Effect of Arachidonic Acid on Lipid Metabolism Gene Expression in Hepatopancreas Cells of Macrobrachium nipponense: Postprint

Authors: Ding Zhili, Cao Fang, Luo Na, Kong Youqin, Zhang Yixiang, Li Jingfen, Ye Jinyun

Date: 2017-10-11T00:00:00+00:00

Abstract

This study aimed to evaluate the effects of arachidonic acid (ARA) concentration in cell culture medium on the viability and lipid metabolism-related gene expression in hepatopancreas cells of Macrobrachium nipponense. Hepatopancreas cells were isolated from M. nipponense and cultured in M199 complete medium for 5 days before being switched to ARA-containing medium with ARA concentrations of 0 (ARA1), 50 (ARA2), 100 (ARA3), 200 (ARA4), and 1,000 mol/L (ARA5). The expression levels of lipid metabolism-related genes were measured at 12 and 24 h, and cell viability was assessed at 24 h. The results showed that primary hepatopancreas cells exhibited good growth and could survive for approximately 15 days when cultured in complete medium; cell viability in the ARA5 group was significantly lower than that in the ARA1 and ARA2 groups at 24 h ($P<0.05$); high ARA concentration reduced the expression levels of Δ4 desaturase (Δ4 FAD), Δ6 desaturase (Δ6 FAD), elongase 6 (Elovl6), class B type I scavenger receptor (SR-BI), fatty acid binding protein 10 (FABP10), and acyl-CoA binding protein (ACBP) genes at 12 and 24 h; at 12 h of ARA treatment, the SR-BI gene expression level in the ARA2 group was significantly higher than that in all other groups ($P<0.05$), the FABP10 gene expression levels in the ARA2 and ARA3 groups were significantly higher than those in the ARA1 and ARA5 groups ($P<0.05$), and the ACBP gene expression level in the ARA3 group was significantly higher than that in all other groups ($P<0.05$); at 24 h of ARA treatment, the SR-BI, FABP10, and ACBP gene expression levels in the ARA2 group were significantly higher than those in all other groups ($P<0.05$). These results indicate that ARA concentration in cell culture medium affects the viability and lipid metabolism-related gene expression in hepatopancreas cells of M. nipponense; excessively high ARA concentration (1,000 mol/L) reduces cell viability, while appropriate ARA concentration (50–100 mol/L) can promote the expression of fatty acid desaturase,

elongase, and fatty acid transport-related genes.

Full Text

Effects of Arachidonic Acid on Lipid Metabolism-Related Gene Expression in Hepatopancreas Cells Isolated from Juvenile Oriental River Prawn, *Macrobrachium nipponense*

DING Zhili[1], CAO Fang[1], LUO Na[2], KONG Youqin[1], ZHANG Yixiang[1], LI Jingfen[1], YE Jinyun[1]*

[1]Zhejiang Provincial Key Laboratory of Aquatic Resources Conservation and Development, Key Laboratory of Aquatic Animal Genetic Breeding and Nutrition, Chinese Academy of Fishery Sciences, College of Life Sciences, Huzhou University, Huzhou 313000, China
[2]College of Fisheries and Life Science, Dalian Ocean University, Dalian 116000, China

Abstract: This experiment was conducted to determine the effects of arachidonic acid (ARA) concentration in culture medium on cell viability and lipid metabolism-related gene expressions of hepatopancreas cells isolated from juvenile oriental river prawn, *Macrobrachium nipponense*. The hepatopancreas cells were dissected from prawns and cultured with complete culture medium for 5 days. After that, cultured cells were incubated in medium supplemented with graded levels [0 (ARA1), 50 (ARA2), 100 (ARA3), 200 (ARA4) and 1 000 mol/L (ARA5)] of ARA. Cell viability at 24 h and gene expressions of lipid metabolism-related genes at 12 and 24 h were examined. The results showed as follows: the hepatopancreas cells showed well growth in complete culture medium, and could survive for 15 days; cell viability was significantly decreased by incubation with higher level (1 000 mol/L) of ARA (ARA5 group) compared with ARA1 and ARA2 groups ($P<0.05$) after 24 h; higher level (1 000 mol/L) of ARA (ARA5 group) caused significant decreases of gene expressions of delta-4 fatty acyl desaturase (Δ4 FAD), delta-6 fatty acyl desaturase (Δ6 FAD), very-long-chain fatty acids-6 (Elovl6), scavenger receptor class B type (SR-B), fatty acid-binding protein 10 (FABP10) and acyl-CoA binding protein (ACBP) of hepatopancreas cells incubation for both 12 and 24 h; after incubation with ARA for 12 h, the gene expression of SR-B of ARA2 group was significantly higher than that of other groups ($P<0.05$), FABP10 gene expression of ARA2 and ARA3 groups was significantly higher than that of ARA1 and ARA5 groups ($P<0.05$), and ACBP gene expression of ARA3 group was significantly higher than that of other groups ($P<0.05$); after incubation with ARA for 24 h, the highest expressions of SR-B , FABP10 and ACBP were observed in ARA2 group, which was significantly higher than those of other groups ($P<0.05$). These findings suggest that ARA can influence cell viability and lipid metabolism-related gene expressions of hepatopancreas cell isolated from *Macrobrachium nipponense*. Cell viability can be decreased by incubation with higher level of ARA (1 000 mol/L). Appropriate levels of ARA (50 to 100 mol/L) can

promote the expressions of genes related to fatty acyl desaturase, elongases of very-long-chain fatty acids and fatty acid transport.

Keywords: *Macrobrachium nipponense*; arachidonic acid; cell culture; gene expression

---

Fatty acids are essential nutrients that maintain cell membrane fluidity and regulate growth performance, lipid metabolism, and immune function [1-2]. Arachidonic acid (20:4n-6, ARA), as an n-6 highly unsaturated fatty acid (HUFA), serves as a precursor for eicosanoids [3], participates in stress and inflammatory responses [4-5], and modulates immune performance [6]. Additionally, ARA and its metabolites can regulate peroxisome proliferator-activated receptor (PPAR) [7], thereby influencing the transcription of lipid metabolism-related genes and regulating fatty acid synthesis and storage [8-10].

Current research on aquatic animals has primarily focused on analyzing the effects of dietary ARA on fish growth performance and body fatty acid composition [5,11-15], stress resistance [5,13], immune performance [15], and metabolism [16-17] through in vivo feeding trials, or on examining ARA effects on cell pathway genes, fatty acid metabolism-related gene expression [18], eicosanoid production [6,18], and immune function [19] through in vitro head kidney cell culture experiments. Additionally, relevant studies on ARA have been conducted in economically important crustaceans such as shrimp and crabs. Xu et al. [20] found that ARA had higher nutritional value than linoleic acid or linolenic acid in Chinese shrimp (*Fenneropenaeus chinensis*). Research on tiger shrimp (*Penaeus monodon*) indicated that ARA supplementation could not improve growth performance when other essential fatty acids in the diet met requirements [21]. Dietary ARA supplementation could alter the expression of immune-related genes in Pacific white shrimp (*Litopenaeus vannamei*) [22]. However, traditional feeding trials are limited by the unified regulation of complex metabolic pathways in vivo and various influencing factors such as feeding conditions and environmental stress, which restrict the investigation of physiological functions and mechanisms of specific nutrients. In vitro cell culture can overcome these limitations [6,23]. Nevertheless, studies on fatty acid nutritional metabolism using in vitro cell culture systems from crustaceans have not been reported.

After absorption, fatty acids are utilized for intracellular triglyceride storage or as fuel for energy metabolism. Numerous studies have demonstrated that fatty acid transport across cell membranes is mediated by a competitive fatty acid transport system involving multiple proteins that significantly facilitate cellular fatty acid uptake and efflux [24-25], such as scavenger receptor class B type (SR-B) from the CD36 scavenger receptor family [26-27] and fatty acid translocase (FAT/CD36) [24]. SR-B can bind various ligands, including modified and unmodified low-density lipoproteins, very low-density lipoproteins, and high-density lipoprotein cholesterol esters [26-27]. Inside cells, fatty acids primarily bind to fatty acid-binding proteins (FABPs), which increases their solubility

and facilitates transport to different sites [28]. Additionally, intracellular acyl-CoA binding protein (ACBP) mainly binds to long-chain acyl-CoA and plays a crucial role in intracellular acyl-CoA transport and acyl-CoA pool formation. ACBP-bound acyl-CoA can be used for phospholipid and triglyceride synthesis or undergo -oxidation to produce ATP [29].

Some organisms can synthesize HUFA from polyunsaturated fatty acids, with fatty acid desaturases and elongases being key enzymes in HUFA synthesis. The desaturases involved in HUFA synthesis mainly include Δ6 desaturase (Δ6 FAD), Δ5 desaturase (Δ5 FAD), Δ4 desaturase (Δ4 FAD), and Δ8 desaturase (Δ8 FAD). In mammals, seven elongases of very-long-chain fatty acids (Elovl1-Elovl7) have been identified to participate in fatty acid elongation, among which Elovl2 and Elovl5 use C18, C20, or C22 PUFA as elongation substrates [30]. Feeding trials in aquatic animals have shown that dietary lipid "quality" or "quantity" can affect the expression of fatty acid desaturase and elongase genes [31-32].

The oriental river prawn (*Macrobrachium nipponense*), also known as freshwater shrimp or river shrimp, is an important freshwater aquaculture species in China and some Southeast Asian countries [33]. Currently, no reports exist on the effects of ARA on growth and nutritional physiology of *M. nipponense*. The hepatopancreas is the primary organ for lipid storage and processing in crustaceans [34] and serves as a sensitive monitor of nutrient metabolism [35-36]. Regarding hepatopancreas cell culture of *M. nipponense*, Liang [37] explored cell culture conditions, and Wang et al. [38] investigated the preliminary effects of linoleic acid on cultured cells, but no follow-up studies have been reported. Therefore, this experiment aimed to culture primary hepatopancreas cells of *M. nipponense* and analyze the effects of different ARA concentrations on hepatopancreas cell viability, fatty acid desaturase and elongase genes (Δ4 FAD, Δ6 FAD, and Elovl6), and fatty acid transport-related genes (SR-B , FABP10, and ACBP). The results provide a theoretical basis for investigating the mechanism of ARA in lipid metabolism and valuable references for studying the metabolism of other nutrients.

1.1 Test Animals

Experimental prawns were purchased from a *M. nipponense* aquaculture farm in Huzhou and acclimated for one week. Healthy prawns with uniform body weight were selected for the experiment.

1.2 Preparation of Complete Culture Medium

The basal medium was M199 culture medium (Gibco, USA) supplemented with 15% fetal bovine serum (Gibco, USA), 200 IU/mL antibiotics (penicillin and streptomycin), 1 g/L glucose, 5.2 g/L NaCl, 1.43 g/L CaCl , 0.05 g/L MgCl , and 0.2 g/L NaHCO . The osmotic pressure was 570 mmol/kg, and pH was 7.0-7.2.

1.3 Preparation of Arachidonic Acid-Bovine Serum Albumin (ARA-BSA) Serum-Free Medium

Ten milligrams of ARA (Sigma, USA) were dissolved in 1 mL anhydrous ethanol, dried under nitrogen gas, and mixed with 32.84 mL M199 culture medium containing 2% BSA (Sigma, USA). The solution was sonicated for 5 min and sterilized through a 0.22 m filter membrane to prepare 1 000 mol/L ARA-BSA M199 stock medium, which was aliquoted and stored at -20 °C. Before the experiment, the stock solution was diluted with M199 containing 2% BSA to prepare culture media at concentrations of 200, 100, and 50 mol/L. All media were supplemented with antioxidant butylated hydroxytoluene (0.01%) and antibiotics (200 IU/mL).

1.4 Isolation and Culture of *Macrobrachium nipponense* Hepatopancreas Cells

Prawns were immersed in 75% ethanol for 3 min and rinsed three times with D-Hanks balanced salt solution. The hepatopancreas was aseptically dissected and washed three times with D-Hanks balanced salt solution containing antibiotics. Tissue was cut into approximately 1 mm^3 pieces and digested with 0.25% trypsin, with repeated pipetting to disperse cells. Digestion was terminated with M199 culture medium containing fetal bovine serum. Cells were centrifuged at 1 000 r/min for 3 min, the supernatant was discarded, and cells were resuspended in complete culture medium. Cell concentration was adjusted to 1×10 cells/mL, and 200 L per well was seeded in 96-well culture plates. Cells were cultured at 27 °C in a 5% CO incubator, observed and photographed daily, with medium changed every 4-5 days.

After stable culture (5 days), the complete culture medium was replaced with medium containing different ARA concentrations. The experiment consisted of five groups with ARA concentrations of 0 (ARA1, control), 50 (ARA2), 100 (ARA3), 200 (ARA4), and 1 000 mol/L ARA-BSA (ARA5). Cells from each group were collected at 12 and 24 h for total RNA extraction for subsequent gene expression analysis.

1.5 Cell Viability Assay

Cell viability was assessed at day 5 using H33342/propidium iodide (PI) staining. One microliter of H33342 and 2 L of PI were added to 200 L cell culture medium, gently mixed, and incubated at 37 °C in the dark for 15 min. Cell viability was then examined under a fluorescence microscope, with live cells appearing blue and dead cells red.

After 24 h incubation with different ARA-BSA concentrations, cell viability was determined using the thiazolyl blue (MTT) cell proliferation and cytotoxicity assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing) according to the manufacturer’ s instructions.

1.6 Total RNA Extraction and cDNA Synthesis

Total RNA was extracted from hepatopancreas cells using a total RNA extraction kit (Beijing Aidlab Biotechnologies Co., Ltd.) according to the manufacturer's protocol. RNA integrity was examined by electrophoresis, and concentration and purity were measured using a nucleic acid-protein analyzer. Total RNA was reverse-transcribed into cDNA using a reverse transcription kit (TaKaRa, Japan). The cDNA was stored at -20 °C for gene expression analysis.

1.7 Quantitative Real-Time PCR (qRT-PCR) Analysis of Gene Expression

Primers for qRT-PCR of Δ4 FAD, Δ6 FAD, Elovl6, SR-B , FABP10, and ACBP genes were designed using online Primer 3 software, and primer sequences are listed in Table 1 . The qRT-PCR reaction volume was 20 L, containing 2 L template, 0.2 L each of forward and reverse primers (10 mol/L), 10 L 2×SYBR Green Premix Ex Taq (TaKaRa, Japan), and 7.6 L double-distilled water (ddH O). Cycling conditions were: 95 °C for 30 s; 40 cycles of 94 °C for 15 s, 58 °C for 20 s, and 72 °C for 20 s. After PCR, melting curves were generated by increasing temperature from 60 °C to 95 °C at 5 °C per 5 s to verify amplification specificity. *-actin* was used as the internal reference to normalize Ct values. Relative expression levels were analyzed using the 2^(-ΔΔCt) method [39] with ARA1 group as the baseline.

Table 1 Primer sequences for qRT-PCR

Primer name	GenBank accession No.	Primer sequence (5' -3')
Δ4 FAD-F	KU922944.1	CCAACCGTTATTTTATGCCC
Δ4 FAD-R		GTGCTCAGAAATAAAGTGGC
Δ6 FAD-F	KU922942.1	GACAGCTGAGAAGATTTTGC
Δ6 FAD-R		CTGGCCGATTTTCTCTAGAA
Elovl6-F	KU953779	TGGTGCACAGTATCATGTAC
Elovl6-R		TCATAGGAAACGTGACACTC
SR-B -F	KP658863	TGCAGTTCTACCTCTTTCAC
SR-B -R		TGTCCTCCCTGAAGAAGTAA
FABP10-F	JN995589	CCAAGCCAACTCTGGAAGTC
FABP10-R	KF896234	GATCTCAACGCTGGCTTCTC
ACBP-F	FL589653.1	CCTAATGATGAGGAGCTG
ACBP-R		GTTGCAATCTCCTACAGTT
-actin-F		GTGCCCATCTACGAGGGTTA
-actin-R		CGTCAGGGAGCTCGTAAGAC

2.1 Hepatopancreas Cell Morphology and Viability

Freshly isolated hepatopancreas cells appeared as single round cells. They began to adhere and proliferate slowly after 2-3 days, forming clusters with good

growth status. Cell morphology is shown in Figure 1 [Figure 1: see original paper]. Cell viability reached approximately 60% at day 5. Primary cultured hepatopancreas cells could survive for about 15 days.

Hepatopancreas cell viability under different ARA concentrations is shown in Figure 2 [Figure 2: see original paper]. After 24 h incubation with different ARA concentrations, cell viability in the ARA5 group was significantly lower than that in ARA1 and ARA2 groups ($P<0.05$). No significant differences were observed among ARA1, ARA2, ARA3, and ARA4 groups ($P>0.05$).

2.2 Effects of Arachidonic Acid on Lipid Metabolism Gene Expression in Hepatopancreas Cells

After 12 h ARA treatment, changes in Δ4 FAD, Δ6 FAD, Elovl6, SR-B , FABP10, ACBP, and ACC gene expressions are shown in Figure 3 [Figure 3: see original paper]. Expression levels of all lipid metabolism genes showed a trend of initial increase followed by decrease with increasing ARA concentration. Specifically, Δ4 FAD expression in ARA2, ARA3, and ARA4 groups was significantly higher than in ARA1 and ARA5 groups ($P<0.05$). No significant differences were observed in Δ6 FAD expression among ARA1, ARA2, and ARA3 groups ($P>0.05$), but ARA2 group showed significantly higher Δ6 FAD expression than ARA4 and ARA5 groups ($P<0.05$). Elovl6 expression was highest in ARA4 group, significantly higher than in ARA5 group ($P<0.05$). SR-B expression was highest in ARA2 group, significantly higher than all other groups ($P<0.05$). FABP10 expression in ARA2 and ARA3 groups was significantly higher than in ARA1 and ARA5 groups ($P<0.05$). ACBP expression in ARA3 group was significantly higher than all other groups ($P<0.05$).

After 24 h ARA treatment, changes in Δ4 FAD, Δ6 FAD, Elovl6, SR-B , FABP10, ACBP, and ACC gene expressions are shown in Figure 4 [Figure 4: see original paper]. The expression trends were similar to those observed at 12 h. Both Δ4 FAD and Δ6 FAD expression levels were higher in ARA2 and ARA3 groups. Elovl6 expression peaked in ARA3 group, significantly higher than all other groups ($P<0.05$). SR-B , FABP10, and ACBP expressions all reached maximum levels in ARA2 group, significantly higher than all other groups ($P<0.05$).

Discussion

Crustacean cell culture has been explored for a considerable time; unfortunately, no crustacean cell line has been established to date, hindering research on various metabolic mechanisms and functions. Studies on shrimp tissue cell culture have found that hemocyte culture is relatively simple, whereas hepatopancreas cell culture is more challenging [40-42]. In this experiment, hepatopancreas cell culture results were similar to those reported by Wang et al. [38], with primary cultured hepatopancreas cells showing good growth status, which is favorable for subsequent experiments.

When investigating the effects of ARA on *M. nipponense* hepatopancreas cells, serum-free BSA was used instead of fetal bovine serum to avoid potential interference from various nutrients including fatty acids present in the serum. MTT assay revealed that high ARA concentration (1 000 mol/L) reduced hepatopancreas cell viability, consistent with Li et al. [19] who reported that high ARA concentrations decreased cell viability in head kidney macrophage cultures. Exogenous fatty acids can enter cell membranes, altering cellular fatty acid composition and membrane physiological properties [43-44]. Particularly, high levels of fatty acids can cause irreversible DNA damage, loss of membrane integrity, altered membrane permeability, and ultimately cell death [45-46].

Fatty acid desaturases and elongases are key enzymes in HUFA synthesis. Studies in higher vertebrates have shown that ARA can generate C22:5n-6 through elongation, Δ6 FAD, and -oxidation, or directly through Δ4 FAD. ARA can also be converted to C20:5n-3 (EPA) via Δ17 desaturase (Δ17 FAD). EPA can be sequentially elongated, Δ6-desaturated, and -oxidized to synthesize C22:6n-3 (DHA), or EPA can be converted to 22:5n-3 and then directly transformed to DHA by Δ4 FAD [47]. This study found that different ARA concentrations and treatment durations altered the expression of key enzyme genes in the HUFA synthesis pathway, with high ARA concentration (1 000 mol/L) decreasing Δ4 FAD, Δ6 FAD, and Elovl6 expression levels, indicating that ARA is an important regulator in the HUFA synthesis pathway of *M. nipponense*. Studies in suckling piglets showed that dietary ARA to DHA ratio could regulate hepatic desaturase gene transcription [48]. In fish feeding trials, Senegalese sole (*Solea senegalensis*) males fed diets containing 0.7%, 2.3%, and 6% ARA showed increased hepatic Elovl5 and Δ4 FAD gene expression [17]. However, studies on grass carp (*Ctenopharyngodon idellus*) demonstrated that fatty acid desaturase and elongase genes were significantly suppressed by dietary ARA levels [10]. These discrepancies may be related to different HUFA synthesis capacities among species and also indicate that ARA levels can significantly affect the expression of HUFA synthesis-related enzyme genes. Although limited data are available on the effects of fatty acid concentrations on HUFA synthesis using in vitro culture systems, this experiment demonstrated that appropriate fatty acid concentrations in vitro can promote the expression of key enzyme genes in the HUFA synthesis pathway of *M. nipponense*.

Few reports exist on the effects of ARA on fatty acid transport-related gene expression. Holen et al. [18] added different combinations of EPA, DHA, and ARA to Atlantic salmon (*Salmo salar*) head kidney cells and found that ARA+EPA upregulated fatty acid translocase CD36 gene expression. SR-B belongs to the CD36 superfamily and plays important roles in maintaining intracellular cholesterol homeostasis, membrane lipid expression, and apoptosis [49-50]. This study found that SR-B expression peaked in the ARA2 group after 12 and 24 h of ARA treatment, suggesting that 50 mol/L ARA is beneficial for maintaining cellular lipid metabolism balance. Studies have shown that fatty acids or acyl-CoA are natural ligands of PPAR that can activate PPAR [51], and SR-B activity can be induced by PPAR and PPAR [52-53]. Therefore, certain con-

centrations of fatty acids in hepatopancreas cell culture medium may regulate SR-B expression through PPAR activation. Studies in hamsters also showed that dietary polyunsaturated fatty acids could increase SR-B gene and protein levels [54]. In vitro culture of bovine mammary epithelial cells demonstrated that lipid metabolism-related gene expression was closely related to fatty acid concentration [55-56]. FABP10 and ACBP are intracellular lipid-binding proteins. After 12 h ARA treatment, FABP10 transcription peaked in ARA2 and ARA3 groups, and ACBP transcription peaked in ARA3 group, indicating that 50-100 mol/L ARA promoted intracellular fatty acid transport at this time point. After 24 h ARA treatment, both FABP10 and ACBP expressions in ARA2 group were significantly higher than other groups, suggesting that 50 mol/L ARA supplementation in *M. nipponense* hepatopancreas cells may be more beneficial for promoting lipid metabolism. Numerous genes related to lipid synthesis, degradation, and metabolism are regulated by PPAR [57]. In vitro cell culture studies showed that PPAR has high affinity for liver-type FABP (L-FABP), indicating that L-FABP can bind to PPAR to regulate long-chain fatty acid metabolism [58]. Similarly, PPAR can also activate ACBP gene expression [59]. Therefore, the mechanism by which appropriate ARA concentrations promote FABP10 and ACBP expression may be similar to that of SR-B . In fact, FABP10 and ACBP are multifunctional proteins that play important roles in immune function [60-61], and studies have shown that appropriate ARA concentrations can modulate immune performance [6]. Therefore, we cannot exclude the possibility that 50-100 mol/L ARA in culture medium enhances immune performance, thereby increasing FABP10 and ACBP gene expression.

In conclusion, ARA concentration in culture medium affects *M. nipponense* hepatopancreas cell viability and lipid metabolism-related gene expression. Excessive ARA concentration (1 000 mol/L) decreases cell viability, while appropriate ARA concentrations (50-100 mol/L) can promote the expression of genes related to fatty acid desaturases, elongases, and fatty acid transport.

References

[1] HIGGS D A, DONG F M. Lipids and fatty acids[M]//STICKNEY R R. *Encyclopedia of Aquaculture*. New York: John Wiley and Sons, 2000: 476-496.

[2] TRICHET V V. Nutrition and immunity: an update[J]. *Aquaculture Research*, 2010, 41(3): 356-372.

[3] BELL J G, SARGENT J R. Arachidonic acid in aquaculture feeds: current status and future opportunities[J]. *Aquaculture*, 2003, 218(1/2/3/4): 491-499.

[4] VAN ANHOLT R D, KOVEN W M, LUTZKY S E, et al. Dietary supplementation with arachidonic acid alters the stress response of gilthead seabream (*Sparus aurata*) larvae[J]. *Aquaculture*, 2004, 238(1/2/3/4): 369-383.

[5] CARRIER J K, WATANABE W O, HAREL M, et al. Effects of dietary arachidonic acid on larval performance, fatty acid profiles, stress resistance, and

expression of Na /K ATPase mRNA in black sea bass *Centropristis striata*[J]. *Aquaculture*, 2011, 319(1/2): 111-121.

[6] FURNE M, HOLEN E, ARAUJO P, et al. Cytokine gene expression and prostaglandin production in head kidney leukocytes isolated from Atlantic cod (*Gadus morhua*) added different levels of arachidonic acid and eicosapentaenoic acid[J]. *Fish & Shellfish Immunology*, 2013, 34(3): 770-777.

[7] QI C, ZHU Y J, REDDY J K. Peroxisome proliferator-activated receptors, coactivators, and downstream targets[J]. *Cell Biochemistry and Biophysics*, 2000, 32(1/2/3): 187-204.

[8] BRASH A R. Arachidonic acid as a bioactive molecule[J]. *The Journal of Clinical Investigation*, 2001, 107(11): 1339-1345.

[9] MARTINS D A, ROCHA F, MARTÍNEZ-RODRÍGUEZ G, et al. Teleost fish larvae adapt to dietary arachidonic acid supply through modulation of the expression of lipid metabolism and stress response genes[J]. *British Journal of Nutrition*, 2012, 108(5): 864-874.

[10] TIAN J J, JI H, OKU H, et al. Effects of dietary arachidonic acid (ARA) on lipid metabolism and health status of juvenile grass carp, *Ctenopharyngodon idellus*[J]. *Aquaculture*, 2014, 430: 57-65.

[11] CASTELL J D, BELL J G, TOCHER D R, et al. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*)[J]. *Aquaculture*, 1994, 128(3/4): 315-333.

[12] BELL J G, CASTELL J D, TOCHER D R, et al. Effects of different dietary arachidonic acid:docosahexaenoic acid ratios on phospholipid fatty acid compositions and prostaglandin production in juvenile turbot (*Scophthalmus maximus*)[J]. *Fish Physiology and Biochemistry*, 1995, 14(2): 139-151.

[13] KOVEN W, BARR Y, LUTZKY S, et al. The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae[J]. *Aquaculture*, 2001, 193(1/2): 107-122.

[14] FOUNTOULAKI E, ALEXIS M N, NENGAS I, et al. Effects of dietary arachidonic acid (20:4n-6), on growth, body composition, and tissue fatty acid profile of gilthead bream fingerlings (*Sparus aurata* L.)[J]. *Aquaculture*, 2003, 225(1/2/3/4): 309-323.

[15] XU H G, AI Q H, MAI K S, et al. Effects of dietary arachidonic acid on growth performance, survival, immune response and tissue fatty acid composition of juvenile Japanese seabass, *Lateolabrax japonicus*[J]. *Aquaculture*, 2010, 307(1/2): 75-82.

[16] VAN ANHOLT R D, SPANINGS F A T, KOVEN W M, et al. Dietary supplementation with arachidonic acid in tilapia (*Oreochromis mossambicus*)

reveals physiological effects not mediated by prostaglandins[J]. *General and Comparative Endocrinology*, 2004, 139(3): 215-226.

[17] NORAMBUENA F, MORAIS S, ESTÉVEZ A, et al. Dietary modulation of arachidonic acid metabolism in senegalese sole (*Solea senegalensis*) broodstock reared in captivity[J]. *Aquaculture*, 2013, 372-375: 80-88.

[18] HOLEN E, HE J Y, ESPE M, et al. Combining eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid, using a fully crossed design, affect gene expression and eicosanoid secretion in salmon head kidney cells in vitro[J]. *Fish & Shellfish Immunology*, 2015, 45(2): 695-703.

[19] LI Q F, AI Q H, MAI K S, et al. In vitro effects of arachidonic acid on immune functions of head kidney macrophages isolated from large yellow croaker (*Larimichthys crocea*)[J]. *Aquaculture*, 2012, 330-333: 47-53.

[20] XU X L, JI W J, CASTELL J D, et al. Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*[J]. *Aquaculture*, 1994, 127(1): 29-40.

[21] GLENCROSS B D, SMITH D M. A study of the arachidonic acid requirements of the giant tiger prawn, *Penaeus monodon*[J]. *Aquaculture Nutrition*, 2001, 7(1): 59-69.

[22] ZHAO L B, WANG X L, HUANG X X, et al. Effects of dietary arachidonic acid level on immune-related gene expression and antibacterial ability of *Litopenaeus vannamei*[J]. *Journal of Fisheries of China*, 2016, 40(5): 763-775.

[23] HOLEN E, WINTERTHUN S, DU Z Y, et al. Inhibition of p38 MAPK during cellular activation modulate gene expression of head kidney leukocytes isolated from Atlantic salmon (*Salmo salar*) fed soy bean oil or fish oil based diets[J]. *Fish & Shellfish Immunology*, 2011, 30(1): 397-405.

[24] ABUMRAD N, COBURN C, IBRAHIMI A. Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm[J]. *Biochimica et Biophysica Acta (BBA): Molecular and Cell Biology of Lipids*, 1999, 1441(1): 4-13.

[25] BONEN A, CHABOWSKI A, LUIKEN J J, et al. Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence[J]. *Physiology*, 2007, 22: 15-29.

[26] ACTON S L, SCHERER P E, LODISH H F, et al. Expression cloning of SR-B , a CD36-related class B scavenger receptor[J]. *The Journal of Biological Chemistry*, 1994, 269(33): 21003-21009.

[27] ACTON S, RIGOTTI A, LANDSCHULZ K T, et al. Identification of scavenger receptor SR-B as a high density lipoprotein receptor[J]. *Science*, 1996, 271(5248): 518-520.

[28] ZIMMERMAN A W, VEERKAMP J H. New insights into the structure and function of fatty acid-binding proteins[J]. *Cellular and Molecular Life Sciences*,

2002, 59(7): 1096-1116.

[29] MANDRUP S, HUMMEL R, RAVN S, et al. Acyl-CoA-binding protein/diazepam-binding inhibitor gene and pseudogenes: a typical housekeeping gene family[J]. *Journal of Molecular Biology*, 1992, 228(3): 1011-1022.

[30] JAKOBSSON A, WESTERBERG R, JACOBSSON A. Fatty acid elongases in mammals: their regulation and roles in metabolism[J]. *Progress in Lipid Research*, 2006, 45(3): 237-249.

[31] KUAH M K, JAYA-RAM A, SHU-CHIEN A C. The capacity for long-chain polyunsaturated fatty acid synthesis in a carnivorous vertebrate: functional characterisation and nutritional regulation of a Fads2 fatty acyl desaturase with Δ4 activity and an Elovl5 elongase in striped snakehead (*Channa striata*)[J]. *Biochimica et Biophysica Acta (BBA): Molecular and Cell Biology of Lipids*, 2015, 1851(3): 248-260.

[32] XIE D Z, CHEN F, LIN S Y, et al. Long-chain polyunsaturated fatty acid biosynthesis in the euryhaline herbivorous teleost *Scatophagus argus*: functional characterization, tissue expression and nutritional regulation of two fatty acyl elongases[J]. *Comparative Biochemistry and Physiology Part B: Biochemistry & Molecular Biology*, 2016, 198: 37-45.

[33] YANG Y, XIE S Q, LEI W, et al. Effect of replacement of fish meal by meat and bone meal and poultry by-product meal in diets on the growth and immune response of *Macrobrachium nipponense*[J]. *Fish & Shellfish Immunology*, 2004, 17(2): 105-114.

[34] HARRISON K E. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review[J]. *Journal of Shellfish Research*, 1990, 9(1): 1-28.

[35] VOGT G, STORCH V, QUINTIO E T, et al. Midgut gland as monitor organ for the nutritional value of diets in *Penaeus monodon* (Decapoda)[J]. *Aquaculture*, 1985, 48(1): 1-12.

[36] ROSAS C, BOLONGARO-CREVENNA A, SÁNCHEZ A, et al. Role of digestive gland in the energetic metabolism of *Penaeus setiferus*[J]. *The Biological Bulletin*, 1995, 189(2): 168-174.

[37] LIANG H. Cell culture of *Macrobrachium nipponense*[D]. Master' s thesis. Baoding: Hebei University, 2001.

[38] WANG H W, LIU R L, GUO M S, et al. Effects of linoleic acid on cultured hepatopancreas cells of *Macrobrachium nipponense*[J]. *Journal of Hebei University (Natural Science Edition)*, 2005, 25(1): 79-83.

[39] LIVAK K J, SCHMITTGEN T D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-ΔΔCt) method[J]. *Methods*, 2001, 25(4): 402-408.

[40] MULFORD A L, AUSTIN B. Development of primary cell cultures from *Nephrops norvegicus*[J]. *Methods in Cell Science*, 1998, 19(4): 269–275.

[41] GEORGE S K, DHAR A K. An improved method of cell culture system from eye stalk, hepatopancreas, muscle, ovary, and hemocytes of *Penaeus vannamei*[J]. *In Vitro Cellular & Developmental Biology: Animal*, 2010, 46(9): 801–810.

[42] JAYESH P, JOSE S, PHILIP R, et al. A novel medium for the development of in vitro cell culture system from *Penaeus monodon*[J]. *Cytotechnology*, 2013, 65(3): 307–322.

[43] CALDER P C. The relationship between the fatty acid composition of immune cells and their function[J]. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 2008, 79(3/4/5): 101–108.

[44] TOCHER D R, DICK J R. Polyunsaturated fatty acid metabolism in cultured fish cells: incorporation and metabolism of (n-3) and (n-6) series acids by Atlantic salmon (*Salmo salar*) cells[J]. *Fish Physiology and Biochemistry*, 1990, 8(4): 311–319.

[45] GORJÃO R, AZEVEDO-MARTINS A K, RODRIGUES H G, et al. Comparative effects of DHA and EPA on cell function[J]. *Pharmacology & Therapeutics*, 2009, 122(1): 56–64.

[46] JI J, ZHANG L, WANG P, et al. Saturated free fatty acid, palmitic acid, induces apoptosis in fetal hepatocytes in culture[J]. *Experimental and Toxicologic Pathology*, 2005, 56(6): 369–376.

[47] TOCHER D R, ZHENG X Z, SCHLECHTRIEM C, et al. Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl Δ6 desaturase of Atlantic cod (*Gadus morhua* L.)[J]. *Lipids*, 2006, 41(11): 1003–1016.

[48] WIJENDRAN V, DOWNS L, SRIGLEY C T, et al. Dietary arachidonic acid and docosahexaenoic acid regulate liver fatty acid desaturase (FADS) alternative transcript expression in suckling piglets[J]. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 2013, 89(5): 345–350.

[49] NECULAI D, SCHWAKE M, RAVICHANDRAN M, et al. Structure of LIMP-2 provides functional insights with implications for SR-B and CD36[J]. *Nature*, 2013, 504(7478): 172–176.

[50] SHEN W J, HU J, HU Z G, et al. Scavenger receptor class B type (SR-B): a versatile receptor with multiple functions and actions[J]. *Metabolism*, 2014, 63(7): 875–886.

[51] SCHOONJANS K, STAELS B, AUWERX J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression[J]. *Journal of Lipid Research*, 1996, 37(5): 907–925.

[52] LOPEZ D, MCLEAN M P. Activation of the rat scavenger receptor class B type gene by PPAR [J]. *Molecular and Cellular Endocrinology*, 2006, 251(1/2): 67–77.

[53] CHINETTI G, GBAGUIDI F G, GRIGLIO S, et al. CLA-1/SR-B is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors[J]. *Circulation*, 2000, 101(20): 2411–2417.

[54] SPADY D K, KEARNEY D M, HOBBS H H. Polyunsaturated fatty acids up-regulate hepatic scavenger receptor B1 (SR-B) expression and HDL cholesteryl ester uptake in the hamster[J]. *Journal of Lipid Research*, 1999, 40(8): 1384–1394.

[55] HU H, WANG J Q, LI F D, et al. Effects of free linolenic acid on mRNA transcription of fatty acid metabolism-related genes in bovine mammary epithelial cells[J]. *Chinese Journal of Animal Nutrition*, 2010, 22(5): 1342–1349.

[56] YONEZAWA T, YONEKURA S, KOBAYASHI Y, et al. Effects of long-chain fatty acids on cytosolic triacylglycerol accumulation and lipid droplet formation in primary cultured bovine mammary epithelial cells[J]. *Journal of Dairy Science*, 2004, 87(8): 2527–2534.

[57] DESVERGNE B, WAHLI W. Peroxisome proliferator-activated receptors: nuclear control of metabolism[J]. *Endocrine Reviews*, 1999, 20(5): 649–688.

[58] HOSTETLER H A, MCINTOSH A L, ATSHAVES B P, et al. L-FABP directly interacts with PPAR in cultured primary hepatocytes[J]. *Journal of Lipid Research*, 2009, 50(8): 1663–1675.

[59] SANDBERG M B, BLOKSGAARD M, DURAN-SANDOVAL D, et al. The gene encoding acyl-CoA-binding protein is subject to metabolic regulation by both sterol regulatory element-binding protein and peroxisome proliferator-activated receptor in hepatocytes[J]. *The Journal of Biological Chemistry*, 2005, 280(7): 5258–5266.

[60] REN Q, DU Z Q, ZHAO X F, et al. An acyl-CoA-binding protein (FcACBP) and a fatty acid binding protein (FcFABP) respond to microbial infection in Chinese white shrimp, *Fenneropenaeus chinensis*[J]. *Fish & Shellfish Immunology*, 2009, 27(6): 739–747.

[61] ZHAO Z Y, YIN Z X, WENG S P, et al. Profiling of differentially expressed genes in hepatopancreas of white spot syndrome virus-resistant shrimp (*Litopenaeus vannamei*) by suppression subtractive hybridisation[J]. *Fish & Shellfish Immunology*, 2007, 22(5): 520–534.

Note: Figure translations are in progress. See original paper for figures.