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Cholesterol Effect on Survival and Development of Larval Mud Crab *Scylla serrata*

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The effect of cholesterol on the survival and development of larval mud crab *Scylla serrata* were examined by feeding larvae with *Artemia* enriched with different level of cholesterol. *Artemia* enriched with four stated levels of cholesterol i.e., 0, 5, 10, and 20 ul/l (Chol 0, 5, 10, and 20). All treatments were mixed with DHA70G at 25 ul/l. All the oil was adjusted to 100 ul/l by adding the oleic acid. Survival rate, intermolt period, and carapace width at the fisrt crab stage of mud crab larvae fed Chol 0, 5, and 10 were higher compared to that of Chol 20 ($P < 0.05$). We suggest that free sterol contained in *Artemia* at 1.37% was harmful to the growth performance of mud crab larvae. This study suggests that mud crab larvae required at least 0.61% cholesterol for maintaining good survival and development and therefore no need to enrich *Artemia* by cholesterol for the practical purpose.

Key words: cholesterol, mud crab, *Scylla serrata*, survival

INTRODUCTION

The previous studies have described the *Artemia* feeding regime for mud crab *Scylla serrata* larvae and found that the zoea 3 stage need *Artemia* to prevent the cannibalism (Suprayudi *et al*. 2002a). We also reported that mud crab larvae require highly unsaturated fatty acid (n-3HUFA) as essential fatty acid (EFA) particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at appropriate levels for maintaining high survival, shorter intermolt period and wider in carapace width during rotifers and *Artemia* feeding (Suprayudi *et al*. 2002b; Hamasaki *et al*. 2002; Suprayudi *et al*. 2004a).

Crustaceans are unable to synthesize sterol from acetate, therefore sterols are essential nutrients for crustaceans (Kanazawa & Teshima 1971). The role of cholesterol is as a cell constituent and a metabolic precursor of steroid hormones and molting hormone as well (Kanazawa *et al*. 1971; Teshima 1997). Some researchers have been demonstrated the essentiality of cholesterol in the diet for crustaceans. D'Abramo *et al*. (1984) reported that *Homarus americanus* required 0.19-0.59% cholesterol in the diets for normal growth. Kanazawa *et al*. (1971) reported that the requirement of prawn *Marsupenaeus japonicus* was 0.5% in the diet. Sheen *et al*. (1994) reported that to achieve maximum weight gain and high survival rate of *Penaeus monodon* required 0.2% of cholesterol in the dietary nutrient. Crayfish, *Pacifastacus liniusculus* required 0.4% cholesterol for survival (D'Abramo *et al*. 1985). Contrary to earlier indications, the supplementation of cholesterol in the diet showed no effect on the survival and growth rate of juvenile prawn *Macrobrachium rosenbergii* (Briggs *et al*. 1988).

Crabs such as other crustaceans have been found to be incapable of synthesizing cholesterol from *de novo* (Van der Oord 1964). However, Teshima (1983) showed that swimming crab *Portunus trituberculatus* have capability to converse of other dietary sterol to cholesterol. Moreover, Teshima *et al*. (1983) reported that for larvae and postlarvae development, the dietary cholesterol was nutritionally superior to other sterols. Ponat and Adelung (1983) reported that the requirement of cholesterol for *Carcinus menas* was 1.5% in the diet. Sheen (2000) reported that *Scylla serrata* at the first crab (by using natural seed) stage fed artificial diets containing 0.5% showed better growth performance. There was no information of cholesterol requirement for mud crab larvae under hatchery condition. Thus, the purpose of the present study was to investigate the requirements of larval mud crab for cholesterol during *Artemia* feeding.

MATERIALS AND METHODS

Larvae Rearing and Feeding. A berried female was put into a cylindrical hatching tank (1000 L) with aerated sea water. After almost eggs completely hatch, larvae were separated by stopping the aeration. Actively swimming larvae were concentrated by using light, then transferred into newly filtered sea water and used in the experiments.

Newly hatched out larvae were reared upon reaching the first crab (FC) stage. Larvae were held in 1l plastic beakers at a stocking density of 30 individuals per beaker.

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Larvae were fed on rotifers (40 ind./ml) once daily, from the first zoea (Z1) stage up to the second zoea (Z2) stage. From Z3 larvae were fed *Artemia* at 1.5 naupplii/ml and at the megalopa stage the density of *Artemia* were increased to 4 nauplii/ml. Every morning before feeding, larvae were transferred into new beakers using a 5 ml pipette. During the transfer, larvae were counted and the developmental stage observed. Larvae were separated into different beakers depending on their larval stage. From the zoea one (Z1) to Z5 larvae were held in sea water, and upon reaching the megalopa stage (MG), the salinity were adjusted to 24‰. Detailed explanation of the methodology used in this experiment, have been reported in our previous study elsewhere (Suprayudi *et al*. 2002a,b; 2004a,b).

Hatching *Artemia* Cyst and Enrichment Methods. The cyst (2 g/l) were incubated in filtered and UV treated sea water at 28-29 °C under continuous strong aeration. After hatching, the nauplii were separated from the empty cyst. Newly hatched *Artemia* were held in 2 l plastic beakers filled filtered seawater at the density of 180-200 nauplii/ml with continuous aeration and water temperature kept at 23 °C. *Artemia* were fed oil emulsion contained 200 µl of oil and 18 hours after feeding *Artemia* were harvested. Samples of *Artemia* for each treatment were frozen after being washed with freshwater. Oil emulsion were prepared by mixing 500 µl of oil and 0.1 g of yolk egg in 100 ml water and mix at 10,000 rotation per minute for 2 minutes as describe by Takeuchi *et al*. (1992).

Experimental Design. Four triplicate treatments were prepared to evaluate the effect of cholesterol during *Artemia* feeding. *Artemia* were enriched with different level of cholesterol (MW = 386.66, Wako pure chemicals0industries, LTD., Osaka, Japan) at 0, 5, 10, and 20 µl (Chol 0, 5, 10, and 20). In all treatments DHA70G (containing 70.7 and 5.2% of EPA and DHA, Nippon Kagaku Shiryo Co., LTD, Hakodate, Japan) were also added at 25 µl to fulfill the essential fatty acid requirement. All the oil in each treatment (Table 1) was adjusted to be 100 µl/l by adding the oleic acid (Tokyo Kasei Kogyo Co., Ltd. Japan).

Evaluating Parameters. Survival rate at each stage, intermolt period to reach each stage, carapace width at FC stage and molting synchronicity to megalopal stage were recorded for crab larvae in this experiment. Survival rate was calculated as the percentage value of larvae that successfully molted from the first zoeal stage. Carapace width was measured from the outer lateral spines and the differences between zoeal stages were distinguished by comparing the distance between eyes, as reported in our previous paper (Suprayudi *et al*. 2004b). In addition, crude lipid, fatty acid composition, and polar and nonpolar lipid fractions of *Artemia* were analyzed.

Table 1. Materials for enrichment of *Artemia*

Treatment abbreviation	Oils (µl/l enrichment media)		
	OA	DHA70G	Cholesterol
Chol 0	75	25	0
Chol 5	55	25	20
Chol 10	35	25	40
Chol 20	60	25	40

Oleic acid ethyl ester (purity < 95%), Triglyceride type of docosahexaenoic acid (containing 70.7% DHA and 5.2% EPA).

Chemical Analysis. Total lipid was extracted from the samples of *Artemia* by Folch method (Folch *et al*. 1957) and separated into polar and nonpolar lipids by using silica cartrige (Sep-Pak Waters Ass., USA). For lipid classes fractionation, both of polar and nonpolar extract samples lipids were spotted into chromatorod (Rod S-III) in volumes of 5 ul and developed with dichloroetane/ chloroform/acetic acid (98/2/0.1, v/v) and chloroform/ methanol/destilated (65/35/4, v/v) as the mobile phase. Furthermore, polar and nonpolar lipids were fractionated by using Iatroscan MK-5 (Iatron Laboratories, Inc, Japan).

Total lipids were saphonified by using 3.3% of KOH in ethanol (15 ml) and heating for 40 min at 80 °C. The saphonified lipid then esterified by using 14% of BF_3 in methanol and heating for 20 min at 80 °C. Fatty acid methyl ester was analyzed by using gas liquid chromatography (Shimadzu, GC-14B) equipped with a silica capillary column (30 m x 0.32 mm x 0.25 µm film thickness) (SUPELCO, Bellefonte, USA). Helium was used as the carrier gas and the pressure was adjusted to 100 kPa. Column, injection port, and detector temperatures were adjusted to 205, 250, and 250 °C respectively. Each peak of fatty acid was identified by the comparing retention times against the standard.

Statistical Analysis. One way ANOVA was utilized to analyze the effects of treatments on survival rate, intermolt period, carapace width, molting synchronicity, fatty acid composition and PC. SNK multiple ranged tests were used to determined differences among means. All the statistical analysis was performed using the SPSS 11.0 computer software package.

RESULTS

Lipid Classes and Selected Fatty Acid. Nonpolar and polar lipid fractions of enriched *Artemia* (Table 2) showed that Chol 0, 5, and 10 had a similar content of sterol esther, but lower compared to Chol 20 ($P < 0.05$). Elevating cholesterol level, linearly increase the free sterol content in enriched *Artemia* ($P < 0.05$). There were no differences in triglycerides, free fatty acid content, monoglycerides and diglycerides, phosphatidilcholine, and phosphatidilethanolamine content in enriched *Artemia* for all treatments ($P > 0.05$). Moreover, all treatments also contain the similar ($P > 0.05$) selected essential fatty acid such EPA and DHA (Table 3).

Survival Rate. The survival rate of mud crab larvae fed enriched *Artemia* containing different level of cholesterol (Table 4) showed that there is no different in survival rate ($P > 0.05$) in all stage of mud crab fed *Artemia* containing free sterol at 0.61 to 0.75% (Chol 0 to 10). However, reduced in survival rate were observed when larvae fed *Artemia* containing free sterol at 1.37% (Chol 20) ($P < 0.05$).

Intermolt Period and Carapace Width. Dietary supplementation of cholesterol significantly affect the intermolt period and carapace width of mud crab larvae to reach the first crab (FC) stage ($P < 0.05$) (Table 5). Mud