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Suplementation Doses Thyroxine Hormone of Broodstock Mud Crab (*Scylla serrata*) During Ovarian Maturation

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Abstract

The aim this research was to increas maturity ovarian of broodstock mub crab (*Scylla serrata*). Mud crab is one commodity yet optimal aquaculture technology. The presumably was to slow the vitellogenesis process stage.The maturity ovarian began by vitelogenin secreted into hemolymph and taken to the ovum to be synthesized into egg yolks.Thethyroxine hormone containing elements, and stored in the follicle assist in the process of yolk absorption. This study used treatment more doses ofthe thyroxine hormone supplementation were with doses 0 µg/BW (control), 0.05 µg/BW; 0.1 µg/BW, and 0.15 µg/BW. The results showed that treatment doses of 0.1 µg/BW has accelerated the fastest of maturity ovarian than others. The analysis of variance that the supplementation of hormone thyroxine were a significant effect (P<0.05) in the acceleration of mature ovarian. The suplementation of thyroxine was increas yolk absorption in vitellogenesis process. It have affectsare an increase concentration of proteinand RNA/DNA during maturity ovary.

Keywords: Mud crab; Maturity ovarian; Thyroxine hormone supplementation

Introduction

The mud crab (*Scylla serrata*) is one of the commercially important crabs and the only species of the genus Scylla in the Indian Ocean. It has both ecological and economic importance to the marine environment and to the coastal fishing villages [1]. However, the production and availability of crab seeds is the main problem in culture of mud crabs. To solve the problem, there is a need to develop a technique to propagate the crab by improving the reproduction of the crab and the survival of the larvae.

Mub crab reproduction required considerable time to produce larvae start from vitellogenesis processes that occur in the body until the embryonic development occurs outside the body. Many factors impact the process were from inside body like hormones and from outside like feed and the environment.

In crustaceans, female reproduction was controlled by a variety of hormonal and neuronal factors [2]. 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. Thyroxine hormone in the circulation of the brood stock can be transferred into the oocyte, the egg and then into the ovary (yolk sac) before ovulation [3]. 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.

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 broodstock and the larvae. The present experiment was designed to study profiles and effect of suplementation the thyroxine hormone to vitellogenesis stages of famele *S. serrata*.

Materials and Methods

Animal

The female 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 [1]. The weigths of female mud crabs used ranged from 350 to 450 g. The female *S. serrata* were classified as vitellogenesis stage 1(immature), vitellogenesis stage 2 (premature) and vitellogenesis stage 3 (mature) according to John and Sivadas [4]. It were reared in fiberglass tank volume 1000-l and had sandy bottom and shelter provided. A berried female was transferred into an 80-l holding tank equipped withflow-through water system.

Experimental design

The hormone thyroxine of experiment derived from levothyroxine sodium tablets/Thyrax (NVorganon, Oss, The Netherland). Each tablet contains 100 mg of thyroxine. Observations of concentration hormone thyroxine started mature mud crabs with vitellogenesis stage 1 (immature) using the ELISA method. The hormone thyroxine supplementation started mature mud crabs with vitellogenesis stage 2 (premature). This experiment consists of 4 triplicate treatments and all treatment of supplementation hormone thyroxineare doses; 0 µg/BW(control); 0.05 µg/BW; 0.1 µg/BW and 0.15 µg/BW. Each treatment was repeated three 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 ml. Thyroxine

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hormone concentrations in the extract of hemolymph from mature mud crabs with vitellogenesis stage 1-3 was determined according to ELISA method (DRG International Inc., USA).

Chemical analysis

Cholesterol concentrations in the ovaries were determined by Lieberman-Burchards method. Phospholipids (PL) and theneutrallipid (NL) concentrations in the ovary were measured by Gas Liquid Chromatography method (GLC) with the method used by Takeuchi. Protein concentration and RNA/DNA in the hemolymph was measured by Nanadrop 2000 Spectrophotometer thermo scintific dengan absorbance 1 pada 280 nm.

Statistical analysis

Thyroxine concentrations in the hemolymph, hepatopancreas, ovary, sponge and larvae and the concentrations of cholesterol, neutral lipid, phospholipid and protein in the ovary were analyzed by the analysis of variance.

Results

Based on observations of thyroxine hormone concentrations in the female *S. serrata* were classified as vitellogenesis stage 1 (immature), vitellogenesis stage 2 (premature) and vitellogenesis stage 3 (mature) showed an increase in concentration during maturity ovary (Table 1).

The result supplementation of thyroxine hormone to female *S. Serrata* haveincreased the concentration itin ovary and based on analysis of variance showed that the results were not significantly (P>0.05) between treatments A and B, but significantly (P<0.05) with treatment C and D (Table 2).

Supplementation thyroxine hormone has effects of protein concentration in ovarian maturity thatis an increase concentration of protein (Table 2). The based on analysis of variance showed that the supplementation thyroxine hormone have improve protein concentration during ovarian maturation process when compared with control (P<0.05).

The results of RNA and DNA showed it improve accordance development maturity ovary. The highest of increase RNA and DNA occurs in the treatment C (hormone dose of 0.1 µg/BW), and the lowest occurred in treatment A (Table 2) (Figure 1).

Concentration of thyroxin hormone was the highest during the beginning of maturity ovarian development. The results (Figure 1) showed a day maturity ovarium development, which starts from vitellogenesis stage II to dispense eggs (berried). In the treatment suplementation hormone thyroxine dose 0.1 µg/BW was the fastest maturity (24 days). In the suplementation it dose 0.15 µg/BW, maturity days decreased to 26 days. The next suplementation dose 0.05 µg/BW needed days maturity to 31 days. The latest day maturity of treatment control was 55 days. Based on the analysis of variance of the addition of multiple doses of the thyroxine hormone in the female *S. serrata* indicates that there is a real effect in the acceleration of mature ovarian stem between treatments. All treatments supplementation thyroxine hormone resulted a significantly (P<0.05) different time maturity ovarium than control. This means that the dose of the supplementation thyroxine hormone can affect acceleration mature ovarium in the process vitelogenesis (Table 3).

Treatment	Vitellogenesis 1	Vitellogenesis 2	Vitellogenesis 3
A	5.0 ^{a} ± 0.21	5.5^{a} ± 0.21	7.1^{a} ± 0.28
B	5.3 ^{a} ± 0.07	7.0^{b} ± 0.35	8.7 ^{b} ± 0.71
C	5.2 ^{a} ± 0.28	9.0^{c} ± 0.21	13.0^{c} ± 0.57
D	5.6 ^{a} ± 0.35	7.9^{b} ± 1.27	8.5^{b} ± 0.35

Means in the same row 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=0,1 µg/BM; D0:15 µg/BM.

Table 1: Concentrations of thyroxine hormone (ng/mL) in the hemolymph, of female mud crabs (*Scylla serrata*) in different vitellogenesis stages.

When the female is ready to spawn, egg cells (oocytes) are forced from the ovaries through the seminal receptacles where they are fertilized. The fertilized eggs, which are about ± 0.25 mm in diameter, are then extruded into a large, cohesive mass or "sponge" that remain attached to the fine hairs beneath the abdomen until they hatch to be a larvae. Concentrations of thyroxine hormones in different treatment of suplementation are presented in Table 3.

The result (Tabel 3) shows that the higher of dose hormone given to female make the higher concentrations of thyroxine were lowered at berried. Based on ANOVA analysis showed significant effect (P<0.05) between the treatment of hormone supplementation to control (Figure 2).

The result of Figure 2 showed that the time (days) development of the embryo to hatching egg. The fastest hatching egg were female crab have supplement thyroxine with a dose 0.15 µg/BW (9 days), the next dose of 0.1 µg/BW (10 days), the thirth dose of 0:05 µg/BW (12 days) and the longest dose of 0 µg/BW (12.5 days). Based on results of statistical analysis showed significantly (P<0.05) between treatments using the hormone thyroxine supplementation with dose 0.15 µg/BW and dose 0.1 µg/BW thancontrol. But not significantly (P>0.05) between treatments several doses of hormone supplementation.

Female fecundity was also observed to determine the effect of the hormone thyroxine supplementation andthe result can be seen in Table 4 below. Fecundity is the number of eggs that can be produced by a mother crab females (Table 4).

The results showed that the highest fecundity values found in crabs that thyroxine hormone supplementation treatment D at dose 0.15 µg/BW (1.86 × 106) and than next dose 0.1 µg/BW, dose 0.05 µg/BW, and control dose 0 µg/BW. Based on the statistical analysis of the overall fecundity of the female crabs were not significantly different (P>0.05).

Discussion

The result of the reaseacth that the thyroxine hormone supplementation of the female mub crab maturity had increase of concentration it to transferred in ovary. This is in accordance with the opinion Ayson and Lam [3] that the thyroxine hormone in the circulation of the broodstock can be transferred into the oocyte,

Treatment	protein (mg/mL)		RNA (ng/µl)		DNA (ng/µl)	
	Vit. II	Vit. III	Vit. II	Vit. III	Vit. II	Vit. III
A (control)	42.46 ± 2.06^{a}	50.38 ± 3.88 ^{a}	21.61 ± 0.74	29.80 ± 4.74	5.97 ± 0.39	11.24 ± 5.09
B	57.42 ± 2.63^{b}	67.91 ± 3.43 ^{b}	26.79 ± 5.21	36.57 ± 1.40	14.39 ± 1.19	16.51 ± 4.29
C	63.71 ± 3.19^{c}	77.94 ± 5.76 ^{c}	31.19 ± 1.48	38.06 ± 5.39	17.83 ± 2.94	19.83 ± 1.71
D	62.97 ± 4.24^{c}	76.35 ± 8.59 ^{c}	22.57 ± 9.29	36.11 ± 1.29	11.83 ± 3.45	17.71 ± 1.52

Table 2: Concentrations of protein and RNA/DNA in hemolymph of female mud crabs (*Scylla serrata*) in different follicle developmental stages.

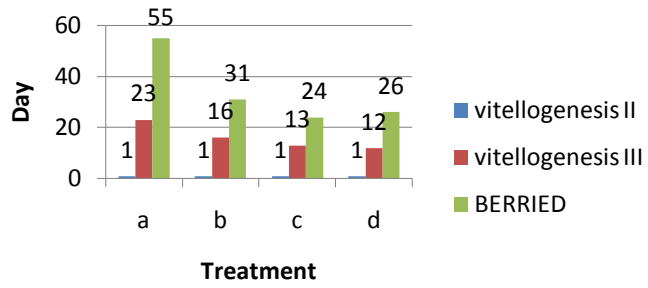

Figure 1: Time (days) of development ovary maturity of female mud crabs (*Scylla serrata*).

No	Treatment	Colour of Sponge	Consentration of thyroxine (ng/mL)
1	A	Orange	86.13 ± 7.21
2	B	Orange	88.30 ± 1.95
3	C	Orange	93.73 ± 2.33
4	D	Orange	96.40 ± 2.39

Table 3: Concentrations thyroxine hormone of egg berriedfemale *S.serrata*.

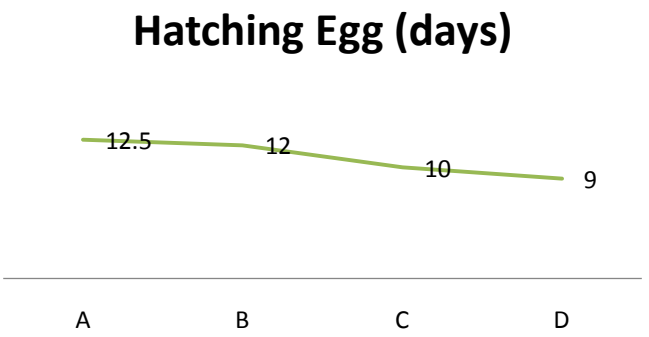

Figure 2: Hatching egg *Scylla serrata*.

	Treatment	Fecundity	Weigth Female (gram)
1	A	1.71 ×10^6 ± 1.64 × 10^5	380 ± 27.84
2	B	1.72 × 10^6 ± 1.61 × 10^5	375 ± 35.00
3	C	1.79 × 10^6 ± 2.66 × 10^5	400 ± 25.00
4	D	1.86 × 10^6 ± 1.30 × 10^5	401 ± 20.00

Table 4: Fecundity of female *S.serrata*.

egg, and then into the ovary (yolk sac) before ovulation. 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 development of the ovaries than to meet the needs of the parent sometimes has to mobilize fat and protein reserves [5]. Supplementation thyroxine hormone with moredoseto female *S. serrata* at vitellogenesis stage II can increase concentration of protein in hemolymph than controls. The results of this study indicate that there are similarities between the patterns of increase in hormone metabolism with ovarian maturity level. It gives an overview of the relationship between speed improvement with mature ovarian metabolites in the broodstock. The suplementation doses of thyroxine hormone in the female *S. serrata* indicates that there is a significantly ($P<0.05$) for the acceleration of maturity ovarian. The better dose of the hormone thyroxine supplementation was dose 0.1 µg/BW. It can more effect acceleration maturity ovarian in the vitellogenesis process than other. Base on Table 4. Showed that the higher the dose of hormone to make the increase in the concentrations of thyroxine derived at the time of berried. Result ANOVA analysis showed significant effect ($P<0.05$) between the treatment of hormone supplementation than control. It has been proven that the thyroxine hormone can be lowered from the broodstock to the embryo. Based on the research results that thyroxine supplementation does not affect of fecundity ($P>0.05$), but more correlated with body weight.

Crustacean reproductive females are controlled by the endocrine system. Cellular actifity occur during the development of the ovary called vitellogenesis process, which is the process by which vitellogenin (Vg), an egg yolk precursor protein, accumulated in the developing oocyte. This leads to changes in the gonads. Vitellogenesis process derived from the granulosa layer of the follicle oocytes are released into the blood, and stimulate the liver to mensintasis vitelogenin. Once synthesized vitelogenin released into the blood, then selectively absorbed by the oocyte. Vitellogenesis is an important step in the maturation of the ovary. Vg can be synthesized in the ovarium and other non-ovarian like hepatopancreas [6].

Thyroid hormone has long been known to have an important role in early development. Either thyroid hormone T3 and T4 that enters the body is brought to the target cell by the plasma proteins. Djojosoebagio [7] suggest that thyroid hormones containing iodine bound elements, and stored in the follicle. This hormone is not directly assist in the process of yolk absorption. One type of thyroid hormone that plays an important role in the metabolism of hormones is thyroxine (T4). Thyroxine is a hormone that is ionized outside the thyroid follicular cells or on the outside of the apical membrane. In follicles, prohormone thyroxine binding to thyroglobulin. It is known that T4 is a hormone an important role to stimulate growth and gonad development. The base on reasearchthat the concentration of the hormone thyroxine supplementation described metabolic activity in line with the maturity ovarian.

The increase of protein and metabolic will be changes affect reproductive performance[8]. Conversely, the decrease of protein for gonad development will be degrade proteins of the body and reduce appearance embryos, which specifically can reduce the availability of nutrients, especially essential amino acids for growth. The result supplementation of thyroxine hormone increased the concentration of protein in the hemolymph compared than control. That mean are similarities between the patterns of increase in hormone metabolism with ovarian maturity level.

Maturity ovarian began by vitelogenesis process, namely the process of yolk formation is characterized by the deposition vitelogenin into the ovum. Vitelogenin secreted into hemolymph and taken to the ovum to be synthesized into egg yolks. Yano [9], states that vitelogenin is a raw material or a precursor of egg yolk proteins are synthesized to mature egg cells (oocytes). Through the bloodstream, vitelogenin be selectively absorbed by a layer of follicle oocytes [10,11]. 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 fusing nuclei break down (GVGD) and ovulation is marked by rupture of the follicle and release of the egg layer into the cavity of the ovaries [10,11]. Egg yolks will be a source of nutrients for embryonic development [12]. The raw material vetelogeninis vitelin, which is synthesized by extra ovarium tissue and it released into hemolymph cause response to Vitellogenin Stimulating Ovarian Hormone (VSOH). Based on the results of the study showed that the suplement of the thyroxine hormone to female *S. serrata* involved vitellogenesis process.

Result on Table 2. The thyroxine hormone supplementation were to the development of RNA and DNA same as the vitellogenesis stage. The effect of thyroid hormone on protein synthesis through mRNA activity given depends on the dose. Thyroid hormones (T3 and T4) in organisms involved in regulation of energy homeostasis and metabolism, protein and lipid. The influence of the thyroid on the synthesis of proteins through RNA activity. The interactions of thyroid hormone and receptor at the core increased activity polymerase enzyme and RNA formation [7]. The increase of transcription RNA and protein synthesis that mean T4 suplementation influced absorption protein in vitelogenin.

Result showed all treatment tofemale crabs not influence fecundity procces. According to Racotta et al. that the weight of broodstock can be effect of the fecundity, spawning frequency, and degree of fertilization.

Conclusion

The treatment of thyroxine hormone supplementation dose of 0.1 µg/BW to broodstock mud crab (*Scylla serrata*) during ovarian maturation provided optimum results in the vitellogenesis process and hatching rate of the ovaries.

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