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Two Δ6-desaturase-like genes in common carp (*Cyprinus carpio* var. Jian): Structure characterization, mRNA expression, temperature and nutritional regulation

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ABSTRACT

Δ6-Desaturase is the rate-limiting enzyme involved in highly unsaturated fatty acid (HUFA) biosynthesis. There is very little information on the evolution and functional characterization of Δ6Fad-a and Δ6Fad-b in common carp (*Cyprinus carpio* var. Jian). In the present study, the genomic sequences and structures of two putative Δ6-desaturase-like genes in common carp genome were obtained. We investigated the mRNA expression patterns of Δ6Fad-a and Δ6Fad-b in tissue, hatching carp embryos, larvae by temperature shock and juveniles under nutritional regulation. Our results showed that the two Δ6Fad genes had identical coding exon structures, being comprised of 12 coding exons, and with introns of distinct size and sequence composition. They were not allelic variants of a single gene. Both Δ6Fad genes were highly expressed in liver, intestine (pyloric caeca) and brain. The Δ6Fad-a and Δ6Fad-b mRNAs showed an increase in expression from newly hatched to 25 days after hatching. The expression levels of Δ6Fad-a were obviously regulated by temperature, whereas Δ6Fad-b was not affected by temperature. The regulation of Δ6Fad-a and Δ6Fad-b in response to dietary fatty acid composition was determined in liver, brain and intestine (pyloric caeca) of common carp fed with diets: diet1with fish oil (FO) rich in n−3 HUFA, diet2 with corn oil (CO, 18:2n−6) and diet3 with linseed oil (LO, 18:3n−3). The differential expression of Δ6Fad-a and Δ6Fad-b genes in liver, brain and intestine in common carps was fed with different oil sources, respectively. Further work is in progress to determine the mechanism of differential expression of the Δ6Fad-a and Δ6Fad-b genes in different tissues and the roles of transcription factors in regulating HUFA synthesis.

1. Introduction

Δ6-Desaturase enzyme is the rate-limiting enzyme involved in HUFA biosynthesis, which is responsible for the first step of the desaturation/elongation process in HUFA synthesis, converting 18:3n−3 (LNA) and 18:2n−6 (LA) to 18:4n−3 and 18:3n−6, respectively. The Δ6-desaturase enzyme is also involved in the synthesis of 22:6n−3 (DHA) from 20:5n−3 (EPA) (Brenner, 1981a,b). Full-length cDNAs for Δ6-desaturases have been isolated from some freshwater fish, including a bifunctional Δ6/Δ5 desaturase from zebrafish (*Danio rerio*) (Hastings et al., 2001) and separate, distinct Δ6 and Δ5 desaturases from Atlantic salmon (*Salmo salar*) (Hastings et al., 2005), and some marine fish, including sea bream (*Sparus aurata*), cod (*Rachycentron canadum*), and turbot (*Scophthalmus maximus*). It is generally thought that freshwater fish have a capacity to convert C_{18} PUFA to $C_{20/22}$ HUFA. But the extent to which fish convert C_{18} PUFA to $C_{20/22}$HUFA varies with species, and is associated with their capacity for fatty acyl desaturation and elongation (Tocher et al., 2003).

The Δ6-desaturase enzyme is involved in the HUFA biosynthesis and is under nutritional and water temperature regulation (Francis et al., 2009; Izquierdo et al., 2008; Tocher et al., 2004; Vagner et al., 2007a,b). It was reported that desaturase transcript level in freshwater fish as well as in mammals increased when FO was replaced by VO in diet (Bell et al., 2001; Tocher et al., 2001, 2002, 2003, 2004, 2006; Zheng et al., 2005). However, some studies have shown that HUFA can inhibit the Δ6-desaturase in freshwater fish as well as in mammals (Garg et al., 1988; Christiansen et al., 1991; Ulmann et al., 1992). Several studies reported that the increase in the degree of fatty acid unsaturation at low temperature related to changes in both desaturase and elongase capacities (Schünke and Wodtke, 1983; Hagar and Hazel, 1985; Wodtke and Cossins, 1991). It has been shown in several freshwater fish species

Abbreviations: HUFA, highly unsaturated fatty acid; Δ6Fad, Δ6 fatty acid desaturase; FO, fish oil; CO, corn oil; LO, linseed oil; LNA, linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; DAH, days after hatching.

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that the Δ6-desaturase activity decreases when temperature increases (Schünke and Wodtke, 1983; Hagar and Hazel, 1985; Tocher et al., 2004). However, less information on its fatty acid desaturation in common carp is available.

The number of chromosome in common carp is twice as that of other carps, so it may have two different genes coding the same protein or peptides in common carp (Ohno et al., 1968). While different enzymatic activities of Δ6Fad-a, Δ6Fad-b and Δ6Fad-c found in Atlantic salmon have been clearly discriminated for their functional roles (Monroig et al., 2010; Zheng et al., 2005). Common carp (*Cyprinus carpio*) is commonly regarded as tetraploid due to its high chromosome number and DNA content. This has led to the hypothesis that common carp may have a complicated mechanism in the regulation in HUFA biosynthesis. In the previous study, we have cloned the two Δ6-desaturase-like genes (termed Δ6Fad-a and Δ6Fad-b) in common carp (Ren et al., 2012). There is still paucity of knowledge on the two Δ6-desaturase-like genes. As a pre-requisite to the roles and mechanisms of the Δ6Fad-a and Δ6Fad-b genes on the HUFA synthesis, we investigated the mRNA expression patterns of Δ6Fad-a and Δ6Fad-b in hatching common carp embryos and larvae, and at the same time, they were also investigated under temperature shock in common carp larvae and under nutritional regulation in common carp juvenile.

The results may be helpful for understanding the evolution and functional characterization of Δ6Fad-a and Δ6Fad-b genes in the biosynthesis of HUFA in common carp, and be beneficial to reveal the molecular mechanism of HUFA biosynthesis in polyploid fish.

2. Materials and methods

2.1. Experiment fish and sample collection

The Common carps were captured from Yi Xing base of Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences. The carp embryos were isolated and raised at 28 °C, then periodically collected at 1, 12, 24, 48, 72 and 96 h post-fertilization (hpf). Carp larvae were bred and sampled at 1, 5, 10, 15, 20, 25 and 30 days after hatching (DAH). The embryos and larvae collected were frozen in liquid nitrogen and stored at −70 °C until RNA extraction.

Samples were collected from common carp juveniles (~40 g) to study tissue distribution of Δ6Fad expression. Intestine, liver, muscle, kidney, heart and brain were dissected from six carps and frozen at −70 °C prior to RNA extraction. Total RNA was extracted using the Trizol Reagent Kit (Takara, Japan), and 2 μg of total RNA reverse transcribed into cDNA using Reverse Transcriptase M-MLV (Takara, Japan). PCR was carried out with primer pairs: Δ6FadaF–Δ6FadaR, Δ6FadbF–Δ6FadbR and β-actinF–β-actinR (Table 1). The PCR products were electrophoresed in 1% agarose gels, and observed with a Kodak Image Station (Kodak, 2000R).

The common carp larvae aged 20 days after hatching bred in six glass tanks (3 replicate tanks for each treatment) were connected to a recirculation system with automatic temperature control, which exposed to high water temperature (32 °C) and low water temperature (10 °C) shock for 96 h. Six common carp larvae sampled on temperature shock of 0 h, 12 h, 24 h, 48 h, 72 h and 96 h from per glass tank were frozen at −70 °C prior to RNA extraction.

The experiment investigating the effects of diet on Δ6Fad expression was performed with common carp juveniles (45–50 g), which were bred in nine glass tanks (3 replicate tanks for each treatment) and were fed three diets with the same basal composition (35% protein and 6% oil) but formulated with different oils including fish oil (FO, rich in 20:4n−6, 22:6n−3 and 20:5n−3), corn oil (CO, rich in 18:2n−6) or linseed oil (LO, rich in 18:3n−3), respectively. At the end of the experiment, liver, brain and intestine (pyloric caeca) of nine fish per dietary treatment were dissected and frozen at −70 °C prior to RNA extraction.

Table 1
Primers for genomic and determining mRNA content of Δ6Fad-a and Δ6Fad-b in common carp (*C. carpio* var. Jian).

Aim	Transcript	Primer	Primer sequence (5′ → 3′)
Genomic cloning	Δ6Fad-a	Δ6Fad-e1F-a	ATGGGTGGCGGAGGACAGCAG
		Δ6Fad-e2R-a	GCTTCATGTATTTCCTCACCAGCGG
		Δ6Fad-e4F-a	GATGGCTGCAGCATGACTTCG
		Δ6Fad-e5R-a	CATGTTGAGCATGTTGACATCCG
		Δ6Fad-e5F-a	GCATCACGCTAAACCGAACGTG
		Δ6Fad-i8R-a	CTCAGCCAGTGAGAATGCATTTGG
		Δ6Fad-e8F	CCTGTGTTACACGCAGTACTACGGTGT
		Δ6Fad-i9R-a	TGACTTAAACCCAGTGCGGCTGT
		Δ6Fad-E9F	CAGATGAGCCACATCCCCATG
		Δ6Fad-E11R	CCTGCAAGGTCTTCTCTTGGTACTTG
		Δ6Fad-i10F-a	TGGCTTCAGTTTATCTTCGGCAT
		Δ6Fad-e12R	GTACGCATCCAGCCAGATTTCTCC
	Δ6Fad-b	Δ6Fad-1eF-b	GTACCAATGGGAGGTTCGGCAC
		Δ6Fad-2eR-b	GAGTTGAAGGTTTGGATGAAATGCATG
		Δ6Fad-2eF-b	CATGCATTTCATCCAAACCTTCAACTC
		Δ6Fad-4iR-b	GCTAATGAGCTGTATTCCTGGAACCATC
		Δ6Fad-4eF-b	AACTCACGATGGGATCACTTACTGC
		Δ6Fad-5eR-b	CGCATTGAGCATGTTGACGTCT
		Δ6Fad-5eF-b	AGACGTCAACATGCTCAATGCG
		Δ6Fad-7eR-b	TACACCGGAATGAGCAGAGGAGG
		Δ6Fad-e8F	CCTGTGTTACACGCAGTACTACGGTGT
		Δ6Fad-i9R-b	GTTGCAACCAGCTAAATGACAGAAATG
		Δ6Fad-E9F	CAGATGAGCCACATCCCCATG
		Δ6Fad-E11R	CCTGCAAGGTCTTCTCTTGGTACTTG
		Δ6Fad-i10F-b	TAGTTTCAGTGCAAAAATGGCTGCTTC
		Δ6Fad-e12R	GTACGCATCCAGCCAGATTTCTCC
Gene expression analysis	Δ6Fad-a	Δ6Fada F	ATCGGACACCTGAAGGGAGCG
		Δ6Fada R	CATGTTGAGCATGTTGACATCCG
	Δ6Fad-b	Δ6Fadb F	GTACCAATGGGAGGTTCGGCAC
		Δ6Fadb R	GAGTTGAAGGTTTGGATGAAATGCATG
	β-actin	β-actinF	CGCCCCAGACATCAGGGTG
		β-actinR	CACAGATCATGTTTGAGACCTTCAACAC

2.2. Genomic cloning for fatty acyl desaturases

Total DNA was extracted from the vein blood of common carp, using the Universal Genomic DNA Extraction Kit Ver.3.0 (Takara, Japan). Alignment of the full-length of Δ6Fad-a and Δ6Fad-b cDNAs with zebrafish genomic DNA sequence of Δ6Fad predicted the intronic position. PCR was performed to amplify genomic DNA fragment of Δ6Fad-a and Δ6Fad-b using primers (Table 1), which were designed on the full-length of Δ6Fad-a and Δ6Fad-b cDNAs and the intronic sequences cloned. PCR amplifications were performed with an initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation step at 94 °C for 30 s, annealing at T_m (annealing temperature according to different primers) for time (annealing time according to size of DNA fragment), and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 8 min. The gel purified PCR products were cloned into the pMD18-T Vector (Takara, Japan) and the positive clones were sequenced in Biosune Inc., China. Finally, the two full-lengths of Δ6Fad genomic DNA were constructed by aligning the genomic DNA fragments.

2.3. RNA extraction and real time quantitative PCR (RT-qPCR)

Total RNA was extracted using the Trizol Reagent Kit (Takara, Japan), and 2 μg of total RNA was reversely transcribed into cDNA using PrimeScript RT-PCR Kit (Takara, Japan). Expression of the genes was measured by real-time quantitative PCR (RT-qPCR) (SYBR Green II) on a Thermal Cycler Dice TP800 sequence detection system (Takara, Japan), using *β-actin* as a housekeeping gene. Primers for gene expression analysis (Table 1) were designed based on the full-length of Δ6Fad-a and Δ6Fad-b cDNAs. Each sample was run in triplicate, and PCR reaction without the addition of template was used as negative controls. The relative mRNA expression level of Δ6Fad-a and Δ6Fad-b in each sample was normalized with *β-actin* expression and calculated with the comparative threshold cycle (Ct) method (Pfaffl, 2001).

2.4. Statistical analysis

For the relative expression ration of each gene in the fish, results were expressed as mean ± standard error of mean (SEM). Differences in the expression of Fad gene were analyzed by one way ANOVA followed by Tukey's multiple comparison. All statistical analyses were computed using SPSS v17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Common carp Δ6Fad-a and Δ6Fad-b gene structure

PCR was performed to amplify genomic DNA fragment of Δ6Fad-a and Δ6Fad-b using primers, which were designed on the full-length of Δ6Fad-a and Δ6Fad-b cDNAs and the intronic sequences cloned. The DNA fragment sequences were spliced and isolated, while the Δ6Fad-a and Δ6Fad-b DNA sequences were cloned both being comprised of 13 exons and 12 introns. Comparison of the cDNAs and genomic sequences for Δ6Fad-a and Δ6Fad-b enabled the identification of an upstream 5′ non-coding exon. The two Δ6Fad genes had identical coding exon structures, being comprised of 12 coding exons, with a high degree of sequence identity (Fig. 1). Identification of these as separate genes was confirmed as each had introns of distinct size and sequence composition. Clearly, they were not allelic variants of a single gene (Table 2). Intron regions could be aligned with sequences of RepBase (http://www.girinst.org/repbase/index.html) entries for various DNA transposons (Fig. 1).

3.2. Δ6Fad-a and Δ6Fad-b gene expression of different tissues

The tissue-specific expressions of Δ6Fad-a and Δ6Fad-b mRNAs were consistently detected in common carp. A high expression was observed in the liver, brain and intestine (pyloric caeca), a weak expression was encountered in the heart, whereas the expression in the kidney and muscle was too low to be detected under the current RT-PCR condition (Fig. 2).

3.3. Temporal expression of Δ6Fad-a and Δ6Fad-b genes during common carp embryonic and larval development

Temporal expressions of two Δ6Fad genes in different embryonic stages were analyzed using RT-qPCR. As for the two Δ6Fad genes, a significant high expression levels were detected at early stages of 1hpf, but there was a significant decrease in the two Δ6Fad gene expressions from 12hpf to 48hpf, and then a significant increase in both gene expressions from 72hpf to 96hpf (Fig. 3).

Table 2
Exon and intron sizes (bp) of Δ6Fad-a and Δ6Fad-b in Common carp (*C. carpio* var. Jian).

	Exon sizes (bp)		Intron sizes (bp)	
	Δ6Fad-a	Δ6Fad-b	Δ6Fad-a	Δ6Fad-b
1	58[a]	55[a]	878	849
2	245[b]	245[b]	1102	1574
3	111	111	1321	1492
4	198	198	221	202
5	102	102	1880	2676
6	126	126	78	87
7	61	61	810	778
8	77	77	108	108
9	98	98	1716	2448
10	97	97	77	76
11	80	80	76	87
12	126	126	1281	904
13	606[c]	570[d]		

[a] Exons are 5′ untranslated regions (UTRs).
[b] Includes a 5′-UTR of 38 bp.
[c] Includes a 3′-UTR of 554 bp.
[d] Includes a 3′-UTR of 518 bp.

Selected stages of 1 to 30 DAH larvae were subjected to RT-qPCR. We demonstrated here an ontogenic-related of the two Δ6Fad genes from 1 DAH larvae onwards, peaking at 20–25 DAH. There was a drop in expression levels at 25–30 DAH stage. From 1 to 10 DAH, the expression levels of Δ6Fad-b were higher than those of Δ6Fad-a, but from the 20–30 DAH larvae, the expression levels of Δ6Fad-a were higher than those of Δ6Fad-b (Fig. 4).

3.4. Expression of Δ6Fad-a and Δ6Fad-b genes under temperature shock

Temporal expressions of the two Δ6Fad genes in common carp larvae exposed to 32 °C and 10 °C were analyzed using RT-qPCR. It was observed that the carp larvae exposed to 32 °C, showed a significant increase in Δ6Fad-a gene expression from 12 h to 24 h, and then a significant decrease from 24 h to 96 h. A significant increase in Δ6Fad-a gene expression was found in common carp larvae exposed to 10 °C from 24 h onwards. However, the expression levels of Δ6Fad-b were not obviously affected by the temperature (Fig. 5).

3.5. Expression of Δ6Fad-a and Δ6Fad-b genes under nutritional regulation

The regulation of Δ6Fad-a and Δ6Fad-b in response to dietary fatty acid composition was determined in liver, brain and intestine (pyloric caeca) of common carp fed with diets containing either FO rich in n−3

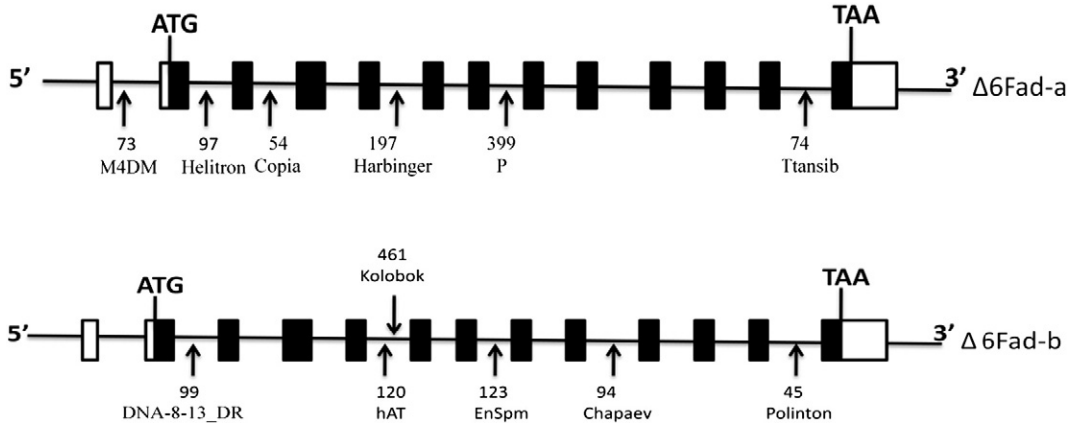

Fig. 1. Structure of Δ6Fad-a and Δ6Fad-b in common carp (*C. carpio* var. Jian). Gene structure was determined from genomic sequence from a single fish. Sequenced coding exons are indicated by black boxes. Sequenced coding introns are indicated by a black line. Non-coding exon regions are indicated by unfilled boxes. The positions, sizes (base pairs) and similarity hit to RepBase (http://www.girinst.org/repbase/index.html) of intron regions for various DNA transposons.

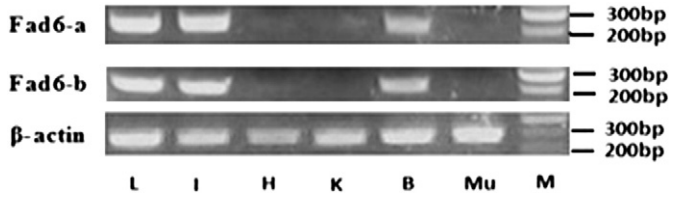

Fig. 2. Tissue-specific expression of Δ6Fad-a and Δ6Fad-b in common carp (*C. carpio* var. Jian) by RT-PCR. L, liver; B, brain; H, heart; K, kidney; I, intestine; Mu, Muscle; and M, marker.

HUFA (EPA and DHA) or VOs rich in C_{18} fatty acids, CO (LA, 18:2n − 6) and LO (LNA, 18:3n − 3). In the liver and brain, there were significantly higher expressions of Δ6Fad-a transcripts in CO-fed common carp compared with the fish fed with FO or LO (Fig. 6, $P < 0.05$). However, in intestine, there was a significantly higher expression of Δ6Fad-a transcripts in LO-fed common carp compared to the fish fed with CO or FO (Fig. 6, $P < 0.05$ and $P < 0.01$). No significant differences were found in transcript levels of Δ6Fad-a in liver and brain between fish fed with FO or LO. The levels of Δ6Fad-b were also higher in liver and intestine of the fish fed with LO compared to the fish fed with CO or FO, but in brain, the result was reverse.

4. Discussion

Genomic characterization showed that the two common carp Δ6Fad genes both have identical genomic coding exon structures consisting of 12 coding exons, with splice and acceptor sites interrupted at identical nucleotide positions within highly conserved codons. They differ in the identity and positions of 5′ non-coding exons, and in size and sequence of 3′ non-coding regions. The genomic coding organization is identical to the previously reported for the Atlantic salmon Δ6Fad-a gene and the human FADS2 (Δ6-desaturase), which also consist of 12 coding exons. Excluding the UTR, the remaining exons are exactly the same size in common carp two Δ6 Fad genes as the salmon and the human Δ6Fad gene (FADS2), with splice and acceptor sites interrupted at similar nucleotide positions, despite the lengths of the introns being very different (Marquardt et al., 2000; Zheng et al., 2005). Although the common carp Δ6-desaturase-like genes show several features, particularly in the intron structure, they may reveal important information on the evolutionary history of Δ6-desaturase-like gene common carp and fish generally. The differential splicing of non-coding exons, and the size and structure of introns may have possible distinct functional consequences. Comparison of the upstream sequences of Δ6Fad-a and Δ6Fad-b revealed a low degree of similarity, with a very different 5′ non-coding exon structure, including the utilization of alternative splice sites, which shows that the two Δ6Fad genes may act as differently functional and PUFA responsive promoters. Based on these observations it is plausible to hypothesize that Δ6Fad-a may be regulated

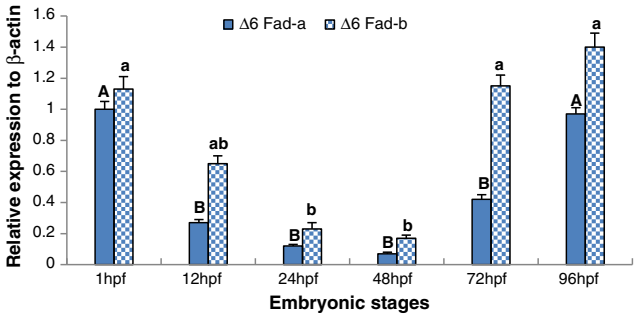

Fig. 3. Relative expression levels of Δ6Fad-a and Δ6Fad-b in different embryonic stages. Expression values were normalized to those of *β-actin*. And data are expressed as mean ± standard error of mean (SEM) (n = 20) from fertilized eggs. Mean value with different alphabets within similar gene are significantly different ($P < 0.05$).

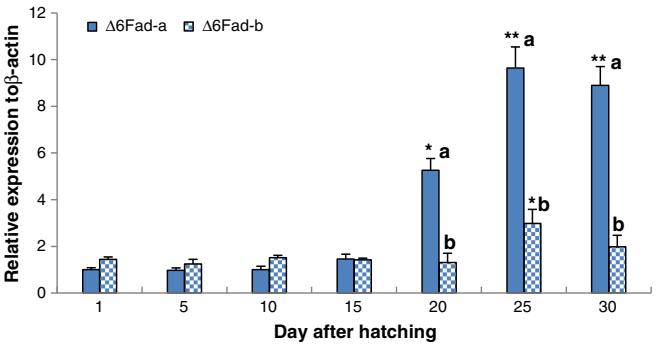

Fig. 4. Relative expression levels of Δ6Fad-a and Δ6Fad-b in different stages of larval developments. Expression values were normalized to those of *β-actin*. And data are expressed as mean ± SEM (n = 6). Asterisks represent significant differences within similar gene, * $P < 0.05$ and ** $P < 0.01$. Different alphabets are significantly different at the same time point ($P < 0.05$).

differently with Δ6Fad-b, which needs to be further clarified. The result is in accordance with that of Δ6Fad-b which may be regulated similarly to Δ6Fad-a, and that Δ6Fad-c is regulated differently, which have been found in Atlantic salmon (Monroig et al., 2010; Zheng et al., 2009a).

In the study, the two Δ6-desaturase mRNAs in common carp were mainly detected in the liver, intestine and brain. In agreement with previous studies on freshwater fish, our results show that the genes in common carp are predominantly expressed in liver, intestine and brain implicating these tissues as the most active in HUFA biosynthesis (Morais et al., 2009). This is consistent with liver and intestine being the major sites of lipid synthesis and distribution. Furthermore, liver and intestine have been described to be the primary tissue for HUFA synthesis in Atlantic salmon (Bell et al., 2001, 2003).

Temporal expression of Δ6Fad-a and Δ6Fad-b in different common carp embryonic stages was analyzed. The expression levels of both genes were found to be decreasing at first, and then increasing, suggesting that Δ6-desaturase-like genes in common carp embryo may come from the matrix at the early stages of 1hpf. Furthermore, the significant increase in both gene expressions from 72hpf to 96hpf was mainly due to embryonic ontogeny. The highlights that the maternal role in HUFA supply to fish embryos are not only the transfer of performed HUFA, but also the transfer of mRNA transcripts that can potentially be translated to active proteins (Mazorra et al., 2003; Rodríguez et al., 1998). The expression of Δ6Fad-a and Δ6Fad-b genes continues to the end of embryogenesis (96 h), and so the pathway could be active throughout to assure the high demands of forming tissues such as brain and retina for HUFA (Monroig et al., 2009). There was an obviously increasing pattern in the expression of Δ6Fad-a compared to Δ6Fad-b in common carp larvae development, which informs the capacity of endogenous HUFA synthesis to support larvae development process, and that the Δ6Fad-a may have played a major role in the above process. An ontogenic gradual increase in fatty acyl desaturase gene expression was also reported in zebrafish and striped snakehead (Monroig et al.,

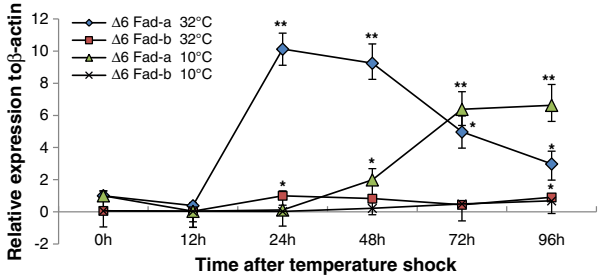

Fig. 5. Temporal expression of Δ6Fad-a and Δ6Fad-b relative to *β-actin* in common carp larvae exposed to 32 °C and 10 °C. Values are mean ± SEM (n = 6). Asterisks represent significant differences from the respective initial values, * $P < 0.05$ and ** $P < 0.01$.

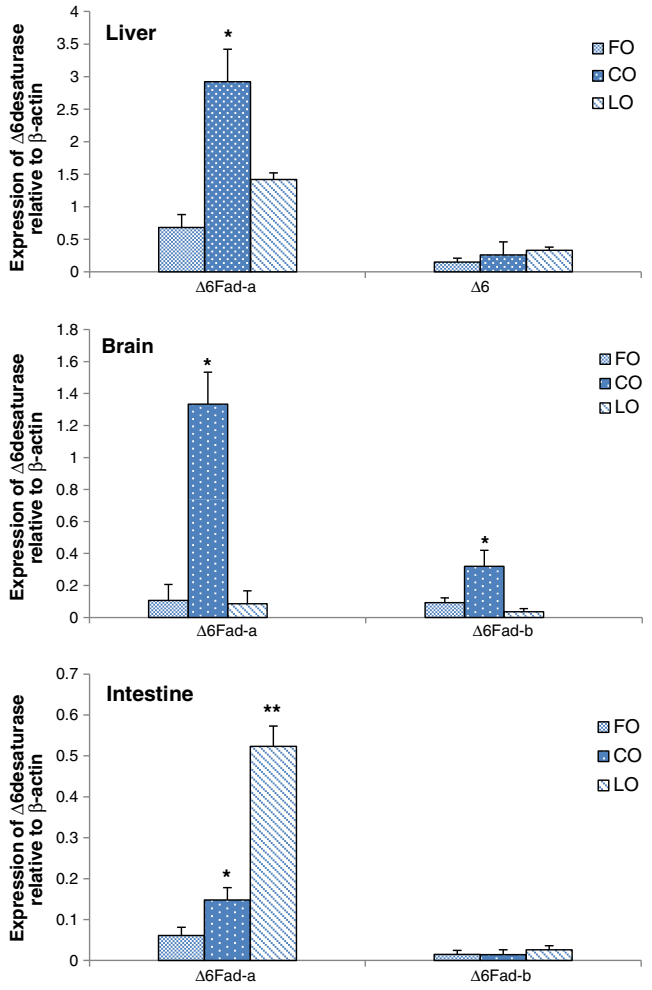

Fig. 6. Relative expression levels of Δ6Fad-a and Δ6Fad-b in the liver, brain and intestine from common carp (*C. carpio* var. Jian) fed diets containing fish oil (FO), corn oil (CO) or linseed oil (LO), respectively. Values are means ± SEM (n = 3). Asterisks represent significant differences within similar gene. * $P < 0.05$ and ** $P < 0.01$.

2009; Ram et al., 2011; Tan et al., 2010). Interestingly, there was a drop in mRNA level in 30DAH common carp larvae. The reason for this is unclear, as several factors including level of dietary HUFA intake and deposition and regulation by transcription factors may play a part. For example, in gilthead seabream larvae, the Δ6Fad appeared to be regulated by dietary HUFA at transcriptional level (Izquierdo et al., 2008). A persistent Δ6Fad mRNA enhancement in European seabass larvae with an n−3 HUFA-deficient larval diet, possible through the control of the peroxisome proliferator-activated receptors (PPARs) (Vagner et al., 2009). The need for HUFA supplementation in larviculture of freshwater fish could be related to HUFA biosynthesis capacity during development (Czesny et al., 1999; Tocher, 2010). Further work to characterize the functional role of Δ6Fad-a will be needed to fully comprehend its expression pattern during larval development and the potential application for larviculture of common carp.

Several studies have reported that low water temperature can increase the Δ6-desaturase gene expression and enzymatic activity in teleosts (Bell et al., 1997; Tocher et al., 2000, 2004). However, the Δ6-desaturase activity decreases in several freshwater fish as water temperature increases (Hagar and Hazel, 1985; Tocher et al., 2004). The Δ6Fad activity was also higher in enterocytes and hepatocytes of rainbow trout kept at 5 °C or 7 °C than in trout kept at 20 °C or 15 °C, respectively (Hagar and Hazel, 1985; Tocher et al., 2004). Similarly, the Δ6Fad activity in liver microsomes has been shown to be 2-fold higher at 16 °C than at 30 °C (Ninno et al., 1974). These results together with the present study suggest that the low temperature can promote the Δ6Fad-a gene transcript level. However, the expression levels of Δ6 Fad-b were not obviously affected by temperature. Determining how the Δ6Fad genes are differentially regulated by temperature will be an interesting challenge, and presumably related to the expression, activities and regulation of transcription factors that could include sterol regulatory element binding protein-1c, liver X receptor and, possibly, peroxisome proliferator activated receptors and retinoid X receptors, or the transcription factors in the Δ6Fad genes promoter (Leaver et al., 2008; Zheng et al., 2009b). Further studies are required to clarify the regulatory mechanism of Δ6Fad-a and Δ6Fad-b gene expressions in common carp by the temperature. The study also showed that the induction timing of Δ6Fad-a gene transcription could depend on temperature. When a temperature change occurred, the response time for gene transcription adaption was 24 h. This result is in accordance with the study (De Torrengo and Brenner, 1976). This phenomenon is known as homeoviscous adaptation, and it ensures that membrane function is unaltered during changes in water temperature (Robertson and Hazel, 1999).

The high expression of Δ6-desaturase in the liver relates to fatty acid metabolism, while the high expression of Δ6-desaturase in the intestine relates to the type of the fatty acids (Bell et al., 2003; Zheng et al., 2009a), and in the brain is consistent with the critical importance of high HUFA levels in neural tissue (Tocher et al., 2006; Zheng et al., 2009a). A Fad with dual Δ5/Δ6 activity involved in HUFA synthesis has been characterized in zebrafish (Hastings et al., 2001). In Atlantic salmon, three Δ6Fad genes have been found that Δ6Fad-a was expressed highly in intestine > liver > brain and Δ6Fad-b was expressed highly in brain > intestine > gill > liver whereas Δ6Fad-c has been expressed being very largely confined to brain. Furthermore, Δ6Fad-c expression showed no nutritional regulation whereas expression of Δ6Fad-a and Δ6Fad-b was significantly increased in liver and intestine, respectively, in fish fed diets with reduced levels of HUFA (Monroig et al., 2010). So the two Δ6Fad genes in common carp may have different functional roles in different tissues. The regulation of Δ6Fad-a and Δ6Fad-b in response to dietary fatty acid composition was determined in liver, brain and intestine (pyloric caeca) of common carp fed with the diets containing either FO rich in n−3 HUFA (EPA and DHA) or VOs rich in C_{18} fatty acids, CO (18:2n−6) and LO (18:3n−3) in the study. The result showed that Δ6Fad-a was the main Fad gene expressed, under nutritional regulated in liver, brain and intestine. In contrast, Δ6Fad-b was lower expressed and nutritionally regulated in that tissue. In the liver, brain and intestine, there was significantly higher expression of Δ6Fad-a transcripts in CO and LO-fed common carp compared with the fish fed with FO. There was a significantly higher expression of Δ6Fad-a transcripts in CO-fed common carp compared with the fish fed with FO and LO, however, in intestine, there was a significantly higher expression of Δ6Fad-a transcripts in LO-fed common carp compared to the fish fed with CO and FO. The different expressions of Δ6fad-a and Δ6fad-b in liver, brain and intestine of common carp fed the diets, with one being regulated by dietary fatty acids. All these results are in accordance with many studies indicating that a high LNA and LA up-regulated Δ6-desaturases gene expression (Francis et al., 2007; Li et al., 2008; Tocher et al., 2004; Turchini et al., 2006), and HUFA can inhibit the Δ6-desaturase in freshwater fish as well as in mammals (Christiansen et al., 1991; Garg et al., 1988; Ulmann et al., 1992). The fact that Δ6fad-a, the highest activity enzyme, is the gene with highest expression in liver, this is consistent with liver being the major tissue for regulating overall body fatty acid metabolism. Further studies are required to clarify the regulatory mechanism of Δ6Fad-a gene expression in common carp by dietary nutrition.

In summary, the present work successfully cloned two Δ6-desaturase-like genes (Δ6Fad-a and Δ6Fad-b) from common carp (*C. carpio* var. Jian). The two Δ6-desaturase-like genes comprise 12 coding exons that are identical to the Atlantic salmon Δ6Fad-a and the human Δ6-desaturase. Our results show that Δ6Fad-a and Δ6Fad-b have distinct expression patterns during the common carp embryonic development, at different larval stages, and under temperature shock and nutritional regulation.

The Δ6Fad-a and Δ6Fad-b mRNAs showed an increase in expression as larvae developed from newly hatched to 25 DAH. Genes for Δ6Fad-a and Δ6Fad-b were expressed in various tissues of common carp, and highly expressed in liver, brain and intestine. The expression levels of Δ6Fad-a were obviously regulated by the temperature, and both Δ6Fad-a and Δ6Fad-b gene expressions in liver, brain and intestine were significantly increased in common carp fed with VO compared with fish fed with FO. The Δ6Fad-a and Δ6Fad-b may have different functional roles in different tissues in common carp, as regulated by dietary nutrition. The Δ6fad-a, the highest activity enzyme, maybe the main gene with higher expression in liver, this is consistent with liver being the major tissue for regulating overall body fatty acid metabolism. Further work is in progress to determine the mechanism of differential expression of the Δ6-desaturase-like genes in different tissues and the roles of transcription factors in regulating HUFA synthesis.

Conflict of interest

All of the authors declare that there are no conflicts of interest.

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