments and 3 repetitions.

Molting frequency data were tested for normality and homogeneity of various data, then analyzed further with ANOVA, before testing. Meanwhile, to determine the effect of treatment on the frequency of molting using a double comparison test, namely the Duncan test (Steel and Torrie, 1981). All data were analyzed using SPSS 16. Meanwhile, to find out the optimum value of a response curve, orthogonal polynomials were obtained using Microsoft Excel 2007.

IV. RESULT AND DISCUSSION

Based on preliminary research that has been obtained, shrimp osmolarity data are 10 ppt (289.20 mOsm / l H_2O) equivalent to postmolt isosmotic, 15 ppt (432.80 mOsm / l H_2O) equivalent to initial intermolt isosmotic, 25 ± 1 ppt (725 , 15 mOsm / l H_2O) is equivalent to the final intermolt isosmotic and 29 ± 1 ppt (820.10 mOsm / l H2O) is equivalent to the isosmotic molt phase.

Based on research that has been carried out for 60 days, obtained data on Osmotic Work Level (TKO), osmolarity *haemolymph*and media osmolarity and frequency of molting. TKO data, haemolymph osmolarity and media osmolarity were obtained at the beginning of the study and at the end of the study and both were conducted at the Coastal Development Laboratory (LPWP), Jepara. Meanwhile, molting frequency data, obtained every 10 days, are used to compare between treatments.

Data on the *L. vannamei* molting frequency during the study are presented in Appendix 6. and the *L. vannamei* molting frequency histogram during the study are presented in **Figure 4.1.**

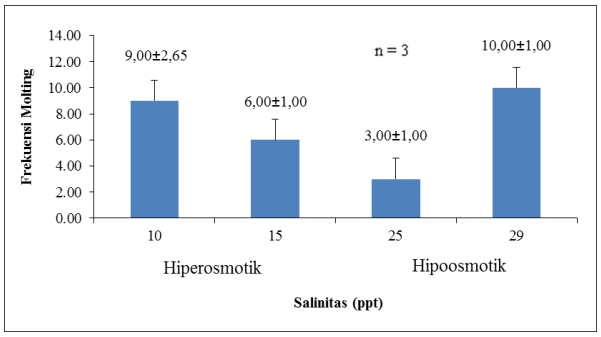

Figure 4.1. Molting Frequency Histogram *L. vannamei* Cultivated and Cultivated at Various Salinity Levels

During 60 days of maintenance, frequency of molting *L. vannamei* the highest occurred in S4 treatment 10 times and the lowest in S3 3 times, while in S1 9 times and S2 occurred 6 times.

The results of normality test and homogeneity of molting frequency at various levels of *L. vannamei* media salinity obtained results of data that spread normally and homogeneously (P> 0.05). This qualifies for further analysis with ANOVA. Based on the ANOVA molting frequency presented in Table 1. it can be seen that salinity affects the molting frequency (P <0.05). The difference between treatments is known by performing the Duncan test, where the test results are presented in **Table 4.1**.

Table 4.1. Duncan Test of *L. vannamei* Molting Frequency which is Affixed and Cultivated at Various Salinity Levels

(I) Salinity	(J) Salinity	Mean Difference (IJ)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
S1	S2	3,000,000 *	1.29099	.499	.0230	5.9770
	S3	6.00000 *	1.29099	.002	3.0230	8.9770
	S4	-1.00000	1.29099	.461	-3.9770	1.9770
S2	S1	-3.00000 *	1.29099	.499	-5.9770	-.0230
	S3	3,000,000 *	1.29099	.499	.0230	5.9770
	S4	-4.00000 *	1.29099	.015	-6.9770	-1.0230
S3	S1	-6.00000 *	1.29099	.002	-8.9770	-3.0230
	S2	-3.00000 *	1.29099	.499	-5.9770	-.0230
	S4	-7.00000 *	1.29099	.001	-9.9770	-4.0230
S4	S1	1.00000	1.29099	.461	-1.9770	3.9770
	S2	4.00000 *	1.29099	.015	1.0230	6.9770
	S3	7.00000 *	1.29099	.001	4.0230	9.9770

* average difference is significant at 0.05

Duncan's test results show that there is a difference in effect between treatments S1-S2, S1-S3, S2-S3, S2-S4, S3-S4, while between treatments S1-S4 there is no difference in influence. The relationship of *L. vannamei*'s molting frequency response that was elaborated and cultivated at various levels of media salinity is presented in **Figure 4.2.**

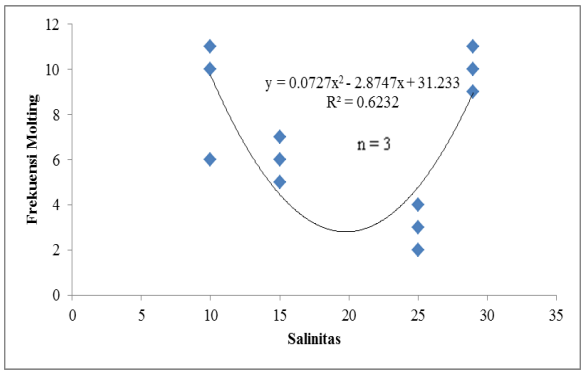

Figure 4.2. Molting Frequency Curve *L. Vannamei* Cultivated and Cultivated at Various Salinity Levels

The *L. vannamei* molting frequency response model at various levels of salinity is as follows:

Y = 0.072x2- 2,874x + 31.32 (R2 = 0.623).....{1}

The equation model can be used to estimate the response of Y (molting frequency) if X (salinity) is known. Based on the results of the above equation, it means that R2 = 0.623 which is 62.3% salinity affects the molting frequency. In Figure 7. it can be seen that the salinity of the S4 treatment (29 ± 1 ppt) is the best salinity range for the molting frequency of *L. vannamei* which is affixed, with a molting frequency of 10 times.

Salinity is one of the environmental factors that influence Molting. Besides salinity, ablation also speeds up the molting process. Molting in shrimp is regulated by 2 hormones viz*Molt Inhibiting Hormone* (MIH) and hormone molting (Hesni *et al.*, 2008). Moting time intervals also become shorter with ablation.

The molting process greatly influences the growth of shrimp, because internally the growth is very dependent on the molting and TKO processes experienced by the shrimp. Shrimp growth will occur if the molting process goes well. Shrimp molting process at high or low salinity requires a lot of time and energy in normalizing the concentration of osmolarity*haemolymph* (Ferraris *et al.*, 1986).

The test results and statistical calculations show that due to different salinity in the media give influence (P <0.05) on the moltig frequency *L. vannamei* which was applied to each treatment and test. Based on the histogram and polynomial frequency graph of molting, the best salinity for the molting frequency of *L. vannamei* which was affixed was in the S4 treatment (29 ± 1 ppt, as well as isoosmotic molt) with a molting frequency of

10 times during the study (60 days), with the fastest molting time interval is 1 day and the longest is 6 days. Decreased duration of the molting cycle, mainly due to the low concentration of MIH caused by ablation and occurs in male and female individuals (Sainz-Hernandes *et al.*, 2008).

In female shrimp molting generally occurs faster than males. According to Haefner Jr. (1969), in shrimp*Septemspinosa crangons* females have better osmoregulatory abilities compared to males. In P. monodon female individuals grow faster than male individuals. This is thought to be due to the ability of osmoregulation, so that female individuals who have better osmoregulation capabilities than males, so that female individuals have more energy for growth, and along with that growth the process of molting in individual females tends to be faster than male individuals.

According to Che Mat (1987), based on the existing molting phase, the molt phase and the beginning of the postmolt are vulnerable points for shrimp life. In this condition the shrimp are in a weak condition, osmoregulation power is not functioning properly and has not been able to perform eating activities properly, thereby increasing the chance of death (*Molt Death Syndrome*).

V. CONCLUSION & RECOMENDATION

The conclusions obtained from the research that have been done show that various levels of isoosmotic media salinity at various molting phases have an influence ($p < 0.05$) on the frequency of molting. The best value of molting frequency is in the treatment of S4 (29 ± 1 ppt) (close to isoosmotic molt) with a molting frequency of 10 times. In the maintenance of *L. vannamei* which is affixed, it should pay attention to the needs of isoosmotic media, ie media with a salinity of 25 ± 1-29 ± 1 ppt (final intermolt isoosmotic range / premolt to molt).

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