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Cloning and characterization of fatty acid-binding proteins (*fabps*) from Japanese seabass (*Lateolabrax japonicus*) liver, and their gene expressions in response to dietary arachidonic acid (ARA)

Houguo Xu [a,b], Yuanqin Zhang [a,c], Chengqiang Wang [a], Yuliang Wei [a], Keke Zheng [a,b], Mengqing Liang [a,b,*]

[a] Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao, Shandong 266071, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Qingdao, Shandong 266237, China
[c] Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, Jiangsu 214081, China

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ABSTRACT

In the present study, putative cDNA of five *fabp* isoforms, i.e., *fabp1*, *fabp2*, *fabp3*, *fabp4*, and *fabp7*, was cloned and characterized from the liver of Japanese seabass (*Lateolabrax japonicus*), and their expression in response to diets with different arachidonic acid (ARA) levels (0.05%, 0.22%, 0.37%, 0.60%, 1.38% and 2.32% of dry matter) was investigated following a feeding trial. The Japanese seabass *fabps* showed high identity to their orthologs in other fish species and mammals. However, a specific *fabp* of Japanese seabass showed much lower identity to other Japanese seabass *fabps*. *fabp1* has high expressions in liver and intestine, whereas *fabp2* is mainly expressed in the gastrointestinal tract. The highest expression level of *fabp3*, *fabp4*, and *fabp7* was observed in muscle, eye, and liver respectively. Different tissue expression patterns of *fabp2*, *fabp4*, and *fabp7* between Japanese seabass and other teleost may indicate specific evolutionary Fabp functions in Japanese seabass. Moderate levels of dietary ARA (0.37–0.60%) enhanced the gene expressions of *fabp1* in liver and intestine, *fabp2* in intestine, and *fabp3* in intestine, whereas excess dietary ARA levels (1.38–2.323%) were ineffective. The highest level of dietary ARA (2.32%) increased only the expression of *fabp3* in muscle compared to the control diet. Gene expressions of *fabp3* and *fabp7* in liver, and *fabp4* in liver, intestine, and muscle were not significantly influenced by dietary ARA. To our knowledge, this is the first study investigating the regulation of *fabp* expressions by dietary ARA.
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1. Introduction

Fatty acid-binding proteins (Fabps), ubiquitous in tissues highly active in fatty acid metabolism, play important roles in lipid uptake and transport, and overall lipid homeostasis (Veerkamp and Maatman, 1995; Massolini and Calleri, 2003). They belong to the conserved multi-gene family of intracellular lipid binding proteins (iLBPs) that are individual genes arising from an ancestral iLBP gene through gene duplication and diversification (Schaap et al., 2002). Different Fabps exhibit differences in ligand specificity (Glatz and van der Vusse, 1990; Veerkamp et al., 1991) as well as affinity for specific fatty acids (Veerkamp et al., 1990).

Fabps have been studied for over 40 years and plenty of articles about them have been published. However, it remains unclear in many aspects, such as why they are so highly and ubiquitously expressed, and what is the difference in function and regulation of different Fabps (Storch and Corsico, 2008). On the other hand, although Fabps have been studied extensively, less data have been reported in fish (Sharma et al., 2004; Jordal et al., 2006; Parmar et al., 2012; Venold et al., 2013; Bayır et al., 2015). Unlike mammals, fish prefer lipids to carbohydrates as their main source of energy (Watanabe, 1982). Moreover, fish is the most important source of long-chain polyunsaturated fatty acids (LC-PUFA), i.e., docosahexaenoic acid (DHA, 22:6n − 3), eicosapentaenoic acid (EPA, 20:5n − 3) and ARA (20:4n − 6), for human consumption. Elucidation of the function and regulation of Fabps in fish, especially in farmed fish, will be beneficial to the efficient production of LC-PUFA from fish.

The present study was aimed to clone and characterize the gene of *fabps* in an important aquaculture species Japanese seabass *Lateolabrax japonicus*. In our previous studies, we have investigate several important proteins in the LC-PUFA homeostasis of Japanese seabass, such as Δ6 desaturase (FADS2) (Xu et al., 2014), sterol regulatory element-binding protein 1 (SREBP-1), and peroxisome proliferator-activated receptors (PPARs) (Dong et al., 2015), as well as their regulation by different lipid sources. Indicated by the difference in the roles and regulation of the previously studied proteins between teleosts and mammals, Fabps are also presumed to have specific properties in fish. Besides the sequence feature and the tissue distribution, the present was also

* Corresponding author at: Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao, Shandong 266071, China.
E-mail address: liangmq@ysfri.ac.cn (M. Liang).

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aimed to investigate the regulation of *fabp* gene expressions by dietary ARA. ARA is one of the essential fatty acids for Japanese seabass reared in seawater. One of our previous studies on Japanese seabass has indicated that ARA has the potential to modulate the lipid deposit and LC-PUFA metabolism (Xu et al., 2010). Other studies also suggested that ARA and its eicosanoid metabolites are high affinity ligands of many Fabps (Raza et al., 1989; Córdoba et al., 1999; Veerkamp et al., 1999; Hanhoff et al., 2002). Therefore, this study investigates the effects of different levels of dietary ARA on the gene expression of *fabp*s in various tissues of Japanese seabass, in order to shed some light on the regulation of marine fish *fabp*s by essential fatty acids in the diets.

2. Materials and methods

2.1. RNA extraction and cDNA synthesis

Total RNA in livers was extracted using RNAiso Plus (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) and reversely transcribed with PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) according to the user manual.

2.2. Cloning and sequencing of fabps

The complete CDS of *fabp3*, *fabp4*, and *fabp7* was obtained from an Illumina sequencing with Japanese seabass liver cDNA (Novogene, Beijing, China; whole data not shown here). The complete mRNA of *fabp1*, of which the complete CDS was not obtain from the Illumina sequencing, was cloned with rapid amplification of cDNA ends (RACE). Specific primers for *fabp1* (*fabp1*-R1, -R2, -F1, and -F2) were designed based on the known *fabp1* sequence fragments from Illumina sequencing to clone the 5′- and 3′-end respectively (Table 1). The SMARTer™ RACE cDNA Amplification Kit (Clontech, California, USA) was used to perform the RACE cloning and the 3′- and 5′-end cDNA templates were synthesized according to the user's manual. The primers were synthesized by TsingKe Biological Technology, Co., Ltd. (Qingdao, China). PCR amplifications were performed on peqSTAR (PEQLAB, Erlangen, Germany). All PCR products were run on a 1.5% agarose gel, and then purified by Zymoclean Gel DNA Recovery Kit (ZYMO RESEARCH, CA, USA). PCR products were cloned into pEASY-T1 simple cloning vector (TransGen, Beijing, China) and sequenced in TsingKe (Qingdao, China). Other details of the PCR amplification were similar with our previous studies (Xu et al., 2014). Since only a short sequence fragment of Japanese seabass *fabp2* was obtained from the Illumina sequencing, specific primers for *fabp2* (*fabp2*-F1, -F2, -R1, and -R2) were also designed based on this *fabp2* sequence fragment and known *fabp2* sequences of other teleost to clone the complete CDS of Japanese seabass *fabp2* (Table 1).

Table 1
Sequences of the PCR primers used in this work.

Primer	Sequences (5′–3′)	Sequence information
fabp1-R1	TGCCGTCACGCTGAACCACAGTCTTG	5′RACE primer
fabp1-F1	ATCCAGAAAGGCAAAGACATCAAGAGC	3′RACE primer
fabp1-R2	GCTCTTGATGTCTTTGCCTTTCTGGAT	5′RACE primer
fabp1-F2	GTCACAGAACTGGTGGATGGGAACA	3′RACE primer
fabp2-R1	ATCCTCTTTGCRTCYACACCT	RT primer
fabp2-F1	ASYTCACCCTCACAGCCAMCAT	RT primer
fabp2-R2	CTTGAAAATCCTCTTTGCRTC	RT primer
fabp2-F2	GAGTACAGCCTTGCAGATGGA	RT primer
fabp1-qF	TGAACTCATCCAGAAAGGCAA	qPCR primer
fabp1-qR	CCGTCACGCTGAACCACA	qPCR primer
fabp2-qF	TTGAGTACAGCCTTGCAGATGG	qPCR primer
fabp2-qR	CAGTTGCTTTCCGTTGTCCTT	qPCR primer
fabp3-qF	ACCCACCACCATCATTTCCTTG	qPCR primer
fabp3-qR	CTCTTTGCCGTCCCATTTCTG	qPCR primer
fabp4-qF	CAAGCCCAATCTGGTGGTGAG	qPCR primer
fabp4-qR	CTATCGTCGGCGGTCGTCT	qPCR primer
fabp7-qF	CGGAGACAAAGTGGTGGTGA	qPCR primer
fabp7-qR	AGATTTGACGTGTCGGTCGT	qPCR primer
β-Actin-F	CAACTGGGATGACATGGAGAAG	qPCR primer
β-Actin-R	TTGGCTTTGGGGTTCAGG	qPCR primer

2.3. Real-time quantitative polymerase chain reaction (qRT-PCR) analysis

Real-time fluorescent quantitative PCR (qRT-PCR) was used to assay the relatively quantitative mRNA expression of *fabp*s in different tissues of Japanese seabass, as well as the gene expressions in fish from different dietary treatments. β-Actin (GenBank accession No. **HE577671.1**) was used as the reference gene. Specific primers for *fabp*s and β-actin were designed using Primer 5.0 based on the *fabp* sequences obtained previously and the β-actin sequences on NCBI (Table 1). The real-time PCR was carried out with SYBR Green Real-time PCR Master Mix (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) in a quantitative thermal cycler (Mastercycler eprealplex, Eppendorf, Germany). The detailed program was similar with Xu et al. (2014). The mRNA expression levels were studied by qRT-PCR method: $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001).

2.4. Feeding trial

A 12-week feeding trial was conducted to investigate the effects of dietary ARA on the *fabp* gene expressions in Japanese seabass. Six diets with similar proximate compositions but graded ARA contents were used in the feeding trial (Table 2). The control diet was formulated using fish meal, soybean meal, and wheat meal as the protein sources, and soy lecithin and tristearin as the lipid sources. An ARA enriched oil (ARA concentration, 41% of total fatty acids (TFA); in the form of triglyceride; Jiangsu Tiankai Biotechnology Co., Ltd., Nanjing, China) was

Table 2
Formulation and proximate composition of the experiment diets (% dry matter).

	Dietary ARA level					
Ingredients	0.05	0.22	0.37	0.60	1.38	2.32
Fish meal	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Wheat meal	30.85	30.85	30.85	30.85	30.85	30.85
Casein	16.00	16.00	16.00	16.00	16.00	16.00
Gelatin	4.00	4.00	4.00	4.00	4.00	4.00
Mineral premix[a]	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix[b]	2.00	2.00	2.00	2.00	2.00	2.00
Attractant[c]	0.30	0.30	0.30	0.30	0.30	0.30
Mold inhibitor[d]	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Soy lecithin	1.20	1.20	1.20	1.20	1.20	1.20
DHA enriched oil[e]	1.50	1.50	1.50	1.50	1.50	1.50
ARA enriched oil[f]	0.00	0.35	0.71	1.45	2.91	5.84
Tristearin[g]	7.00	6.65	6.29	5.55	4.09	1.16
Proximate composition						
Crude protein	43.64	43.53	44.40	44.36	43.39	44.13
Crude lipid	11.32	11.07	11.09	11.24	11.50	11.22
Ash	6.89	6.94	6.81	6.98	6.92	6.83

[a] Mineral premix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; $Ca(IO_3)_2 \cdot 6H_2O$ (1%), 60 mg; $CaH_2PO_4 \cdot H_2O$, 10 g; zoelite, 8.485 g.

[b] Vitamin premix (mg or g/kg diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12} (1%), 10 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol (50%), 240 mg; ascorbic acid, 5 g; choline chloride (50%), 5 g; wheat middling, 4.47 g.

[c] Attractant: glycine and betaine.

[d] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

[e] DHA enriched oil: DHA content, 43% of total fatty acid; in the form of triglyceride; Jiangsu Tiankai Biotechnology Co., Ltd., Nanjing, China.

[f] ARA enriched oil: ARA content, 46% of total fatty acid; in the form of triglyceride; Jiangsu Tiankai Biotechnology Co., Ltd., Nanjing, China.

[g] Tristearin: HUDONG article of everyday use Co., Ltd., Jiaxing, China.

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