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The influence of dietary arachidonic acid on growth, fatty acid profile and sex steroid hormones of F_2 generation Chinese sturgeon (*Acipenser sinensis*)

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ABSTRACT

An 22-week feeding trial was performed to evaluate the effects of dietary arachidonic acid (ARA) on the growth performance, tissue fatty acid, ARA metabolites, sex steroid hormones and steroidogenesis-related gene expression in F_2 generation Chinese sturgeon. Four diets with different ARA levels (0.0 %, 0.5 %, 1.0 % and 2.0 % in the diets) were formulated. The results showed that dietary ARA did not affect final body weight (FBW), weight gain rate (WGR), feed conversion ratio (FCR), survival rate (SR), hepatosomatic index (HSI), viscerosomatic index (VSI), condition factor (CF) and gonadalsomatic index (GSI) ($P > 0.05$), while tissue ARA deposition was significantly effected by the dietary ARA ($P < 0.05$). In the ovary and liver, the metabolites of prostaglandin E_2 (PGE_2) and 5-hydroxyeicosatetraenoic acid (5-HETE) increased significantly with increasing dietary ARA ($P < 0.05$). In the muscle, the contents of the two metabolites in the 1.0 % ARA diet were significantly higher than that the 0.0 % and 2.0 % ARA diets ($P < 0.05$). Significantly decreased liver and ovary estradiol (E_2), testosterone (T) levels were found in the 0.0 % ARA diet compared with 2.0 % ARA diet ($P < 0.05$). In the ovary and liver, the contents of vitellogen (Vtg) increased significantly with increasing dietary ARA ($P < 0.05$). E_2 and Vtg contents in the serum of fish fed the 1.0 % ARA diet were significantly higher than the 0.0 % ARA diet ($P < 0.05$). These results indicated that dietary ARA did not affect growth, but ARA levels greater than 1 % in the diets plays vital roles in the ARA deposition, metabolism, steroid regulation and Vtg synthesis in F_2 generation female Chinese sturgeon. The best efficiency was achieved 1 % ARA, based on roles or cost effective, which were beneficial to promote ovary development.

1. Introduction

Lipids have a variety of functions, as they provide energy, essential fatty acids, and fat-soluble nutrients and are essential for the biological structure and normal function of cells (Bautista-Teruel et al., 2011; Bou et al., 2017). Notably, lipids play vital roles in the process of gonadal development in fish (Izquierdo et al., 2001; Johnson, 2009). Compared to the volume of studies on the reproductive regulatory impact of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), there are relatively few studies on n-6-LC-PUFAs, primarily arachidonic acid (ARA) (Xu et al., 2017; Shehata et al., 2020). The roles of ARA in spawning performance, egg quality, offspring quality and oocyte maturation are well documented in some aquatic animals (Zhou et al., 2011; Coman et al., 2011; Xu et al., 2017; Khajeh et al., 2017; Shehata et al., 2020; Nhan et al., 2020; Fei et al., 2020). ARA influences steroidogenesis in fish species (Norambuena et al., 2013; Xu et al., 2017). Ovarian development, is a very important process in fish reproduction, and it is accompanied by changes in plasma steroid levels (Miura et al., 2007). Steroid hormones also drive ovarian maturation and are indicators of oocyte maturation in fish (Lubzens et al., 2010; Reading et al., 2018). However, information on the regulatory role of ARAs in fish steroidogenesis is very scarce (Norambuena et al., 2013; Xu et al., 2017). Data on the functions of ARA in steroidogenesis in artificially cultured endangered Chinese sturgeon are very limited. Chinese sturgeon, *Acipenser sinensis*, is a Grade I protected animal in China that was listed as a critically endangered (CR) species on the International Union for the Conservation of Nature (IUCN) Red List in 2010 (Wei, 2010). In response, the Chinese government and scientific researchers made substantial efforts related to sturgeon conservation in recent decades. Although artificial propagation techniques to obtain F_2 generations were

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fully developed in the past few years (Guo et al., 2011; Wei et al., 2013), these attempts did not always successfully produce larvae (He et al., 2020). This lack of success can be attributed to many factors, such as the 14–26 years for female Chinese sturgeons to reach maturity, the low maturity rate among females, the scarcity of females with ovaries developing to stage III or IV, the reduction in broodstock fertility in long-term freshwater breeding environments and the poor germplasm quality of offspring individuals (Wei et al., 2013; Luo et al., 2020). A method of regulating gonadal development in artificially cultured endangered Chinese sturgeon is urgently needed. Previous studies indicated that ARA is a key nutrient that is absorbed and used by Chinese sturgeon for gonadal development and juvenile growth (Liu et al., 2014; Zhou et al., 2017). Leng et al. (2019) also reported that an 18 % dietary lipid level was beneficial for oocyte growth and lipid deposition in oocytes via the regulation of enhanced lipid metabolism and ARA metabolism, and 14 % of dietary lipids benefited steroid hormone synthesis. He et al. (2020) further suggested that enhanced steroid synthesis, and unsaturated fatty consumption contributed to ovarian maturation in Chinese sturgeon. Researchers reported that freshwater may not meet the entire requirements of this group of LC-PUFAs in their diet, which may be responsible for reproductive failure (Tocher et al., 2003). Therefore, supplementation with appropriate LC-PUFAs in the diet, particularly during the maturity stage in freshwater fish, is absolutely essential because of their important structural and functional roles in the gonads (Asil et al., 2017). Current, knowledge of the relationship between dietary ARA and sex steroid hormones and gonadal steroidogenesis-related gene expression in Chinese sturgeon is limited. Therefore, the present study investigated the effects of dietary ARA on growth performance, morphological parameters, tissue fatty acid composition, ARA metabolites, sex steroid hormones and ovary steroidogenesis-related gene expression in F_2 generation Chinese sturgeon. This study provides a theoretical basis for the nutritional regulation of steroidogenesis in Chinese sturgeon and enriches our knowledge of ovary development in artificially cultured endangered sturgeons.

2. Materials and methods

2.1. Ethics statement

All fish handling and experimental procedures were approved by the Laboratory of Animal Experimental Ethics Inspection of the Laboratory Animal Centre, Yangtze River Fisheries Research Institute.

2.2. Experimental diet

Four isonitrogenous and isoenergetic diets were formulated by adding 0–2 % ARA to achieve different ARA levels, namely, 1.31 % (0.0 %), 3.91 % (0.5 %), 7.30 % (1.0 %) or 13.70 % (2.0 %) of the total fatty acids in the sturgeon feed. Peruvian fishmeal, soybean meal, casein and gelatin were used as the protein sources in the feed. Fish oil and ARA oil were used as lipid sources, and wheat flour was used as the carbohydrate source. The ARA contents of the diets were manipulated by adding different levels of ARA oil. ARA oil was purchased from Fuxing Biotechnology Co., Ltd, Hubei, China. ARA oil produced by the fermentation method, *Mortierella alpine*, was used in oil production. Its content was 44.88 %, and the main fatty acid profile of the ARA oil contained 8.91 % palmitic acid, 6.03 % stearic acid, 5.34 % oleic acid, 2.62 % linoleic acid, 0.13 % linolenic acid, 0.26 % eicosapentaenoic acid and 0.40 % docosahexaenoic acid. The formulation and fatty acid compositions of the experimental diets are presented in Tables 1 and 2, respectively. After all of the dry ingredients were thoroughly mixed, approximately 20 % water by mass was added to obtain a wet dough. The wet dough was made into pellets of 5.8 mm in diameter and approximately 12.0 mm in length using a particle feed machine (KL-210; Nongle Machinery Co., Ltd, Hunan, China) and dried at room temperature with an electrical fan. The dried pellets were stored at −20 °C until

Table 1
Formulation and ingredients of the basal diet (%).

Ingredients	Dietary inclusion of ARA level (% of diet)			
	0.00	0.50	1.00	2.00
Peruvian fish meal	37.00	37.00	37.00	37.00
Soybean meal	12.00	12.00	12.00	12.00
Casein	8.00	8.00	8.00	8.00
Gelatin	2.00	2.00	2.00	2.00
Fish oil	10.00	9.50	9.00	8.00
ARA-enriched oil	0.00	0.50	1.00	2.00
Wheat flour	21.00	21.00	21.00	21.00
Choline chloride	0.20	0.20	0.20	0.20
Monocalcium phosphate	1.00	1.00	1.00	1.00
Vitamin premix	1.00	1.00	1.00	1.00
Mineral premix	2.00	2.00	2.00	2.00
Yeast	1.00	1.00	1.00	1.00
Micro- cellulose	2.80	2.80	2.80	2.80
Carboxymethylcellulose sodium	2.00	2.00	2.00	2.00
Proximate analysis (%)				
Moisture	10.21	10.36	10.21	9.88
Crude protein	42.67	42.17	41.90	42.34
Crude lipid	13.93	13.50	13.21	13.80

*Vitamin premixture provided the following per kg of diet: vitamin, B_1 50 mg; vitamin B_2, 200 mg; vitamin B_6, 50 mg; vitamin B_{12}, 20 mg; folic acid, 15 mg; vitamin C, 325 mg; calcium pantothenate, 400 mg; inositol, 1500 mg; *D*-biotin (2%), 5 mg; niacin, 750 mg; vitamin A, 2.5 mg; vitamin E, 160 mg; vitamin D3, 2.0 mg; vitamin K_3, 20 mg.
*Mineral premixture provided the following per kg of diet: Ca $(H_2PO4)_2$, 1800 mg; KH_2PO_4, 1350 mg; NaCl, 500 mg; $MgSO{\cdot}7H_2O$, 750 mg; $NaH_2PO_4{\cdot}2H_2O$, 650 mg; KI, 1.5 mg; $CoSo_4{\cdot}6H_2O$, 2.5 mg; $CuSo_4{\cdot}5H_2O$, 15 mg; $ZnSO_4{\cdot}7H_2O$, 350 mg; $FeSO_4{\cdot}7H_2O$, 1250 mg; $MnSO_4{\cdot}4H_2O$, 80 mg; Na_2SeO_3, 6 mg.

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

Fatty acid	Dietary inclusion of ARA level (% of diet)			
	0.00	0.50	1.00	2.00
C12:0	0.11	0.11	0.11	0.10
C14:0	5.86	5.75	5.34	4.61
C15:0	0.65	0.64	0.60	0.53
C16:0	21.64	21.28	20.51	19.02
C17:0	0.58	0.57	0.55	0.52
C18:0	4.23	4.39	4.61	4.83
C20:0	0.35	0.38	0.41	0.45
C22:0	0.13	0.27	0.49	0.85
ΣSFA	33.56	33.38	32.61	30.91
C14:1	0.21	0.21	0.19	0.15
C16:1	6.77	6.56	5.96	5.17
C17:1	0.89	0.91	0.79	0.73
C18:1n-9	15.46	14.83	14.36	13.10
C20:1	2.69	2.55	2.34	2.05
C22:1	3.21	3.02	2.72	2.30
ΣMUFA	29.25	28.07	26.36	23.50
C18:2n-6	4.03	4.29	4.78	5.29
C18:3n-6	0.32	0.49	0.67	0.94
C20:4n-6 (ARA)	1.31	3.91	7.30	13.70
C22:4n-6	0.47	0.43	0.40	0.34
Σn-6PUFA	6.13	9.12	13.14	20.27
C18:3n-3	1.11	1.09	1.04	1.09
C18:4	1.95	1.84	1.68	1.43
C20:5n-3 (EPA)	10.84	10.19	9.63	8.20
C22:6n-3 (DHA)	14.84	14.38	13.53	11.85
Σn-3PUFA	29.31	27.49	25.87	22.57
DHA/EPA	1.37	1.41	1.40	1.45
EPA/ARA	8.27	2.61	1.32	0.60
Σn-3/Σn-6	4.78	3.02	1.97	1.11

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

they were fed to the sturgeon.

2.3. Fish and feeding trial

The F_2 generation Chinese sturgeons were cultured in concrete pools (length: 14 m, width: 7 m, depth: 1 m) in a continuous groundwater flow system with continuous aeration at Taihu Experimental Station, Yangtze River Fisheries Research Institute in Jingzhou, Hubei Province. The tank volume was 98 m^3, and the density was approximately 3.27 kg/m^3. Prior to the experiment, 72 specimens (average body weight: 4.17 ± 0.22 kg) that were four years of age and had gonads in developmental stage II were randomly selected. The selection of stage II Chinese sturgeon gonads was primarily based on age and body weight, as described in Conservation Biology of Chinese Sturgeon (Wei et al., 2019). The fish were randomly placed into 12 indoor concrete pools (diameter: 3 m; depth: 0.5 m; n = 18/treatment; female: male = 1: 1).

Sex was identified using PCR amplification with a pair of female-specific primers (unpublished) in our laboratory, the amplified band was shown in Supplementary 1. During the feed trial, the water flow rate was approximately 2 L/min, the water temperature was 19.65 ± 0.95 °C, the dissolved oxygen content of the water was 6.83 ± 0.54 mg/L, the water pH was 8.02 ± 0.06, the NH_4-N concentration of the water was 0.33 ± 0.19 mg/L, the iron concentration of the water was 0.06 ± 0.01 mg/L and the turbidity was 1.26 ± 0.41 NTU. The experimental fish were hand-fed twice daily (8:00 and 20:00) for 22 weeks. The feeding rate was approximately 1% of the fish body weight and adjusted according to the status of the fish and weather conditions.

2.4. Sample collection and analysis

At the termination of the feeding trial, 6 female fish from each ARA treatment group were randomly selected and anaesthetized with MS-222 (GREENHX Biological Technology Co, Ltd., Beijing, China). The fish body weight and body length were measured to calculate the CF. The following parameters were measured at the end of 22 weeks using the following equations:

Weight gain rate (WGR, %) = 100 × (final weight (kg) - initial weight (kg)/ initial weight (kg).

Feed conversion ratio (FCR) = dry feed intake/wet weight gain.

Hepatosomatic index (HSI, 100) = 100 × liver weight (g)/body weight (g).

Viscerosomatic index (VSI, 100) = 100 × viscera weight (g)/body weight (g).

Condition factor (CF, 100) = 100 × body weight (g)/body length^3 (cm).

Survival rate (SR, 100) = 100 × final survival number/initial survival number.

Gonadalsomatic index (GSI, 100) = 100 × ovary weight (g)/body weight (g).

Blood samples were obtained from the caudal vein using a 5-mL syringe, and the fish caudal veins were excised without contaminating the internal organs with blood. The blood samples were placed in a 4 °C refrigerator overnight, and centrifuged (3000 *g*/min 4 °C, 10 min) to collect serum samples for the detection of serum sex hormones. The serum sex hormones E_2 and T were determined using an automatic biochemical analyzer (REMISOL_DX1800) with microparticle chemiluminescence. The visceral organs were extracted, and the liver and ovary tissues were separated and weighed to calculate the HSI, VSI and GSI. Liver, muscle and ovary samples were collected and cryopreserved at −80 °C for fatty acid composition, sex steroid, PGE_2, 5-HETE, Vtg and gene expression analyses. Ovarian samples were fixed in a 4% paraformaldehyde solution for 24 h, then transferred to 70 % ethanol for histological section analysis. Hematoxylin-eosin was used to stain ovarian tissues. Ovarian tissues were dehydrated and embedded in paraffin to obtain sections with a thickness of 3−4 μm. Images were captured using a Nikon Eclipse E100 (Tokyo, Japan) microscope equipped with a Nikon DS-U3 (Tokyo, Japan) imaging system. Six images were taken from each experimental fish in two visual fields. Three of these images were taken at a magnification of ×200, and the other images were taken at a magnification of ×400.

The PGE_2, 5-HETE, E_2, T, and Vtg contents in the different tissues were detected using enzyme-linked immunosorbent assay (ELISA) test kits (Meimian Biotech, Jiangsu, China) according to the manufacturer's instructions. The weighed samples were homogenized in a PBS solution (pH 7.2–7.4). Grinding was performed using an automatic sample rapid grinding machine (JxFSTPRP-24). The diameter of the grinding ball was 0.2 mm (2 pieces) and 1 mm (1 piece). The homogenization frequency was 90 Hz, and the time was 90 s. The supernatants were collected after centrifugation for 15 min at 1000 *g*/min and added to the testing sample wells for further reactions according to the instructions in the operating manual. The absorbance at 450 nm was detected using a microplate reader (Rayto RT-6100, Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China), and the detailed methods of estrogen determination are shown in the supplementary 2.

The moisture contents of the liver, muscle and ovary and of the diets were analyzed using vacuum freeze-drying and oven drying at 105 °C for 3 h. The crude protein (Kjeldahl method, N × 6.25) and crude lipid (following Soxhlet extraction with petroleum ether) in the diets were detected using AOAC methods (1995). The following detection methods were used for fatty acids in the diets and tissues. Approximately 0.6 g feed samples (1.9 g tissue samples) were placed in 50 ml triangular bottles, 15 ml of a mixed solvent (chloroform: methanol = 2:1) was added, and oscillation extraction and filtration were performed. The mixed solvent (10 ml) was added twice, and oscillation extraction and filtration were repeated twice. The fluids remaining on the filters were combined and dried via evaporation. A NaOH methanol solution (0.5 mol/L) was added to 2 ml of the evaporated filtrate. The mixture was held in a 60 °C water bath for 30 min then cooled. Two milliliters of a 25 % BF_3 methanol solution was added to the mixture, followed by a period of 20 min at 60 °C in a water bath and cooling. Two milliliters each of C_6H_{14} and NaCl solution were added, and oscillation extraction and static layering were performed. The upper solution was extracted for analyses using a gas chromatograph (Shimadzu GC-2030) with a capillary column (DB-WAX 30 M; ID 0.32 mm). The temperatures of the injector and detector were set at 250 °C. High- purity nitrogen was used as the carrier gas at a flow rate of 3 ml/min. The H_2 gas flow rate was 40 ml/min. The air flow rate was 400 ml/min, and the diversion ratio was 1:10. Individual methyl esters were identified via comparison to known standards. The results of identified fatty acids are presented as the area percentage of detected total fatty acids.

Total RNA was extracted using TRIzol reagent (Invitrogen) and treated with RNase-free DNase I (Fermentas). The quality of RNA was detected at 260 and 280 nm. cDNA was synthesized using oligo (dT) 18 primers, following the instructions of the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) as described previously (Luo et al., 2017). The PCR primer sequences are listed in Du et al. (2018) and Wu et al. (2020). Detailed information about the primers is provided in Table 3. Real-time PCR was performed using KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems). The reaction mixture and reaction procedure for qPCR were performed as described previously (Luo et al., 2018). The comparative CT method ($2^{-\Delta\Delta CT}$) described by Livak and Schmittgen (2001) was used to calculate the gene expression values. The result for each gene was normalized to the expression of the reference gene β-actin. The fold change in gene expression was obtained via comparison of the normalized gene expression level in a treated group with the corresponding untreated group (defined as 1).

2.5. Statistical analysis

All data are presented as the means ± SE (standard error) and were subjected to one-way ANOVA after the data normality and homogeneity were verified using IBM 22.0 (SPSS, Michigan Avenue, Chicago, IL,

Table 3
Primer used for real-time PCR analysis.

Gene	Forward primer (5-3,)	Reverse primer (5-3,)	Annealing temperature (°C)
fshr	ACCTGGCCTTTGCAGACCTGT	CTCCAGCTGCATCGCATAGGTGA	60
lhr	CGCCAGTCTGCCACTCTATTGCAT	ACATCCTGGGCAACGACTTCCT	60
hsd3b1	CCTCTGCCACTGCTGTTGTTCCT	AGCATCCTCCCAGCTGTACTTTGG	60
hsd17b1	AGTTCCATCTTCCACGGTCAGCTT	CCAGTCTGCAGCACGTCGACCC	60
cyp19a1	GATAGCAGCACCTGACACCA	GCAGTTTCCTCTCAACCTCG	57
star	GAGACCGCAGGCAACATCGT	GCCGTTCTCCGCTCTGATGA	63.2
β-actin	CCTTCTTGGGTATGGAATCTTGC	CAGAGTATTTACGCTCAGGTGGG	57

Abbreviations: *fshr*, follicle stimulating hormone receptor; *lhr*, luteinizing hormone receptor; *hsd3b1*, 3b1-hydroxysteroid dehydrogenase; *hsd17b1*, 17b1-hydroxysteroid dehydrogenase; *cyp19a1*, cytochrome p450 aromatase; *star*, steroidogenic acute regulatory protein.

USA). If the data did not exhibit homogeneity of variance, a nonparametric test (Kruskal-Wallis) was used for the comparison. When significant differences between treatments were identified, Tukey's honestly significant differences (HSD) test was used to compare the mean values, and $P < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Growth performance and morphological parameters

The different contents of dietary ARA did not affect the WGR, FCR, HSI, VSI, CF, SR and GSI of experimental fish ($P > 0.05$) (Table 4).

3.2. Fatty acid profiles in the muscle, liver and ovary

The fatty acid profiles of the muscle, liver and ovary tissues of the experimental fish are shown in Tables 5 and 6. The content of ARA (C20:4n-6) in the liver increased significantly with increasing dietary ARA ($P < 0.05$). The Σtotal saturated fatty acid (SFA), Σtotal monounsaturated fatty acid (MUFA), C20:5n-3 (EPA), C22:5n-3, C22:6n-3 (DHA), Σtotaln-3 polyunsaturated fatty acid (n-3PUFA) contents, and the ratios of n-3/n-6 and EPA/ARA in the liver decreased with increasing dietary ARA, and significant differences in these parameters were found between the 0.0 % and 2.0 % ARA diets ($P < 0.05$). However, the contents of C18:2n-6 and Σtotal n-6 polyunsaturated fatty acids (n-6 PUFAs) in the liver increased with increasing dietary ARA, and significant differences in these parameters were found between the fish fed the 0.0 % and 2.0 % ARA diets ($P < 0.05$).

The different ARA diets did not affect the contents of SFAs, C18:2n-6, C22:4n-6, C18:3n-3, C20:5n-3, C22: 5n-3, C20:6n-3 and Σn-3PUFAs in muscle ($P > 0.05$). However, the accumulation of ARA and Σn-6PUFAs in the muscle increased with increasing dietary ARA. The ARA and Σn-6PUFA contents in the muscles of the fish fed the 1.0 % and 2.0 % ARA diets were higher than the fish fed the 0.0 % and 0.5 % ARA diets ($P > 0.05$). The ΣMUFA contents and ratios of n-3/n-6 and ARA/EPA in the muscle decreased with increasing dietary ARA. The lowest ΣMUFA content and ratios of n-3/n-6 and ARA/EPA were found in the fish fed the 2.0 % ARA diet, and significant differences in these parameters were observed between fish fed the 0.0 % and 2.0 % ARA diets ($P < 0.05$).

The different ARA diets did not affect the contents of C16:0, ΣSFAs, C18:1n-9, C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3, C22:6n-3, and Σn-3PUFAs in the ovary ($P > 0.05$). The ΣMUFA content and ratios of n-3/n-6 and ARA/EPA decreased with increasing dietary ARA, and significant differences in these parameters were found between the 0.0 % and 2.0 % ARA diets ($P < 0.05$). The accumulation of ARA, C18:2n-6 and Σn-6PUFAs increased with increasing dietary ARA, and significant differences were observed in these parameters between the 0.0 % and 2.0 % ARA diets ($P < 0.05$). The content of C22:4n-6 was the lowest in the ovary of the fish fed the 2.0 % ARA diet, and significant differences in ovary C22:4n-6 content were observed between fish fed the 0.5 % and 2.0 % ARA diets.

3.3. ARA-related metabolites in the liver, muscle and ovary

The contents of ARA-related metabolites in the liver, muscle and ovary tissues of the experimental fish are shown in Table 7. The PGE_2 and 5-HETE contents in the ovary and liver reached their highest levels in fish fed the 2.0 % ARA diet and were significantly higher than fish fed the 0.0 % and 0.5 % ARA diets ($P < 0.05$).

The trends of these two metabolites in the muscle were not consistent with the liver and ovary. The contents of the two metabolites in muscle first increased then decreased at 2 % ARA dietary inclusion. The contents of PGE_2 and 5-HETE in the muscles of fish fed the 1.0 % ARA diet were significantly higher than those in the muscles of fish fed the 0.0 % and 2.0 % ARA diets ($P < 0.05$).

3.4. Tissue hormones and Vtg contents

The impact of dietary ARA on the tissue hormone and Vtg contents of the experimental fish are shown in Table 8. The E_2, T and Vtg levels in the ovaries of the fish fed the 2.0 % ARA diet were higher than the ovaries of the fish fed the 0.0 % ARA diet ($P < 0.05$). There were no

Table 4
Effects of dietary ARA on growth performance and morphological parameters on F_2 generation Chinese sturgeon.

Index	Dietary inclusion of ARA level (% of diet)				ANOVA (*P*)
	0.00	0.50	1.00	2.00	
[a]Initial weight (kg)	4.10 ± 0.15	4.31 ± 0.08	4.21 ± 0.17	4.06 ± 0.10	0.570
[a]Final weight (kg)	6.37 ± 0.36	6.61 ± 0.35	5.84 ± 0.26	6.12 ± 0.10	0.331
[a]WGR (%)	57.57 ± 3.11	53.95 ± 5.79	39.38 ± 2.03	51.59 ± 4.45	0.059
[a]FCR	2.01 ± 0.21	2.05 ± 0.21	2.69 ± 0.08	2.12 ± 0.14	0.068
[b]HSI (%)	1.41 ± 0.09	1.51 ± 0.13	1.73 ± 0.15	1.62 ± 0.19	0.447
[b]VSI (%)	4.67 ± 0.15	4.60 ± 0.32	5.31 ± 0.54	4.09 ± 0.47	0.231
[b]CF (%)	0.88 ± 0.04	0.87 ± 0.02	0.80 ± 0.03	0.87 ± 0.04	0.289
[a]SR (%)	100.00 ± 0.00	100.00 ± 0.00	94.44 ± 5.56	100.00 ± 0.00	0.441
[b]GSI (%)	0.13 ± 0.00	0.09 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	0.053

Data are presented as means ± SE (n = 6), data in the same line with different superscript letters indicate a statistical significance ($P < 0.05$).
[a] Indicated that the data were obtained from the females and males fishes.
[b] Indicated that the data were obtained from the female fishes.

Table 5
Effects of dietary ARA on liver and muscle main fatty acid composition (% total fatty acids) of F_2 generation female Chinese sturgeon*.

Fatty acid	Dietary inclusion of ARA level (% of diet)							
	0.00 Liver	0.50	1.00	2.00	ANOVA (*P*)	0.00 Muscle	0.50	1.00	2.00	ANOVA (*P*)
C16:0	16.50 ± 0.52^{a}	16.74 ± 0.35^{a}	15.21 ± 0.22^{ab}	13.75 ± 0.15^{b}	0.001	21.65 ± 1.40^{a}	21.66 ± 0.72^{a}	18.84 ± 0.36^{a}	18.10 ± 0.25^{a}	0.059
∑SFA	25.82 ± 0.26^{a}	26.40 ± 0.48^{a}	24.39 ± 0.51^{ab}	23.59 ± 0.52^{b}	0.009	31.59 ± 2.07^{a}	31.67 ± 0.97^{a}	27.85 ± 0.68^{a}	27.28 ± 0.14^{a}	0.055
C18:1n-9	27.28 ± 1.03^{a}	25.35 ± 0.60^{ab}	25.38 ± 0.36^{ab}	23.45 ± 0.13^{b}	0.017	24.50 ± 0.90^{a}	22.05 ± 0.72^{ab}	20.90 ± 0.48^{bc}	19.08 ± 0.14^{c}	0.002
∑MUFA	40.86 ± 1.02^{a}	37.63 ± 0.79^{b}	36.49 ± 0.44^{b}	33.06 ± 0.02^{c}	0.000	34.79 ± 1.07^{a}	30.12 ± 1.42^{b}	28.61 ± 0.50^{b}	26.43 ± 0.50^{b}	0.002
C18:2n-6	5.51 ± 0.07^{ab}	5.23 ± 0.22^{a}	6.05 ± 0.22^{bc}	6.61 ± 0.10^{c}	0.002	5.24 ± 0.32^{a}	4.95 ± 0.52^{a}	5.98 ± 1.28^{a}	5.13 ± 0.14^{a}	0.754
C20:4n-6 (ARA)	1.05 ± 0.03^{a}	4.01 ± 0.13^{b}	7.28 ± 0.12^{c}	13.08 ± 0.10^{d}	0.000	2.22 ± 0.12^{a}	5.76 ± 0.60^{b}	9.33 ± 0.40^{c}	14.41 ± 0.23^{d}	0.000
C22:4n-6	0.56 ± 0.02^{a}	0.55 ± 0.02^{a}	0.56 ± 0.02^{a}	0.53 ± 0.04^{a}	0.855	0.41 ± 0.04^{a}	0.42 ± 0.02^{a}	0.49 ± 0.00^{a}	0.43 ± 0.02^{a}	0.206
∑n-6PUFA	7.12 ± 0.06^{a}	9.79 ± 0.31^{ab}	13.89 ± 0.15^{bc}	20.23 ± 0.13^{c}	0.000	7.87 ± 0.41^{a}	11.13 ± 1.06^{a}	15.80 ± 0.91^{b}	19.97 ± 0.38^{c}	0.000
C18:3n-3	1.28 ± 0.03^{a}	1.17 ± 0.01^{b}	1.17 ± 0.03^{ab}	1.14 ± 0.01^{b}	0.016	0.79 ± 0.09^{a}	0.64 ± 0.02^{a}	0.78 ± 0.07^{a}	0.75 ± 0.01^{a}	0.356
C22:5n-3	3.51 ± 0.08^{a}	3.51 ± 0.15^{a}	3.32 ± 0.08^{ab}	2.76 ± 0.18^{b}	0.009	2.02 ± 0.26^{a}	1.97 ± 0.02^{a}	1.99 ± 0.06^{a}	1.90 ± 0.05^{a}	0.934
C20:5n-3 (EPA)	7.67 ± 0.38^{a}	7.32 ± 0.20^{ab}	6.26 ± 0.19^{bc}	4.92 ± 0.40^{c}	0.001	8.36 ± 0.99^{a}	8.56 ± 0.54^{a}	7.37 ± 0.07^{a}	6.07 ± 0.19^{a}	0.054
C22:6n-3 (DHA)	12.86 ± 0.52^{a}	12.56 ± 0.04^{a}	11.88 ± 0.20^{ab}	10.02 ± 0.25^{b}	0.001	13.88 ± 1.29^{a}	14.77 ± 0.78^{a}	15.78 ± 0.63^{a}	14.82 ± 0.50^{a}	0.512
∑n-3PUFA	25.32 ± 0.92^{a}	24.56 ± 0.33^{a}	22.63 ± 0.30^{a}	18.85 ± 0.74^{b}	0.000	25.05 ± 2.61^{a}	25.94 ± 1.27^{a}	25.92 ± 0.65^{a}	23.55 ± 0.32^{a}	0.654
∑n-3/∑n-6	3.56 ± 0.15^{a}	2.51 ± 0.10^{ab}	1.63 ± 0.02^{bcd}	0.93 ± 0.04^{c}	0.000	3.16 ± 0.18^{a}	2.36 ± 0.13^{b}	1.66 ± 0.13^{c}	1.18 ± 0.01^{c}	0.000
EPA/ARA	7.31 ± 0.29^{a}	1.83 ± 0.11^{b}	0.86 ± 0.03^{c}	0.37 ± 0.03^{c}	0.000	3.75 ± 0.39^{a}	1.50 ± 0.07^{b}	0.79 ± 0.03^{bc}	0.42 ± 0.02^{c}	0.000
DHA/EPA	1.68 ± 0.02^{a}	1.72 ± 0.05^{ab}	1.90 ± 0.08^{ab}	2.06 ± 0.14^{b}	0.039	1.67 ± 0.05^{a}	1.73 ± 0.03^{a}	2.14 ± 0.07^{b}	2.45 ± 0.15^{b}	0.001

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
* Data are presented as means ± SE (n = 3), data in the same line with different superscript letters indicate a statistical significance ($P < 0.05$).

significant differences in ovary E_2, T and Vtg levels in the fish fed the 1.0 % and 2.0 % ARA diets ($P > 0.05$).

The E_2 and T contents in the liver showed similar trends. The highest levels of E_2 and T were found in the livers of fish fed the 1.0 % and 2.0 % ARA diets and were significantly higher than fish fed the 0.0 % and 0.5 % ARA diets ($P < 0.05$). The Vtg levels in the livers of fish fed the 1.0 % ARA diet were higher than the fish fed the 0.0 % ARA diet ($P < 0.05$), and no significant differences were observed between the fish fed the 1.0 % and 2.0 % ARA diets ($P > 0.05$).

The different dietary ARA contents did not affect serum T levels ($P > 0.05$). The serum E_2 levels in fish fed the 1.0 % ARA diet were significantly higher the 0.0 % ARA diet ($P < 0.05$), and no significant differences in E_2 levels were observed between the fish fed the diets supplemented with 1% and 2% ARA ($P > 0.05$). The Vtg levels of the fish fed the 1.0 % ARA diet were higher than the fish fed the 0.0 % and 0.5 % ARA diets ($P < 0.05$), and no significant differences were observed between the fish fed the 1.0 % and 2.0 % ARA diets ($P > 0.05$).

3.5. Ovary steroidogenesis-related gene expression

Fig. 1 shows the effects of dietary ARA levels on the mRNA expression of ovary steroidogenesis-related genes. The expression of the luteinizing hormone receptor (*lhr*) gene in the ovaries of fish fed the 1.0 % and 2.0 % ARA diets was higher than the fish fed the 0.0 % ARA diet ($P < 0.05$), and no significant differences in *lhr* expression were observed between the fish fed the 0.0 % and 0.5 % ARA diets ($P > 0.05$). There were no significant differences in the follicle stimulating hormone receptor (*fshr*), 3b1-hydroxysteroid dehydrogenase (*hsd3b1*), 17b1-hydroxysteroid dehydrogenase (*hsd17b1*), steroidogenic acute regulatory protein (*star*), or cytochrome p450 aromatase (*cyp19a1*) mRNA expression levels between the fish fed the 0.0 % ARA diet and the fish fed the ARA-supplemented diets ($P > 0.05$).

3.6. Histology of ovaries

As shown in Fig. 2, the histology of Chinese sturgeon ovaries in our experiment was performed at stage II, and it was characterized by the appearance of primary oocytes, but cortical alveoli and many lipid droplets did not appear in the cytoplasm.

4. Discussion

The roles of ARA in growth performance, spawning performance, egg quality, offspring quality and oocyte maturation are well demonstrated in marine species (Xu et al., 2010; Luo et al., 2012; Stuart et al., 2018; Torrecillas et al., 2018; Nhan et al., 2020). However, there are relatively few studies on freshwater species, likely because it is assumed that the essential fatty acid requirements of freshwater fish may be met by providing alpha linolenic acid and/or linoleic acid (Bell and Sargent, 2003), and their ARA requirement, if any, is very low and is met by a small amount. Fortunately, some studies also showed that dietary ARA supplementation improved the growth performance, reproductive performance and physiological characteristics of freshwater species, such as female rice field eel (*Monopterus albus*) (Zhou et al., 2011), grass carp (*Ctenopharyngodon idellus*) (Ji et al., 2011), gibel carp (*Carassius auratus gibelio*) (Chen et al., 2011), blue gourami (*Trichopodus trichopterus*; Pallas, 1770) (Asil et al., 2017), juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Ma et al., 2017) and female yellow catfish (Fei et al., 2020). The results of the present study showed that dietary ARA levels did not affect the growth performance and morphological parameters of the experimental fish at the end of the experimental period. Similar results were observed in grass carp (Tian et al., 2014), blue gourami (Asil et al., 2017) and malabar red snapper (*Lutjanus malabaricus*) fingerlings (Chee et al., 2019). Notably, several studies showed that dietary ARA supplementation had positive effects on the growth of juvenile Japanese seabass (*Lateolabrax japonicus*) (Xu et al., 2010), juvenile goby

Table 6
Effects of dietary ARA on ovary fatty acid composition (% total fatty acids) of F_2 generation Chinese sturgeon*.

Fatty acid	Dietary inclusion of ARA level (% of diet)				ANOVA (*P*)
	0.00	0.50	1.00	2.00	
C16:0	19.67 ± 0.39^{a}	19.17 ± 0.63^{a}	19.00 ± 0.75^{a}	17.32 ± 0.23^{a}	0.066
∑SFA	28.52 ± 0.55^{a}	29.02 ± 0.28^{a}	27.83 ± 1.25^{a}	25.90 ± 0.53^{a}	0.074
C18:1n-9	25.07 ± 0.73^{a}	23.38 ± 1.46^{a}	24.98 ± 1.16^{a}	20.59 ± 0.64^{a}	0.053
∑MUFA	39.06 ± 1.12^{a}	34.70 ± 0.89^{ab}	35.94 ± 1.25^{a}	30.77 ± 0.83^{b}	0.003
C18:2n-6	4.82 ± 0.04^{a}	4.51 ± 0.12^{a}	5.65 ± 0.28^{b}	5.69 ± 0.19^{b}	0.003
C20:4n-6 (ARA)	1.53 ± 0.06^{a}	6.41 ± 2.24^{ab}	8.31 ± 0.34^{bc}	13.16 ± 0.28^{bc}	0.001
C22:4n-6	0.45 ± 0.02^{ab}	0.49 ± 0.01^{a}	0.43 ± 0.03^{ab}	0.39 ± 0.01^{b}	0.036
∑n-6PUFA	6.79 ± 0.10^{a}	11.42 ± 2.21^{ab}	14.39 ± 0.52^{bc}	19.23 ± 0.22^{c}	0.000
C18:3n-3	0.93 ± 0.04^{a}	0.81 ± 0.10^{a}	0.79 ± 0.07^{a}	0.93 ± 0.02^{a}	0.363
C22:5n-3	2.28 ± 0.12^{a}	2.07 ± 0.21^{a}	1.71 ± 0.18^{a}	1.88 ± 0.12^{a}	0.149
C20:5n-3 (EPA)	8.14 ± 0.49^{a}	7.34 ± 1.03^{a}	5.70 ± 0.67^{a}	6.23 ± 0.34^{a}	0.128
C22:6n-3 (DHA)	12.37 ± 0.79^{a}	12.42 ± 0.76^{a}	10.92 ± 1.19^{a}	11.00 ± 0.59^{a}	0.474
∑n-3PUFA	23.71 ± 1.32^{a}	22.63 ± 2.02^{a}	19.13 ± 2.09^{a}	20.04 ± 1.04^{a}	0.256
∑n-3/∑n-6	3.49 ± 0.14^{a}	2.18 ± 0.53^{b}	1.33 ± 0.14^{b}	1.04 ± 0.05^{b}	0.001
DHA/EPA	1.52 ± 0.07^{a}	1.73 ± 0.15^{a}	1.92 ± 0.06^{a}	1.77 ± 0.01^{a}	0.079
EPA/ARA	5.33 ± 0.18^{a}	1.51 ± 0.56^{b}	0.69 ± 0.10^{b}	0.47 ± 0.02^{b}	0.000

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
* Data are presented as means ± SE (n = 3), data in the same line with different superscript letters indicate a statistical significance ($P < 0.05$).

Table 7
Effects of dietary ARA on the main metabolites of ovary, liver and muscle in the F_2 generation female Chinese sturgeon*.

Index	Dietary inclusion of ARA level (% of diet)				ANOVA (*P*)
	0.00	0.50	1.00	2.00	
Ovary					
PGE_2 (ng/g)	5.10 ± 0.19^{a}	5.92 ± 0.48^{ab}	7.14 ± 0.38^{bc}	8.34 ± 0.34^{c}	0.000
5-HETE (ng/g)	4.88 ± 0.35^{a}	6.07 ± 0.32^{ab}	6.36 ± 0.39^{b}	8.01 ± 0.28^{c}	0.000
Liver					
PGE_2 (ng/g)	3.23 ± 0.34^{a}	3.51 ± 0.39^{ab}	4.93 ± 0.50^{bc}	5.47 ± 0.31^{c}	0.001
5-HETE (ng/g)	3.11 ± 0.36^{a}	3.99 ± 0.30^{ab}	5.43 ± 0.36^{c}	5.14 ± 0.32^{bc}	0.000
Muscle					
PGE_2 (ng/g)	4.11 ± 0.24^{a}	5.23 ± 0.30^{a}	6.71 ± 0.42^{b}	4.41 ± 0.42^{a}	0.000
5-HETE (ng/g)	3.62 ± 0.23^{a}	4.57 ± 0.44^{ab}	5.28 ± 0.22^{b}	3.88 ± 0.31^{a}	0.005

Abbreviations: prostaglandins, PGE_2; 5-hydroxyeicosatetraenoic acid, 5-HETE.
* Data are presented as means ± SE (n = 6), data in the same line with different superscript letters indicate a statistical significance ($P < 0.05$).

Table 8
Effects of dietary ARA on sex steroid hormones and Vtg contents of F_2 generation female Chinese sturgeon*.

Index	Dietary inclusion of ARA level (% of diet)				ANOVA (*P*)
	0.00	0.50	1.00	2.00	
Ovary					
E_2 (pmol/g)	0.35 ± 0.01^{a}	0.45 ± 0.02^{b}	0.50 ± 0.02^{b}	0.51 ± 0.03^{b}	0.000
T (ng/g)	2.01 ± 0.08^{a}	2.47 ± 0.10^{ab}	2.49 ± 0.17^{ab}	2.70 ± 0.14^{b}	0.010
Vtg (ug/g)	2.11 ± 0.24^{a}	2.40 ± 0.11^{ab}	2.85 ± 0.15^{bc}	3.51 ± 0.14^{c}	0.000
Liver					
E_2 (pmol/g)	0.26 ± 0.02^{a}	0.31 ± 0.02^{a}	0.41 ± 0.03^{b}	0.49 ± 0.01^{b}	0.000
T (ng/g)	1.48 ± 0.14^{a}	1.49 ± 0.13^{a}	2.05 ± 0.10^{b}	2.28 ± 0.14^{b}	0.000
Vtg (ug/g)	1.37 ± 0.20^{a}	1.73 ± 0.19^{ab}	2.33 ± 0.21^{bc}	2.56 ± 0.11^{c}	0.001
Serum					
E_2 (pg/mL)	17.33 ± 3.11^{a}	27.83 ± 7.13^{ab}	39.17 ± 6.30^{b}	24.00 ± 2.19^{ab}	0.046
T (ng/mL)	0.53 ± 0.09^{a}	0.49 ± 0.04^{a}	0.29 ± 0.04^{a}	0.54 ± 0.09^{a}	0.087
Vtg (ng/mL)	231.24 ± 13.61^{a}	310.06 ± 19.75^{b}	392.81 ± 19.03^{c}	344.41 ± 10.87^{bc}	0.000

Abbreviations: estradiol, E_2; testosterone, T; vitellogen, Vtg.
* Data are presented as means ± SE (n = 6), data in the same line with different superscript letters indicate a statistical significance ($P < 0.05$).

(*Synechogobius hasta*) (Luo et al., 2012), juvenile European sea bass (*Dicentrarchus labrax*) (Torrecillas et al., 2018) and juvenile yellow catfish (Ma et al., 2017). However, it is strange that dietary ARA supplementation had negative effects on the growth of larval atlantic cod (*Gadus morhua*) (Bransden et al., 2005), juvenile pacific white shrimp (*Litopenaeus vannamei*) (Araújo et al., 2020) and black sea urchin (*Diadema setosum,* Leske, 1778) (Nhan et al., 2020). All of these results indicate that the effects of dietary ARA on different aquatic animals are

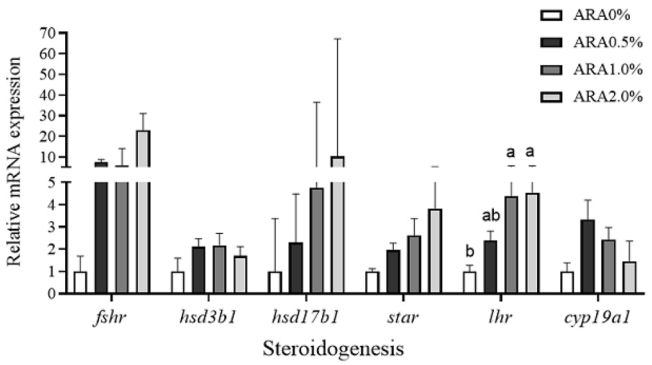

Fig. 1. Effects of dietary ARA on ovary steroidogenesis-related gene expression in F_2 generation Chinese sturgeon. Values are expressed as means ± SE (n = 6). Different letters indicate significant differences ($P < 0.05$).
Abbreviations: *fshr*, follicle stimulating hormone receptor; *lhr*, luteinizing hormone receptor; *hsd3b1*, 3b1-hydroxysteroid dehydrogenase; *hsd17b1*, 17b1-hydroxysteroid dehydrogenase; *cyp19a1*, cytochrome p450 aromatase; *star*, steroidogenic acute regulatory protein.

not consistent, and the dietary ARA requirements of specific species should be evaluated individually, without reference to other species. These discrepancies in ARA requirements are related to many factors, such as genetic lineage, species-specific factors, life phases, culture systems and conditions, and especially diet composition. Ratios of LC-PUFAs (primarily ARA, EPA and DHA and different fatty acid series (primarily n-3/n-6) directly influence metabolic processes and consequently growth performance (Bell and Sargent, 2003; González-Félix et al., 2003; Glencross, 2009; Araújo et al., 2020).

The liver, muscle, and gonadal adipose tissues of sturgeons accumulate lipids that play important facilitative roles in the onset of puberty, when energy is diverted from somatic growth to gonadal development (Webb and Doroshov, 2011). C16:0 was the most abundant SFA in the three tissues in the present study, which was similar to other sturgeons (Caprino et al., 2008). Li et al. (2014) reported that the higher amount of C16:0 in wild eggs than in cultured eggs might be used to compensate for the low content of other SFAs, which may indicate that C16:0 plays an important role as a compensatory fatty acid in Chinese sturgeon. Similarly, 18:1n-9 was the most dominant MUFA in the three tissues, which is consistent with Song et al. (2014) and Zhou et al. (2017). Notably, Liu et al. (2014) found that the contents of C18:1n-9 were higher in wild eggs than cultivated eggs, which may be explained by the fact that fish tissues are rich in C18:1n-9, which plays a vital role in supplying energy for fish activities (Caprino et al., 2008). We also found that the SFA contents in tissue were lower than the contents in the diets, but that the MUFA contents showed the opposite trend. Yuan et al. (2015) found similar results. This finding may be related to the excessive level of SFAs provided in the diets for the experimental fish, and the fact that SFAs may have been metabolized preferentially. Some of the SFAs may have been desaturated and elongated to form MUFAs (Yuan et al., 2015). Dietary ARA was notably incorporated into the muscle, liver and ovary tissues in our study, and similar results were also found in Japanese seabass (Xu et al., 2010), grass carp (Tian et al., 2017), malabar red snapper (Chee et al., 2019) and juvenile pacific white shrimp (Araújo et al., 2020). However, the contents of ARA in the female gonads were lower than the male gonads in cultured Chinese sturgeon with gonadal development at stage II (Zhou et al., 2017). This result may indicate insufficient deposition of ARA in the gonads of female Chinese sturgeon, which also further suggests that ARA inclusion in the diet is necessary. Wirth et al. (2002) reported that sturgeon could not convert linoleic acid into ARA, which is consistent with our study. Certainly, it could not be excluded that the synthetic substrate was insufficient or the synthetic ability of the female Chinese sturgeon was weak. Asil et al. (2017) also recently challenged the generally accepted hypothesis that freshwater fish met their HUFA requirements via the elongation and desaturation of precursors and suggested that blue gourami broodstock need to receive at least 1% ARA in their maturation diet to improve reproduction. With respect to the accumulation of other HUFAs in tissues, the EPA and DHA

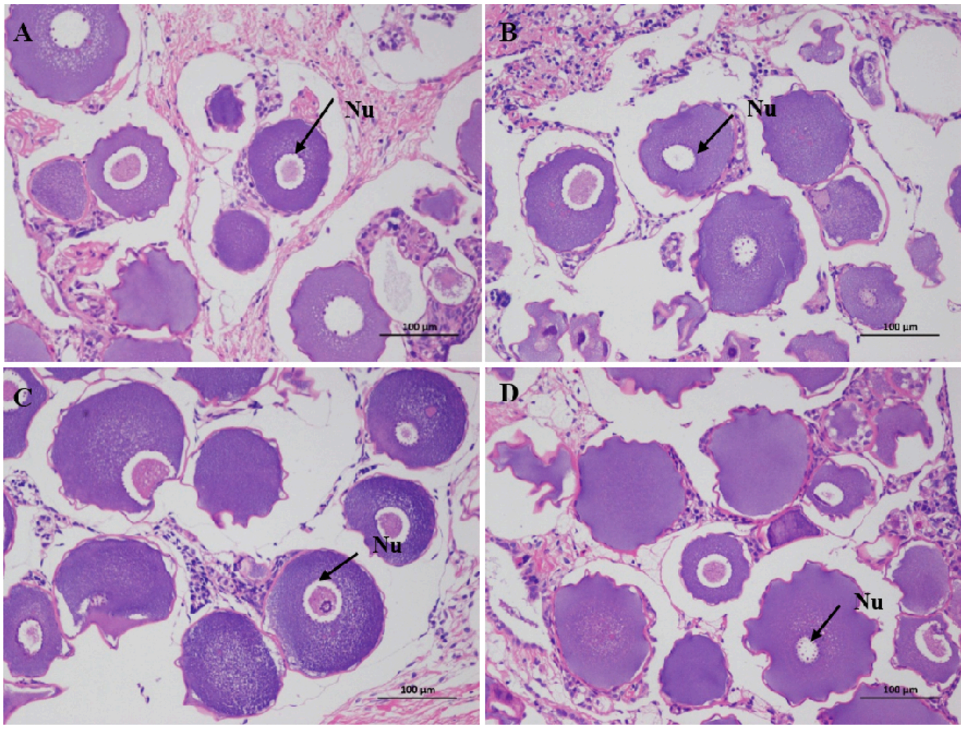

Fig. 2. Histology of F_2 generation Chinese sturgeon ovaries at stage II (H&E, original magnification×200, bars 100 μm).
Abbreviations: nucleus (Nu).
A: 0.0 % ARA levels; B: 0.5 % ARA levels; C: 1.0 % ARA levels; D: 2.0 % ARA levels.

concentrations in muscle were relatively high and less sensitive to dietary ARA changes, which is consistent with Xu et al. (2017). The ratio of DHA to EPA in the liver and muscle tissues tested in this study increased with increasing dietary ARA, and the changing trend of the ratio of EPA to ARA in the tissue was consistent with the diets because F_2 generation Chinese sturgeon are seemingly able to regulate the ratios of DHA to EPA and EPA to ARA in their bodies.

The trend of eicosanoid contents in the ovary and liver in our study was similar to the trend of ARA accumulation in the different tissues, and this result may have been due to the increased availability of substrates for ARA metabolism (Tian et al., 2017). Furne et al. (2013) and Tian et al. (2017) also found that dietary ARA increased the eicosanoid contents in tissues. ARA is metabolized to form prostaglandins, which are involved in steroid production and follicle maturation in fish (Norambuena et al., 2013). The results of this study indicated that dietary supplementation with ARA had a positive effect on the production of eicosanoids in endangered female Chinese sturgeon and suggest that appropriate dietary supplementation with ARA is necessary. Notably, the eicosanoid contents in the muscle tended to increase initially then decrease with increasing dietary ARA levels, which suggests that dietary ARA levels greater than 1 % have an inhibitory effect on eicosanoid production. This effect deserves further study. Notably, Norambuena et al. (2013) reported no obvious difference in the level of PGE_2 in Senegalese sole (*Solea senegalensis*) fed different diets with different levels of ARA.

The levels of E_2, T and Vtg in the liver and ovary of experimental fish fed the 2.0 % ARA diet were significantly higher than those in fish fed the 0.0 % ARA diet. The E_2 and Vtg levels in the serum of fish fed the 1.0 % ARA diet were significantly higher than the fish fed the 0.0 % ARA diet. All of these results revealed that dietary supplementation with 1.0 % ARA promoted the synthesis of sex steroid hormones and Vtg, and these results are consistent with Fei et al. (2020). However, these results are inconsistent with Norambuena et al. (2013) who found that the contents of E_2 in females did not change with changing concentrations of ARA. Similarly, Xu et al. (2017) reported that dietary ARA reduced serum E_2 contents in immature and mature female tongue sole (*Cynoglossus semilaevis*). In vitro studies also obtained inconsistent results (Van Der Kraak and Chang, 1990; Mercure and Van Der Kraak, 1995). All of these results indicate that ARA plays a vital role in regulating sex steroid hormones in fish species but its role varies depending on the fish gender and developmental stage.

The conversion of cholesterol to estrogen in follicles is a complex process that is regulated primarily by the steroid metabolic pathway involving *star*, *lhr*, *cyp17a1*, *cyp19a1* and *ar* (Flück and Pandey, 2017; Du et al., 2018). The steroidogenesis-related gene mRNA expression of *lhr* and *star* tended to increase with the increase in the dietary ARA level in the study. This finding is consistent with Fei et al. (2020). However, these results were not similar to Xu et al. (2017) who reported that dietary supplementation with lower ARA levels stimulated the expression of *star* in immature ovaries, and serum E_2 production did not increase in ARA-supplemented diets. The mRNA expression of the *cyp19a1* gene tended to increase first then decrease with increasing dietary ARA levels, which is inconsistent with Xu et al. (2017) and Fei et al. (2020). *Hsd17b1* and *hsd3b1* are important steroidogenic enzymes involved in the biosynthesis of estrogen. Normal follicular development at certain physiological *fsh* concentrations may depend on androgen-stimulated increases in *fshr* (Luo and Wiltbank, 2006). No significant differences were observed between the groups related to the gene expression of *hsd3b1* and *hsd17b1*, which is consistent with Fei et al. (2020). Notably, Xu et al. (2017) found differential regulation of the above genes in mature ovaries and immature ovaries. Although these results differed between the different fish species studied, they suggest that dietary supplementation with ARA promotes or inhibits the expression of some steroidogenesis-related genes related to the feedback regulation of ARA effects by steroid hormones and/or gonadotropin (Xu et al., 2017; Fei et al., 2020). These results were also confirmed by in vivo and in vitro experiments (Mercure and Van Der Kraak, 1995; Xu et al., 2017).

5. Conclusions

In conclusion, the present study suggests that ARA incorporation levels greater than 1 % in the diets plays vital roles in the ARA deposition, metabolism, steroid regulation and Vtg synthesis in F_2 generation female Chinese sturgeon. The best efficiency was achieved 1 % ARA, based on roles or cost effective, which were beneficial to the promote ovary development.

Authors statement

All authors declare that this paper has not been submitted to any other journal for publication. The authors can confirm that the study has no actual or potential conflicts of interest to report. All authors have read and agree to the contents of the manuscript, and consent to its publication. To conform to the high publication standards of your journal, a professional language editing service that specializes in scientific manuscripts has already edited the attached paper.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2021.100818.

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