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INDUCTION OF GONADAL MATURATION OF POND CULTURED MALE TIGER SHRIMP, *Penaeus monodon* WITH DIFFERENT DOSAGES OF GONADOTROPIN RELEASING HORMONE ANALOGUE AGAINST EYE STALK ABLATION

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ABSTRACT

Very low naturally mating rate of pond-reared tiger shrimp broodstock is probably due to the slow maturation of the male stock. The aim of this study was to evaluate the salmon gonadotrophin releasing hormone analoque (sGnRHa) in stimulating the gonadal maturation of male stock of pond-reared tiger shrimp. The treatments were three dosages of sGnRHa at 0.1 (OV-1), 0.2 (OV-2), and 0.3 (OV-3) mL/kg of shrimp weight and control was eye stalk ablation (AB). The sGnRHa was administered via injection three times with one week interval. Male stocks with average initial body weight of 82.1 g were randomly distributed into four of 10 m^3 concrete tanks, 26 males for each tank. Variables observed were performances of spermatophores and profiles of amino acid and fatty acid of muscle of the male stocks. After induction, number of male maturing indicated by spermatophores releasing from terminal ampullas was higher in shrimp induced with OV-1 (80.8%) compared to control which was only 46.1%. Furthermore, shrimp treated OV-2 had the highest spermatophore weight of 0.16 g compared to control (0.11 g) and other two groups. Amino acid profiles improved as the dose of sGnRHa increased up to 0.2 mL/kg from 61.23% for ablated male becoming 71.27% for OV-2. Total fatty acid also tended to improve by increasing the dose of hormone injection, however, the ablated male had higher total fatty acid content than that of OV-1. The present finding demonstrated that the dose of sGnRHa to stimulate the gonadal maturation of pond-reared male tiger shrimp could be applied at range between 0.1-0.2 mL/kg of shrimp weight.

KEYWORDS: sGnRHa; hormone dosage; spermatophore; reproduction; tiger shrimp

INTRODUCTION

Very low naturally mating rate of tiger shrimp broodstock in captive remains the main constrainst for domestication of this penaeid species. Immatured spermatophore produced by male stock could be the reason why broodstock cannot mate naturally in pond (Laining *et al*., 2015a) or it is related to the infertility of sperm releasead by the pond-reared male stock (Pongtippatee *et al*., 2007). Jiang *et al*. (2009) reported that culture condition influenced the reproductive traits of males of *P. monodon* at the same age and size. In addition, several studies previously reported the low hacthing of eggs spawned from domesticated stocks of *P. monodon* (Menasveta *et al*., 1994; Coman *et al*., 2007).

Eye stalk ablation is a hormone manipulation which has been widely practised by commercial hatcheries in order to induce gonadal maturation of female stock (Primavera *et al*., 1978). This technique has been reported as the most successful hormonal manipulation for inducing maturation of penaeids in captivity (Primavera, 1978; Sellars *et al*., 2006; Coman *et al*., 2007). However, this technique has several negative effects on the female stock itself. Eye stalk ablation could cause heavy stress or even kill the shrimp during the application and moreover, it was detected that total amino acid in carcass of ablated female was significantly lower than that of injected gonadotropin hormone (Laining *et al*., 2015a). It was suspected that eye stalk ablation changed the physiological process of the broodstock that decreased its ability to synthesize amino acid. An alternative to eye stalk ablation for female broodstock is to use synthetichormone either via injection (Alfaro *et al*., 2004; Wongprasert *et al*., 2006) or oral administration (Kagawa *et al*., 2013). Our recent study dealing with wild female tiger shrimp broodstock showed that injection of pregnat mare serum gonadotropin (PMSG) hormone combined with dopamin

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antagonistat dose of 0.3 mL/100 g of shrimp weight resulted in a higher number female spawn of 82% which was higher compared to 60% for ablated female. However, hatching rate of the eggs spawned was higher (52%) at ablated female than that of injected with the combined hormones which was around 39% (Laining *et al*., 2015a).

Other hormone which has been widely applied for stimulating the gonadal maturation of fish is the salmon gonadotropin releasing hormon analogue (sGnRHa, commercial name Ovaprim) (Cejko *et al*., 2012; Genz *et al*., 2014; Jamroz *et al*., 2008; Zadmajid, 2016; Zarski *et al*., 2009). This commercial hormone is a liquid peptide preparation that contains sGnRHa 20 μg/mL and domperidone 10 mg/mL. Application of this hormone has not been tried so far for the shrimp gonadal maturation in particular the pond-reared male tiger shrimp broodstock. Therefore, evaluation of sGnRHa to improve the reproductive performances of the pond-reared male tiger shrimp was necessary to be carried-out. The aim of this study was to assess the influence of different levels of sGnRHa in stimulating the gonadal maturation of pond-reared male tiger shrimp, *Penaeus monodon* broodstock.

MATERIALS AND METHODS

Pond-Reared Male Broodstock of Tiger Shrimp

Matured size of male tiger shrimps used for the hormonal manipulation were provided by rearing early prematured shrimp in concrete ponds at experimental pond unit of Tiger Shrimp Hatchery of Research Institute for Coastal Aquaculture (RICA) located in Barru Regency. Tiger shrimp with initial body weight of 32.0 ± 1.1 g were stocked into two of 1,000 m^2 concrete ponds at stocking density of one shrimp/m^2. The age of the shrimp at stocking were approximately six months. During culture, shrimp were fed commercial diet for tiger shrimp enriched with 0.45% carotenoids mixture (Laining *et al*., 2015b) and 0.1% stable vitamin C (Laining *et al*., 2014a). Shrimp were fed four times a day at 07:00, 13:00, and 19:00 at rate of 2%-5% of biomass for around five months until reaching maturation stage. Selected matured male shrimps from each pond were transferred into quarrantined tanks for one week maintance before being used for the treatments.

Induction of Gonadal Maturation with sGnRHa Injection

Induction of gonadal development of the male stock was carried-out at Shrimp Hacthery of RICA from September until November 2015. The treatments were three different dosages of sGnRHa at 0.1 (OV-1), 0.2 (OV-2), and 0.3 (OV-3) mL/kg of shrimp weight and as the control was male with eyed ablation (AB). These doses are recommended by the producer as indicated in the package of the hormone. The sGnRHa was administered via injection three times with one week interval following the previous protocol dealing with wild tiger shrimp female broodstock (Laining *et al*., 2015a).

Matured male tiger shrimps with 11 months of age were selected and transferred from quarrantine area into maturation room at nucleous centre of the hatchery. Males were selected based on weight and morphologically appearances. Male stocks with average initial body weight of 82.1 g (minimum weight of 60 g) were randomly distributed into four of 10 m^3 concrete tanks with density of 26 males for each tank. Shrimp in each tank were tagged on the eyestalk for individual identification. After a week adaptation at maturation tanks, all males in the three tanks were injected the sGnRHa except for those were just molted, their injections were delayed until their shell become normally hard. At the same time males from controlled tank were ablated using steriled scissor. A week after the last injection, males were electrically shocked (Diwan & Joseph, 2009) to release their spermatophores from ampula terminalis using transformer connected with two electrodes. The edge of the electrodes were placed carefully near gonopores at the base of the fifth pereiopods while turning on the transformer set at 15 V, 7 mA for 1-5 seconds stimulating the shrimp to ejaculate its spermatophores (Laining *et al*., 2014c). Male whose spermatophore released from its terminal ampullas was assumed to be maturing males. A week after first electrical shocked ejaculation, males were repeatedly shocked to determine the number of rematuration within two weeks assessment with one week interval.

During maturation development periode, all shrimps were fed fresh feed (squid and clam) at the same portion combined with commercial-enriched pellet diets at a rate of 40%:60% (Laining *et al*., 2014b). The pellet applied during the maturation phase was similar to that was used for the prematuration stage. Feed was given at the rate of 2%-2.5% of biomass four times a day at 08:00, 12:00, 16:00, and 20:00 hours.

Variables Observed and Chemical Analysis

Variables observed were number of male releasing spermatophores through electrical shock and weight of the spermatophores produced at maturation and re-maturation stages. Proximate analyses and

micro-nutrient profiles of shrimp muscle post-induction including amino acid and fatty acid content were also determined. Proximate analyses were carried out according to AOAC (1999) methods. Briefly, moisture was analysed after drying the samples at 105°C for 16 hours using oven (Memmert, Germany). Crude protein was determined according to micro-Kjeldahl procedure and lipid was extracted using chloroform and methanol. Ash was analyzed using muffle furnace at 550°C (Barnstead, Thermolyne, CA, USA). The amino acid profile of shrimp fillet was analysed using high performance liquid chromatography (HPLC) in a Shimadzu 20A HPLC (Tokyo, Japan) whereas fatty acid analysis were carried out using Gas Chromatography (Shimadzu 2010 plus, Tokyo, Japan). Data collected during induction period were descriptively discussed.

RESULTS AND DISCUSSION

Growth and Survival Rate of Tiger Shrimp During Prematuration Stage

The growth of tiger shrimp during prematuration stage is illustrated by Figure 1. During 150 days culture, the shrimp tended to grow liniearly showing that the culture environment was still in the proper condition even at the fifth months of the rearing periode. Survival rate of the shrimp was 36.9% ± 2.5% which was normal value for tiger shrimp.

Male Gonadal Maturation and Weight of Spermatophores

Number of males maturing and rematuring indicated by spermatophores released through electrical shock post-hormonal induction are presented in Table 1. After three times injection of sGnRHa, males injected with 0.1 mL/kg (OV-1) had the highest number of male releasing spermatophores (80.8%) among the treatments. Increase of sGnRHa injection dose up to 0.3 mL/kg tented to negatively adverse the spermatophore production indicated by decreased number of male releasing spermatophores after electrical shock which was the lowest for OV-3 (42.3%). However, percentage of maturing male induced with the OV-2 was 46% which was similar to control ablation (AB). Furthermore, number of rematuring male was highest at male induced with OV-1 (23.1%) followed by OV-2 (19.2%) and the lowest at OV-3 which was only 7.7%.

Results found in the present study demonstrated the positive effects of sGnRHa in stimulating gonadal maturation of pond-reared male tiger shrimp in which injection of 0.1 mL/kg (OV-1) produced the highest spermatophores up to 80%, two times higher than that of OV-2 and OV-3 as well as the control ablation. This finding demonstrated that the sGnRHa which has positive effects on various finfish reproductive performances including the male (Cejko *et al*., 2012; Cejko & Kejszeff, 2016; Krol *et al*., 2009; Zadmajid, 2016) also showing a benefit on reproductive traits of male tiger shrimp indicating that the role of certain reproductive hormones on both finfish and crustacean might have a similarity. Several hormones found in fish had been reported to be also indentified on crustacean. Gonadotropin releasing hormone (GnRH) found in lamprey and octopus also play a role on ovarian maturation of vannamei shrimp

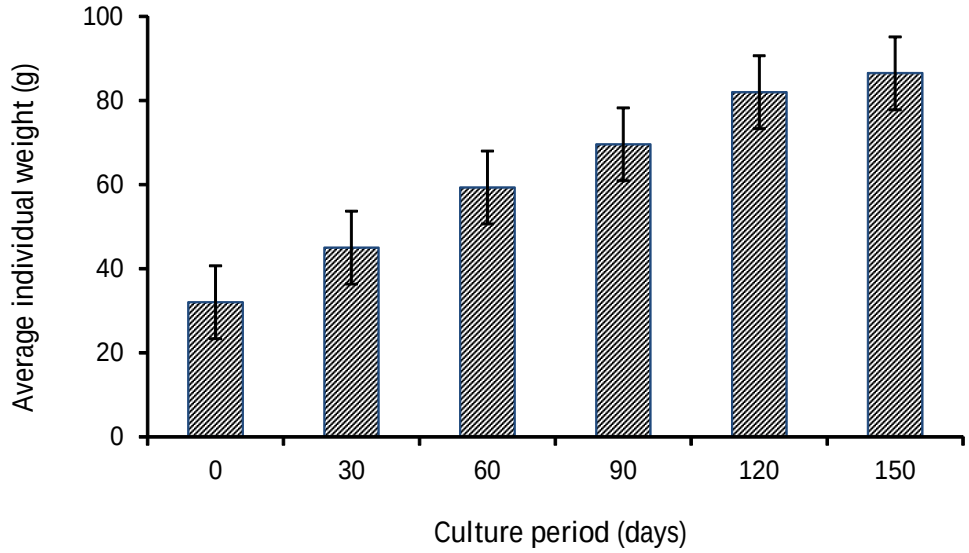

Figure 1. Pattern of weight increment of tiger shrimp during 150 days of prematuration stage culture in concrete pond

(Tinikul *et al*., 2014). Huang *et al*. (2008) reported that a follicle-stimulating hormone-luteinizing hormone (FSH-LH) like substances was detected in thoracic ganglion and brain of *Portunus trituberculatus*. Furthermore, this study showed that eye-stalk ablation could also enhance gonadal maturation of male similar to that of female shrimp. Eye stalk ablation has been widely practised by commercial hatcheries as hormonal manipulation for ovary maturation of female stock as explained earlier.

Increase of sGnRHa dose of injection up to 0.3 mL/kg caused a depletion of males maturing indicated by the lower number of males releasing spermatophore after electrical shock, even lower than control ablation. This is revealed the negative effect of the hormone when injected at higher dose. Similar trend was assessed on wild tiger shrimp female stock when induced with gonadotropine hormone of pregnant mare stimulating gonadotropin (PMSG) combined with antidopamin (AD) where number of female maturing after four times injection was lower than that of three times injection (Laining *et al*., 2015a).

Shrimp induced with OV-2 had the heaviest spermatophore (0.16 g) compared to that of control (0.11 g) and other two groups as presented in Table 2. At the first maturation, weight of spermatophores produced by males injected with OV-2 had the biggest size of spermatophores with weight of approximately 0.155 g among treatments. Moreover, the weight of spermatophores produced at rematuration were generally smaller than the first maturation which ranged from 0.09-0.11 g. Although induction of gonadal maturation with SGnRHa at 0.1 mL/kg resulted in higher number of male maturing compared to ablation, size of spermatophores produced improved when dose of sGnRHa increaed up to 0.2 mL/kg. These data may imply that the optimum level of the sGnRHa for the induction is in the range of 0.1-0.2 mL/kg.

In the present study, testes development and spermatophore characteristics such as number of sperm cells within a spermatophores and sperm abnormality were not evaluated, however, the effectiveness of sGnRHa in stimulating the testicular development and maturation was shown in longspine scraper, *Capoeta trutta*, a freshwater cyprinid species (Zadmajid, 2016). In addition, clear signs of spermiation and lobules filled with a large number of spermatozoa also demonstrated in testes from sGnRHa treated fish by identifying the testes morphological differences compared to HCG-injected groups. A stimulatory effect of this hormone on germ cell pro-

Table 1. Number of males producing spermatophores during maturation and rematuration after induction with SGnRHa and ablation

Treatments*	∑ Males (shrimp)	Mean and range of body weight of male (g)	∑ Male releasing spermatophore after electrical shock at first maturation (shrimp)	∑ Male releasing spermatophore at rematuration (shrimp)
AB	26	84.1 (66-116)	12 (46.1%)	3 (11.5%)
OV-1	26	89.8 (82-104)	21 (80.8%)	6 (23.1%)
OV-2	26	76.1 (64-94)	12 (46.1%)	5 (19.2%)
OV-3	26	78.3 (64-98)	11 (42.3%)	2 (7.7%)

* AB = eye stalk ablation; OV-1 = injection at 0.1 mL/kg; OV-2 = injection at 0.2 mL/kg; OV-3 = injection at 0.3 mL/kg

Table 2. Weight of spermatophores after sGnRHa induction at first maturation and rematuration of pond-reared male stock tiger shrimp

Treatments*	∑ Males stocked (shrimp)	Mean and range of body weight of male stocked (g)	Weight of spermatophore released at first maturation (g/shrimp)	Weight of spermatophore released at rematuration (g/shrimp)
AB	26	84.1 (66-116)	0.110 ± 0.037 (n=12)	0.110 ± 0.035 (n=3)
OV-1	26	89.8 (82-104)	0.097 ± 0.026 (n=21)	0.090 ± 0.019 (n=6)
OV-2	26	76.1 (64-94)	0.155 ± 0.035 (n=12)	0.094 ± 0.025 (n=5)
OV-3	26	78.3 (64-98)	0.094 ± 0.098 (n=11)	0.100 ± 0.042 (n=2)

* AB = eye stalk ablation; OV-1 = injection at 0.1 mL/kg; OV-2 = injection at 0.2 mL/kg; OV-3 = injection at 0.3 mL/kg

liferation and spermatozoid differentiation has been also reported in marine fish the golden rabbitfish (Komatsu *et al*., 2006) and freshwater species of Senegalese sole (Guzman *et al*., 2011) and yellow catfish (Zhuo *et al*., 2012).

Muscle Proximates Composition, Amino Acid, and Fatty Acid Profiles

Proximates composition of male muscle sampled at the end of gonadal development evaluation is presented in Table 3. Muscle proximates composition in particular protein and lipid content tended to increase with the increase of sGnRHa injection dose up to 0.2 mL/kg and further decreased at 0.3 mL/kg. Ablated male had the lowest protein content in fillet of 72.1% and the highest was detected in male injected with OV-3 of 86.6%. Lipid content in fillet showed a similar trend in which its content increased by increasing the dose level of the sGnRHa from 3.4%, 4.5%, to 5.8% for OV-1, OV-2, and OV-3, respectively whereas lipid content of fillet of ablated shrimp was 4.6%.

Amino acid profile of fillet of pond-reared male tiger shrimp post-induction with sGnRHa is presented in Table 4. Male without injection (ablated shrimp) had the lowest total amino acid content of 61.23% and tended to improved with the increase of sGnRHa injection dose up to 0.2 mL/kg. Injection of sGnRHa at OV-3 seemed to decrease the total amino acid content in fillet (61.47%). The increased protein content and total amino acid in sGnRHa-injected males and PMSG-injected female might be directly influenced by the hormone themselves because both hormones belong to peptide/protein group (Sherwood & Adams, 2005). The increased protein and total amino acid content in the unablated shrimp muscle may also indicate that hormone injection did not negatively affect the phisiological process of the shrimp in sintesize the amino acid. Similar trend was previously investigated on wild tiger shrimp induced with PMSG combined with AD where ablated female had lower total amino acid in whole body of 57.78% compared to induced female of 61.41% (Laining *et al*., 2015a). The effect of eyestalk ablation is not on a single hormone such as gonadotropin inhibitory hormone (GIH), but rather affects several physiological processes (Bray & Lowrence, 1992).

Interestingly, total fatty acids content in muscle of male stocks after induction were increased with the increase of the sGnRHa particularly for OV-2 (5.8711% of total lipid) and improved to 6.8017% for OV-3 treatment. These values were generally higher compared to control (eyed ablated male) at 3.6013% and OV-1 group which was only 2.952%. The fatty acid profiles based on the lipid classes are generally improved in particular PUFA by increaing the dose of the injection as indicated by Table 5. It is difficult to explain about this finding, but this might also indicate that physiological process in shrimp and other crustaceans including fatty acid metabolism negatively influenced by eye stalk ablation.

Generally, reproduction of fish in captivity can be controlled by environmental manipulation such as photoperiod, water temperature, or spawning substrate. However, in certain case it may be impossible to stimulate the required environmental parameters for natural reproductive performance like water depth and water hydraulics. In case of tiger shrimp and also other closed thelycum penaeids, lower mating rate occurred naturally remains the main issues in utilizing the cultured broodstock in captivity, and the reason of this phenomenon is still unclear. However, artificial insemination by implanting spermatophores into female thelycum can be the alternative solution in increasing the fertiled egg of this shrimp family. Therefore, spermiation control become important in order to synchronize the production of sperm with female oocyte maturation through hormonal manipulation as demonstrated in the present study.

Table 3. Proximates composition (% dry basis) of fillet of pond-reared tiger shrimp post-induction with sGnRHa*

Nutrien	AB	Dose of sGnRHa		
		OV-1	OV-2	OV-3
Crude protein	72.1	81.4	86.6	83.4
Crude lipid	4.6	3.4	4.5	5.8
Fiber	0.2	0.3	0.4	0.7
Ash	8.3	8.5	7.9	10
NFE	14.9	6.4	0.6	0.1

* AB = eye stalk ablation; OV-1 = injection at 0.1 mL/kg; OV-2 = injection at 0.2 mL/kg; OV-3 = injection at 0.3 mL/kg

Table 4. Amino acid profiles (% dry basis) of fillet of pond-reared tiger shrimp post-induction with sGnRHa*

Fatty acid	AB	Dose of sGnRHa injection		
		OV-1	OV-2	OV-3
Amino acid				
Aspartic acid	6.79	6.95	7.24	6.22
Glutamic acid	11.77	12.18	13.16	10.72
Serine	2.70	2.77	3.14	2.74
Histidine	1.11	1.11	1.35	1.26
Glycine	4.07	5.30	5.93	5.13
Threonine	2.51	2.49	3.04	2.63
Arginine	5.59	6.08	7.30	5.98
Alanine	4.38	4.19	4.72	4.00
Tyrosine	2.39	2.12	2.31	2.23
Methionine	1.74	1.83	1.91	1.64
Valine	2.89	2.89	3.27	2.89
Phenylalanine	2.63	2.61	3.12	2.72
I-leucine	2.71	2.75	3.17	2.80
Leucine	4.97	5.08	5.83	5.08
Lysine	5.00	4.68	5.78	5.44
Amino acid total	**61.23**	**63.02**	**71.27**	**61.47**

Table 5. Fatty acid profile (% of lipid, dry basis ± SD of duplicates) of muscle of male broodstock post-induction with sGnRHa*

Parameters	AB	Dose of sGnRHa injection		
		OV-1	OV-2	OV-3
Total omega 3	0.3380 ± 0.0065	0.3080 ± 0.0030	0.6473 ± 0.0007	0.7130 ± 0.0035
Total omega 6	0.2224 ± 0.017	0.1749 ± 0.0063	0.4506 ± 0.0037	0.4322 ± 0.0022
Total omega 9	0.1735 ± 0.0067	0.1703 ± 0.0025	0.2390 ± 0.0065	0.3108 ± 0.0040
Unsaturated fatty acid	0.8111 ± 0.0274	0.7130 ± 0.0099	1.4438 ± 0.0125	1.6597 ± 0.0129
Saturated fatty acid	0.7440 ± 0.0125	0.4471 ± 0.0102	0.7312 ± 0.0125	1.0004 ± 0.0172
Mono-unsaturated fatty acid (MUFA)	0.2508 ± 0.0066	0.2302 ± 0.0006	0.3460 ± 0.0155	0.5005 ± 0.0074
Poly-unsaturated fatty acid (PUFA)	0.5603 ± 0.0129	0.4829 ± 0.0094	1.0979 ± 0.0030	1.1592 ± 0.0055
Arachidonic acid (ARA)	0.1632 ± 0.0115	0.1176 ± 0.0080	0.2679 ± 0.0022	0.3182 ± 0.0006
Docosahexanoic acid (DHA)	0.1484 ± 0.0014	0.1490 ± 0.0064	0.2747 ± 0.0097	0.4148 ± 0.0001
Eicosapentanoic acid (EPA)	0.1896 ± 0.0036	0.1590 ± 0.0034	0.3727 ± 0.0104	0.2929 ± 0.0031

CONCLUSION

Hormonal manipulation using sGnRHa at level of 0.1 mL/kg produced 80.8% of pond-reared maturing male which was higher than that of control ablation of 46.1%. Spermatophores was bigger at dose of 0.2 mL/kg shrimp (0.155 g) and at this dosage, muscle protein and total amino acid content was also higher at level of 86.6%and 71.27%, respectively. Dose of sGnRHain inducing gonadal maturation of pond-reared male tiger shrimp could be applied at range of 0.1-0.2 mL/kg of shrimp weight.

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