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EFFECTIVY OF THYROXINE HORMONE SUPLEMENTATION IN THE OVARIAN MATURATION OF FEMALES MUD CRAB *(SCYLLA SERRATA)*

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ABSTRACT

The aim of this research was to study the effectiveity of thyroxine hormone to increase ovarian maturition of broodstock mud crab (*scylla serrata*). The thyroxine hormone has been tested to increase survival and growth of larvae fish but have yet on crab. The weight of broodstock mub crab was 350-400 g. The thyroxine hormone used in this research of levothyroxine sodium tablets production by NV organon, Oss, The Netherland and each tablet have contains 100 µg of thyroxine. This study used treatment with more doses of the thyroxine hormone supplementation were; with doses 0 (control), 0.05ug/BW (low dose); and 0.5ug/BW (high dose). The analysis of variance that the suplementation thyroxine hormone low dose of broodstock mud crab were significant effect ($P<0.05$) in the acceleration ofovary maturation and survival.

Key words: Thyroxine hormone, ovarian maturation, broodstock mud crab

INTRODUCTION

The mud crab (*Scylla serrata*) is one of the commercially important crab. It have beena high fecundity but low survival of larvae. The low survival of mud crab larvae made phase into its own problems in the development technology of hatchery. The presumably cause the reproduction processes of broodstock were long time start from development of the ovaries, embryos to larval development.

Reproduction of male crustaceans is controlled by the endocrine system. The cellular events that occur during the development of the ovary called vitellogenesis process. The female reproduction controlled by a variety of hormonal and neuronal factors (Subramoniam, 2000). These hormones include the neuropeptide hormones, such as the gonad stimulating hormone, and the vitellogenin inhibiting hormone which have an agonist-antagonist effect, respectively, on vitellogenesis. Thyroid hormone is required by all cells in the body to stimulate enzyme synthesis required for cellular metabolism, especially for synthetic anabolism processes (Ayson and Lam, 1993). Thyroid hormones indirectly facilitate the absorption of the vitellogenin from the circulation into the developing oocyte to form

yolk. Thyroxine hormone can easily enter the target cell through the cell membrane. This hormone has long has been known to have an important role in early development. Thyroxine hormone in the circulation of the broodstock can be transferred into the oocyte, the egg and then into the ovary (yolk sac) before ovulation (Ayson and Lam, 1993).

The research of this hormone in mud crab has never been done. Similarly, the physiological condition of the hormone thyroxine is not widely revealed, both the brood stock and the larvae. The present experiment was designed to study the effectivity of thyroxine hormone to vitellogenic stages of brood stock *S.serrata*.

MATERIALS AND METHODS

Animal: The brood stock mud crab (*Scylla serrata*) of various maturation stages were obtained from traditional ponds and mangroves in Tarakan Island, North Borneo, Indonesia. The identification of *Scylla serrata* was conducted according to the description of Kennan *et al.*, (1998). The weights of female mud crabs used ranged from 350 to 400g. The brood stock S. *serrata* were used asvitellogenic stage 2 (maturing) according to John and Sivadas (1978). It were reared in fiber glass tank volume 1000-l and sandy bottom.

Experimental design: The hormone thyroxine of experiment derived from levothyroxine sodium tablets/Thyrax (Nvorganon, Oss, The Netherland). Each tablet contained 100µg of thyroxine. The hormone thyroxine supplementation started of mud crabs with vitellogenic stage 2 (maturing). This experiment consists of 3 triplicate treatments and all treatment of supplementation hormone thyroxine are doses; 0ug/BW(control); 0.05ug/BW (lower doses) and 0.5ug/BW (higher doses). Each treatment was repeated six (6) times. The supplementation hormone thyroxine with injection is done once the appropriate dose and carried between the legs of the road and swimming legs used spuit 1.0ml. Parameters observed in this study include:

Time of ovarian development began from vitellogenic stage 2: Observation of the ovarians maturation of female mud crab was noted after every four days according to John and Sivadas (1978).

Time of incubation: Time was the incubation time (days) required for embryo, calculated up to the female when spawning release larvae.

Survivalrate of female: Survival rate of female was calculated based on the formula S=Nt / Nox100% reported by (Effendie, 1997).

Resistance of larvae: Resistance of larvae carried through starvation. Larvae from each treatment were kept in a container with volume 10 liters density of 5 larvaes L^{-1}.

Chemical analysis*:* Cholesterol concentrations in the ovaries were determined by Lieberman-Burchards method (Burke, *et al.*, 1974). Phospholipids (PL) and the neutral lipid (NL) concentrations in the ovary were

measured by Gas Liquid Chromatography method as used by Husek, (2005). Protein concentration in the hemolymph was measured by Nanadrop 2000 Spectrofotometer thermo scintific dengan Absorbansi 1 pada 280 nm.

Statistical analysis*:* Parameters like time of ovarian development, incubation, survival rate of female, and result of cholesterol, neutral lipid, phospholipid and protein were analyzed by the analysis of variance.

RESULTS

Females mud crabs (*Scylla serrata)* in this experiment were collected from caught of fishermenon around mangrove of the traditional farms. Females *Scylla serrata* have been selected and have the maturity level for research.

Observations of the ovarian maturation were conducted by observing morphological shapes and colors. Ovarian morphology after suplementation thyroxine hormone can be seen in Figure 1.

A B C

Figure-1: The ovarium maturation of vitellogenic stage 2 (A = control, B= 0,05 ug/BW and C= 0.5ug/BW).

Figure 1 shows the ovarian maturation of female mud crab in process vitelogenic stage 2 with sample control and supplementation thyroxine hormone. Wherever, the treatment of a low dose (treatment B) of the thyroxine hormones showed the same ovarium color with the control is yellow but the treatment with high-dose (treatment C) showed the orange ovarium color.

Based on Figure 2, the the ovarian maturation of brood stock mud crab in process vitelogenic stage 3. There are the difference colors and size of ovarium in the treatment A, B and C. The difference colors and size of ovarium cause effect suplementation thyroxine hormones with low and high doses.

A **B** **C**

Figure-2: The ovarium maturation of vitellogenic stage 3 (A = control, B= 0,05 ug/BW and C= 0.5ug/BW

Thyroxine hormone was increased absorption yolk during vitellogenic process and the effects uplementation of thyroxine hormone on the brood stock *S.serrata* wereincreased the time of ovarian maturation.

The results (Table-1) shown ovarium maturation of brood stock *Scylla serrata,* starts from vitellogenec stage 2 to berried. In the treatment suplementation hormone thyroxine dose 0.05 µg/BW was the faster maturity than control. The suplementation low dose 0.05 µg/BWneeded 8 days to mature ovary from vitellogenic stage 2 to vitelogenic stage 3 than the control needed 12 days, while the suplementation high dose 0.5 µg/BW needed 2 day after that all female dead. The suplementation low dose 0.05 µg/BW needed 38 days to maturity ovary from vitellogenic stage 3 to berried than control need 42 days.Based on the analysis of variance of the suplementation thyroxine hormone in the female *S. Serrata* indicates that there is a real effect in the acceleration of mature ovarian. The treatments supplementation thyroxine hormone low dose resulted a significantly ($P<0.05$) time maturity ovarium than control . This means that the dose of the supplementation thyroxine hormone can be affect acceleration mature ovarium in the process vitelogenesis.

Table-1: Data (mean ± SD) of development ovary maturity of female *Scylla serrata(days*)

Treatment	Vitellogenic stage 2-3	Vitellogenic stage 3-Berried	SR (%)
A	12.7±1.49[a]	42±00.0 [a]	16.7 [a]
B	8.25±0.43[b]	38.3±3.77 [b]	50[b]
C	2*[c]	0	0

Means in the same column with the same superscripts under source of treatments and control are significantly different ($P<0.05$).*Treatment doses; A= 0* µg/BM; B= 0,05 µg/BM; C= 0,5 µg/BM.

Based on observations on the survival of male maturity mudcrab (Table-1), it appears that the suplementation thyroxine hormone low-dose shown asignificant effect (P <0.05) with control but was be deadly if the dose of thyroxine hormone given in high quantities.

The results showed that the profiles of cholesterol, neutral lipid, phospholipid and protein in the ovary were not changed dramatically during vitellogenic stage or ovarian maturation as compared to those of thyroxine hormone (Table 2). The concentrations of cholesterol, phospholipid and protein of female mature with suplementation thyroxine hormone low-dose, in the ovary were low during vitellogenic stage 2and slightly increased during vitellogenic stage 3. Based on the results of ANOVA analysis showed a significant difference (P <0.05) between treatments.

Table-2: Data (mean ± SD) The concentration of protein, cholesterol and phospholipid on ovarium maturition of female mud crabs

	Protein (µg/mL)		Cholesterol (mg/gr)		Phosfolipid (mg/gr)	
	Vitellogenic stage 2	Vitellogenic stage 3	Vitellogenic stage 2	Vitellogenic stage 3	Vitellogenic stage 2	Vitellogenic stage 3
A	42.46^{a} ±2.06	50.38^{a} ±3.88	2.52^{a} ±0.08	3.66^{a} ±0.31	2.30^{a}±0.46	2.84^{a}±0.88
B	63.71^{b} ±3.19	77.94^{b} ±5.76	2.68^{b} ±0.03	3.84^{b} ±0.24	2.06^{a}±0.46	3.49^{b}±0.46

Means in the same column with the same superscripts under source of treatments and control are significantly different (P<0.05).*Treatment doses; A= 0* µg/BM; B= 0,05 µg/BM

The test resistance larvae from female each treatments done through starvation or gratification. Larvae from each treatment maintained in container a volume 10 liter and the density of 6 species L^{-1}. During the test period the larvae are not fed. The number of larvae survive expressed by percent (%),interval performed testing since 12 hours until no more larvae were alive (Table 3).

Results of larvae quality shown that the suplementation of the thyroxine hormone in the female maturity increase the survival of mud crab larvae. Larvae were derived from the female who got a low dose of thyroxine hormone treatment has a longer survivability is about 84 hours than the controls (Table 3).

Table-3: The survival rate of larvae *Scylla serrata* without feed (hour)

Treatment	Times (hour)								
	0	12	24	36	48	60	72	84	96
A SR (%)	100	95	78.3	60	18	13	5	0	0
B SR (%)	100	91.7	71.7	65	45	25	16.7	8.3	0

The water quality treatment during the study was maintained where the temperature range around 28-30^{o}C, about salinity range around 28-30ppt and DO around 5.4-6.8ppm.

DISCUSSION

Ovarian maturation process (vitelogenesis) on mud crab is the process absorption vitelogenin by ovary which came from the hepato-pancreas. Yano (1992), states that vitelogenin is a raw material or a precursor of egg yolk proteins are synthesized to mature egg cells (oocytes). Through the blood stream, vitelogenin be selectively absorbed by a layer of follicle oocytes (Zairin, 2003; Yaron and Sivan, 2006). This process is known as vitelogenesis, while the next is the final maturation process in which there is movement to the edge of the egg nucleus, or germinal vesicle break down (GVBD) and ovulation is marked by rupture of the follicle and release of the egg layer into the cavity of the ovaries (Zairin, 2003; Yaron and Sivan, 2006). Result in Table 1 shown that the suplementation of thyroxine hormone low dose helps the absorption process vitelogenin by ovary so the time to reach the final ovarian maturation faster than the controls. Egg yolks will be a source of nutrients for embryonic development (Silversand *et al*., 1993). Thyroxine is a hormone to stimulate the growth and development of the gonads. The supplementation of thyroxine hormone illustrated the higher metabolic activity at the maturity level of the female. The suplementation of thyroxine hormone low dose can increase the survival of female maturity than control. The high doses were used to increase mortality of female (Table-1). Djojosoebagio (1990) reported that the suplementation of the hormone thyroxine in excessive have disturb the function of organs body so that the organs will showed impeded performance.

The function of thyroxine hormones was an auxiliary metabolic processes and absorption nutrient. It have been evidenced by the use of high concentration of cholesterol, phosfolipid and protein during ovarian development (Table 2). The deposition of thyroxine and vitellogenin, as indicated by cholesterol, neutral lipid, phospholipid and protein in the ovary increased with the advance stage of ovarian maturation. The yolk proteins synthesized during vitellogenic stage 2 and stage 3 are immediately released from the hepatopancreas into the hemolymph. Furthermore the yolk proteins in the hemolymph are then transported into the ovary causing the increased concentrations of yolk protein in the ovary during vitellogenic stage (Subramoniam, 2000).

Thyroxine hormone supplementation also affects was the concentration of protein in ovarian maturity level. During the period of gonad development, protein is needed for growth and developments of the ovaries than to meet the needs of the parent sometimes have to mobilize fat and protein reserves (Altiner, 2006). Supplementation thyroxine hormone with more doses to female S. serrata at

vitellogenesis stage 2 can increase concentration of protein in hemolymphthan controls (Table 2).

The results of this study indicated that there is similarity between the patterns of hormone metabolism increase with the maturity level of the ovary. This was an overview of the relationship between the acceleration of blood improvement with ovarian maturation. This is in accordance of Clowes *et al.,* (2003) results that the increase of protein and metabolic rate showed effect on reproductive performance.

The concentration of lipid of cholesterol, phospholipids and neutral lipid were increase in accordance to development of ovarian maturity (Table 2). The concentrations of lipid in female *S.serrata* received supplementation thyroxine hormone were higher than controls. This proves that the thyroxine hormone with optimum dose was optimize the absorption of lipid when vitelogenesis process. This is in accordance of Teshima *et al.,* (1983) reported that there is always an increase lipid concentration of immature to mature ovaries, the level is maintained at mature ovaries and decreased after spawning.

Effect of hormone suplementation indicated on larvae tested with starvation process where larvae reared until death without feed. The result (Table 3) shown that larvae from the addition of thyroxine treatment, have a better quality of life than the larvae control. This proves that the suplementation of thyroxine hormone have been improved the quality of female and larvae.

CONCLUSION

The supplementation of thyroxine hormone with low dose (0.05µg/ BW) was effective to increase ovarian maturation of female mud crab (*Scylla serrata*) than control.

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