USE OF SPONGE, *Callyspongia basilana* EXTRACT AS ADDITIVE MATERIAL ON TIGER SHRIMP CULTURE

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ABSTRACT

Blue shrimp disease is one of the main problems in tiger shrimp culture. It reduces shrimp quality which eventually will decrease its market price. Blue shrimp is caused by deficiency of nutrition and additive materials such as carotene and other nutrient which function as vitamin source for important metabolic processes and formation of color profile in shrimp and fish. The aims of this study were to study the application effect of carotenoid extract of sponge *Callyspongia basilana*, as an additive material on the ability of shrimp to get back to normal state after suffering blue shrimp disease and survival rate of shrimp and to find out the optimal concentration of sponge carotenoid extract to cure the diseased shrimp. This study was consisted of two steps namely; (1). Extraction of sponge carotenoid by maseration and fractionation using acetone and petroleum ether solvents and (2), the application of carotenoid extract on the diseased shrimp. The research was arranged in a complete randomized design with four experiments consisted of (A). Control (without carotenoid extract); (B), (C), and (D) carotetoid extract addition of 3 mg/L, 6 mg/L, and 9 mg/L respectively with three replication each. The test animal used were blue diseased tiger shrimp with the density of 15 ind./container having 7.5–9.5 cm in size and the average weight of 5.5–10.0 g. The study showed that *Callyspongia basilana* carotenoid extract was able to change blue diseased shrimp to be normal within six days at the concentration of 9 mg/L. The highest survival rate was found in the experiment D (93.3%). Meanwhile, the lowest was obtained by the control population (13.3%) and the other two treatments were 80.0% (C) and 73.3% (B). The average of water quality parameters such as temperature, dissolved oxygen, pH, salinity, nitrite, and ammonia were in the suitable range for the growth and survival rate of tiger shrimp.

KEYWORDS: sponge, *Callyspongia basilana*, carotenoid, and blue shrimp

INTRODUCTION

One of the main challenges in tiger shrimp culture in intensive ponds was the occurrence of blue shrimp disease. This condition can reduce the quality of harvested shrimp and has a direct effect to shrimp price which can be reduced up 50% from its normal price (Ahmad, 1998). Blue shrimp is actually not included as a type of disease. On the shrimp body, there are several pigments such as blue, red, brown, or bearer substance of other colors (chromatophora). Blue shrimp has less body fitness, and is easy to experience stress and sometimes exhibits listlessness, thin surface, and coarse skin (McVeg, 1989).

Blue shrimp is mainly caused by the deficiency of nutrition and additive material like carotene (low astaxhantine carotene), poor environmental/water quality factor and soil (Baticados, 1988). Blue shrimp is also caused

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by unfulfilled β-carotene need so that for mation of natural pigmen in shrimp body is obstructed (McVeg, 1989). Blue shrimp has been documented with other names such as pale coloration, blue disease, sky blue shrimp disease, and blue shell syndrome.

Blue shrimp case can be avoided by using feed containing high carotenoid substance and vitamin A. The efforts to prevent the case have been done namely; by addition of β-carotene of carrot, potato, melon, and yellow corn (Kompyang *et al*., 1989) and the usage of *Leucaena glauca* leaves as a vitamin A source (Sulaiman & Kabangnga, 1990). However, the use of these two vitamin A sources has been less effective because of the structure and characteristic difference of these carotenoids. Based on these information, it is required other alternatives such as carotenoid isolated from sponge. Previous studies reported that sponge contains carotenoid containing a complex carotenoid structure and high structure variation which can be used by water biota (Scheuer, 1995). Other investigations also found that several species of sponge such as *Callyspongia basilana* (Figure 1), *Thionella* sp., and *Xestospongia* sp. were known to have dominant carotenoid with the common name of astaxanthin, adonixanthin, and suberixanthin (Rosmiati *et al*., 2005). The aplication of these sponge carotenoid needs to be investigated to know its effect on the change speed of blue shrimp to get back to normal state and survival rate of Tiger shrimp.

Figure 1. *Callyspongia basilana*

MATERIALS AND METHODS

Materials

Sponge *Callyspongia basilana* was collected from waters off Bakki and Barrang Lompo Islands, South Sulawesi using scuba gear. Sponge was placed directly in a cool box and brought to the Biotechnology laboratory of the Research Institute for Coastal Aquaculture (RICA), Maros. Blue shrimp was harvested from the RICA research pond in Punaga village, Takalar. The test animal having the size of 7.5–9.5 cm and weight of 5.5–10.0 g were carried to the wet laboratory of the experiment instalation owned by RICA in Maranak Village, Maros using a big container filled with sea water and continuously earated.

Methods

Extraction of Sponge Carotenoid

Fresh sponge was cut in small sizes by using cutter and weighted 2 kg for each sample. The sample was smashed by a grinder and repeatedly extracted with aceton till the residue was colorless. The acetone extract was filtered and collected for further separation. Before fractionation, this acetone extract was concentrated by evaporator to decrease its solvent. The concentrated acetone extract was repeatedly fractionated with petroleum ether. The petroleum ether containing the carotenoid was transferred to a separation funnel for saponification. This process was done by adding 10% alcoholic KOH for 5 minutes at room temperature. Produced carotenoid extract was dried with Na_2SO_4 anhidrous and evaporated till free from any solvent.

Application of Carotenoid Extract on Blue Shrimp

Blue shrimp was adapted for 24 hours in an aerated container before tested. The shrimp were then placed in containers with the density of 15 ind./container. Each container was filled with 30 L sea water with salinity of 38 ppt and equipped with aerator. This experiment used Complete Random Design with four treatments and three replications as follows;

A. Control (without carotenoid extract);
B. Addition of 3 mg/L carotenoid extract;
C. Addition of 6 mg/L carotenoid extract; and
D. Addition of 9 mg/L carotenoid extract

During rearing period, shrimp were fed twice daily with the dosage of 10% of body mass in the morning and in the afternoon. Feed for experiment B and C was also added with 0.3 g carotenoid/50 g feed binded using progol of 5 g/kg feed. Meanwhile, control was without addition of carotenoid extract.

The change of blue shrimp to get back to normal state was monitored every week for four weeks. The survival rate was counted at the end of the experiment based on the formulation of Effendie (1979). Resulted data were analyzed by variation print using smallest real difference at confidence level of 95% (Suhardjono, 1973). Water quality parameters were also measured including temperature, dissolved oxygen, pH, salinity, nitrite, and ammonia.

RESULTS AND DISCUSSION

Blue Color Changes to Normal State

The application of sponge carotenoid extract was able to restore the color of blue shrimp to normal color (Figure 2). The time needed by the diseased shrimp to change back to normal shrimp was shown in Table 1.

From the table, it can be seen that all treatments of carotenoid extract were able to normalize the blue shrimp at different times. The fastest change of blue shrimp to get back to normal on the first week which was on the sixth day of the treatment was achieved by treatment D although three individuals had not changed at the period. Overall, numbers of blue shrimp that were able to get back to normal state reaching a total of 100% for all treatments except control occurred in the fourth week. Furthermore, the control population showed no color change until the end of the study.

Results of the observations showed that visually, blue shrimp transformed into normal in stages from blue to gray and eventually became normal. Blue discoloration of shrimp from all three treatments was vary widely, namely in the first week, treatment D exhibited that the shrimp into the normal average one individual, in the second week of the 7 individuals, the third week of the 5 individuals and in the fourth week of the all normal. Followed by treatment C, in the first week there was no change in shrimp, in the second week of the 4 individuals, and also in its third week and finally changed all in the fourth week. The same condition was displayed by treatment B with less number of blue shrimp transforming into normal state.

On the basis of the results presented in Table 1, it is indicated that the higher concentration of extract used, the faster the blue shrimp turned to normal. The blue shrimp turned into normal were suspected because of fulfillment of carotenoid (astaxanthin substance) which can be absorbed by the exoskeleton of shrimp from the sponges extract. Based on the thin layer chromatography analysis, it was clear that *Callyspongia basilana* extract does not only contain astaxanthin but also β-karoten exhibited by a yellow spot under ultra violet light with the value of Rf = 0.33 (by the standard of β-carotene used) with the wave length of 366 nm. Karrer & Solmssen (1953) reported that *Callyspongia* sp. generally contains two types

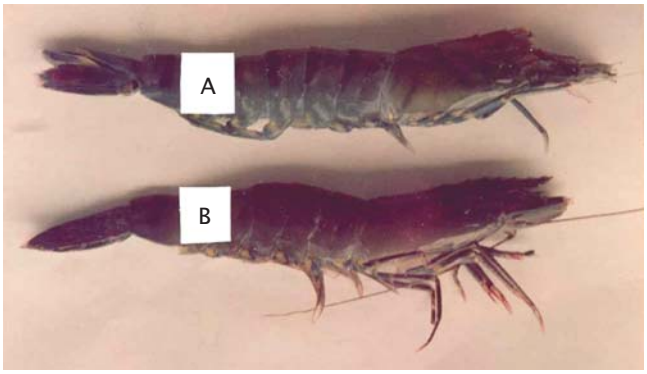

Figure 2. Shrimp which is still blue (A); Shrimp which has turned into a normal shrimp (B)

Table 1. *Penaeus monodon* blue color changes to back to normal in every week during the study

Treatments initial number of shrimp (ind.)		Blue color changes to be normal (ind.) in every week				Final number of shrimp (ind.)
		I	II	III	IV	
A1	15	0	0	0	0	0
A2	15	0	0	0	0	0
A3	15	0	0	0	0	0
Average		**0**	**0**	**0**	**0**	**0**
B1	15	0	3	3	6	12
B2	15	0	3	6	0	9
B3	15	0	6	3	3	12
Average		**0**	**4**	**4**	**3**	**11**
C1	15	0	6	3	6	15
C2	15	0	6	3	0	9
C3	15	0	6	6	0	12
Average		**0**	**6**	**4**	**2**	**12**
D1	15	3	6	6	0	15
D2	15	0	6	6	0	12
D3	15	0	9	3	3	15
Average		**1**	**7**	**5**	**1**	**14**

of major carotenoids which are β-carotene and astaxanthin. This astaxanthin substance has the properties of light absorption, which can change the color of the product (Baticados, 1988; Kompyang *et al*., 1989). The use of sponge containing 9 mg/L carotenoid extract was more effective than that of anti-blue diet at doses of 12.3 g/kg feed which was the fastest treatment to normalize the blue shrimp in the second week (Tompo & Tjaronge, 2005).

Fingerprint analysis of the three varieties showed that there were significant different results among the treatments ($P<0.01$). Treatment A significantly differed with treatment B and C, while treatments B, C, and D differed very strong with the control.

The histology analysis showed that before the shrimp were given carotenoid extract, it was very clearly seen the spreading of blue pigment in the shrimp body (Figure 3) and after the experiment, the existences of the red pigment (astaxanthin) which spread and caused blue pigment to concentrate (Figure 4) were visible. This condition means that blue pigment in the crustacean's body is not dominant or does not appear (Lockwood, 1967).

Survival Rate

The survival rate of tiger shrimp at the end of the experiment among the four treatments displayed different values (Table 2). The highest survival rate (93.3%) was achieved by treatment D. The two other treatments given carotenoid extract exhibited a lower survival rate which were 80% and 73.3% for treatment C and B respectively. Meanwhile the lowest value was showed by the control which was only 13.3%. This value gave an indication that the higher sponge carotenoid extract concentration, the higher survival rate of the shrimp was.

Mostly, mortality was caused by shrimp's cannibalism behavior which occurs during moulting period. During this period, shrimp are in a weak condition in which the skin becomes smoother, so it is an easy prey for other shrimp. Shrimp's cannibalism was previously reported by Wyban & Sweenly (1988). They reported

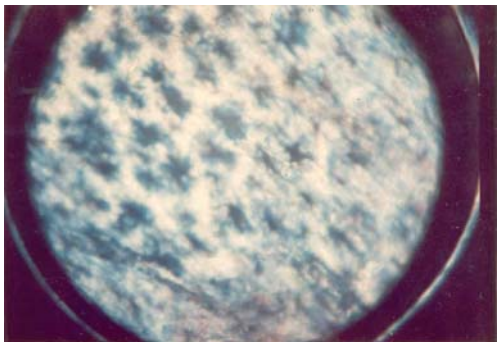

Figure 3. The spread of blue pigment in the shrimp meat tissue before the treatment

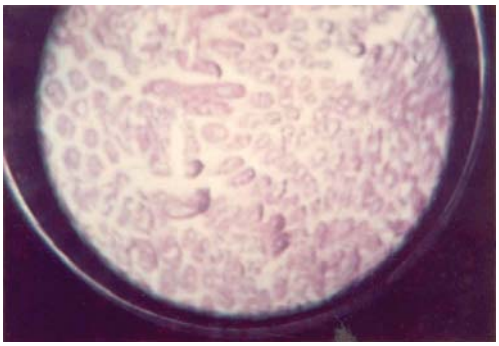

Figure 4. The spread of the blue pigment to red pigment in the shrimp meat tissue after the treatment

Table 2. Percentage of the survival rate of Tiger shrimp at the end of the experiment

Replication	Treatments			
	A	B	C	D
1	20	80	100	100
2	0	60	60	80
3	20	80	80	100
Average	**13.3**	**73.3**	**80**	**93.3**

that prawn is a kind of animal which has a high nature of cannibalism causing a high mortality. The highest mortality shown by the control was the consequence that the shrimp are not in normal condition. The blue shrimp were less resistant and showed a smooth skin surface. In addition, the prawn sometimes exhibited apathy which is to be preyed upon other shrimp (Figure 5). On the other hand, almost all treated shrimp had turned into normal state by which their skin surface became coarse and had the agile nature.

Figure 5. Died blue shrimp

The fingerprint analysis revealed that treatment D and C were significantly different with the control (A) and showed no significant difference with treatment B, while B treatment itself displayed no significant difference with the control (A).

The water quality parameters during the experiment such as temperature, dissolved oxygen, pH, salinity, nitrite, and ammonia were shown in Table 3.

From Table 3, the average water quality parameters during the study were suitable for tiger shrimp survival (Poernomo, 1979; Muslim, 1987; Manik & Mintarjo, 1983; Suyanto & Hardjono, 1986; Poernomo, 1988), except for salinity (38–44 ppt). Although measured salinity showed a high value, reared shrimp were still able to grow well. It means that shrimp had the ability for tolerating the high salinity as it was at the initial stage of the experiment where

Table 3. Range of water quality parameters for all treatments during the experiment

Parameters	Range	Average
Temperature (°C)	27.1-26.8	26.5
Dissolved oksygen (mg/L)	6.3-4.0	5.1
pH	8.2-7.3	7.7
Salinity (ppt)	38-44	41
Nitrite (mg/L)	0.02-0.03	0.025
Ammonia (mg/L)	0.01-0.05	0.03

shrimp were successfully to adapt for 24 hours on the high salinity and temperature.

CONCLUSION AND RECOMMENDATION

Conclusion

Addition of *Callyspongia basilana* carotenoid extract as additional feed ingredient for tiger shrimp is able to normalize the blue diseased shrimp back to normal state within one week (day six) with a concentration of 9 mg/L. Sponge carotenoid extract with concentration 9 mg/L also provides the highest shrimp survival of 93.3%.

Recommendation

Further research should be carried out using higher carotenoid extract concentrations to determine the best concentration of sponge carotenoid extract to effectively accelerate change in color of blue shrimp to back to normal in a short time without any effect on the survival of tiger shrimp.

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