Chapter 13
Effects of Water Temperature and Nutritional Manipulation on the Expression of Liver-Type Fatty Acid-Binding Protein (L-FABP) Gene in Golden Pompano *Trachinotus ovatus* Larvae

Haijun Wei, Shengjie Zhou, and Mingjun Fu

Abstract The liver fatty acid-binding protein (L-FABP) is a 14-kDa cytoplasmic protein that has the function of binding long-chain fatty acids with high affinity. L-FABP cDNA was 604 bp in golden pompano, and its expression level varies from ages and tissues. Temperature and nutrition can significantly regulate the expression level of L-FABP in *Trachinotus ovatus*. On 12 and 18 days post hatch, the maximum expression appeared in fish larvae at 29 °C. The maximum expression of L-FABP was observed in fish fed with *Artemia* nauplii enriched with Algamac 3080, and the minimum expression was observed in fish fed with *Artemia* nauplii enriched with *Nannochloropsis*. This chapter addresses the expression of the L-FABP gene in *Trachinotus ovatus* larvae under different nutritional and environmental conditions. This study suggests that L-FABP can be used as a potential indicator to evaluate the digestive function of fish larvae during early development.

Keywords Liver-type fatty acid-binding protein · Nutrition · Temperature · *Trachinotus ovatus*

H. Wei · S. Zhou
Tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Sanya, China

M. Fu (✉)
College of Life Science, Longyan University, Fujian, China
e-mail: fu.mj@lyun.edu.cn

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13.1 Introduction

The fatty acid-binding proteins (FABPs) belong to a multigene family with 14–16 kDa molecular mass, which could combine with fatty acids or other organic dissolved substances in eukaryotic organisms (Borchers et al. 1989, 1997; Kanda et al. 1989; Alvite et al. 2008). The length of FABPs was 126–137 amino acids, and it varies from species to species (Pelsers et al. 2005; Chen and Shi 2009). FABPs can protect cells from the cytotoxic effects of free fatty acids, target specific metabolic pathways, mediate the transport of free fatty acids, modify lipid metabolism enzymes, and participate in fatty acid signaling in the nucleus (Besnard et al. 2002; Storch and McDermott 2009; Lowe et al. 1987). According to the physiological characteristics of different tissues, different types of FABP fulfilled the specific functions (Banaszak et al. 1994; Veerkamp et al. 1991, 1993); therefore, FABPs have been named after the first mammalian tissue from which they were isolated, for instance, heart, adipose, myelin, intestine, and liver tissue.

Veerkamp and Maatman (1995) pointed out that a 14 kDa cytoplasmic protein, liver FABP (L-FABP), could bind long-chain fatty acids with high affinity. The complete primary structures of L-FABPs have been determined in some nonmammalian vertebrates, such as catfish, frogs, chick, and shark (Di Pietro et al. 1996; Schleicher and Santome 1996; Baba et al. 1999; Cecilian et al. 1994; Medzihradszky et al. 1992). Furthermore, mammalian L-FABPs, a small cytosolic protein in many tissues including kidney, liver, and small intestine, play an important character in intracellular fatty acid metabolism and trafficking (Her et al. 2003).

Due to rapid growth, strong suitability, and adaptability, *Trachinotus ovatus* has become a suitable species for culture (Ma et al. 2014). According to Storch and McDermott (2009), L-FABP can intervene the transport of free fatty acids to target specific metabolic pathways, and it can improve fingerling quality and our knowledge of the nutrition requirement and digestive ontogeny in fish larvae (Ma et al. 2012). Consequently, this chapter aims to discuss the L-FABP expression during the development in the first 18 DPH of golden pompano *T. ovatus* and the effects of temperature and nutritional manipulation on L-FABP gene expression. Such information will improve our understanding of the digestive organs of *T. ovatus* and provide potential indicators to evaluate digestive function during early development of fish larvae.

13.2 Cloning and Sequencing of L-FABP Gene cDNA

The Primer 5.0 (Premier Biosoft International, Palo Alto, CA, USA) was used for designing the gene cloning primers (Table 13.1) based on the unpublished *T. ovatus* transcriptome sequences in our lab. The full length of L-FABP cDNA (GenBank accession No. MF034872) from *T. ovatus* was 604 bp, including a UTR of 154 bp, a 3′-UTR of 69 bp, and a 281 bp ORF encoding a 126 amino acids polypeptide with a

Table 13.1 Sequences of primers (Zhou et al. 2019)

Primers	Sequence (5′–3′)	Amplicon sizes (bp)
EF-1α-qF	CCCCTTGGTCGTTTTGCC	101
EF-1α-qR	GCCTTGGTTGTCTTTCCGCTA	
L-FABP-F	ATTGCGATGGGACCCC	539
L-FABP-R	TTAACTTCACTGCCAAGTT	
L-FABP-qF	CAAGGACATCAAGCCAATTACTG	100
L-FABP-qR	AATGGTAAAGGAATTGGTCACAG	

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  1 GTTACTCATTACACATTGCGATGGGACCCCTTTGCCTTCCAGTATAAGAAGGTTTGGTAG  60
 61 CACATTCACATTCTCCACATTGTGTTGAGCTTCACACAGCTGTCTCAGCCTCCACTCCAC 120
121 TTTGGTGAAGGAGATCCCAGACCTTCTAGAGAAGatggacttcaatggaacatggcaggt 180
  1                                   M  D  F  N  G  T  W  Q  V   9
181 ttactctcaggagaattacgagtcgttcctcagggccatggaactcccagaagatgtcat 240
 10  Y  S  Q  E  N  Y  E  S  F  L  R  A  M  E  L  P  E  D  V  I  29
241 caagatggccaaggacatcaagccaattactgagatcaaacagagtggcaatgactttgt 300
 30  K  M  A  K  D  I  K  P  I  T  E  I  K  Q  S  G  N  D  F  V  49
301 tgtcacctccaagacccctggaaagtctgtgaccaattcctttaccattggtaaggaggc 360
 50  V  T  S  K  T  P  G  K  S  V  T  N  S  F  T  I  G  K  E  A  69
361 tgaaatcaccaccatggacggcaagaagctcaagtgcatcgtcaatctggagggtggcaa 420
 70  E  I  T  T  M  D  G  K  K  L  K  C  I  V  N  L  E  G  G  K  89
421 aatggtgtgcaagactggcaagttctgccacatccaagagctcaagggaggagagatggt 480
 90  M  V  C  K  T  G  K  F  C  H  I  Q  E  L  K  G  G  E  M  V  109
481 tgagacattgaccatgggctcaacaactctcgtcaggaagagcaaaaagatgtaaACTTG 540
110  E  T  L  T  M  G  S  T  T  L  V  R  K  S  K  K  M  *        126
541 GCAGTGAAGTTAACCAATGTCTAATAAAAGTGTTGCTTGATAACAAAAAAAAAAAAAAA 600
601 AAAA                                                         604
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Fig. 13.1 Nucleotide sequence and deduced amino acid of *L-FABP* gene in *T. ovatus* (Zhou et al. 2019)

point of 8.73 theoretical isoelectric, and a weight 14.06 kDa predicted molecule (Fig. 13.1). The deduced protein sequence has the characteristics of cytoplasmic fatty acid-binding protein, as shown in the multiple sequence alignment, and this domain was found in all detected sequences (Fig. 13.2). Multiple sequence alignments showed that the L-FABP of *T. ovatus* was highly identical with other known orthologs (Fig. 13.2). The L-FABP sequence of *T. ovatus* ginseng is 76.61% identical to zebra fish liver bile acid-binding protein (PDB ID: 2qo4). Then, there are ten antiparallel β-sheets forming a hydrophobic pocket.

Table 13.2 shows the multiple sequence alignment of some known L-FABP family with the deduced amino acid sequences of L-FABP genes. The predicted amino acid sequence of L-FABP genes from *T. ovatus* had high identity and similarity with *Epinephelus coioides* (95.2% and 97.6%, ADG29164.1) and had different similarity (62.2–98.4%) and identity (40.9–84.1%) with other species (Table 13.2). Similar to the FABP of other species, the L-FABP in golden pompano can actively participate in the transport of fatty acids and other fat-soluble substances

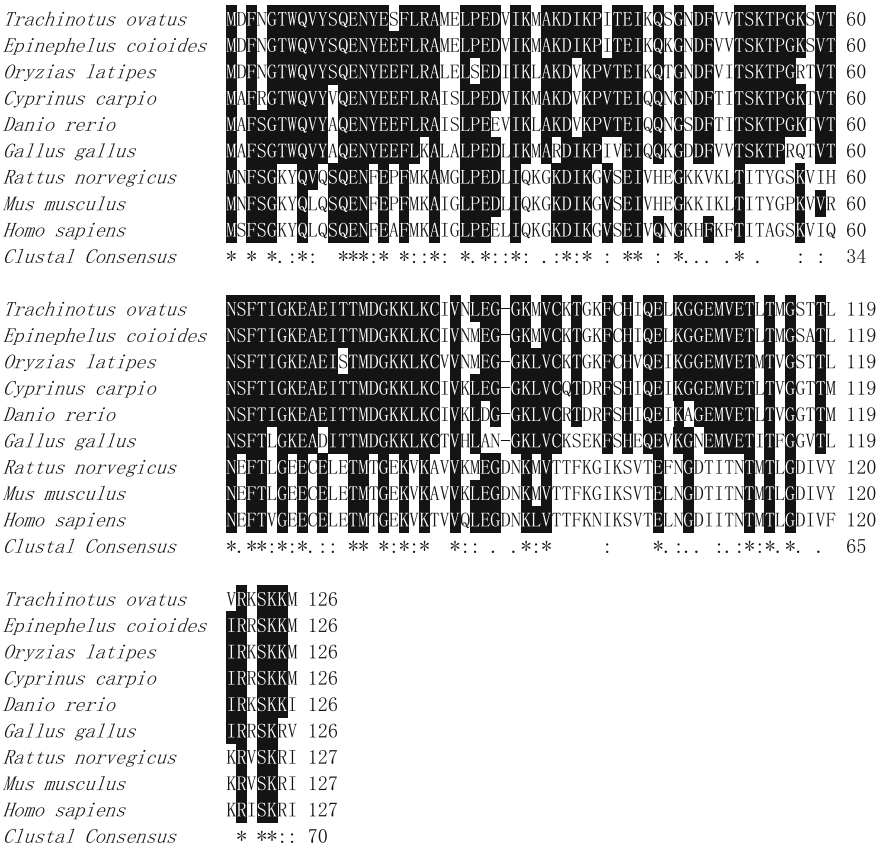

Fig. 13.2 Aligned of L-FABP with other known homologous H-FABP amino acid sequences in *T. ovatus* (Zhou et al. 2019)

Table 13.2 Multiple sequence alignment of L-FABP genes in golden pompano (Zhou et al. 2019)

Species	Accession NO.	AA	Similarity (%)	Identity (%)
Trachinotus ovatus	Present study	126	–	–
Epinephelus coioides	ADG29164.1	126	97.6	95.2
Oryzias latipes	XP_004078356.1	126	98.4	84.1
Cyprinus carpio	ACA64701.1	126	92.1	80.2
Danio rerio	NP_694492.1	126	92.9	76.2
Gallus gallus	NP_989965.1	126	87.3	70.6
Rattus norvegicus	NP_036688.1	127	62.2	40.9
Mus musculus	NP_059095.1	127	62.2	41.7
Homo sapiens	NP_001434.1	127	63.8	40.9

in cells, assigning fatty acids to different metabolic pathways (Hsu and Storch 1996; Andre et al. 2000; Venold et al. 2013; Storch and Corsico 2008).

13.3 Expression of L-FABP Genes in *T. ovatus*

On 18 DPH, the expression level of *T. ovatus* L-FABP gene in the heart, muscle, stomach, intestine, eye, spleen, head kidney, gill, and brain was similar and significantly lower than that in the liver ($P < 0.01$, Fig. 13.3). The expression level of L-FABP gene has been observed from the embryo stage to adult stage in zebra fish (Her et al. 2003). The study is rare on the expression level of L-FABP gene during early life of commercially cultured larval fish. During the embryogenesis of chicks and Japanese quails, a small amount of L-FABP mRNA is identified in the liver and intestinal tissues (Murai et al. 2009). The L-FABP gene expression level was low at hatching, but it continued to increase significantly from 0 DPH to 4 DPH (Fig. 13.3). The expression of L-FABP rapidly raised starting from 4 DPH and reached a high level and remained at a stable level until 18 DPH when the experiment was complete. Such expression pattern suggests that the *T. ovatus* L-FABP gene in larvae expressed before the digestive tract developed, as the digestive system of *T. ovatus* expressed before the development of the digestive tract, as the digestive system was immature at hatch, and a mature digestive system emerged around 15 DPH (Ma et al. 2014). Additionally, the upregulation of L-FABP expression may be connected with the uptake of dietary fatty acids after a fully mature digestive tract developed in *T. ovatus* larvae (Ma et al. 2014).

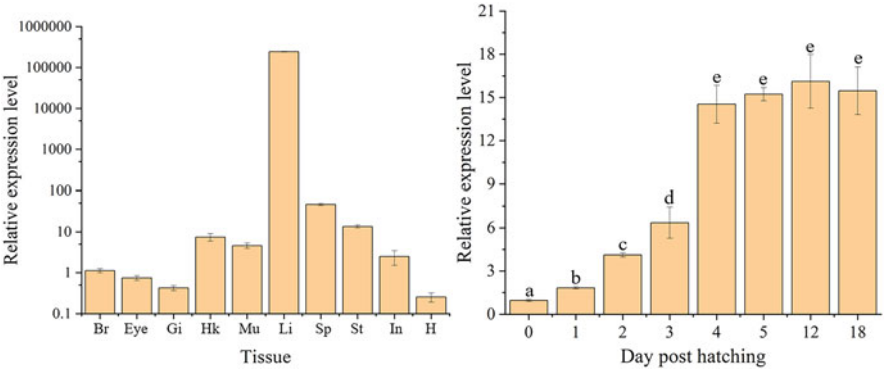

Fig. 13.3 Tissue and ontogenetic expression of L-FABP in *T. ovatus* larvae (Zhou et al. 2019)

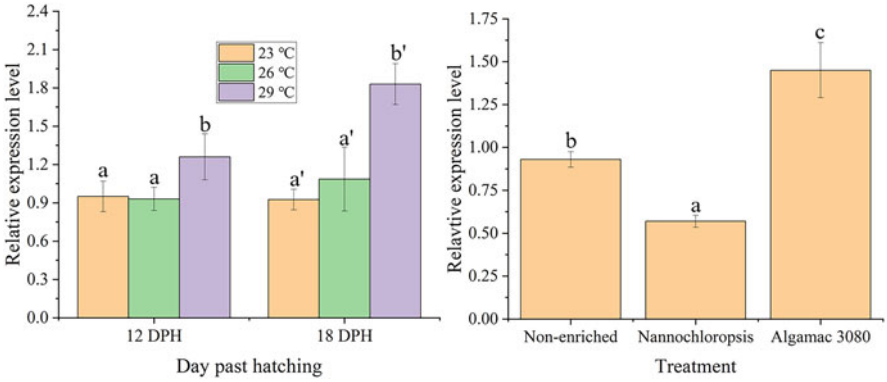

Fig. 13.4 Effects of temperature and nutrient enhancement on the expression of L-FABP gene in *T. ovatus* larvae (Zhou et al. 2019)

13.4 Temperature and Nutrient Enhancement Regulates the Expression of L-FABP Genes

Although genetic factors can control fish growth, fish development is also regulated by environmental parameters. As an essential environmental factor, the temperature can cause significant impact in fish metabolism and feeding activity (Ma et al. 2014), and water temperature can significantly affect the digestive function of fish larvae (Liu et al. 2017; Hevrøy et al. 2012). Fatty acid metabolism and fatty acid composition of fish can be regulated by temperature (Kemp and Smith 1970; Skalli et al. 2006; Farkas et al. 1980), but it is not clear whether temperature could impact the expression level of L-FABP gene in the early developmental stage of larval fish. In golden pompano, the expression level of the L-FABP gene has significant difference in different water temperatures on 12 and 18 DPH (Fig. 13.4). Compared to 12 DPH, a higher expression level of the L-FABP gene was noticed at 18 DPH, which may reveal the developmental process of the digestive tract in fish larvae, as the digestive system of *T. ovatus* seems to be more functional at 18 DPH (Ma et al. 2014).

FABP can affect gene regulation and activation of peroxisome proliferator-activated receptors, leading to the decline of the expression of lipid-related genes (Tan et al. 2002; Lawrence et al. 2000). Stimulation is not always the primary determinant as it may only stimulate the expression of the L-FABP gene slightly (Atsushi et al. 2009). In addition, it may be caused by the start of first feeding after yolk absorption, and the L-FABP gene expression level did not alter after incubation (Atsushi et al. 2009). In golden pompano, the L-FABP gene expression level had significant difference in different nutritional enhancement. The highest expression level of the L-FABP gene was observed in the Algamac 3080 treatment group, while the lowest expression level was found in the *Nannochloropsis* group (Fig. 13.4). This expression may have a parabolic relationship with the diet total saturated fatty

acid content. In the Algamac 3080 group, the high level of diet fatty acid content may facilitate the expression of the L-FABP gene in *T. ovatus* (Yang et al. 2015).

13.5 Conclusion

The expression of L-FABP gene in *T. ovatus* was significantly affected by temperature and nutrient treatments. The tissue-dependent and time-dependent expressions of the L-FABP gene in larval fish are essential for understanding the ontogeny and growth of fish during their early stage. The monitoring of L-FABP gene expressions in larval *T. ovatus* may serve as an effective indicator to assess the response of fish to the change of nutritional and environmental conditions during fish early development.

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