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Effect of estradiol on hepatopancreatic lipid metabolism in the swimming crab, *Portunus trituberculatus*☆

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ABSTRACT

Estradiol is an important sex steroid hormone that involved in regulation of animal lipid metabolism. However, the effect of estradiol on lipid metabolism in swimming crab (*Portunus trituberculatus*) is unclear. The present study investigated the effect of four concentrations of exogenous estradiol (0, 0.01, 0.1 and 1 μg g^{-1} crab weight) on the expression levels of lipid metabolism-related genes, lipid composition and histology of hepatopancreas in the *P. trituberculatus*. The results showed that the mRNA levels of *carnitine palmitoyltransferase I* and *II* (*CPT-I* and *CPT-II*) increased significantly at the low concentrations (0.01 μg g^{-1} and 0.1 μg g^{-1}), while decreased significantly in the highest concentration (1 μg g^{-1}). The mRNA levels of *acyl-CoA oxidase* (*ACOX*), *fatty acid transport protein* (*FATP*), *fatty acid-binding protein* (*FABP*), *diacylglycerol acyltransferase 1* (*DGAT1*) and *acetyl-CoA carboxylase* (*ACC*) were significantly down-regulated. The transcripts of *fatty acid synthase* (*FAS*) and *fatty acyl desaturase* (*FAD*) decreased significantly only in 1 μg g^{-1} treatment. All estradiol treatments (0.01, 0.1 and 1 μg g^{-1}) had significantly higher percentages of 20:4n6, 20:5n3 and 22:6n3, but lower percentages of total monounsaturated fatty acids and polar lipids than the control treatment (0 μg g^{-1}). Histological observations indicated the size of B cell became larger under estradiol treatment. The results indicated that estradiol promoted lipid catabolism in the hepatopancreas of *P. trituberculatus*.

1. Introduction

Estradiol, an important sex steroid hormone, plays a vital role in crustacean reproductive development (Michael et al., 2016). In most crustaceans, estradiol promotes the process of vitellogenesis and ovarian development (Yano and Hoshino, 2006; Rodríguez et al., 2002). Moreover, it was also reported that estradiol involved in regulating growth, immune and metabolic processes (Avtanski et al., 2014; Khan and Ansar, 2016; Hussain et al., 2015). However, the results of previous studies were not consistent for the effects of estradiol on the lipid metabolism in mammal (Kenagy et al., 1981; Wohlers and Spangenburg, 2010). For example, estradiol-induced the synthesis of triglyceride by increasing the concentration of fatty acid-binding protein (Ockner et al., 1980). Similarly, estradiol also increased triglyceride secretion in the rat (Kenagy et al., 1981). However, recent studies showed that the exposure of female mice to estradiol led to down-

Abbreviations: *CPT-I* and *CPT-II*, *carnitine palmitoyltransferase I* and *II*; *ACOX*, *acyl-CoA oxidase*; *FATP*, *fatty acid transport protein*; *FABP*, *fatty acid-binding protein*; *DGAT1*, *diacylglycerol acyltransferase 1*; *ACC*, *acetyl-CoA carboxylase*; *FAS*, *fatty acid synthase*; *FAD*, *fatty acyl desaturase*; FAME, fatty acid methyl esters; SFA, saturated fatty acid; ΣSFA, total saturated fatty acids; MUFA, monounsaturated fatty acid; ΣMUFA, total monounsaturated fatty acids; PUFA, polyunsaturated fatty acid; ΣPUFA, total monounsaturated fatty acids; ΣHUFA, total highly unsaturated fatty acids; Σn-3 PUFA, total n-3 PUFA; Σn-6 PUFA, total n-6 PUFA; E cell, embryonic cell, B cell, blister-like cell; R cell, resorptive cell; F cell, fibrillar cell; ER, estrogen receptor; PPAR, peroxisome proliferation activator receptors; SREBP1c, sterol regulatory element-binding protein 1c; ERR, estrogen related receptor

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regulation of the expression of lipogenic genes in the adipose tissue (Wohlers and Spangenburg, 2010). Furthermore, estradiol increased the fat oxidation in skeletal muscle inducing the inhibition of lipogenesis in adipose depots and muscle (Jones et al., 2006; Oosthuyse and Bosch, 2012). In fish, it is well documented that estradiol increased hepatic lipogenesis in the liver (Sharpe and Maclatchy, 2007). To date, little information was achieved on the effect of estradiol on lipid metabolism in crustaceans.

The lipid classes of crustaceans include triacylglycerides, phospholipids, free fatty acid and sterols. Triacylglycerides was one of their main energy storage forms in most organisms (Kumar et al., 2018). Phospholipids, fatty acids and cholesterol were integral components of cell membranes, while some can act as precursors of necessary hormones for growth and reproduction (D'Abramo, 1989). It was reported previously that lipid deposition was related to lipid synthesis and oxidation rate, which were regulated by many genes (Cai et al., 2016; Liu et al., 2018). In vertebrates, a number of studies demonstrated that the estrogen would cause the changes of lipid level by regulating the expression levels of lipid metabolism-related genes, including the *fatty acid transport protein* (*FATP*), *fatty acid-binding protein* (*FABP*), *acetyl-CoA carboxylase* (*ACC*) and *fatty acid synthase* (*FAS*) (Smith and Muscat, 2005; D'Eon et al., 2005; Fu et al., 2009). Similar to vertebrates, these genes were also proven involving in the lipid metabolism of crustaceans (Sargent, 1989; Honda et al., 2016; Yan et al., 2015).

Hepatopancreas is the center of lipid metabolism in crustaceans. It performs the functions similar to mammal intestinal, hepatic, pancreatic and adipose tissue, which was being somehow analogous to insect fat body (Vogt, 1994; Gilbert and Chino,1974; Arrese et al., 2001) or spider midgut diverticula (Laino et al., 2009). The stored nutrients, particularly lipids, in hepatopancreas were transported to the gonads and other tissues for energy expenditure during the reproductive stages (Cheng et al., 1997; Yao et al., 2008; Jiang et al., 2009). Therefore, the hepatopancreas of crustacean was an ideal organ for studying lipid changes. The swimming crab, *Portunus trituberculatus*, is an important marine-culture crab widely distributing in the coastal water area of East Asia, including Korea, Japan, Philippine and China (Hamasaki et al., 2006). The present study aimed comprehensively explore the effects of exogenous estradiol on the expression levels of lipid metabolism-related genes, lipid composition, and histology of hepatopancreas in female *P. trituberculatus*. The results will provide valuable information for exploring the molecular mechanism of the lipid metabolism regulation of estrogen in crustaceans.

2. Materials and methods

2.1. Animals source and culture conditions

The female *P. trituberculatus* were obtained from the outdoor earthen ponds in Qidong research base of Shanghai Fisheries Research Institute, Jiangsu, China. The crabs (body weight: 175 ± 25 g) which had finished the puberty molt were selected and acclimated for a week in the indoor circulating water system. After acclimation, 120 healthy, intact and active females with immature ovaries were further selected for formal experiments. During the experiment, the crabs were individually cultured in the culture boxes (Length × Width × Depth = 33 × 27.5 × 35 cm). In the bottom of each culture box, it was divided two parts: feeding area (the feeds were put on the board of this area and hiding area (after feeding, the crab will hide in the sand of this area). A layer of 5–6 cm sand was provided at the bottom of hiding area for each culture (Pan et al., 2015). All culture boxes were floating in four concrete tanks (Length × Width × Depth = 5.8 m × 2.4 m × 1.8 m) by attaching foam to the surrounding of the boxes. There were many uniform holes on the walls of each culture box that allow water exchange with the concrete tanks, which were connected to a recirculating system to maintain the water quality. The filtration medium used for the recirculating system composed of polyester wool, activated charcoal, corallite and gravel, which were located in four connecting smaller reservoir tanks, respectively.

During the experiment, all crabs were fed commercial formulated diets (Zhejiang Alpha Feed Co. Ltd; protein: 41%, lipid: 11%) at a ratio of 1–3% of body weight per day, and feeding ratio was adjusted on feed residues. After 2–3 hours of feeding, the uneaten feeds were removed for each culture box every day while the layer sand in each box was weekly replaced with clean sand. During the experiment, the salinity was 25 ‰, while temperature ranged from 14 to 24 °C. The water quality was maintained at: ammonia-N < 0.5 mg L^{-1}; nitrite < 0.10 mg L^{-1}; DO > 5 mg L^{-1} and pH 7.0–9.0.

2.2. Experimental design and sampling procedures

Four estradiol treatments, 0, 0.01, 0.1 and 1 μg g^{-1} crab weight, were set up and each treatment had thirty replicate crabs. The lower injection dose (0.01 μg g^{-1} crab weight) of estradiol is around 10 times of the highest physiological concentration (0.001 μg g^{-1}, Feng, 2011; Lu et al., 2018) in the hemolymph during the crustacean ovarian maturation cycle of female *P. trituberculatus*. The estradiol dose of 0 μg g^{-1} crab weight was served as the control treatment, which was received the same volume of solvent (ethanol/saline = 1:1, v/v) (Koskela et al., 1992; Rodríguez et al., 2002; Coccia et al., 2010). Prior to the injection, the crabs were weighed individually and three stocking estradiol solution was prepared. The estradiol (Sigma, E2758-1g; CAS: 50-28-2, purity ≥98%) was dissolved with ethanol and diluted in the crab saline solution to reach the final concentration according to previous studies (Rodríguez et al., 2002). The injection volume (0.5 μl g^{-1} crab weight) and interval (1 time $week^{-1}$) were followed the previous study (Koskela et al., 1992; Rodríguez et al., 2002; Reddy et al., 2006). The estradiol was injected into arthrodial membrane at the base of the swimming-leg of females. The injection was carried on the 0, 7, 14, 21 and 28 days respectively (Koskela et al., 1992; Reddy et al., 2006), and the experiment was terminated after 35 days of culture experiment.

Ten crabs were sampled randomly from each treatment and weighed at the end of the experiment. Then all crabs were anesthetized on ice and dissected to obtain hepatopancreas. Two small pieces of hepatopancreas were samples: one was quickly frozen by liquid nitrogen for total RNA extraction, and the other one was fixed in Bouin's solution for histological sections. The remaining tissue was then stored in −40 °C for the later lipid analysis.

2.3. RNA isolation and real-time quantitative PCR

The expression levels of the nine candidate genes critical for the process of lipid metabolism (*carnitine palmitoyltransferase I* (*CPT-I* and *II*), *acyl-CoA oxidase* (*ACOX*), *FATP*, *FABP*, *diacylglycerol acyltransferase 1* (*DGAT1*), *FAS*, *ACC*, *fatty acyl desaturase* (*FAD*)) were analyzed by real-time quantitative PCR (qPCR) in four treatments. The cDNA sequence of *CPT-1*, *CPT-II*, *ACOX*, *FATP*, *DGAT-I*, *FAS* and *ACC* genes were obtained from the ovarian transcriptome data of *P. trituberculatus*, while the cDNA sequence of *FABP* and *FAD* were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/). The gene-specific primers were designed using Primer premier 5.0 (Table 1). *18S rRNA* was used as an endogenous control (Wang et al., 2015). Amplification efficiencies of all genes were > 96%.

Total RNA was extracted from the hepatopancreas of different treatments using the RNAiso Plus reagent (9109, Takara Bio, Japan) according to the manufacturer's recommendations. The quality and concentrations were determined by 1% agarose gel and ultraviolet spectrophotometer Q5000 (Quawell Q5000, USA), respectively. Subsequently, the first-strand cDNA was synthesized using a reverse first strand cDNA synthesis kit (RR036A, Takara Bio, Japan). The qPCR used a FAST-7500 system (ABI-7500, ThermoFisher, Singapore) with SYBR Premix Ex Taq (RR420A, Takara Bio, Japan). PCR reactions were

Table 1
Primers and their sequences used in this experiment.

Primer name	Sequence (5′–3′)	Source	NCBI Accession no.
CPT-I-F	CGAGCCATGGTGAGCAGTAAGA	experimental design	Unpublished data
CPT-I-R	TGTATTGGCTTGGATCTGTTGC	experimental design	Unpublished data
CPT-II-F	CATCATCATCTCCACCTCAACTCTTC	experimental design	Unpublished data
CPT-II-R	CAGCCCAATTCGTCATCCCGTA	experimental design	Unpublished data
ACOX-F	CAGCATGCTGAAGGACGGTAAT	experimental design	Unpublished data
ACOX-R	GGTAGATGTGATGGTTGGGGAG	experimental design	Unpublished data
FATP-F	ACATCATTTACAACCCCCTTCC	experimental design	Unpublished data
FATP-R	CTTCTTGGCCTCAATCCAGAAC	experimental design	Unpublished data
FABP-F	GGAGTGGCTGGCTGCTGTTGGTA	(Ding et al., 2017)	KU950355.1
FABP-R	AAGGTGGAGCCAGACAGGTTCA	(Ding et al., 2017)	KU950355.1
DGAT1-F	GGAAAAGGCCTGGAAATTCTTC	experimental design	Unpublished data
DGAT1-R	CTCTCTTCTCCGCTTGTGTGAT	experimental design	Unpublished data
FAS-F	CAACCTCACCCTGAAACCTCAA	experimental design	Unpublished data
FAS-R	CACCCTCCCCCTTATTACCATC	experimental design	Unpublished data
ACC-F	CGTGTTCCTCAACTTTGTCCC	experimental design	Unpublished data
ACC-R	TTGTCTTGCTGTTTGGCGTCT	experimental design	Unpublished data
FAD-F	CCATGATGGAGACAAGATGAGAGA	(Shi et al., 2015)	KP288227.1
FAD-R	GGTCAACAGTGGGCAGGAGAT	(Shi et al., 2015)	KP288227.1
18S-F	TCCAGTTCGCAGCTTCTTCTT	(Wang et al., 2015)	KF266707.1
18S-R	AACATCTAAGGGCATCACAGACC	(Wang et al., 2015)	KF266707.1

performed as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 30 s. Ten biological and three technical replicates were performed. The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ methods (Livak and Schmittgen, 2001).

2.4. Lipids analysis

Prior to lipids analysis, all frozen hepatopancreas samples were freeze-dried for lipid extraction. The total lipids were extracted with chloroform-methanol (2:1, v/v) and analyzed based on the method described by Folch-Pi (1957). The neutral lipids and polar lipids were firstly separated by one developing solvent system (hexane/diethyl ether/formic acid = 42/28/0.3, v/v/v). Then the lipid fractions were quantified using an Iatroscan MK-6s TLC-FID analyzer (Iatron Laboratories Inc., Tokyo, Japan) according to the method described by Wu et al (2007). Lipid classes of the hepatopancreas were quantified for total polar lipids (including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and others phospholipids) and neutral lipids (including triacylglycerol, free fatty acids and cholesterol). The level of total neutral lipids and polar lipids were expressed as the percentage of total lipids (% total lipids).

For fatty acids analysis, fatty acid methyl esters (FAME) were prepared by transesterification with boiling 14% borontrifluoride/methanol (w/w) following the method of Morrison and Smith (1964). Fatty acid methyl esters were analyzed by fame ionization detection after injecting a sample into a Thermo Trace GC Ultra gas chromatograph fitted with a 100 m × 0.25 mm ID (0.2 μm film thickness) Supelco SP-2560 capillary column (Supelco, Inc., Billefonte, PA, USA). Injector and detector temperatures were kept at 260 °C. The column temperature was initially held at 70 °C, followed by an increase at a rate of 50 °C min^{-1} to 140 °C and held for 1 min, then increased at a rate of 4 °C min^{-1} to 180 °C and held for 1 min. It was then further increased at 3 °C min^{-1} to the final temperature of 225 °C and held for 30 min until all FAME had been eluted. The carrier gas was nitrogen with the flow velocity at 1 mL min^{-1}. Peaks were identified by comparing retention times with known standard (Sigma-Aldrich Co., St. Louis, MO, USA). Fatty acids profile was expressed as percentage of each fatty acid to the total fatty acids (% total fatty acids) based on the area percentage.

2.5. Histology

The hepatopancreas sections were prepared according to the method described by Yao et al (2008). The hepatopancreas from sampled crabs was fixed in Bouin's solution for 24 h, and then dehydrated through a series of gradient ethanol solutions and cleared with xylene before being embedded in paraffin blocks. The blocks were trimmed and sectioned at 5–7 μm thickness using a rotary microtome (Leica RM2125RTS, Leica Microsystems, Bannockburn, IL, U.S.A.) and stained with hematoxylin-eosin. Histological observations were performed under a light microscope (Leica DM2500, Leica Microsystems) equipped with an automated Leica digital camera system (Leica DFC295, Leica Microsystems) and image manager software (Leica Application Suite version 3.8.0, Leica Microsystems).

2.6. Data analysis

Data were presented as the mean ± standard error (SE). Homogeneity of the variance of relevant data was tested with Levene's test. When necessary, an arcsine-square root or logarithmic transformation was performed prior to analysis. Statistical analysis was conducted using one-way ANOVA and differences were determined using Tukey's multiple range test. When normal distribution and/or homogeneity of variances were not achieved, the data were subjected to the Kruskal-Wallis H nonparametric test, followed by the Games-Howell nonparametric multiple comparison tests. P-value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 19.0 software (SPSS, Chicago, USA).

3. Results

3.1. Effects of estradiol on the expressions of lipid metabolism-related genes

In order to evaluate the role of estradiol on lipid metabolism of female *P. trituberculatus*, the expression levels of genes involved in lipid metabolism were detected by qPCR. The results showed that estradiol significantly up-regulated the expression levels of *CPT-I* and *CPT-II* in the low concentrations treatments (0.01 μg g^{-1} and 0.1 μg g^{-1}), while significantly down-regulated the expression levels of *CPT-I* and *CPT-II* in the high concentration treatment (1 μg g^{-1}) (Fig. 1a, b). The expression levels of *ACOX*, *FATP* and *FABP* significantly decreased in a dose-dependent manner after estradiol treatments compared to the control (Fig. 1c, d, e).

The expression levels of *DGAT1* and *ACC* significantly decreased in a dose-dependent manner by estradiol treatments (Fig. 2a, c). However, the expression levels of *FAS* and *FAD* showed no significant difference in the low concentrations of estradiol treatments (0.01 μg g^{-1} and

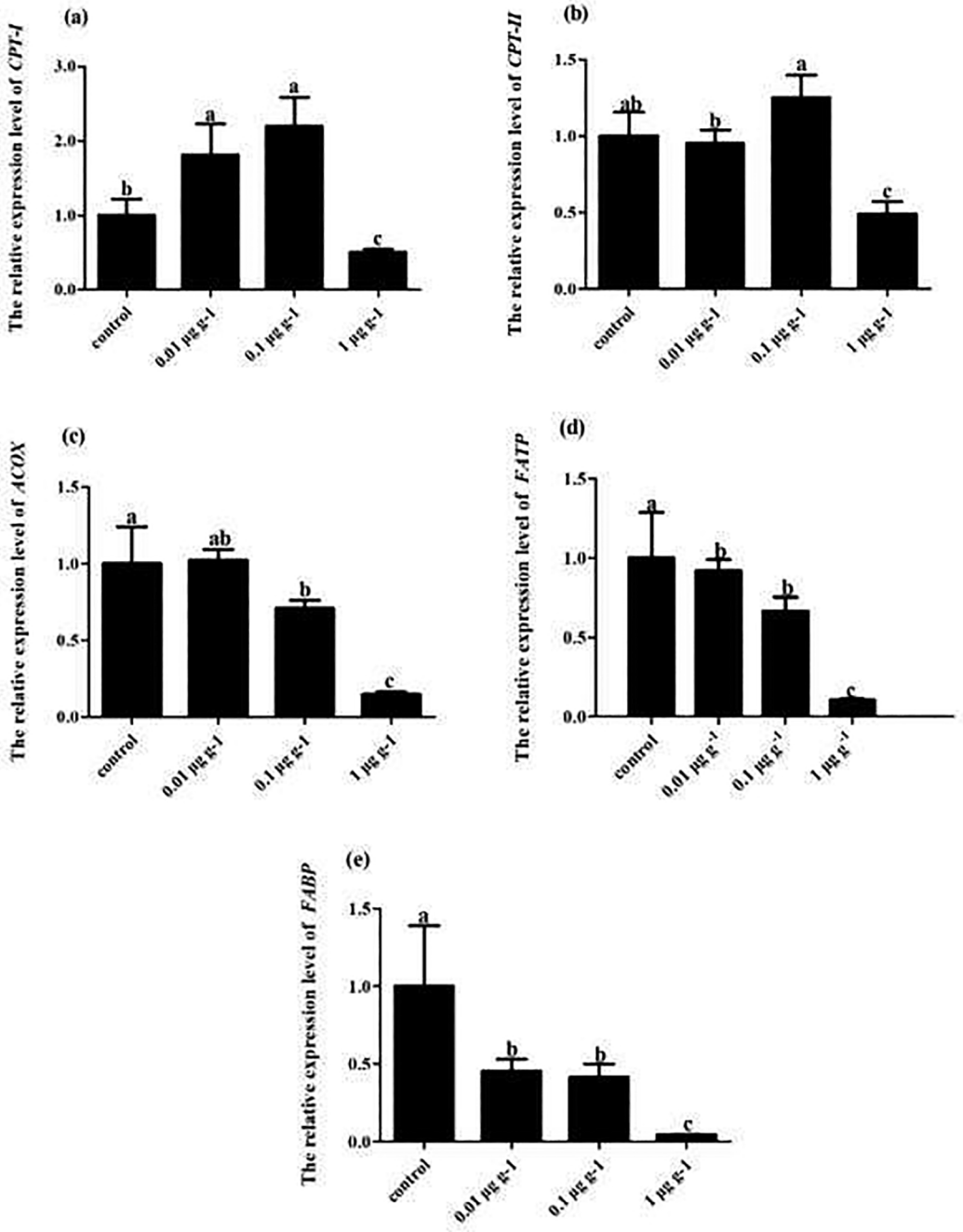

Fig. 1. The expression of fatty acids β-oxidation and transport related genes in hepatopancreas of *P. trituberculatus* by different concentrations of exogenous estradiol. The columns with different letter indicate significant difference ($P < 0.05$). The same as below.

0.1 μg g^{-1}) while significantly decreased in the highest concentration of estradiol treatment (1 μg g^{-1}) (Fig. 2b, d).

3.2. Lipid composition of hepatopancreas

The total lipids, neutral lipids and polar lipids contents in the hepatopancreas of the female *P. trituberculatus* injected with different concentrations of estradiol were presented in Table 2. The level of polar lipids in the hepatopancreas decreased significantly in three estradiol treatments compared to the control group. There was no significant difference among the four treatments for the total lipids and the percentage of neutral lipids.

The fatty acids profile in the hepatopancreas was shown in Table 3. Most of the SFA and total saturated fatty acids (ΣSFA) showed a slightly decreasing trend with the estradiol concentration increasing, but there was no significant difference among the four estradiol treatments. Most monounsaturated fatty acid (C16:1, C17:1n7, C18:1n7) had the lowest levels in 0.1 μg g^{-1} estradiol treatment. Compare to the control (0 μg g^{-1}), all estradiol treatments (0.01, 0.1 and 1 μg g^{-1}) had significantly lower levels of total monounsaturated fatty acids (ΣMUFA), while no significant difference was found among the three estradiol treatments. All estradiol treatments had significantly higher levels of three major polyunsaturated fatty acid (PUFA, i.e. 20: 4n6, 20: 5n3 and 22: 6n3) and ΣPUFA than the control treatment.

3.3. Histology of hepatopancreas

Fig. 3 showed the hepatopancreatic histology for all treatments.

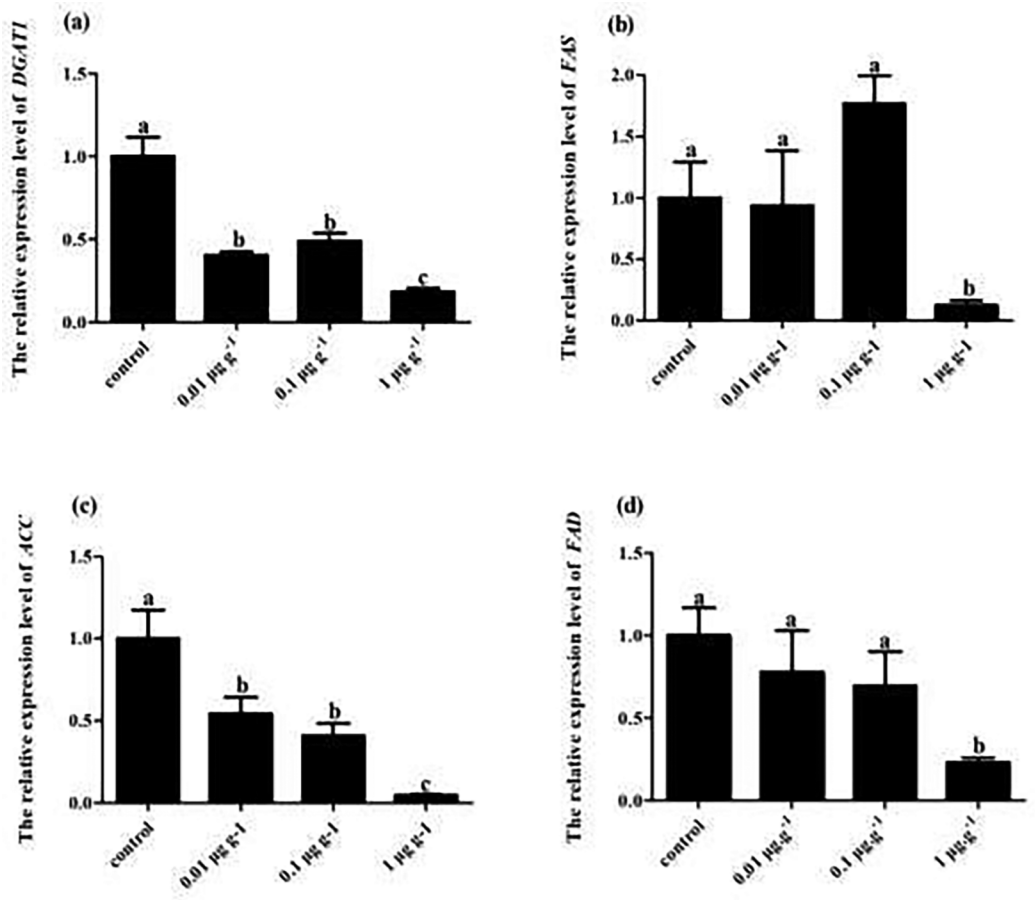

Fig. 2. The expression of lipogenic related genes in hepatopancreas of *P. trituberculatus* by different concentrations of exogenous estradiol.

After injected with different concentrations of estradiol, the walls of hepatopancreatic tubules appeared less fragile (Fig. 3b, c, d) compared to the control (Fig. 3a). Moreover, the size of B cell became larger in the hepatopancreas for all three estradiol treatments (Fig. 3b, c, d) than the control treatment (Fig. 3a). However, the hepatopancreatic histology showed no significant changes among three estradiol treatments.

4. Discussion

Several studies have indicated that lipid plays a vital role in cell structure and the energy supply for physiological processes, including growth and reproduction (Wall et al., 2010; Pasquevich et al., 2011). Estrogen treatment caused the change of lipid level by regulating the expression levels of lipid metabolism-related genes, which has been proved in previous studies (Smith and Muscat, 2005; D'Eon et al., 2005; Fu et al., 2009). In present study, the genes involved in fatty acids β-oxidation were first detected, including *CPT* and *ACOX*. The CPT-I and ACOX were the rate-limiting enzymes of mitochondrial and peroxisomal fatty acids β-oxidation, respectively (Kerner and Hoppel, 2000; Hunt et al., 2002). The results showed that the mRNA levels of *CPT* significantly increased at the low concentrations of estradiol treatments (0.01 μg g^{-1} and 0.1 μg g^{-1}), while significantly decreased in the highest concentration (1 μg g^{-1}). Similarly, Campbell et al (2003) reported that the level of *CPT-I* was increased by 7-fold in skeletal muscle of ovariectomized rats under the estradiol treatment compared with the control. It was speculated that the low concentrations of estradiol could promote the mitochondrial fatty acids β-oxidation. In addition, the transcript of *CPT* was significantly down-regulated in 1 μg g^{-1} estradiol treatment, which could maybe that the dose of this treatment (1 μg g^{-1}) was too high. Gower et al (2002) reported that the higher doses of estradiol suppress the expression level of *CPT-I* in the liver. Moreover, estradiol treatment down-regulated the mRNA level of *ACOX* in a dose-dependent manner in this study. Similar results were also found in the brown trout (Madureira et al., 2016). Those results suggested a negative correlation between estrogenic input and the expression level of *ACOX*. Overall, estradiol regulates fatty acid mitochondria and peroxisomal β-oxidation differently, because estradiol can regulate the transcript of *CPT* and *ACOX* by up-regulating PPAR in vertebrates (Campbell et al., 2003; Madureira et al., 2016).

In addition to the genes involved in fatty acids β-oxidation, the

Table 2
Total lipids (% wet weight) and neutral lipids and polar lipids (% total lipids) in the hepatopancreas of female *P. trituberculatus* injected with different concentrations of estradiol.

	control	0.01 μg g^{-1}	0.1 μg g^{-1}	1 μg g^{-1}
Total lipid (%)	32.50 ± 1.22	27.79 ± 2.19	27.40 ± 1.34	32.56 ± 1.82
Neutral lipids (%)	89.01 ± 2.45	93.78 ± 0.74	92.75 ± 1.23	91.08 ± 1.29
Polar lipids (%)	10.37 ± 1.62[a]	5.93 ± 0.76[b]	6.55 ± 0.52[b]	8.64 ± 1.03[b]

Values are presented as mean ± SE. Different superscript letters within a same row indicate significantly different ($P < 0.05$).

Table 3
Fatty acids profile (% of total fatty acids) in the hepatopancreas of female *P. trituberculatus* injected with different concentrations of estradiol.

	control	0.01 μg g^{-1}	0.1 μg g^{-1}	1 μg g^{-1}
C14:0	2.58 ± 0.13	2.48 ± 0.08	2.51 ± 0.17	2.46 ± 0.10
C15:0	0.72 ± 0.02	0.70 ± 0.03	0.68 ± 0.02	0.69 ± 0.01
C16:0	14.71 ± 0.47	14.33 ± 0.25	14.16 ± 0.29	14.17 ± 0.30
C17:0	0.95 ± 0.02^{a}	0.84 ± 0.04^{b}	0.91 ± 0.03^{ab}	0.91 ± 0.03^{ab}
C18:0	4.42 ± 0.24	4.32 ± 0.19	4.26 ± 0.21	4.43 ± 0.14
C20:0	0.48 ± 0.03	0.44 ± 0.01	0.46 ± 0.03	0.46 ± 0.03
C21:0	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
C22:0	0.38 ± 0.03	0.37 ± 0.01	0.37 ± 0.03	0.34 ± 0.02
C24:0	0.32 ± 0.01	0.30 ± 0.02	0.29 ± 0.03	0.28 ± 0.01
ΣSFA	24.66 ± 0.93	23.89 ± 0.35	23.74 ± 0.67	23.82 ± 0.55
C14:1n5	0.24 ± 0.00	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02
C16:1	9.04 ± 0.22^{a}	8.52 ± 0.37^{ab}	7.97 ± 0.31^{b}	8.54 ± 0.25^{ab}
C17:1n7	0.93 ± 0.01^{a}	0.86 ± 0.05^{ab}	0.82 ± 0.01^{b}	0.88 ± 0.01^{ab}
C18:1n9	21.32 ± 0.04^{a}	19.10 ± 0.60^{c}	20.24 ± 0.44^{ab}	19.92 ± 0.12^{bc}
C18:1n7	6.45 ± 0.07^{a}	5.81 ± 0.08^{b}	5.76 ± 0.12^{b}	6.05 ± 0.15^{b}
C20:1n9	3.43 ± 0.01^{a}	2.86 ± 0.06^{b}	3.01 ± 0.07^{b}	2.93 ± 0.09^{b}
C22:1n9	0.50 ± 0.03^{b}	0.53 ± 0.05^{a}	0.44 ± 0.02^{ab}	0.39 ± 0.01^{b}
ΣMUFA	41.91 ± 0.36^{a}	37.89 ± 0.83^{b}	38.46 ± 0.60^{b}	38.93 ± 0.30^{b}
C18:2n6	1.36 ± 0.02	1.29 ± 0.02	1.35 ± 0.06	1.43 ± 0.12
C20:2n6	1.08 ± 0.03	1.03 ± 0.05	1.16 ± 0.05	0.92 ± 0.17
C18:3n3	0.38 ± 0.02^{b}	0.57 ± 0.02^{a}	0.51 ± 0.07^{a}	0.56 ± 0.010^{a}
C20:3n3	0.41 ± 0.02	0.40 ± 0.01	0.43 ± 0.03	0.42 ± 0.020
C20:3n6	0.10 ± 0.00^{b}	0.11 ± 0.00^{a}	0.19 ± 0.00^{a}	0.11 ± 0.00^{a}
C20:4n6	1.36 ± 0.04^{b}	1.70 ± 0.06^{a}	1.73 ± 0.07^{a}	1.64 ± 0.06^{a}
C20:5n3	4.02 ± 0.13^{b}	5.31 ± 0.23^{a}	5.10 ± 0.14^{a}	5.08 ± 0.17^{a}
C22:5n3	1.08 ± 0.06^{b}	1.38 ± 0.05^{a}	1.36 ± 0.01^{a}	1.27 ± 0.040^{a}
C22:6n3	10.92 ± 0.99^{b}	13.70 ± 0.62^{a}	13.14 ± 0.20^{a}	13.11 ± 0.62^{a}
ΣPUFA	20.73 ± 1.20^{b}	25.49 ± 0.78^{a}	24.89 ± 0.38^{a}	24.54 ± 0.77^{a}
n-3PUFA	16.82 ± 1.17^{b}	21.36 ± 0.75^{a}	20.53 ± 0.32^{a}	20.44 ± 0.80^{a}
n-6PUFA	3.91 ± 0.03^{b}	4.13 ± 0.91^{ab}	4.36 ± 0.10^{a}	4.10 ± 0.20^{ab}
n-3/n-6	4.30 ± 0.27^{b}	5.17 ± 0.19^{a}	4.72 ± 0.10^{ab}	5.04 ± 0.37^{ab}
ΣHUFA	18.99 ± 1.23^{b}	23.63 ± 0.77^{a}	23.02 ± 0.37^{a}	22.56 ± 0.73^{a}

Values are presented as mean ± SE. Different superscript letters within a same row indicate significantly different ($P < 0.05$).

mRNA levels of genes associated with long-chain fatty acid transport and fatty acid uptake and utilization (Schaffer and Lodish, 1994; Li et al., 2011) were also detected, such as *FATP* and *FABP*. Estradiol treatment significantly down-regulated the mRNA levels of *FATP* and *FABP* in a dose-dependent manner. However, Oosthuyse and Bosch (2012) reported that estrogen stimulated the expression of *FATP* and *FABP* to promote long chain fatty acid (LCFA) uptake in skeletal muscle. The possible reason for the contradictory results was the tissue-specific regulation of estradiol. Previous studies have reported that the estradiol had different effects on lipid metabolism in different tissues (Campbell and Febbraio, 2001; Beckett et al., 2002).

Due to the lipid deposition is also related to lipid synthesis rate, this study analyzed the expression levels of lipogenic related genes, including *DGAT, FAS, ACC, FAD*. The FAS and ACC catalyzed the *de novo* synthesis of fatty acids (Wakil et al., 1983; Davis et al., 2000), while the FAD catalyzed the first desaturation step in the long chain polyunsaturated fatty acids (LC-PUFA) biosynthesis pathway (Almudena et al., 2009; Li et al., 2014). The DGAT was the committed step in the biosynthesis of triacylglycerols (Yen et al., 2008). In this study, the mRNA levels of *DGAT1* and *ACC* significantly decreased among three estradiol treatments, while the mRNA levels of *FAS* and *FAD* were significantly down-regulated only in 1 μg g^{-1} injection. Consistent with the findings in ovariectomized rats that estradiol could suppress liver lipogenesis by significantly reduced the expression of *DGAT1* (Zhu et al., 2014) and *FAS* and *ACC* (D'Eon et al., 2005; Gao et al., 2006). The biosynthesis pathway of fatty acids is very complex, involving multiple factors (Ganguly, 1960). For example, FAS catalyzes the conversion of Malonyl-CoA to palmitate (Wakil et al., 1983) different from the role of ACC in *de novo* synthesis of fatty acids. Only the highest estradiol treatment could down-regulated the transcripts of *FAS* and *FAD*, which might be due to the different sensitivity of genes. Those results indicated that estradiol not only decreased triacylglycerols synthesis but also reduced the synthesis of fatty acids.

The effect of estradiol on lipid composition in hepatopancreas was also analyzed in present study. The results showed that estradiol treatments significantly decreased the content of polar lipids and in the

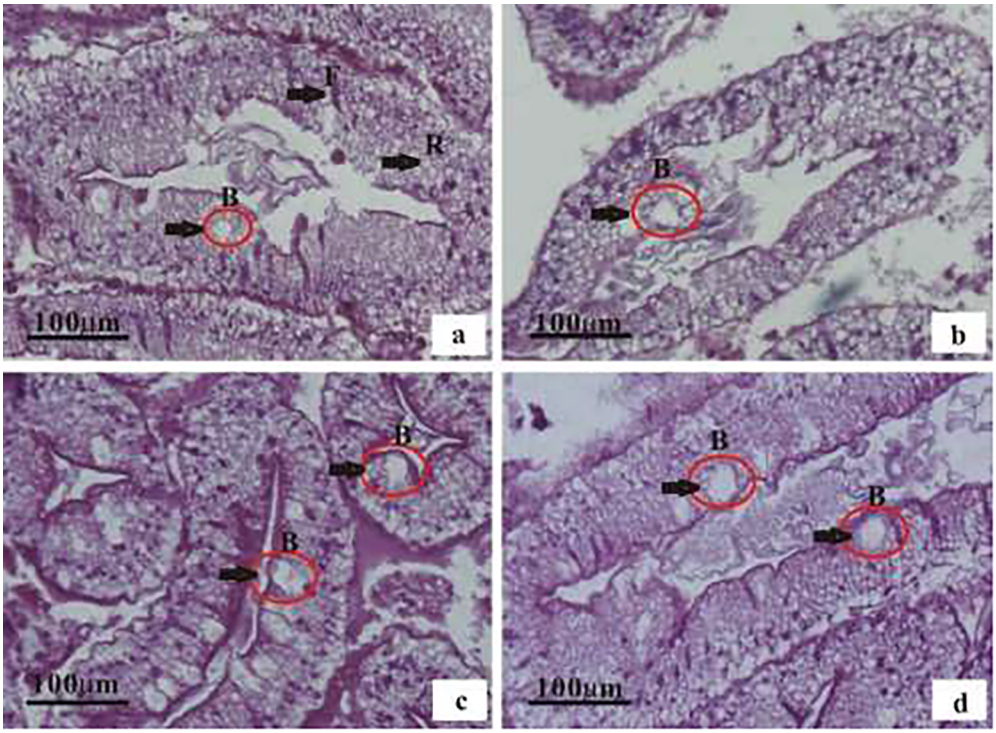

Fig. 3. The hepatopancreatic histology of female *P. trituberculatus* injected with different concentrations of estradiol. (a) 0 μg g^{-1} estradiol treatment (control); (b) 0.01 μg g^{-1} estradiol treatment; (c) 0.1 μg g^{-1} estradiol treatment; (d) 1 μg g^{-1} estradiol treatment. F, fibrillar cell; B, blister-like cell; R, resorptive cell.

hepatopancreas, which were inconsistent with the results in the normal rats (Kenagy et al., 1981; Ferreri and Naito, 1977). Previous studies demonstrated that the polar lipids in hepatopancreas were transported by hemolymph to ovary for the synthesis of vitellin during the ovarian development in crustacean (Cheng et al., 1997). Moreover, the fatty acids composition in crustaceans were regulated by many factors such as species, developmental stage, nutritional status, dietary content and season (Rosa and Nunes, 2004). In this study, ΣMUFA decreased significantly after the estradiol treatments, while the ΣPUFA showed a significantly increasing trend. It has been demonstrated ΣMUFA decreased significantly various from 0.05 to 0.5 $\mu g\, g^{-1}$ estradiol concentrations, while ΣPUFA showed the different trend (Coccia et al., 2010; Zhao et al., 2014). The reason may be the dose of estradiol and species differences, which need to be further studied. Furthermore, it is well known that the SFA and MUFA were used for energy supply in aquatic animals (Jezierska et al., 2010). Therefore, the decrease of MUFA in hepatopancreas may be oxidized by the organism to provide energy.

The hepatopancreas is a major lipid storage organ and plays an important role in lipid metabolism in crustaceans (Wouters et al., 2001). Previous studies have shown that the hepatopancreas of *P. trituberculatus* is composed of multiple blind-end tubules. The tubule walls consist of four kinds of cells, the embryonic cell (E cell), blister-like cell (B cell), resorptive cell (R cell) and fibrillar cell (F cell) (Yao et al., 2008). It is generally accepted that the E cells are undifferentiated cells, differentiating into three other cells. The main roles of B cells are the intracellular digestion and transport of nutrient, while the roles of R cells in the hepatopancreas are the absorption and storage of lipid droplets (Loizzi and Peterson, 1971; Hopkin and Nott, 1980). F cells synthesize digestive enzymes, which is then discharged into the hepatopancreatic lumen (Vogt et al., 1989). In the present study, similar to the results in the shrimp (Zhao et al., 2014), the size of B cell in hepatopancreas became larger after estradiol injection. This may be the increased fatty acid content causing the larger size of B cells (Zhao et al., 2014). The PUFA performed vital roles in maintaining the permeability and fluidity of cell membranes (Vance and Vance, 2002). The increase in ΣPUFA caused by estradiol treatment might explain the walls complete of hepatopancreatic tubules.

Cellular estrogen signaling was mediated primarily via the estrogen receptor (ER), a family of nuclear hormone receptor-type transcription factors (Qiu et al., 2017). In mammals, it was demonstrated that estrogen function mainly through binding estrogen receptor to stimulate the genomic expression of certain other nuclear hormone receptors ((PPAR), sterol regulatory element-binding protein 1c (SREBP1c)) and downstream targets to promote long chain fatty acid (LCFA) uptake, mitochondrial shuttling and β-oxidation (Smith and Muscat, 2005; D'Eon et al., 2005). In this study, the expression levels of lipid metabolism-related genes were regulated by estradiol, suggesting that a class of receptors similar to ER may also exist in crustaceans. To date, the ER gene which considered lost during the evolution of arthropods has not been functionally identified for any crustacean species (Thornton et al., 2003). However, the estrogen related receptor (ERR) with high sequence homology with the vertebrate ER has been found in some crustacean species (Zhao, 2016; Lu et al., 2016). It has also been reported that ERR1 was prominently expressed in tissues with a high capacity for fatty acids β-oxidation such as skeletal muscle and brown adipose tissue, suggesting that it involved in regulating the cellular energy balance (Giguère, 1999). Therefore, it was speculated that estradiol regulated the lipid metabolism in crustacean through the ERR-mediated signaling pathway. This remains to be further studied.

5. Conclusions

The present study showed that estradiol treatment influenced lipid metabolism in the hepatopancreas of *P. trituberculatus* at different levels. Specifically, estradiol treatment altered the gene expression pattern related to fatty acids β-oxidation and lipogenesis. Moreover, estradiol treatment decreased the percentages of polar lipids and ΣMUFA and increased the ΣPUFA levels and the size of B cells in the hepatopancreas. The results indicated that estradiol promoted lipid catabolism in the hepatopancreas of *P. trituberculatus*.

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