The Effect of Natural Feeding Enrichment Using Beta Carotene on Stress Resistance and Survival Rate of Blue Swimming Crab (*Portunus pelagicus*) Larvae

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Abstract- One of the problems in blue swimming crab (*Portunus pelagicus*) hatchery is low survival rate of larvae, especially at the zoea and megalopa stages. This study aims to determine the optimum beta carotene dose on the survival rate of blue swimming crab larvae. The study was carried out at the crab hatchery in the Brackish Water Aquaculture Center (BPBAP), Galesong District, Takalar Regency, South Sulawesi. The crab larvae are maintained in a 40L plastic container filled with 24L of seawater (32ppt) salinity with density of 50 larvae/L. This experiment was designed with a completely randomized design (CRD) consisting of 4 (four) and 3 replications, and therefore, the experiment consisted of 12 experimental units. The treatment used different beta carotene doses in rotifer and artemia. The dosage used is 0ppm, 5ppm, 10ppm and 15ppm of beta carotene. The results of the variance analysis showed that the treatment had a very significant effect ($P < 0.01$) on the stress resistance and survival rate of blue swimming crab larvae. The highest level of stress resistance and survival rate was obtained at enrichment at a dose of 10 mg/L and the lowest at the dose 0 ppm of beta carotene.

Index Terms- *Portunus pelagicus*, beta carotene, rotifer, artemia, cumulative stress index, survival rate.

I. INTRODUCTION

The higher demand for crab meat (*Portunus pelagicus*) needs adequate and sustainable availability that can be met by intensive aquaculture. The development of crab aquaculture requires the availability of sufficient seeds to supply the demand of farmers. The presence of crab hatcheries is a solution for the supply of sufficient crab seeds. Production of crab seeds faces the problem of still low survival. The results of trials conducted by Prastyanti et al (2017) et al. resulted in survival rates of crab seeds to crab stadia ranging from 7.78-12.89%. The number of mortality of crab larvae in metamorphoses process due to feed quality and low-stress resistance (Suprayudi et al., 2006, Niklani A, 2013).

The critical phase of larval rearing is in the phase where the yolk of the larva runs out and begins to require exogenous nutrient intake (Watanabe and Kiron, 1994). According to Nikhlani (2013) larval deaths occur a lot when changing the feed phase from the egg yolk to the intake of nutrients from the outside. This phase is a critical phase in the rearing of crab larvae, so all the supporting factors for the survival of crab larvae need to be considered, one of which is exogenous nutrition.

Efforts to increase crab survival, especially in the zoea stage, can be done by increasing the quality of natural feed through enrichment. Beta carotene is a hydrocarbon compound that is widely found in plants. Among the many important functions of beta carotene are related to provitamin A, which in turn affects embryonic development, properly growth, and vision. Beta carotene also shows anticancer and antioxidant properties (Berman et al., 2014). The antioxidant properties of beta-carotene can increase resistance to stressors during rearing.

Several studies on the use of beta carotene have been carried out. The use of carotenoid type astaxanthin and beta carotene as pigmentation in *Penaeus japonicus kuruma* shrimp has a positive influence on their survival (Chien and Jeng, 1992). The enrichment of rotifer with beta carotene for feed fish larvae of Japanese parrot fish (*Oplegnathus fasciatus*) results in higher survival in larvae compared to controls (Tachibana et al., 1997). The use of beta carotene enriched in rotifer supports the success of the Japanese parrot hatchery (*Oplegnathus fasciatus*) in the Nagasaki Municipal Fisheries Center where anti-infectives and beta-carotene antioxidants have a positive effect by producing larvae that are resistant to pathogenic viruses and bacteria (Tachibana et al., 1997).

One natural ingredient that has high beta carotene content is carrots (Marlyati, 2012). Some procedures are usually performed in extracting beta carotene, namely the operation of sorting and destruction of ingredients, suppressing juice, protein coagulation, sedimentation, centrifugation and extraction with organic solvents, filtration, dyeing, evaporation, and crystallization (Radomska and Harasym, 2018). According to Wingqvist (2012) extraction methods of beta carotene include traditional boiling, reflux, Soxhlet, and pressurized fluid extraction methods.

Until now, information about the use of beta carotene, especially those sourced from carrots to increase survival, the rate of metamorphosis and stress resistance in crab larvae is still limited.

II. METHOD

This research was carried out in the blue swimming crab hatchery unit of the Takalar Brackish Aquaculture Center, Indonesia, from October to February 2018. Extraction and analysis of beta carotene from carrots was conducted at the BPBAP Takalar Chemical Physics Test Laboratory using the Soxhlet method.

The animals tested are blue swimming crab larvae hatched in BPBAP Takalar hatchery. The larvae are stocked with a density of 50 head/L. 12 test containers tanks with a volume of 40L filled 24L of seawater (32ppt) salinity which had been treated for sterilization of sea water. The container is equipped with aeration to supply oxygen during the rearing.

The test feed used during the rearing of crab larvae is rotifer and artemia which has been enriched with beta carotene as a result of carrot extraction. Rotifer is obtained from the massive culture in the natural feed production unit of the Takalar Brackish Aquaculture Fisheries Center. Natural food types of artemia obtained from the results of hatching of commercially produced Inve artemia cysts, that has been enriched with beta carotene from carrots. The density of the rotifer is 500,000 ind/L and nauplius artemia is 300,000 ind/L, enrichment is carried out in containers with a capacity of 3L which are equipped with aeration for 6 hours. Carrot extraction is done by using a soxhlet tool (Wingqvist, 2012; Ismail, 2013). Analysis of beta-carotene using a spectrophotometer according to the method used by Takaeuchi et al. (1995) has been modified.

The parameters observed were stress resistance and survival rate. Stress resistance of crab larvae was tested by osmotic shock namely crab larvae were put into 0 ppt saline water. Cumulative stress index (CSI) is calculated by modifying the formula used by Ress et al (1994). with the following formula:

$CSI = D_5 + D_{10} + \ldots + D_{60}$

Description:

CSI = Cumulative stress index

D_5, D_{10}, D_{60} = Number of stressed larvae at a certain time (minutes)

The survival rate is calculated using the following:

$$S = \frac{Nt}{No} \times 100$$

Description:

S = survival rate (%)

NO = Number of crab larvae at the beginning of the study

Nt = Number of crab larvae at the end of the study

The data obtained will be analyzed using variance analysis (ANOVA) and will be continued with W-Tukey's further test (Steel and Torrie, 1993). As supporting data during rearing, water quality measurements are carried out for several water quality parameters including temperature, salinity, dissolved oxygen, pH and ammonia.

III. RESULTS AND DISCUSSION

Results

The average content of beta-carotene rotifer, artemia and crab larvae are presented in Table 1.

Table 1. The average content of beta-carotene in rotifers, artemia, and crab larvae

Dosage of beta-carotene (ppm)	Average content		
	rotifer (ppm) ± SD	artemia (ppm) ± SD	Crab larvae (ppm) ± SD
0	0.643 ± 0.13^c	2.307 ± 0.18^c	0.423 ± 0.11^c
5	3.271 ± 0.28^b	5.261 ± 0.10^b	2.267 ± 0.16^b
10	7.239 ± 0.29^a	9.217 ± 0.19^a	4.797 ± 0.27^a
15	4.488 ± 0.21^b	7.385 ± 0.50^b	3.259 ± 0.21^b

Description: Numbers followed by different letters in the average column show significantly different based on the Tukey test

Table 2. Average of *cumulative stress index* (CSI)

Dosage of beta-carotene (ppm)	*cumulative stress index*) ± SD
0	117.33 ± 1.15^a
5	117.00 ± 1.0^a
10	112.33 ± 0.58^b
15	113.33 ± 0.58^b

Description: Numbers followed by different letters in the average column show significantly different based on the Tukey test

Table 3. Average of survival rate of crab larvae

Dosage of beta-carotene (ppm)	Average of survival rate of crab larvae (ppm) ± SD
0	42.78 ± 1.03^c
5	51.58 ± 10.56^b
10	59.36 ± 4.79^a
15	56.72 ± 5.00^b

Description: Numbers followed by different letters in the average column show significantly different based on the Tukey

IV. DISCUSSION

Discussion

The enrichment of rotifer with beta-carotene has an impact on the increase in beta-carotene content in rotifers. The results of the analysis of variance showed a very significant effect ($p < 0.01$). W-Tukey's further test results showed that each treatment showed significant differences compared to controls or rotifers without the addition of beta-carotene. ($p < 0.05$). The highest beta-carotene content in rotifers is the dose of 10 ppm which is 7,239 ppm. These results showed a significant increase in beta-carotene content compared to rotifer with a dose of 0 ppm (control) with a beta-carotene content 0.643 ppm.

The results of variance analysis showed that different doses had a very significant effect on the content of beta-carotene in artemia, rotifers and crab larvae. In the W-Tukey further test

the value of the beta-carotene content between treatments in artemia nauplius, rotifer and crab was significantly different ($p < 0.05$). The highest beta-carotene content was obtained at a dose of 10 ppm.

Low content of beta-carotene in rotifers and artemia at a dose of 0 ppm is due to the absence of beta-carotene in the culture medium. The content of beta-carotene in rotifers in the enrichment of 0 ppm beta-carotene originates from the intake of chlorella containing beta-carotene (Kristiyaningrum, et al. 2013).

The results of the analysis showed that the content of beta-carotene in the body of crab larvae increased after being given natural feed enriched with beta-carotene during the study. The highest beta-carotene content was obtained in crab larvae with the treatment of natural feed enriched with beta-carotene at a dose of 10 ppm which is equal to 4,988 ppm. The greater beta-carotene content compared to other treatments is supported by natural feed intake that has been enriched at optimal doses during rearing. The lowest beta-carotene content in the treatment dose of 0 ppm beta-carotene because there is no enrichment of beta-carotene in the natural feed.

Cumulative stress index testing (CSI) was conducted to determine the resistance of crab larvae to drastic changes of conditions in environmental. One cause of stress (stressor) is a change in salinity. Analysis of the variety of blue swimming crab larvae showed a significant difference between the crab larvae who received natural feed intake and beta-carotene enrichment compared to controls. Stress resistance is indicated by the low-stress response shown by larvae after receiving a stressor. According to Bendich (1993), the potential of carotenoid (beta-carotene) can improve immune function by increasing the modulation of macrophages and lymphocyte activation. In addition, beta carotene plays a role in cooling antioxidants and singlet oxygen, pro-vitamin A activity and increasing regulation of DNA expression. The existence of stress will have a negative influence on the physiological balance of crab larvae which will accelerate the flow of energy. The 0 ppt salinity stress given to the test animals gave a difference in rearing media concentration and body fluid concentration which resulted in blue swimming crab larvae conducting osmoregulation to maintain body fluid concentration as a result of diffusion and osmose (Ernawati, 2017).

The results of variance analysis showed that the provision of natural feed enriched with beta carotene with various doses had a very significant effect on the survival of crab larvae ($P < 0.01$). W-Tukey's advanced test showed significant differences between treatments ($P < 0.05$).

The highest survival obtained at a dose of 10 ppm is associated with a higher level of stress resistance compared to other treatments. Stress resistance in larvae will reduce mortality rates in larvae caused by changes in environmental conditions. describe stress resistance of crab larvae to changes in the environment.

V. Conclusion

Rotifer and nauplius of artemia enriched with beta-carotene as natural feed for blue swimming crab larvae can increase the content of beta-carotene in natural feed and blue swimming crab larvae. A dose of 10 ppm beta carotene gave the best cumulative stress index results with a value of 112.33 and the highest survival of the crab larvae was 59.36%.

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