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INVERTEBRATE REPRODUCTION & DEVELOPMENT
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Identification of steroid hormones and fatty acids during gonadal maturation of spiny lobster *Panulirus homarus*

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ABSTRACT

Information on steroid hormones and fatty acids that play roles in lobster reproduction is still very limited although the data are indispensable to seed production in hatchery. The study was designed to identify steroid hormones and fatty acids during gonadal maturation of spiny lobster (*Panulirus homarus*). Pyrolysis GCMS was used to identify steroid hormones and fatty acids and compared their concentrations between sexes and treatments. Samples from 6 male and 18 female spiny lobsters were used with different treatments. Male spiny lobsters were treated with and without thyroxine injection. Female spiny lobsters were treated with and without eyestalk ablations during mature and immature gonad developments. Androst-5-en-17-one,3β (androst) and estran-3-one,17β (estran), two steroid hormones were identified at different levels of gonadal maturity of spiny lobsters. High concentrations of androst and estran were detected in the male spiny lobsters treated with thyroxine injections. Estran has limited roles in stimulating gonadal development in male spiny lobsters. Androst was also found in the gonad of mature female spiny lobsters, but with low concentrations. Estran showed high concentrations in female brood stock of spiny lobsters during oogenesis stages both without eyestalk ablation and with ablation of one or two eyestalks, except in the immature female gonads. It was found that stearic acid was the highest and dominant fatty acid in mature male spiny lobster. Stearic acid, oleic acid, palmitic acid and caprylic acid were fatty acids with high concentrations in immature and mature female spiny lobsters. After 30 days in captivity, only stearic acid and oleic acid were found dominantly in eyestalk ablated mature female spiny lobsters. AQ2

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Introduction

Reproduction in crustaceans is controlled by endocrine factors and nutritional conditions (Subramoniam 2011). The roles of hormones and fatty acids in reproduction are reflected in gonadal maturation technology that has been applied practically in hatcheries to produce post larvae (Wilder et al. 2010). Eyestalk ablation is a conventional technique applied to male and female spiny lobsters (*Panulirus homarus*) to accelerate gonad maturity, increase feed intake, and its conversion related to reproduction, and more specifically to shorten the duration from intermoult to premoult stages (Radhakrishnan and Vijayakumaran 1984; Vijayakumaran and Radhakrishnan 1984; Fernandez and Radhakhrisnan 2016).

Another gonadal maturation technology is the injection of sex steroids hormones of vertebrates. Kirubagaran et al. (2005) reported two steroid hormones namely estradiol and progesterone during ovarian maturation in spiny lobster. The steroid hormones play roles in gonadal maturation, shorten maturation period, increase matured female, and extend the periods of fertilisation in spiny lobsters (*P. interruptus*) (Nan et al. 2015).

Nandi (1967) explained that the quantitative and qualitative analysis of hormones and the release of hormones from the gland were the activities that can link the important functions of steroid hormone and nutrition in reproduction. Nutrients such as lipid, protein, carbohydrate, vitamin and minerals are important factors for maturation process of crustacean (Harrison 1990). Lipid is a major energy source in marine invertebrate, involved in many important processes including growth, moult and reproduction (Yan et al. 2017). Nutrients requirements of brood stock can be traced on the basis of changes in the composition of the corresponding materials when the maturation process occurs in the crustacean gonads (Harrison 1990). Lack of information on steroid hormone and fatty acids in spiny lobster gonadal maturation is due to the limited utilisation of this species in commercial mariculture,

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especially in hatchery. This study was designed to identify the types and levels of steroid hormones and fatty acids during gonadal maturation in spiny lobster.

Materials and methods

Spiny lobster origin and rearing

Immature and mature male and female spiny lobsters used in the experiment were obtained from Krui, West Coast Residence, Lampung Province, Indonesia. Mature male spiny lobsters with body weights range of 145–152 g, immature female spiny lobsters with body weights range of 126–134 g, and mature female spiny lobsters with body weights range of 148–174 g were handled with sea sand, ice, and packed in paper box, and then transported by car about six hours to Main Center for Marine Aquaculture or MCMA (Balai Besar Perikanan Budidaya Laut) in Pesawaran Residence, Lampung Province, Indonesia. Permit clearance was obtained from Fish Quarantine and Fish Quality Products Inspection Office of Lampung Province to use spiny lobsters for research purposes. Four fibres illuminated plastic tanks each with the size of 200 × 100 × 50 cm were used for rearing of spiny lobsters. The tanks were filled with sea water with 40 cm height, continuously changed, and aerated. Fresh squid or fish meats were used as feeds given twice a day at 08 am and 05 pm with the level of 3–5% of body weight. The experimental tanks were siphoned and cleaned two times daily at 07 am and 04 pm. For a shelter of spiny lobster in captivity, each tank was provided with 10 PVC pipes each with 6-inch diameter and 30 cm length.

Spiny lobster treatments

Spiny lobster of 24 individuals consisting of 6 males and 18 females were used in the experiment. Mature male spiny lobsters, each with body weight of 145–152 g, were identified or marked individually by the number written on the paper covered with transparent waterproof plastic and tighten into the tail by rubber band. All experimental male spiny lobsters were reared in one tank. The observations for male spiny lobsters were divided into two parts.

Part I. The Effect of Thyroxine Injection on Gonadal Maturation of Male Spiny Lobsters

Two mature male spiny lobsters were used to study the effect of thyroxine hormone injection on gonadal maturation. The dosages of thyroxine hormone used were 0 and 0.1 µg/g body weight. Two experimental male spiny lobsters were used in this test (each dose of thyroxine injection only used one male spiny lobster). Levothyroxine sodium tablets (Thyrax N.V. Organon, Oss. The Netherlands) with thyroxine concentration of 100 µg in one tablet was used as a source of thyroxine hormone in this study. Stock solution for thyroxine hormone injection was made by crushing one tablet of Thyrax into powder form and dissolved in 100 µL of physiological NaCl (0.9 g NaCl/100 µL sterile ddH_2O) as a stock solution and then a serial dilution was made to obtain the required concentrations. Thyroxine hormone at a dose of 0 µg/g was used 1 mL of physiological NaCl for injection without thyroxine. Gonads from two male spiny lobsters injected with thyroxine at doses of 0 and 0.1 µg/g body weights, respectively, were collected 8 days after thyroxine injections.

Part II. The Effect of Thyroxine Injection on the Growth of Male Spiny Lobster

Four gonad mature male spiny lobsters were used to study the effect of thyroxine hormone injection on the growth of male spiny lobster. The doses of thyroxine injection used were 0; 0.1; 0.2; and 0.5 µg/g body weight. Thyroxine hormone stock solution and injection doses were similar with those used in Part I above. Gonads from four male spiny lobsters injected with thyroxine at doses of 0; 0.1; 0.2; and 0.5 µg/g body weights were isolated eight days after thyroxine injection.

A total of 18 female spiny lobsters were used and the treatments were divided into three parts.

Part I. Effect of one eyestalk ablation on immature female spiny lobster

The first part consisted of six immature female spiny lobsters with body weight range of 122–134 g. The experimental female spiny lobsters were divided into two treatments i.e., without eyestalk ablation and with one eyestalk ablation. Each treatment consisted of three immature female spiny lobsters.

Part II. Effect of one eyestalk ablation on mature female spiny lobster

The second part consisted of six mature spiny lobsters with body weight range of 148–152 g. The mature female spiny lobsters were divided into two treatments i.e., without eyestalk ablation and with one eyestalk ablation. Each treatment consisted of three mature female spiny lobsters.

Part III. Effect of one and two eyestalk ablation on mature female spiny lobster

The mature spiny lobsters were treated with two stages of treatment of eyestalk ablation. This part of experiment used six matured female spiny lobsters with body weight range of 163–174 g. In the beginning of the experiment, or on day one of treatment, all of six experimental matured female spiny lobsters were eyestalk ablated. After 30 days of one eyestalk ablation (on day 31), three of experimental mature female spiny lobsters previously ablated with one eyestalk were continued with the ablation of the other eyestalk to obtain a two eyestalk ablation.

All matured female groups of experimental spiny lobsters were reared in three different tanks and marking methods were used similar to those used in male spiny lobster. From the first day of rearing or maintenance, the gonad was taken by following the changes in the three stages of oogenesis. Practically, the condition and stage of oogenesis phases was confirmed by bending the cephalothorax of female spiny lobster and highlighting it with a flask light. The changes in the colour of the gonads were main indicators used to determine the phases of oogenesis. After the gonad reached the oogenesis, the spiny lobster was dissected without considering the duration of maintenance to reach gonad maturity. In female spiny lobsters without eyestalk ablation that were difficult to reach peak of oogenesis phases, feeding level was increased to 8% of body weight/day and duration of rearing was extended to 14 days.

Ovaries were taken in each phase of three phases of oogenesis in three groups of experimental female spiny lobsters. Three phases of oogenesis were primary vitellogenesis, secondary vitellogenesis and maturation that were confirmed by the histology of ovaries. Determination of three phases of oogenesis and histological analysis were conducted by following the methods of Subramoniam (2017a) and Shields and Boyd (2014) (Figure 1). Clove oil at a dose of 10 ml/L of sea water was used as an anaesthetic agent. Eyestalk ablation was conducted by cutting the eyestalk by using a sterile scalpel and sanitised the cut eyestalk with iodine to protect from pathogens infection. Gonad samples from male and female groups of experiments were kept in −80°C until analysis with a pyrolysis GCMS.

Pyrolysis gas chromatography mass spectrometry analysis

Approximately 1 µg of gonad was weighed with microbalance and pyrolysed at 400°C. The products of pyrolysis were analysed by GCMS. Gas Chromatography separations were carried out with a GCMS-QP2010 (Shimadzu, Tokyo) plus instrument equipped with an rt x 5 ms capillary column (length of 60 cm, diameter of 0.25 mm, and thickness of 0.25µm). Chromatographic separation was achieved by the following temperature program: 50°C for 5 min, then it was raised to 280°C for 50 min with pressure of 101 kPa. Helium was the carrier gas at 0.85 mL/min with total flow of 46.5 mL/min and flow program mode at a linear velocity of 23.7 cm/sec, and purge flow of 3.0 mL/min. Split injection mode used was the ratio of 1:50. Mass spectra was set with ion source at 200°C and interface at 280°C with solvent cut time of 1.5 min. Detector temperature used was 280°C and compound identification was based on comparison of mass spectra with WILEY7 library database. Compounds with the highest similarity (>90%) were

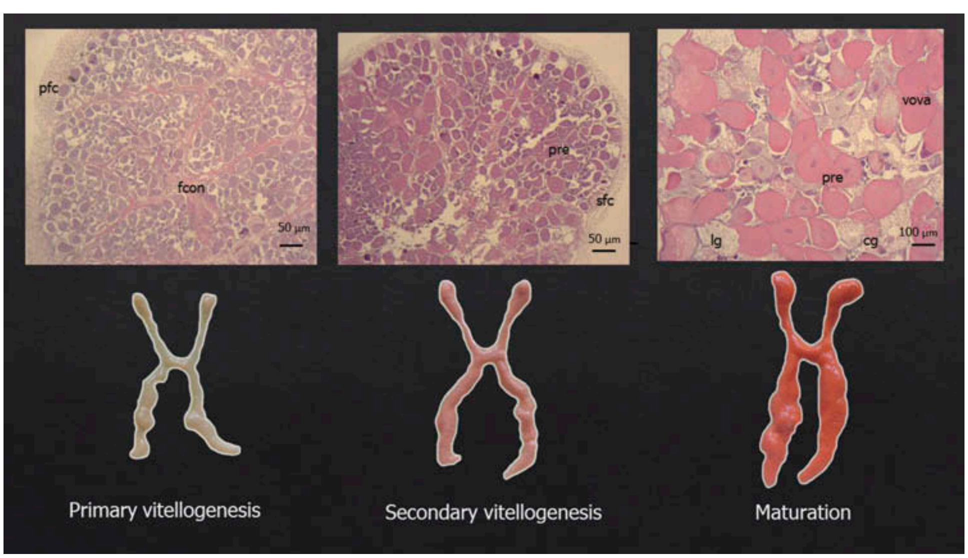

Figure 1. Oogenesis stages of spiny lobster (*Panulirus homarus*) ovaries used in this study. pfc: primary follicular cell; fcon: fibrous connective tissue; sfc: secondary follicular cell; pre: previtellogenic oocyte; lg: lipid globule; vova: vitellogenic oocyte; cg: cortical granule.

identified as steroid hormones and fatty acids data were selected from five ranks of compounds similarity available from library database.

Data analyses

WILEY7 library database was able to identify structural and molecular weights of steroid hormones and fatty acids. Due to the focus on steroid hormones and fatty acids identification, two treatments on male spiny lobster used were with and without thyroxine injection. It means the effects of thyroxine injection on growth and gonadal maturation and their doses of injection were ignored. Identified steroid hormones that appeared more than one concentration were presented as mean ± SD.

Fatty acids identifications were conducted, due to their roles as main nutrients involved in gonadal maturation. Further, comparisons of concentrations of those above fatty acids were conducted between male and female gonads and between treatments. Fatty acids that appeared more than one concentration were presented as mean ± SD. In order to compare similar fatty acids among treatments with exception to steroid hormones, concentration was grouped into three categories i.e., high (>10%), moderate (5–10%), and low (<5%).

Results

Pyrolysis GCMS showed the ability to identify steroid hormones and fatty acids during gonadal maturation of spiny lobsters. Two steroid hormones found in this study were androst-5-en-17-one,3β (androst) and estran-3-one,17β (estran). Pyrolysis GCMS also provided structures and molecular weights of these two steroids hormones (Figure 2).

During gonadal maturation of male spiny lobsters, androst showed a high concentration in control spiny lobsters without thyroxine injection (2.00 ± 0.09%) compared to those injected with thyroxine (0.31 ± 0.00%). Estran was also found in gonadal mature male spiny lobsters without thyroxine injection with concentrations of 0.74 ± 0.00% and 1.00 ± 0.07% in mature male spiny lobsters with thyroxine injection (Table 1).

Concentrations of estran decreased in immature female spiny lobsters with one eyestalk ablation and without eyestalk ablation in different stages of oogenesis. Without eyestalk ablation, the immature female spiny lobsters showed an inconsistent decrease in estran concentrations within different stages of oogenesis i.e., primary vitellogenesis (8.14 ± 0.50%), secondary vitellogenesis (3.74 ± 0.69%), and maturation (4.88 ± 0.57%). However, immature female spiny lobsters with one eyestalk ablation also showed a consistent decrease in the concentrations of estran in different stages of oogenesis i.e., primary vitellogenesis (13.79 ± 2.59%), secondary vitellogenesis (4.61 ± 1.49%), and maturation stage (3.26 ± 0.66%) (Table 1). Androst was not detected in immature female spiny lobster.

Different from the immature female spiny lobster, high concentrations of estran and androst were detected in mature female spiny lobsters. Mature female spiny lobsters without eyestalk ablation showed a pattern of increase in estran concentrations with the stage of oogenesis i.e., primary vitellogenesis (0.13 ± 0.00%), secondary vitellogenesis (5.30 ± 0.00%), and maturation stage (5.56 ± 0.81%). Mature female spiny lobster with one eyestalk ablation also showed an increase in estran from 2.35 ± 0.66% during primary vitellogenesis to 10.86 ± 3.10% during maturation stage. In this group of mature female spiny lobster with one eyestalk ablation, during secondary vitellogenesis, estran and androst

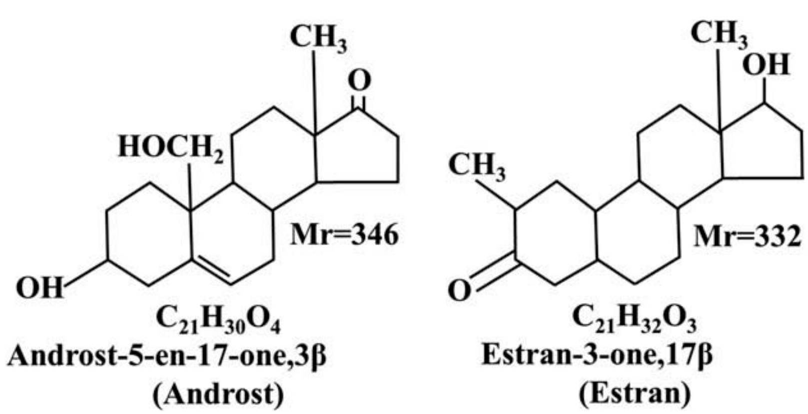

Figure 2. Structures and molecular weights of androst-5-en-17-one,3β (androst) and estran-3- one,17β (estran). Two steroid hormones in immature and mature gonads of male.

Table 1. Two steroid hormones androst-5-en-17-one,3β (androst) and estran-3-one,17β (estran) concentration (%) during gonadal maturation of spiny lobster (*Panulirus homarus*) in different treatments.

No	Sexes	Treatments	Concentration of androst (%)			Concentration of estran (%)		
1	Male	Without thyroxine injection	2.00 ± 0.09			0.74 ± 0.00		
2	Male	Thyroxine injection	0.31 ± 0.00			1.00 ± 0.07		
			Concentration of androst (%) during oogenesis phases			Concentration of estran (%) during oogenesis phases		
			Primary vitellogenesis	Secondary vitellogenesis	Maturation	Primary vitellogenesis	Secondary vitellogenesis	Maturation
3	Female	Immature and without eyestalk ablation	Not detected	Not detected	Not detected	8.14 ± 1.50	3.74 ± 0.69	4.88 ± 0.57
4	Female	Immature and one eyestalk ablation	Not detected	Not detected	Not detected	13.79 ± 2.59	4.61 ± 1.49	3.26 ± 0.66
5	Female	Mature and without eyestalk ablation	Not detected	Not detected	Not detected	0.13 ± 0.00	5.30 ± 0.00	5.56 ± 0.81
6	Female	Mature and one eyestalk ablation	Not detected	0.37 ± 0.00	Not detected	2.35 ± 0.66	1.37 ± 0.00	10.86 ± 3.10
7	Female	Mature and one eyestalk ablation after 30 days in captivity	0.84 ± 0.00	Not detected	1.11 ± 0.00	2.59 ± 0.68	3.93 ± 0.94	5.17 ± 1.47
8	Female	Mature and two eyestalk ablation after 30 days in captivity	Not detected	Not detected	Not detected	2.52 ± 0.00	7.07 ± 1.63	7.85 ± 1.73

showed low concentrations i.e., 1.37 ± 0.00% and 0.37 ± 0.00%, respectively (Table 1).

Matured female spiny lobsters with one and two eyestalk ablation after 1 month in captivity and three stages of oogenesis showed variations of estran and androst concentrations. Mature female spiny lobsters with one eyestalk ablation showed increases in the concentrations of estran with the stages of oogenesis i.e., primary vitellogenesis (2.59 ± 0.00%), secondary vitellogenesis (3.93 ± 0.94%), and maturation stage (5.17 ± 1.47%). Concentrations of androst were also detected to increase from primary vitellogenesis (0.84 ± 0.00%) to maturation stage (1.11 ± 0.00%). Two-eyestalk ablated mature female spiny lobsters showed the increase in estran concentrations during oogenesis stages i.e., primary vitellogenesis (2.52 ± 0.00%), secondary vitellogenesis (7.07 ± 1.63%), and maturation stage (7.85 ± 1.73%) (Table 1).

Variations of fatty acids concentrations were detected during gonadal maturation of spiny lobster treated without thyroxine injection and with thyroxine injection. Results of experiment showed that deposition of fatty acids in the gonads of mature male spiny lobsters was dominated by stearic acid with a high concentration (13.05 ± 2.37%; 10.84 ± 1.13%). Fatty acids with low concentrations in this treatment were oleic acid (2.83 ± 0.55%), pentadecanoic acid (1.80 ± 0.00%), cyclopropane pentanoic acid (1.49 ± 0.00%), hexyl oleate (1.35 ± 0.00%), and docosaenoic acid (1.83 ± 0.00%) (Table 2).

In immature female spiny lobsters without eyestalk ablation, fatty acids found in high concentrations were stearic acid (16.47 ± 0.00; 22.32 ± 0.00%), oleic acid (25.96 ± 4.31%), and palmitic acid (11.33 ± 0.00%). Octadecatrienoic acid (2.60 ± 0.00%), cyclopropentanoic acid (2.59 ± 0.00%), prepenoic acid (1.15 ± 0.00%), pentadecanoic acid (0.80 ± 0.00%), nonadecanoid acid (1.15 ± 0.00%), and cyclopentaneundecanoid acid (0.59 ± 0.00%) were identified with low concentrations. Fatty acids with high concentrations in immature female spiny lobster with one eyestalk ablation were caprylic acid (24.08 ± 0.00%) and oleic acid (10.90 ± 0.85; 27.08 ± 8.74%). Low concentration in immature female spiny lobster with one eyestalk ablation were docosenoic acid (4.89 ± 0.00%), hexanoic acid (1.34 ± 0.00%), and undecenoic acid (1.11 ± 0.00%) (Table 3).

In mature female spiny lobsters without eyestalk ablation, fatty acids deposition showed a great variation. Fatty acids that were found with high and moderate concentrations during secondary vitellogenesis and maturation stages were cyclopentaneundecanoid acid (15.62 ± 0.00%), stearic acid (9.63 ± 0.00; 16.82 ± 0.00%), and oleic acid (12.35 ± 0.00%). Fatty acids with low concentrations were octadecanoic acid (4.50 ± 0.53%),

Table 2. Identified fatty acids concentration (%) during gonadal maturation of male spiny lobster (*Panulirus homarus*).

No	Treatments (number of sample)	Body weight (g)	Maturity	Identified fatty acids	Concentration (%)
1	Without thyroxine injection (2)	145;150	Mature	Octadecanoid acid/Stearic acid	13.05 ± 2.37
				Pentadecanoid acid,14-methyl-,methyl ester	1.80 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	1.49 ± 0.00
				9-Octadecenoid acid, hexyl ester/Hexyl oleate	1.35 ± 0.00
				13-Docosenoic acid, methyl ester	1.03 ± 0.00
2	Thyroxine injection (4)	148–152	Mature	Octadecanoid acid/Stearic acid	10.84 ± 1.13
				9-Octadecenoid acid/Oleic acid	2.83 ± 0.55

prepenoic acid (1.32 ± 0.00%), docosenoic acid (0.79 ± 0.00%), and butanoic acid (1.53 ± 0.00%). Fatty acids with moderate and low concentrations during primary vitellogenesis were palmitic acid (5.78 ± 0.00%), oleic acid (4.98 ± 0.00%), ethyl palmitate (3.32 ± 0.00%), and prepenoic acid (0.07 ± 0.00%) (Table 3). Mature female spiny lobsters with one eyestalk ablation showed a variation. In this group of mature female spiny lobsters with one eyestalk ablation, concentrations of palmitic acid (15.55 ± 0.00; 10.12 ± 0.00; 17.24 ± 0.00%), cyclopentaneundecanoid acid (13.72 ± 0.00%), and oleic acid (11.39 ± 0.00%) were dominantly high. In this group of mature female spiny lobsters with one eyestalk ablation, fatty acids showing moderate concentrations were cyclopropane pentanoic acid (5.40 ± 0.61%), eicosatrienoic acid (4.20 ± 0.00%), and decanoic acid (5.33 ± 0.00%). In this group of mature female spiny lobsters with one eyestalk ablation, fatty acids with low concentrations during secondary vitellogenesis and maturation stage were AQ10 pentadecanoid acid (2.44 ± 0.83%), octadecanoid acid (1.62 ± 0.00%), and prepenoic acid (0.38 ± 0.00; 0.44 ± 0.00%) (Table 3).

AQ11 After one month in captivity, mature female spiny lobsters with one and two eyestalk ablations showed distinctly different fatty acids concentrations. In mature female spiny lobsters with one and two eyestalk ablations, fatty acids showing high concentrations were stearic acid (17.78 ± 0.00; 15.81 ± 0.00; 11.04 ± 0.00; 14.39 ± 0.00%) and oleic acid (16.74 ± 0.00; 18.80 ± 0.00; 13.01 ± 0.00%) followed by a variety of moderate and low concentrations of fatty acids. In contrast, fatty acids during maturation stage were dominated only by stearic acid 16.45 ± 0.00; 11.95 ± 0.00%) and oleic acid (9.77 ± 0.00; 11.33 ± 0.00%) and no other fatty acid was detected during maturation stage. In mature female spiny lobsters with one and two eyestalk ablations, fatty acids showing moderate and low concentrations were cyclopropanepentanoic acid (8.74 ± 0.00; 4.98 ± 1.23; 4.53 ± 0.75), octadecatrienoic acid (1.33 ± 0.00; 2.33 ± 0.00), pentadecanoic acid (1.32 ± 0.00; 2.86 ± 0.00), and prepenoic acid (1.45 ± 0.00) (Table 3).

Discussion

Analytical platform of GCMS has advantages and disadvantages in determining metabolites samples (Young and Alfaro 2018). The combination between platforms such as pyrolysis GCMS may support more advantages that can be used to obtain broad analysis coverage including hormones and fatty acids identifications with very limited sample numbers. The use of pyrolysis GCMS is robust and convenient to produce the results rapidly. Pyrolysis GCMS has been applied for exploring the natural active compounds from biological materials (Martinez-Balmori et al. 2013). The use of pyrolysis GCMS is able to find new products (Ghalibaf et al. 2017). This study proved the benefits of pyrolysis GCMS in identification of steroid hormones and fatty acids in different stages of gonad maturation and treatments.

Hormonal regulation of reproduction in spiny lobster is debatable due to the limited studies previously conducted to improve spiny lobster hatchery production (Subramoniam 2017b). Progesterone and 17β-estradiol are hormones recorded to affect vitellogenesis (Subramoniam and Kirubagaran 2010). This present study found two new steroid hormones that had roles in gonadal maturation and vitellogenesis of male and female spiny lobsters that were different from vertebrate-type sex steroid hormones published so far. In general, steroids are available in two main forms i.e., the ecdysteroids and the vertebrate-type sex steroids (Subramoniam 2017b). Distinctly, the difference in the roles of these two main steroids is ecdysteroid controls moulting process and gonadotrophic hormones and vertebrate-type sex hormones have main roles in stimulating vitellogenesis (Subramoniam 2011).

The results of our present study showed that concentrations of androst and estran fluctuated with sexes and stages of gonad maturity. These new steroid hormones have positive roles in gonadal maturation of spiny lobster. Androst was abundantly found in male spiny lobster and estran expression was much more frequent in female spiny lobster. Moreover, estran was detected in male spiny lobster and androst was also detected in female spiny lobster. Both hormones were

Table 3. Identified fatty acids concentration (%) during gonadal maturation of female spiny lobster (*Panulirus homarus*).

No	Treatments (number of sample)	Body weight (g)	Maturity	Identified fatty acids	Concentration (%)
1	Without eyestalk ablation during primary vitellogenesis (1)	128	Immature	Octadecanoid acid/Stearic acid	22.32 ± 0.00
				9,12,15-Octadecatrienoic acid,methyl ester	2.60 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	2.59 ± 0.00
				2-Prepenoic acid,2-methyl,2-(dimethylamino) ethyl ester	1.15 ± 0.00
				Pentadecanoid acid,4,6,10,14-tetramethyl-,methy ester	0.80 ± 0.00
2	Without eyestalk ablation during secondary vitellogenesis (1)	126	Immature	9-Octadecenoid acid/Oleic acid	25.96 ± 4.31
				Octadecanoid acid/Stearic acid	16.47 ± 0.00
				Nonadecanoid acid, ethyl ester	1.15 ± 0.00
				Cyclopentaneundecanoid acid	0.59 ± 0.00
3	Without eyestalk ablation during maturation (1)	134	Immature	Hexadecanoic acid/Palmitic acid	11.33 ± 0.00
				9-Octadecenoid acid/Oleic acid	4.02 ± 0.00
4	One eyestalk ablation during primary vitellogenesis (1)	132	Immature	13-Docosenoic acid,methyl ester	4.89 ± 0.00
5	One eyestalk ablation during secondary vitellogenesis (1)	125	Immature	Octanoid acid/Caprylic acid	24.08 ± 0.00
				9-Octadecenoid acid/Oleic acid	10.90 ± 0.85
				Hexanoic acid	1.34 ± 0.00
6	One eyestalk ablation during maturation (1)	130	Immature	9-Octadecenoid acid/Oleic acid	27.08 ± 8.74
				2-Undecenoic acid	1.11 ± 0.00
7	Without eyestalk ablation during primary vitellogenesis (1)	148	Mature	Hexadecanoic acid/Palmitic acid	5.78 ± 0.00
				9-Octadecenoid acid/Oleic acid	4.98 ± 0.00
				Hexadecanoid acid, ethyl ester/Ethyl palmitate	3.32 ± 0.00
				2-Prepenoic acid,2-methyl,2-(dimethylamino) ethyl ester	0.07 ± 0.00
8	Without eyestalk ablation during secondary vitellogenesis (1)	152	Mature	Cyclopentaneundecanoid acid	15.62 ± 0.00
				Octadecanoid acid/Stearic acid	9.63 ± 0.00
				9,12,15-Octadecatrienoic acid,methyl ester	4.50 ± 0.53
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	2.09 ± 0.47
				2-Prepenoic acid,2-methyl,2-(dimethylamino) ethyl ester	1.32 ± 0.00
				13-Docosenoic acid, methyl ester	0.79 ± 0.00
9	Without eyestalk ablation during maturation (1)	151	Mature	Octadecanoid acid/Stearic acid	16.28 ± 0.00
				9-Octadecenoid acid/Oleic acid	12.35 ± 0.00
				Butanoic acid,2((trifluoroacthyl)amino)-,butyl ester	1.53 ± 0.00
				2-Prepenoic acid,2-methyl,2-(dimethylamino) ethyl ester	0.44 ± 0.00
10	One eyestalk ablation during primary vitellogenesis (1)	152	Mature	Hexadecanoic acid/Palmitic acid	15.55 ± 0.00
				Cyclopentaneundecanoid acid	13.72 ± 0.00
				Cyclopropane pentanoic acid,2-undercyl-,methyl ester, trans	5.40 ± 0.61
11	One eyestalk ablation during secondary vitellogenesis (1)	150	Mature	9-Octadecenoid acid/Oleic acid	11.39 ± 0.00
				Hexadecanoic acid/Palmitic acid	10.12 ± 0.00
				8,11,14-Eicosatrienoic acid, methyl ester	4.20 ± 0.00
				Pentadecanoid acid,14-methyl-,methyl ester	2.44 ± 0.83
				11-Octadecanoic acid, methyl ester	1.62 ± 0.00
				2-Prepenoic acid,2-methyl,2-(dimethylamino) ethyl ester	0.38 ± 0.00
12	One eyestalk ablation during maturation (1)	149	Mature	Hexadecanoic acid/Palmitic acid	17.24 ± 0.00
				Decanoic acid, 1-methylethyl ester	5.33 ± 0.00
				6,9,12-Octadecatrieonic acid, methyl ester	2.92 ± 0.00
				9-Octadecen-12-ynoic acid, methyl ester	2.06 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	1.65 ± 0.28
				9-Octadecanoic acid, methyl ester	1.58 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	1.25 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	0.45 ± 0.00

(*Continued*)

Table 3. (Continued).

No	Treatments (number of sample)	Body weight (g)	Maturity	Identified fatty acids	Concentration (%)
13	One eyestalk ablation during primary vitellogenesis after 30 days in captivity (1)	169	Mature	Octadecanoid acid/Stearic acid	17.78 ± 0.00
				9-Octadecenoid acid/Oleic acid	16.74 ± 0.00
				9,12,15-Octadecatrienoic acid, methyl ester	1.69 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	1.33 ± 0.00
				Phosphorothioc acid,O,O-diisopropyl ester, S-ester-N	1.11 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	0.90 ± 0.00
				Octadecanoic acid, phenylmethyl ester	0.67 ± 0.00
14	One eyestalk ablation during secondary vitellogenesis after 30 days in captivity (1)	174	Mature	9-Octadecenoid acid/Oleic acid	18.80 ± 0.00
				Octadecanoid acid/Stearic acid	15.81 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	4.98 ± 1.23
				9,12,15-Octadecatrienoic acid, methyl ester	1.60 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	1.33 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	0.69 ± 0.00
15	One eyestalk ablation during maturation after 30 days in captivity (1)	163	Mature	Octadecanoid acid/Stearic acid	16.45 ± 0.00
				9-Octadecenoid acid/Oleic acid	9.77 ± 0.00
16	Two eyestalk ablation during primary vitellogenesis after 30 days in captivity (1)	172	Mature	Octadecanoid acid/Stearic acid	11.04 ± 0.00
				Cyclopentaneundecanoid acid	8.74 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	4.53 ± 0.75
				12,15-Octadecadiynoic acid, methyl ester	2.33 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	1.45 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	1.32 ± 0.00
17	Two eyestalk ablation during secondary vitellogenesis after 30 days in captivity (1)	170	Mature	Octadecanoid acid/Stearic acid	14.39 ± 0.00
				9-Octadecenoid acid/Oleic acid	13.01 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	2.86 ± 0.00
18	Two eyestalk ablation during maturation after 30 days in captivity (1)	168	Mature	Octadecanoid acid/Stearic acid	11.95 ± 0.00
				9-Octadecenoid acid/Oleic acid	11.33 ± 0.00

detected in low concentrations and showed their limited roles in stimulating gonadal development in different sexes of spiny lobsters.

Injections of thyroxine hormone in male spiny lobster affect androst and estran concentrations. Concentrations of androst in male spiny lobsters with thyroxine injection were lower compared to those without thyroxine injection. Distinctly, estran concentration was higher in male spiny lobster with thyroxine injection compared to those without thyroxine injection. These results showed that the roles of androst and estran were related to the thyroxine injection as was indicated by the decreased androst concentration in the male gonads. These facts showed that androst as a stimulator of gonad development in male spiny lobster was affected by thyroxine hormone. The relation between thyroxine hormone and steroid hormone remain unclear. However, within these results it is shown that thyroxine suppressed steroid hormone synthesis. If this condition continues, faster growth rate caused by thyroxine injection in male spiny lobster is obviously necessary for maturation. In Decapoda, thyroxine hormone functions to stimulate somatic growth in *Penaeus monodon* (Pillai et al. 1987) and *Macrobrachium rosenbergii* (Roustaian and Gaik 2006).

Estran has a role in the mature females compared to immature forms. This condition was shown by the concentrations of estran in immature female spiny lobsters even with one eyestalk ablation that were not able to increase compared to the increase in its concentration in mature female spiny lobsters. The observation of Quackenbush (1994) supported this result that small female lobsters at the first stage of gonad maturity delayed their reproductions by allowing moulting process to obtain larger sizes. During vitellogenesis stages and treatments, mature female spiny lobsters showed fluctuated concentrations of androst and estran. Yan et al. (2017) also showed that steroids concentrations in the gonad during reproductive cycle tended to fluctuate due to biotic factors (maturation, reproductive, food availability and age) and abiotic factors (photoperiodicity, temperature, pH and dissolved oxygen). Since those biotic factors were relatively similar during the experiment, in this study it was assumed that abiotic factors played roles in influencing the level of vitellogenesis that further affected the expressions of androst and estran.

Androst and estran were detected in low concentrations in mature female with one eyestalk ablation both during a short-time exposure and a long-time exposure (after 1 month in captivity). In contrast, androst was not found in all vitellogenesis stages in mature female spiny lobsters without eyestalk ablation and those with two eyestalk ablations after one month in captivity. The practice of eyestalk ablation involves the losing of endogenous moult and vitellogenesis inhibiting hormone (VIH) and lead to the induction of ovarian maturation (Kumar et al. 2018). It is shown that one eyestalk ablation also suppressed androst and estran during vitellogenesis phases. Probably, the availability of vitellogenesis stimulating hormone (VSH) reduced androst and estran into a basal level. These facts need to be clarified in the future study to clarify the relation among androst, estran, VIH and VSH. Quackenbush (1994), Subramoniam and Kirubagaran (2010), and Subramoniam (2011) reported that, VIH and VSH showed inhibitory and stimulatory effects on ovarian growth, vitellogenesis, and yolk regulations of lobsters. In addition, it is also needed to confirm the importance of one eyestalk ablation in mature spiny lobsters. In the present study it was found that female spiny lobsters without eyestalk ablation could produce optimal maturation stage during treatment with high sterol diets. Due to the pressure of animal right it is necessary to support the other option like feeding with high cholesterol diets to replace eyestalk ablation.

In mature female spiny lobsters without eyestalk ablation and with two eyestalk ablation, estran increased two folds followed by vitellogenesis stages. The peak concentration of estran was reached during secondary vitellogenesis and maturation stage. It is shown that cholesterol from feed is converted into estran by the hepatopancreas and further transported to the ovaries during vitellogenesis similar to those found by Fairs et al. (0, 1990)) in *Nephrops norvegicus* and *Penaeus monodon*. Spiny lobster is able to convert exogenous cholesterol obtained from the diet into sex steroids such as progesterone, 17-hydroxyprogesterone, androstenedione, testosterone and moulting hormones such as ecdysterone (Kanazawa and Teshima 1971; Burns et al. 1984).

Fatty acids accumulations that were found during gonadal maturation of male and female spiny lobsters had high variety. Feed such as squid and fish meat that were given to spiny lobster during this study contained a lot of lipid. Metabolism of lipid in crustacean is located in the hepatopancreas as the main site and the products were then accumulated in the muscle and the ovaries (Swevers et al. 1991). Garofalaki et al. (2006) showed that fatty acid contents of the muscle and hepatopancreas of *P. vulgaris* were low, but phospholipids were available abundantly in these organs. Concentration of lipid in the hepatopancreas was found to decrease as a result of its mobilisation into the gonads during vitellogenesis and increasing lipid accumulation in the ovaries at oogenesis phases (Iromo et al. 2014). These facts suggest that ovarian vitellogenin is synthesised in the hepatopancreas, and later transported into the ovaries (Yan et al. 2017).

Fatty acids compounds were also found abundant and showed no single fatty acid that played a dominant role in the gonadal maturation. Stearic acid is the most dominant fatty acid found in mature male spiny lobster on two different treatments of thyroxine hormone. This means that injection of thyroxine is not related to the elevation of stearic acid concentration in the gonads of mature male spiny lobsters. Immature female spiny lobsters showed a more complex condition in terms of fatty acids accumulation. There are stearic acid, oleic acid, and palmitic acid in spiny lobsters without eyestalk ablation. Moreover, only caprylic acid and oleic acid were dominant fatty acids found in spiny lobster with one eyestalk ablation. Fatty acids depositions in the gonad of female spiny lobsters with mature gonads had high varieties. Fatty acids that were also found with high varieties were caprylic acid and oleic acid. Mature female spiny lobsters with one eyestalk ablation had different fatty acids patterns where palmitic acid, cyclopentaneundecanoid acid and oleic acid were the most abundant in the ovaries. In addition, for a long time exposure of eyestalk ablation, only two fatty acid classes were found *i.e.*, stearic acid and oleic acid.

This study showed a low variety of nutrients identified in immature female compared to mature female spiny lobsters. Physiological changes caused by fatty acids variation are related to hormonal changes in female brood stock (Yano 1998). It was found that fatty acids deposition in the gonad was more diverse in female spiny lobsters without eyestalk ablation compared to female spiny lobsters with one and two eyestalk ablation. Harrison (1990) supported the results of this study that when maturation was induced by eyestalk ablation it would be able to accelerate hormonal and metabolic change that would stimulate ovarian development to reach its peak. It means there is a low variety in nutrient but with high concentration for specific purposes such as egg yolk deposition.

Many new fatty acids class that were found in this study may improve our understanding and knowledge to modify feed composition for brood stock reproduction in spiny lobster. In addition, these results will fill the gap of information required for further development of spiny lobster mariculture industry.

Disclosure statement

No potential conflict of interest was reported by the authors.
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