

# Temperature regulates fatty acid desaturase and elongase at the transcriptional level and modulates the fatty acid profile in the early stage of the common carp (*Cyprinus carpio*)

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**Abstract:** This study explored the regulatory effect of temperature stress on fatty acid biosynthesis in the early stage of common carp (*Cyprinus carpio*) based on  $\Delta 6$ -fatty acids desaturase ( $\Delta 6FAD$ ) and elongase-5 (*ELOVL5*) gene expression and fatty acid composition. One-day-old carp larvae were selected, and after seven days of acclimatisation at 25 °C, the larvae were subjected to temperature stress for 96 h in water at 32 °C or 10 °C. In the post-larval stage, 30-day-old carp juveniles were selected and, after seven days of acclimatisation at 25 °C, were subjected to temperature stress for seven days in water at 32 °C or 10 °C. The results showed that common carp larvae could rapidly and highly express  $\Delta 6FAD$  and *ELOVL5* genes within 48 h at high temperature (32 °C) compared with the 0 h group ( $P < 0.05$ ), while gene expression began to gradually increase after 48 h at low temperature (10 °C). There was a significant improvement in C22:6n-3 and C20:5n-3 after 96 h at low temperature compared with the 0 h group ( $P < 0.05$ ). In common carp juveniles, the  $\Delta 6FAD$  gene in the intestine, brain and liver was sensitive to low temperature, but the *ELOVL5* gene in the intestine, brain and liver of common carp was sensitive to high temperature. The low temperature increased the amount of highly unsaturated fatty acids (HUFA) in the common carp juveniles. The results indicated that temperature could regulate the expression of the  $\Delta 6FAD$  and *ELOVL5* genes for HUFA production, as well as for participation in the biosynthesis of fatty acids in the body during the early development of common carp. The results of this study help clarify the regulatory effects of temperature on fatty acid biosynthesis during the early development of common carp.

**Keywords:**  $\Delta 6$ -fatty acids desaturase; elongase-5; gene expression; highly unsaturated fatty acids

Highly unsaturated fatty acids (HUFA) such as docosahexaenoic acid (DHA; 22:6n-3), arachidonic acid (ARA; 20:4n-6) and eicosapentaenoic acid (EPA; 20:5n-3) play important roles in the metabolism of animals, including the maintenance of cell membrane integrity and as cell signalling and tran-

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scription factor ligands (Jump 2002; Morais et al. 2012; Prchal et al. 2018). The HUFA in the bodies of fish mainly comes from feed supply and its own biosynthesis. As with all vertebrates, fish cannot synthesise linoleic (LA, 18:2n-6) and  $\alpha$ -linolenic (ALA, 18:3n-3) due to their lack of  $\Delta 12$  and  $\Delta 15$  desaturases. So, linoleic acid and  $\alpha$ -linolenic acid are commonly added to feed as essential fatty acids for freshwater fish. It is widely believed that freshwater fish can convert C18 polyunsaturated fatty acids (PUFA) to HUFA (Cook and McMaster 2004) but different fish have different synthesis abilities, mainly due to differences in their fatty acid desaturases and fatty chain elongases (Tocher 2010). Fatty acid desaturases can introduce double bonds into fatty acids, while fatty chain elongases can extend fatty acid chains (Jakobsson et al. 2006). Among these, the  $\Delta 6$ FAD and ELOVL5 genes encode the key enzymes of HUFA biosynthesis,  $\Delta 6$ -fatty acids desaturase and elongase-5, respectively.

Water temperature is considered to be a very important abiotic factor affecting the entire life cycle of fish including growth, development and reproduction (Atwood et al. 2003; Tan et al. 2016; Barriviera et al. 2021; Ge et al. 2021). As ectotherms, fish can adapt to natural temperatures and change with seasonal temperature fluctuations. The internal temperature of fish largely reflects the ambient environmental temperature. Fish counteract the effects of fluctuations in the environmental temperature on the properties and function of their cell membranes by remodelling membrane lipids. This process, known as homeoviscous adaptation, ensures the maintenance of membrane functions (Fadhlaoui and Couture 2016).

Cell membranes are composed of a lipid bilayer, which is highly sensitive to temperature fluctuations. This sensitivity is due to the effects of temperature on membrane lipids and, consequently, on the proteins embedded in the membranes (Fadhlaoui and Couture 2016). Additionally, when fish are exposed to cold water temperatures, their fatty acid composition changes to adapt to the cold environment (Hsieh et al. 2007). Several studies have also investigated the effects of variations in adaptation temperature on cell membrane PUFA composition and consistently reported that cold temperatures led to an increase in cell membrane polyunsaturation (Grim et al. 2010; Fadhlaoui et al. 2018). Low temperature significantly increased the content of monounsaturated fatty acids

(MUFA) and PUFA (mainly n-3 fatty acids) in the liver of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*; Liu et al. 2018).

Desaturases and elongases are the key enzymes involved in the fatty acid synthesis and remodelling pathways. It has been demonstrated that cold-challenged fish display an upregulation of these enzymes to restore the fluidity of cold-rigidified membranes (Tocher et al. 2004). The early developmental stage of fish, from fertilisation to the start of exogenous feeding, is a critical period for the growth of the body and the rapid development of organs (Liu et al. 2020; Tshering et al. 2021). During this period, growth and development are completely dependent on endogenous nutrients. Among these, HUFA is required for the development of fish embryos and larvae. However, little is known about endogenous HUFA biosynthesis systems and their roles during the early development of fish under different temperatures.

The common carp (*Cyprinus carpio*) is a typical freshwater fish. It is found in almost all the freshwater regions of the northern hemisphere. To date, to the best of our knowledge, there has been little research on the effects of temperature on endogenous HUFA biosynthesis during the early development of the common carp. The present study aimed to elucidate the regulation patterns of the HUFA biosynthetic pathway under different temperatures. The results will help clarify the regulatory effects of temperature on fatty acid biosynthesis during the early development of common carp.

## MATERIAL AND METHODS

### Experimental animals and design

The experimental carp were taken from a local common carp breeding farm in Mengjin County, Luoyang City, China. Ten groups of female and male parents with the same genetic background, size and age were selected for artificial spawning and then hatched. The common carp larvae were hatched for one day and adapted to an indoor temperature-controlled circulating water system. During the period of adaptation, sufficient oxygen supply was ensured and soya milk was fed three times a day (8:00, 12:00 and 16:00). The water temperature was  $25.0 \pm 1.0$  °C, the dissolved oxygen  $\geq 6$  mg/l, the ammonia nitrogen  $< 0.01$  mg/l and the pH about 7.6–7.8. After seven days

of adaptation, 360 healthy common carp larvae were selected and divided into six parallel groups. Three groups were high-temperature groups ( $32.0 \pm 1.0^\circ\text{C}$ ) and three groups were low-temperature groups ( $10.0 \pm 1.0^\circ\text{C}$ ). The initial water temperature of all the experimental groups was controlled at  $25.0 \pm 1.0^\circ\text{C}$ . In the experiment, six carp larvae were randomly taken from each fish tank at 0, 12, 24, 48, 72 and 96 h, placed into the freezing tube, quick-frozen in liquid nitrogen and then frozen at  $-80^\circ\text{C}$  for subsequent analysis of the fatty acid composition and the expression of  $\Delta 6\text{FAD}$  and  $\text{ELOVL5}$  genes. The 0 h group was the control group.

The 30-day-old carp juveniles were adapted in the indoor recirculating water culture system, fed with 2–3% of their body weight every day over three feedings (8:00, 12:00 and 16:00). Common carp juvenile commercial feed was purchased from the Tongwei Feed Co., Ltd (Chengdu, China). After seven days of adaptation, 180 healthy carp juveniles were selected and divided into six parallel groups. Three groups were high-temperature groups ( $32.0 \pm 1.0^\circ\text{C}$ ) and three groups were low-temperature groups ( $10.0 \pm 1.0^\circ\text{C}$ ) with temperature-controlled circulating water. Each parallel group had 30 fish. At the beginning of the experiment, three fish were randomly taken from each tank and the liver, brain and intestine were removed. Samples from the same fish were put in the same tube and then frozen at  $-80^\circ\text{C}$  for the initial group. At 1, 4 and 7 days, the liver, brain and intestine of three fish were taken from each temperature treatment group, and stored as above. The fish were quickly frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  for later experiments.

### RT-qPCR of $\Delta 6\text{FAD}$ and $\text{ELOVL5}$ mRNA in common carp

The samples collected at the end of the experiment were subjected to total RNA extraction

(Trizol Reagen Kit, Takara, Japan), followed by nucleic acid detection and 2  $\mu\text{g}$  of total RNA was taken for reverse transcription into cDNA (PrimeScript RT-PCR Kit, Takara, Japan). Gene expression was measured by real-time quantitative PCR (RT-qPCR) using  $\beta\text{-actin}$  as the internal reference gene. The design of primers (Table 1) for the  $\Delta 6\text{FAD}$  and  $\text{ELOVL5}$  genes in the experiment was according to the method of Ren et al. (2020). The mRNA expression level of genes in the samples was normalised to  $\beta\text{-actin}$  expression and the relative gene expression was calculated using the comparative threshold cycle (Ct) method.

### Fatty acid analysis

An appropriate amount of sample was freeze-dried for 48 h. After fully grinding it in a mortar, 0.1 g of the sample was transferred into a 12 ml threaded glass tube. Methanolic potassium hydroxide solution (1 M, 3 ml) was added and the mixture was heated in a  $75^\circ\text{C}$  water bath for 20 min and then cooled to room temperature. Then, methanolic hydrochloric acid solution (2 M, 3 ml) was added and the mixture was heated in a  $75^\circ\text{C}$  water bath for 20 minutes. Finally, *n*-hexane (1 ml) was added, the mixture was shaken and then left to stand for stratification. The supernatant was taken and analysed by GC-MS. The relative content of each fatty acid was calculated by the area normalisation method.

### Data analysis

All data are presented as the mean  $\pm$  SEM. The experimental data were analysed by one-way analysis of variance (one-way ANOVA) using SPSS v18.0 (SPSS Inc., Chicago, IL, USA) and the Tukey method was used for multiple comparisons.  $P < 0.05$  indicated a significant difference.

Table 1. Primers used in the experiment

Primers	Sequence (5'→3')	Annealing temperature ( $^\circ\text{C}$ )
$\Delta 6\text{FAD}$ -F	ATCGGACACCTGAAGGGAGCG	59.9
$\Delta 6\text{FAD}$ -R	CATGTTGAGCATGTTGACATCCG	57.6
$\text{ELOVL5}$ -F	GTCCTGACCATGTTCCAGACATCTTG	59.7
$\text{ELOVL5}$ -R	CTGTAAGCGGACGAGGTGTCGTC	59.7
$\beta\text{-actin}$ -F	CGCCCCAGACATCAGGGTG	58.3
$\beta\text{-actin}$ -R	CACAGATCATGTTTGAGACCTTCAACAC	59.0

## RESULTS

### Effects of temperature stress on the expression of $\Delta 6FAD$ and $ELOVL5$ genes in common carp larvae

As shown in Figure 1, compared with at 0 h (control group), after 96 h in 32 °C water, the expression of the  $\Delta 6FAD$  gene of common carp larvae was higher from 24 to 48 h, while the expression of the  $ELOVL5$  gene was higher from 12 to 24 h ( $P < 0.05$ ). The expression of both the  $\Delta 6FAD$  and  $ELOVL5$  genes of carp larvae in 10 °C water significantly decreased from 12 to 24 h and significantly increased from 48 to 72 h (Figure 1). No larvae died after 96 h of temperature stress treatment. The results showed that the temperatures were within the tolerance range of the larvae.

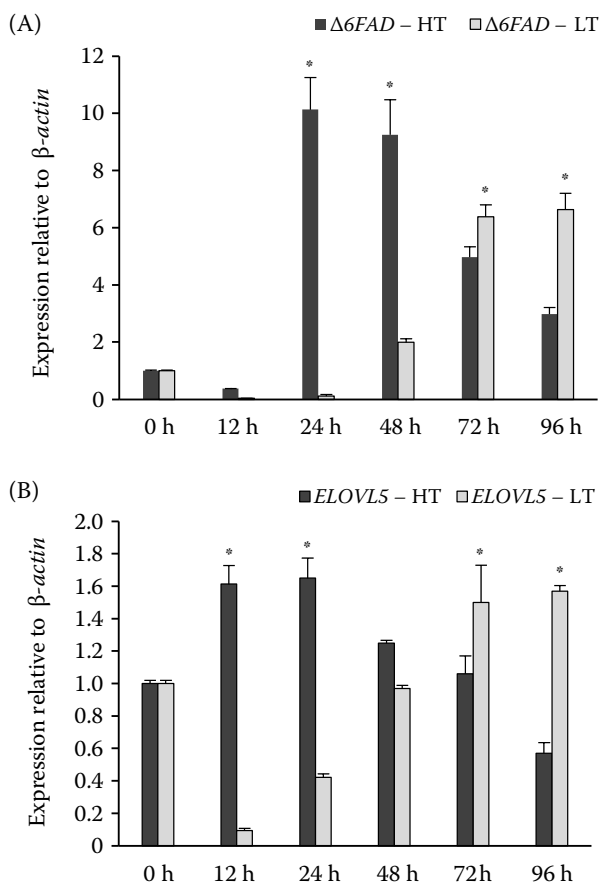


Figure 1. Temporal expression of  $\Delta 6FAD$  (A) and  $ELOVL5$  (B) relative to  $\beta$ -actin in common carp larvae exposed to high temperature (HT, 32 °C) and low temperature (LT, 10 °C)

\*Significant differences from the respective initial values (0 h or control group),  $P < 0.05$

### Effects of temperature stress on the fatty acid profile in common carp larvae

As shown in Table 1, compared with at 0 h (control group), the C18:2n-6 content of the carp larvae did not change significantly from 0 to 96 h under high-temperature stress (32 °C) but significantly decreased under low temperature (10 °C) stress at 96 h ( $P < 0.05$ ). The content of C20:4n-6 (ARA) increased significantly at 48 and 96 h for both high-temperature and low-temperature stress ( $P < 0.05$ ). The content of C18:3n-3, C20:5n-3 and C22:6n-3 increased significantly after 96 h of low-temperature stress but the reverse was true under high-temperature stress ( $P < 0.05$ ). The total content of n-3 fatty acids was significantly higher after 96 h of low-temperature stress compared to high-temperature stress ( $P < 0.05$ ), while there was no significant difference in the total content of n-6 fatty acids (Table 2).

### Effects of different water temperatures on $\Delta 6FAD$ and $ELOVL5$ in different organs of common carp juveniles

Figure 2 shows the expression of the  $\Delta 6FAD$  gene in the liver, brain and intestine of the juvenile carp under temperature stress for seven days. At the end of the experiment, the expression levels of the  $\Delta 6FAD$  gene in the liver, brain and intestine were higher compared with the control group. The expression of the  $\Delta 6FAD$  gene in the intestine and brain at low temperature was significantly higher than that at high temperature on the 7<sup>th</sup> day, while the expression of the  $\Delta 6FAD$  gene in the liver appeared on the 4<sup>th</sup> day ( $P < 0.05$ ). Figure 3 shows the expression of the  $ELOVL5$  gene in the liver, brain and intestine of the juvenile carp under temperature stress for seven days. The expression of the  $ELOVL5$  gene in the liver, intestine and brain at high temperature was significantly higher than that at low temperature on the first day ( $P < 0.05$ ). No juvenile carp died after seven days of temperature stress treatment, indicating that the temperatures were within the tolerance range of the juveniles.

### Effects of temperature stress on the fatty acid profile in common carp juveniles

After seven days of temperature stress treatment in 32 °C or 10 °C water, the composition

Table 2. Fatty acids profiles (%) of common carp larvae exposed to temperature stress for 96 h (mean  $\pm$  SEM)

Fatty acids	0 h	Low temperature (10 °C)		High temperature (32 °C)	
		48 h	96 h	48 h	96 h
14:0	0.76 $\pm$ 0.02	0.80 $\pm$ 0.12	0.85 $\pm$ 0.32	0.81 $\pm$ 0.15	0.82 $\pm$ 0.23
16:0	26.2 $\pm$ 1.23	27.1 $\pm$ 2.21	27.2 $\pm$ 1.36	27.4 $\pm$ 1.98	27.3 $\pm$ 1.56
16:1	4.61 $\pm$ 0.53	4.97 $\pm$ 1.23	4.78 $\pm$ 0.68	4.87 $\pm$ 0.54	4.54 $\pm$ 0.34
18:0	4.74 $\pm$ 0.47	5.11 $\pm$ 0.98	5.20 $\pm$ 0.75	4.71 $\pm$ 0.82	5.10 $\pm$ 0.75
18:1	21.4 $\pm$ 2.31	21.6 $\pm$ 1.56	21.4 $\pm$ 2.01	22.1 $\pm$ 1.24	21.3 $\pm$ 1.36
20:0	0.14 $\pm$ 0.02	0.15 $\pm$ 0.01	0.16 $\pm$ 0.03	0.13 $\pm$ 0.04	0.11 $\pm$ 0.01
20:1	0.89 $\pm$ 0.03	0.90 $\pm$ 0.03	0.87 $\pm$ 0.05	0.91 $\pm$ 0.21	0.82 $\pm$ 0.14
18:2n-6	6.32 $\pm$ 0.56 <sup>a</sup>	6.12 $\pm$ 0.26 <sup>a</sup>	5.41 $\pm$ 0.75 <sup>b</sup>	6.73 $\pm$ 0.23 <sup>a</sup>	6.25 $\pm$ 0.54 <sup>a</sup>
18:3n-6	0.36 $\pm$ 0.05	0.32 $\pm$ 0.03	0.29 $\pm$ 0.03	0.35 $\pm$ 0.02	0.33 $\pm$ 0.01
20:4n-6	3.32 $\pm$ 0.12 <sup>b</sup>	4.02 $\pm$ 0.45 <sup>a</sup>	4.50 $\pm$ 0.25 <sup>a</sup>	4.54 $\pm$ 0.22 <sup>a</sup>	4.20 $\pm$ 0.11 <sup>a</sup>
18:3n-3	1.82 $\pm$ 0.06 <sup>a</sup>	1.56 $\pm$ 0.06 <sup>b</sup>	1.48 $\pm$ 0.42 <sup>b</sup>	1.45 $\pm$ 0.04 <sup>b</sup>	1.54 $\pm$ 0.03 <sup>b</sup>
20:5n-3	1.45 $\pm$ 0.05 <sup>b</sup>	1.66 $\pm$ 0.06 <sup>a</sup>	1.78 $\pm$ 0.08 <sup>a</sup>	1.41 $\pm$ 0.01 <sup>b</sup>	1.36 $\pm$ 0.02 <sup>b</sup>
22:5n-3	0.39 $\pm$ 0.02	0.47 $\pm$ 0.04	0.43 $\pm$ 0.05	0.49 $\pm$ 0.01	0.53 $\pm$ 0.03
22:6n-3	4.67 $\pm$ 0.32 <sup>b</sup>	4.98 $\pm$ 0.78 <sup>b</sup>	6.14 $\pm$ 0.48 <sup>a</sup>	5.34 $\pm$ 0.24 <sup>b</sup>	3.86 $\pm$ 0.35 <sup>c</sup>
Total SFA	31.8 $\pm$ 1.45	33.2 $\pm$ 1.23	33.4 $\pm$ 1.42	33.2 $\pm$ 1.20	33.3 $\pm$ 0.98
Total MUFA	26.9 $\pm$ 0.96	27.5 $\pm$ 1.12	27.0 $\pm$ 1.25	27.9 $\pm$ 1.56	26.7 $\pm$ 1.85
Total n-3	8.33 $\pm$ 0.42 <sup>b</sup>	8.67 $\pm$ 0.36 <sup>b</sup>	9.83 $\pm$ 0.45 <sup>a</sup>	8.70 $\pm$ 0.14 <sup>b</sup>	7.29 $\pm$ 0.18 <sup>c</sup>
Total n-6	9.98 $\pm$ 0.11	10.5 $\pm$ 0.15	10.2 $\pm$ 0.13	11.6 $\pm$ 0.22	10.8 $\pm$ 0.14

MUFA = monounsaturated fatty acid; SFA = saturated fatty acid

<sup>a-c</sup>Different lowercase letters on the same line represent significant difference ( $P < 0.05$ )

of C18:2n-6 and C18:3n-3 and their respective PUFA (C20:4n-6, C20:5n-3 and C22:6n-3) were measured in the carp juveniles. The levels of C20:4n-6, C20:5n-3 and C22:6n-3 in juveniles exposed to 10 °C water were higher than those in the carp exposed to 32 °C water. The levels of saturated fatty acids (SFA) and MUFA following exposure to 32 °C water were higher than those following exposure to 10 °C water but the levels of n-3 PUFA and n-6 PUFA following exposure to 32 °C water were lower than those following exposure to 10 °C water (Table 3).

## DISCUSSION

Several studies have reported that temperature can affect the saturation of fatty acids in fish cell membranes (Ninno et al. 1974; De Torrenco and Brenner 1976; Hagar and Hazel 1985; Ruyter et al. 2003), with low temperatures better increasing the unsaturation of cell membrane phospholipids than high temperatures, so that the cell membrane has better fluidity (Cossins 1983; Schunke

and Wodtke 1983; Cossins and Lee 1985; Tiku et al. 1996). The content of unsaturated fatty acids in fish is higher at low temperatures, which may be related to the effect of temperature on the activity of desaturase and fatty acid chain elongase (Wodtke and Cossins 1991). Nutrient and ambient temperature can regulate HUFA synthesis in Atlantic salmon (*Salmo salar*) by regulating changes in fatty acid desaturase and fatty acid elongase, of which fatty acid desaturase plays a major role (Zheng et al. 2005). In some fish, the activity of  $\Delta 6$ -desaturase decreased with increasing temperature.  $\Delta 6$ -desaturase activity in rainbow trout (*Oncorhynchus mykiss*) enterocytes and hepatocytes at 5 °C and 7 °C was higher than that at 20 °C and 15 °C, respectively (Tocher et al. 2004). The activity of fatty acid desaturase in the liver microsomes of catfish (*Silurus asotus*) at 14–15 °C was higher than that at 29–30 °C (De Torrenco and Brenner 1976).

To date, there have been few reports on the effect of temperature stress on fatty acid elongase in fish. In this study, the variations in  $\Delta 6FAD$  and *ELOVL5* gene expression in carp were significantly

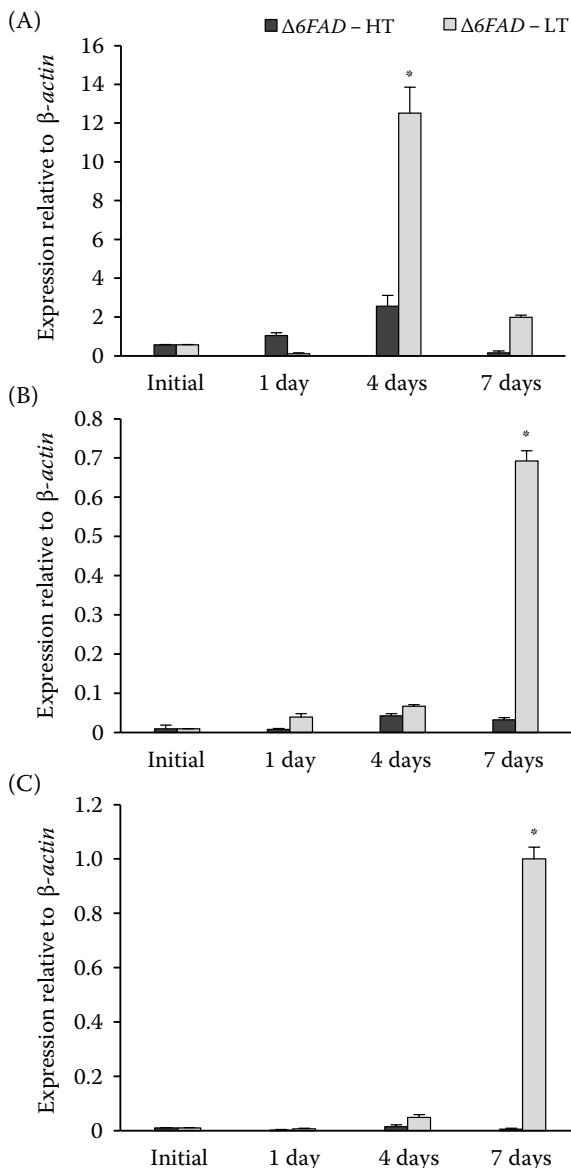


Figure 2. Expression of  $\Delta 6FAD$  relative to  $\beta$ -actin in liver (A), brain (B) and intestine (C) from common carp juvenile at the initial, 1<sup>st</sup> day, 4<sup>th</sup> day and 7<sup>th</sup> day after temperature treatment

HT = high temperature (32 °C); LT = low temperature (10 °C)

\*Significant differences from the respective initial values (day 0 or control group),  $P < 0.05$

different at high and low temperatures, with significantly greater sensitivity to high temperature than to low temperature. The gene expression levels of  $\Delta 6FAD$  and  $ELOVL5$  in carp larvae were highest at 24 h following exposure to high-temperature water (32 °C). It is possible that, in the high-temperature environment, the larvae suffered high-temperature stress at the initial stage

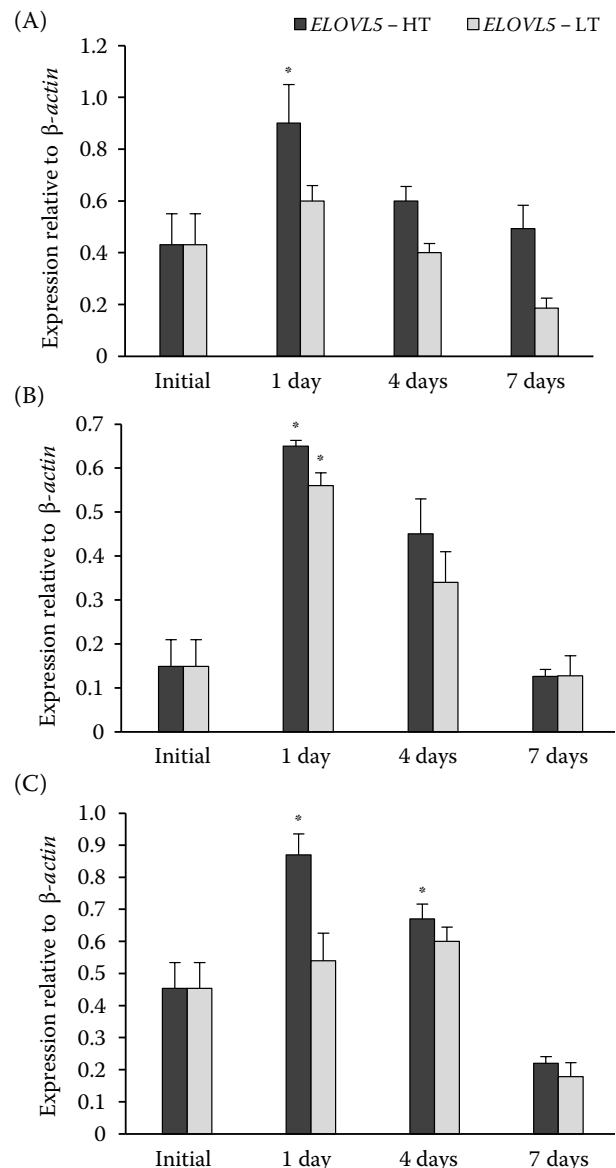


Figure 3. Expression of  $ELOVL5$  relative to  $\beta$ -actin in liver (A), brain (B) and intestine (C) from common carp juvenile at the initial, 1<sup>st</sup> day, 4<sup>th</sup> day and 7<sup>th</sup> day after temperature treatment

\*Significant differences from the respective initial values (day 0 or control group),  $P < 0.05$

and needed a large amount of HUFA to increase their ability to resist this stress. The  $\Delta 6FAD$  and  $ELOVL5$  genes in the larvae stage of common carp were activated quickly under high temperature stress and the high levels of gene expression lasted about 12–48 hours.

The expression of the  $\Delta 6FAD$  and  $ELOVL5$  genes in common carp larvae following exposure to 10 °C water increased gradually from 48 hours. Interestingly, the  $\Delta 6FAD$  and  $ELOVL5$  genes were



Table 3. Fatty acids profiles (%) of common carp juvenile exposed to temperature stress for seven days (mean  $\pm$  SEM)

Fatty acids	Day 0	Day 7 LT (10 °C)	Day 7 HT (32 °C)
14:0	0.87 $\pm$ 0.14	0.78 $\pm$ 0.11	0.82 $\pm$ 0.17
16:0	25.31 $\pm$ 2.42	26.21 $\pm$ 2.47	28.42 $\pm$ 3.15
16:1	6.42 $\pm$ 1.10	6.68 $\pm$ 1.25	6.83 $\pm$ 1.46
18:0	3.34 $\pm$ 0.43	3.17 $\pm$ 0.68	3.24 $\pm$ 0.32
18:1	34.14 $\pm$ 4.33	34.25 $\pm$ 4.13	36.67 $\pm$ 3.58
20:0	0.10 $\pm$ 0.00	0.21 $\pm$ 0.03	0.25 $\pm$ 0.01
20:1	2.50 $\pm$ 0.13	1.77 $\pm$ 0.25	3.76 $\pm$ 0.12
18:2n-6	15.31 $\pm$ 0.76 <sup>a</sup>	14.17 $\pm$ 0.45 <sup>b</sup>	12.72 $\pm$ 0.31 <sup>b</sup>
18:3n-6	0.34 $\pm$ 0.06	0.43 $\pm$ 0.08	0.24 $\pm$ 0.04
20:4n-6	4.21 $\pm$ 0.31	4.65 $\pm$ 0.42	4.34 $\pm$ 0.52
18:3n-3	0.40 $\pm$ 0.02	0.32 $\pm$ 0.01	0.31 $\pm$ 0.04
20:5n-3	0.19 $\pm$ 0.01	0.35 $\pm$ 0.02	0.15 $\pm$ 0.01
22:5n-3	0.32 $\pm$ 0.01	0.23 $\pm$ 0.02	0.20 $\pm$ 0.05
22:6n-3	4.11 $\pm$ 0.21 <sup>a</sup>	5.40 $\pm$ 0.46 <sup>a</sup>	3.24 $\pm$ 0.28 <sup>b</sup>
Total SFA	29.58 $\pm$ 1.45	30.37 $\pm$ 1.42	32.87 $\pm$ 1.23
Total MUFA	43.07 $\pm$ 0.96 <sup>b</sup>	42.49 $\pm$ 1.25 <sup>b</sup>	47.30 $\pm$ 1.12 <sup>a</sup>
Total n-3	5.02 $\pm$ 0.42 <sup>a</sup>	6.30 $\pm$ 0.45 <sup>a</sup>	3.73 $\pm$ 0.36 <sup>b</sup>
Total n-6	19.88 $\pm$ 0.11 <sup>a</sup>	19.25 $\pm$ 0.13 <sup>a</sup>	17.30 $\pm$ 0.15 <sup>b</sup>

HT = high temperature; LT = low temperature; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid

<sup>a,b</sup>Different lowercase letters on the same line represent significant difference ( $P < 0.05$ )

highly expressed after 48 h under low-temperature stress. The fatty acid composition of the common carp larvae revealed a significant increase in C22:6n-3 and C18:3n-3 after 24–48 h under low-temperature stress; however, the levels of C18:2n-6 and C18:3n-3 decreased. These results indicated that common carp larvae can synthesise C22:6n-3 and C20:4n-6 from C18:3n-3 and C18:2n-6 under low-temperature stress. It is speculated that the endogenous C22:6n-3 and C20:4n-6 supply in the carp larvae was complemented by a remarkable increase in the expression of  $\Delta 6FAD$  and  $ELOVL5$  to transform the C<sub>18</sub> PUFA into C20–22, possibly to ensure sufficient C22:6n-3 and C20:5n-3 for neural and visual development as well as organogenesis.

Some studies have suggested that temperature affects the activity of fatty acid desaturase, which varies between tissues and organs (Tocher et al. 2004; Skalli et al. 2006). In the rainbow trout study, for example, intestine cells had the highest fatty acid desaturase activity at 7 °C and liver cells had

the highest fatty acid desaturase activity at 11 °C. In the present study, there were significant differences in the regulation of the  $\Delta 6FAD$  and  $ELOVL5$  genes in the liver, brain and intestine of common carp during seven days of temperature stress. The expression level of the  $\Delta 6FAD$  gene was higher under low-temperature stress than under high-temperature stress. However, the expression level of the  $ELOVL5$  gene was higher under high-temperature stress than under low-temperature stress. This was an interesting finding that requires further exploration. Additionally, the timing of up-regulation of  $\Delta 6FAD$  gene expression was different in different organs. At low temperature,  $\Delta 6FAD$  gene expression peaked on the 4<sup>th</sup> day in the liver but on the 7<sup>th</sup> day in the intestine and brain. The relative expression level in the liver was significantly higher than that in the brain and intestine, indicating that the liver played an important role in fatty acid synthesis. However, the  $ELOVL5$  gene in the liver, intestine and brain was simultaneously highly expressed after one day of high-temperature stress, which was significantly different from the  $\Delta 6FAD$  desaturase gene. The fatty acid composition of the common carp juveniles revealed a significant increase in C22:6n-3 and C20:4n-6 after seven days under low-temperature stress, which indicated that a suitable low temperature could increase the amount of HUFA.

## CONCLUSION

In conclusion, in the larval stage of common carp, the  $\Delta 6FAD$  and  $ELOVL5$  genes were activated quickly at high temperature. And there was a significant improvement in C22:6n-3 and C20:5n-3 after 96 h at low temperature. In common carp juveniles, the  $\Delta 6FAD$  gene in the liver, brain and intestine was sensitive to low temperature but the  $ELOVL5$  gene in the liver, brain and intestine was sensitive to high temperature. The low temperature also increased the amount of HUFA in the common carp juveniles. These results indicated that, during the early development of carp, temperature regulates the expression of key genes for HUFA biosynthesis and also participates in the biosynthesis of fatty acids in the body. These results help clarify the regulatory effect of temperature on fatty acid biosynthesis during the early development of common carp.

## Conflict of interest

The authors declare no conflict of interest.

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