Aquaculture 444 (2015) 93–98

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effect of dietary n − 3 LC-PUFAs on plasma vitellogenin, sex steroids, and ovarian steroidogenesis during vitellogenesis in female silver pomfret (*Pampus argenteus*) broodstock

Shiming Peng *, Quanxin Gao, Zhaohong Shi, Chenjie Zhang, Jiangang Wang, Fei Yin, Yanliang Zhang

East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China

ARTICLE INFO

Article history:
Received 17 January 2015
Received in revised form 27 March 2015
Accepted 28 March 2015
Available online 3 April 2015

Keywords:
Silver pomfret
Broodstock
Vitellogenin
Sex steroids
Steroidogenesis

ABSTRACT

We investigated the effects of dietary n − 3 LC-PUFAs on plasma vitellogenin (Vtg), sex steroids levels and ovarian steroidogenesis during vitellogenesis in female silver pomfret (*Pampus argenteus*) broodstock. Broodstock were fed four iso-nitrogenous, iso-energetic, and iso-lipidic experimental diets for 185 d: FO (100% fish oil), FSO (70% fish oil and 30% soybean oil), SFO (30% fish oil and 70% soybean oil), and SO (100% soybean oil) with n − 3 LC-PUFA levels of 5.18, 4.01, 3.02, and 2.22%, respectively. The results revealed that plasma FSH levels increased at vitellogenesis ($P < 0.05$) and slightly decreased at post-vitellogenesis in all four groups. However, plasma LH levels continuously increased during vitellogenesis and significantly increased at post-vitellogenesis ($P < 0.05$). The FO and FSO groups had significantly higher FSH and LH levels than the SO group at vitellogenesis and post-vitellogenesis. Plasma E2 and T levels continuously increased during vitellogenesis in all four groups. However, the SO group had the lowest plasma E2 and T levels during vitellogenesis. Plasma Vtg levels significantly increased at vitellogenesis and post-vitellogenesis ($P < 0.05$). FO and FSO had significantly higher Vtg levels than SO ($P < 0.05$) during vitellogenesis. *Cyp19α1α* and *erα* expression levels significantly increased at vitellogenesis and post-vitellogenesis ($P < 0.05$), with no significant differences between the vitellogenesis and post-vitellogenesis, except in the SO group. *Cyp19α1α* and *erα* expression levels decreased in SO at vitellogenesis. In conclusion, dietary n − 3 LC-PUFAs affect ovarian steroidogenesis in silver pomfret by altering plasma FSH and LH levels and ovarian *Cyp19α1α* gene expression levels during vitellogenesis. High dietary n − 3 LC-PUFAs promote ovarian development in silver pomfret, by increasing E2 secretion and Vtg synthesis.

1. Introduction

It has been reported that n − 3 long-chain polyunsaturated fatty acids (n − 3 LC-PUFAs) such as eicosapentaenoic acid (EPA, 20:5n − 3) and docosahexaenoic acid (DHA, 22:6n − 3) play critical roles in cell permeability and enzyme activity (Li et al., 2005; Zakeri et al., 2011). Marine teleosts cannot synthesize LC-PUFAs from 18-carbon precursors due to a deficiency in Δ5-desaturase (Bruce et al., 1999). Therefore, it is imperative that teleost diets contain LC-PUFAs. The importance of LC-PUFAs, especially n − 3 LC-PUFAs, in broodstock nutrition has been extensively studied. Dietary n − 3 LC-PUFAs affect spawning performance (Zakeri et al., 2011), egg and larval quality (Li et al., 2005), and egg fatty acid composition of broodstock (Li et al., 2005; Vassallo-Agius et al., 2001; Zakeri et al., 2011). While most studies have focused on the effects of diet on body composition, growth performance, egg quality, and larval survival rate (Li et al., 2005; Rennie et al., 2005; Zakeri et al., 2011), few studies have investigated the effects of nutrients on hormone synthesis during gonadogenesis.

Pituitary gonadotropin hormone (GtH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) are involved in gametogenesis and sexual maturation in teleosts (Swanson et al., 2003). In salmonid fish, FSH is primarily involved in vitellogenesis and spermatogenesis, whereas LH triggers maturation, ovulation, and spermiation (Nagahama and Yamashita, 2008). In response to GtH, cholesterol is converted into testosterone (T) and 17β-estradiol (E2). E2 stimulates hepatic vitellogenin (Vtg) synthesis, which is essential in oocyte development (Lubzens et al., 2010). In *Oncorhynchus mykiss* (Walbaum) rainbow trout, FSH levels increase during vitellogenesis and decrease during the final maturation concomitant with increasing LH levels (Gomez et al., 1999; Prat et al., 1996). Sex steroid hormones play important roles in vertebrate reproduction. In several teleost species, E2 and T, which are produced in gonadal tissues under the control of GtH, are essential in gametogenesis (Nagahama and Yamashita, 2008).

In humans, fatty acids, especially PUFAs, are cell signaling molecules, structural compounds, and energy sources. PUFAs are crucial for

Abbreviations: n − 3 LC-PUFAs, n − 3 long-chain polyunsaturated fatty acids; FO, 100% fish oil diet; FSO, 70% fish oil and 30% soybean oil diet; SFO, 30% fish oil and 70% soybean oil diet; SO, 100% soybean oil diet; GtH, gonadotropin hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, 17β-estradiol; T, testosterone; Vtg, vitellogenin; *Erα*, estrogen receptor alpha; HPG, hypothalamic pituitary gonadal.

* Corresponding author. Tel.: +86 21 65684690-8150; fax: +86 21 65812067.
E-mail address: pengshiming@hotmail.com (S. Peng).

http://dx.doi.org/10.1016/j.aquaculture.2015.03.031
0044-8486/

mediating immunological, metabolic, and endocrine signals within the uterine-fetal-placental unit (Benassayag et al., 1999). PUFAs are precursors of cell signaling molecules (e.g., eicosanoids), and there is evidence that they regulate membrane signal transduction pathways (Graber et al., 1994) and steroid hormone action (Benassayag et al., 1999), by modulating the binding of estrogen, progesterone, and glucocorticoids to their intracellular receptors (Benassayag et al., 1986; Haourigui et al., 1995; Vallette et al., 1991). Therefore, PUFAs act as endogenous modulators of key enzymes involved in steroid metabolism (Nunez et al., 1995). Previous studies on fish reproduction have focused on lipids as energy sources; their roles in other physiology processes have been overlooked in spite of the fact that PUFAs and their metabolites produced from cyclooxygenase and lipoxygenase have modulatory effects on steroid metabolism (Nunez et al., 1995), which in turn control ovulation, steroidogenesis (Sorbera et al., 2001), and fecundity rates (Bogevik et al., 2014; Navas et al., 1997; Zhang et al., 2013).

Silver pomfret (*Pampus argenteus*) is a novel aquaculture species in China (Peng et al., 2012, 2013). In spite of important technical developments and intense research in silver pomfret, some bottlenecks still limit the expansion of its industry. One of these limitations is the lack of a suitable formulated diet for broodstock, which leads to the poor quality of gonads in cultured broodstock. The effects of diet on the body composition and growth performance of juvenile silver pomfret have been extensively studied (Almatar and James, 2007; Hossain et al., 2010; Peng et al., 2012, 2013); however, there is little evidence on the effects of diet on the reproductive system. The objective of this study was to investigate the effects of dietary n − 3 LC-PUFAs on ovarian steroidogenesis by analyzing the plasma levels of FSH and LH and measuring the expression levels of ovarian *Cyp19α1α* and hepatic *erα* (estrogen receptor alpha) in female silver pomfret broodstock. Plasma levels of E2, T, and Vtg during vitellogenesis were analyzed.

2. Materials and methods

2.1. Experimental diets

Four iso-nitrogenous, iso-energetic, and iso-lipidic (16% lipids) experimental diets were formulated: (1) 100% fish oil (FO), (2) 70% fish oil and 30% soybean oil (FSO), (3) 30% fish oil and 70% soybean oil (SFO), and (4) 100% soybean oil (SO; Table 1). The fatty acid compositions of the experimental diets are shown in Table 2. Fish meal and soybean meal were used as protein sources; fish oil and soybean oil were used as the lipid sources. Dietary protein and lipid concentrations were approximately 50% and 16% (dry-weight basis), respectively; n − 3 LC-PUFA concentrations of FO, FSO, SFO, and SO were 5.18, 4.01, 3.02, and 2.22%, respectively. All ingredients were thoroughly mixed and mechanically extruded in 2-mm pellets. The pellets were oven-dried at 25 °C to obtain a moisture level of approximately 100 g × kg^{-1} and stored in airtight plastic bags at −20 °C.

Table 1
Ingredient and proximate composition of the experimental diets.

	Experimental diets			
	FO	FSO	SFO	SO
Ingredients (g/kg)				
Fish meal[a]	610	610	610	610
Soybean meal[a]	160	160	160	160
Wheat flour[a]	100	100	100	100
Anchovy oil	100	70	30	0
Soybean oil	0	30	70	100
Vitamin premix[b]	20	20	20	20
Mineral premix[c]	10	10	10	10
Proximate composition (%)				
Crude protein	49.62	49.95	49.73	50.14
Crude fat	16.29	16.36	16.46	16.02
Ash	10.43	9.98	10.12	10.01
n − 3 LC-PUFA[d]	5.18	4.01	3.02	2.22

[a] Proximate composition as % dry weight. Fish meal: 67% crude protein, 10% crude lipid; soybean meal: 43% crude protein, 1.9% crude lipid; wheat flour: 12% crude protein, 1.6% crude lipid.
[b] Supplied (mg × kg^{-1} diet): myo-inositol, 400; nicotinic acid, 150; calcium pantothenate, 44; riboflavin, 20; pyridoxine hydrochloride, 12; menadione, 10; thiamine hydrochloride, 10; retinyl acetate, 7.3; folic acid, 5; biotin, 1; cholecalciferol, 0.06; cyanocobalamin, 0.02; L-ascorbic acid, 400; Dl-α-tocopherol acetate, 500.
[c] Supplied (kg^{-1} diet): KH_2PO_4, 22 g; $FeSO_4 \cdot 7H_2O$, 1.0 g; $ZnSO_4 \cdot 7H_2O$, 0.13 g; $MnSO_4 \cdot 4H_2O$, 52.8 mg; $CuSO_4 \cdot 5H_2O$, 12 mg; $CoSO_4 \cdot 7H_2O$, 2 mg; KI, 2 mg.
[d] Calculated from lipid content × Σ n − 3 LC-PUFA.

2.2. Experimental design

Female silver pomfret (1 y of age) was assigned to one of the four diets (with three replicates each), with 20 fish per 16-m^3 tank. Fish were fed to satiation twice a day at 09:00 and 17:00 for 185 days (from early October to early April) prior to spawning. The tanks were continuously aerated; dissolved oxygen levels were >7 mg × L^{-1}. Water was replaced with fresh water once a day (09:00). Temperature and salinity were 16–23 °C and 25–28, respectively.

2.3. Sample collection

Fish from each treatment were sampled on November 20, 2013, February 15, 2014, and April 8, 2014 to evaluate pre-vitellogenesis (stage II), vitellogenesis (stage III) and post-vitellogenesis (stage IV), respectively. In each sampling, three fish from each tank were anesthetized with ethyl *m*-aminobenzoate methanesulfonate (MS-222) at 0.1 g × L^{-1} following a 24-h fast. Blood was collected by caudal puncture using pre-heparinized syringes fitted with 5-ml needles and centrifuged at 12000 × *g* for 5 min at room temperature. Plasma was stored at −20 °C. Ovary and liver sections were excised, mixed with 2 ml of RNA Later™ (Qiagen, Germany), incubated overnight at 4 °C, and stored at −80 °C. Each kind of sample from each tank was pooled for further analyses.

2.4. Biochemical analyses

2.4.1. FSH, LH, E2, T, and Vtg levels

Plasma levels of FSH, LH, E2, T and Vtg were measured by ELISA (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). In brief, 40 μl plasma sample, 10 μl biotinylated anti-antibodies (anti-FSH, LH, E2, T and Vtg, respectively) and 50 μl streptavidin-HRP were added to each well, mixed gently and then incubated at 37 °C for 60 min. After incubation, supernatant was discarded and each well was washed 5 times with washing buffer, waiting for 30 s and discarding the supernatant. Then, 50 μl solutions A and B were added in this sequence to each well, mixed gently and incubated at 37 °C in the dark for 10–15 min. To stop the reaction, 50 μl of stop buffer was applied to each well. Plates

Table 2
Fatty acid composition (% of total fatty acids) of the experimental diets.

Fatty acids	Experimental diets			
	FO	FSO	SFO	SO
Saturated	29.10	28.86	27.01	25.06
Σ MUFAs	28.94	28.19	28.08	27.36
C18:2n6	7.41	11.15	20.75	27.71
C18:3n6	0.18	0.24	0.28	0.30
C20:4n6	3.13	1.69	0.71	0.63
Σ n − 6 PUFAs	10.72	13.08	21.74	28.64
C18:3n3	1.23	1.94	2.94	3.62
C20:3n3	0.23	0.20	0.16	0.14
C20:5n3	14.92	12.06	8.65	5.97
C22:5n3	1.81	1.51	1.18	0.92
C22:6n3	12.82	10.75	8.34	6.82
Σ n − 3 PUFAs	31.00	26.45	21.27	17.46
n − 3/n − 6	2.89	2.02	0.98	0.61
Σ n − 3 LC-PUFAs	29.78	24.51	18.33	13.84