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Molecular cloning, mRNA expression, and nutritional regulation of a fatty acyl Δ6-desaturase-like gene of the Manchurian trout, *Brachymystax lenok* (Pallas)

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Key words: *Brachymystax lenok*; fatty acyl delta-6 desaturase-like; cloning; fish oil; sunflower oil; linseed oil

Abstract

The fatty acyl delta-6 desaturase (Δ6-desaturase) is a key rate-limiting enzyme in the biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFAs). To study the effects of different oil sources in the feed on the nutritional regulation of Δ6-desaturase-like gene mRNA expression, the full-length cDNA of the Δ6-desaturase-like gene was cloned from the liver of Manchurian trout (*Brachymystax lenok*). A 9-week feeding trial was performed, and the fish received diets with three different oil sources: fish oil (FO), sunflower oil (SO), and linseed oil (LO). The results showed that the 2448 bp long full-length cDNA contained an open reading frame (with a length of 1365 bp), encoding 454 amino acids. Gene expression analysis indicated that Δ6-desaturase-like gene mRNA is widely distributed throughout different tissues, with highest expression levels in both the liver and the brain. The linolenic acid (ALA) and linoleic acid (LA) compositions were highest in LO and SO diets, respectively. The eicosapentaenoic acid and docosahexaenoioc acid composition in LO and SO diets were significantly lower than in the FO diet. Fish fed with SO and LO showed significantly higher liver expression levels of Δ6-desaturase-like gene mRNA than those fed with FO. This indicates that SO and LO in the diet affected the nutritional regulation of the Δ6-desaturase-like gene mRNA in the liver of the Manchurian trout. In addition, these data suggest that the Manchurian trout has the ability to synthesize long-chain unsaturated fatty acids (LC-PUFAs) from ALA and LA.

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Introduction

Long-chain polyunsaturated fatty acids (LC-PUFAs) are important for both growth and development in fish, and provide required metabolic energy (Glencross 2009). Omega-3 (n-3) LC-PUFAs, especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoioc acid (DHA; 22:6n-3), in the feed are known to exert an important effect on growth, survival, ontogenesis, pigmentation, and both the development and functionality of the brain, vision, and nervous system in fish (Vagner and Santigosa 2010; Tocher 2015). In addition, DHA and EPA play important roles in both enzyme activity and cell permeability (Zakeri et al. 2011). In general, fresh-water fish can obtain DHA and EPA from PUFA endogenous biosynthetic pathways and external pathways such as vegetable and dietary fish oils. Multiple desaturases and elongases are involved in PUFA endogenous biosynthetic pathways (Vagner and Santigosa 2010; Emery et al. 2013). Among these enzymes, the fatty acyl delta-6 desaturase is a key rate-limiting enzyme that is active during the process of LC-PUFA biosynthesis. Δ6-desaturase is responsible for the first step in the LC-PUFA synthesis pathway, where it converts linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) to 18:4n-3 and 18:3n-6, respectively. In addition, Δ6-desaturase also catalyzes the conversion from 24:5n-3 to 24:6n-3, which is required for the synthesis of DHA (22:6n-3) (Sprecher 2000; Tanomman et al. 2013).

At present, to provide biological information on the enzymatic activities of Δ6-desaturase, the Δ6-desaturase gene has been widely cloned and studied in both fresh-water and marine-water fish, including, the Zebrafish (*Danio rerio*) (Hastings et al. 2001); the Atlantic salmon (*Salmo salar*) (Zheng et al. 2005); the Rainbow trout (*Oncorhynchus mykiss*) (Seiliez et al. 2001); the Atlantic cod (*Gadus morhua*) (Zheng et al. 2009); the Nile tilapia (*Oreochromis niloticus*) (Tanomman et al. 2013); and Chu's croaker (*Nibea coibor*) (Huang et al. 2017). Several studies have shown that the Δ6-desaturase gene mRNA levels are regulated by nutrition, which in turn can affect LC-PUFA synthesis (Bendiksen et al. 2003; Tocher et al. 2006). ALA (18:3n-3) and LA (18:2n-6) are essential fatty acids for fresh-water fish. Due to the higher 18:3n-3 and 18:2n-6 inclusions in linseed oil (LO) and sunflower (SO), respectively, LO and SO have become two important vegetable oils for fresh-water fish. Similar to mammals, the Δ6-desaturase expression level in fresh-water fish can be increased when fish oil is replaced by vegetable oil in the diet (Zheng et al. 2005; Tocher et al. 2006).

The Manchurian trout, *Brachymystax lenok* (Pallas), is a cold-water salmonid fish that is distributed throughout eastern Siberia and areas of Mongolia, Kazakhstan, Korea, and China. It is a high-interest aquaculture fresh-water fish in China due to its high nutritional value and disease resistance. Recently, Chinese researchers have studied the effects of a variety of feed ingredients on the levels of lipid and n-3 LC-PUFA in the Manchurian trout (Zhang et al. 2009; Xu et al. 2015; Chang et al. 2017). However, whether vegetable oil in the feed affects the expression of Δ6-desaturase-like gene mRNA, and thus affects the synthetics of n-3 LC-PUFA, remain poorly understood and should be examined in the Manchurian trout. For this purpose, this study cloned, sequenced, and genetically characterized the full-length cDNA of Δ6-desaturase-like gene from the Manchurian trout for the first time. Additionally, the tissue distribution of Δ6-desaturase-like mRNA expression was evaluated, as well as changes to the expression of Δ6-desaturase-like mRNA levels in the liver caused by dietary oil sources. The obtained results provide basic molecular biological information on the Δ6-desaturase gene, its expression pattern, and nutritional regulation. This provides a foundation for further research into its role in PUFA biosynthesis, nutrition, and physiology of the Manchurian trout.

Materials and Methods

Manchurian Trout.

Fifteen Manchurian trout used for the conducted experiments were sourced from the Fengcheng Base of Cold-water Fish Research (China). Six healthy fish (body mass approximately 300 g each) were euthanized with MS-222 (100 mg L^{-1}) and the heart, liver, stomach, ovary, intestine, gill, spleen, brain, muscle, and eyes were collected and

rapidly frozen in liquid nitrogen followed by storage at -80 °C until further use for RNA extraction. These tissue samples were used for Δ6-desaturase-like gene cloning and tissue distribution studies.

Experimental diets.

The Manchurian trout studied in this experiment received a basic diet for two weeks prior to the start of the experiment. The initially designed basic diet formulation included 39.5% crude protein and 18.5% crude lipid. The feed was made as previously described by Chang et al. (2017). Ingredients and proximate composition of the diets are presented in Table 1. Three diets with different oil sources were prepared by either adding fish oil (FO), sunflower oil (SO), or linseed oil (LO) to the base diets. The fatty acid composition of the diets is shown in Table 2.

Procedure of feeding experiment.

The feeding experiments were performed in a recycling water system at the Cold Water Fish Culture Laboratory of the Inner Mongolia University for Nationalities. Prior to the trial, Manchurian trout did not receive any food for 24 h. Healthy fish (average body mass: 6.43 ± 0.02 g) were selected and randomly stocked into nine breeding buckets (150 L/ per bucket), with 20 fish per bucket. The three diets were randomly assigned in triplicate to the buckets (each diet for three buckets). During the experimental period, fish were fed to apparent satiation twice per day (07:00 and 17:30). The feeding experiment lasted for nine weeks. The water temperature was 15 ± 2 °C and the dissolved oxygen level was > 7.5 mg/L.

Sample collection.

At the end of the trial, the Manchurian trouts were starved for 24 h prior to harvest. Four fish from each bucket were euthanized with MS-222 (100 mg L^{-1}), the liver and muscle tissues were collected, rapidly frozen in liquid nitrogen, and stored at -80 °C until further use.

RNA isolation and cDNA synthesis.

Total RNA from the organs of the Manchurian trout was extracted using RNA iso Plus (Takara, China) and treated with gDNA Eraser (Takara, China). Then, 1 μg of total RNA was reverse transcribed into cDNA using the PrimeScript 1st Strand cDNA Synthesis Kit (Takara) following the manufacturer's instructions. The quality and concentration of the total RNA were evaluated by agarose-gel electrophoresis (1%) and spectrophotometer at 260 and 280 nm (NanoDrop 2000, Thermo Scientific, USA), respectively.

Cloning cDNA for the full-length Δ6-desaturase gene.

The cDNA of Δ6-desaturase was cloned using primers designed via BLASTx analysis of homologous genes from *Salmo salar* (GenBank accession no: NP_001117047.1) and *Oncorhynchus mykiss* (GenBank accession no: NP_001117759.1). A pair of the specific gene primers FAD6-F1 and FAD6-R1 (Table 3) was designed for sequence verification based on Primer 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/) to amplify Δ6-desaturase cDNA using the Takara TksGfiex DNA Polymerase (Takara, China). Then, other primers (see Table 3) were designed based on the core Δ6-desaturase cDNA sequences via rapid amplification cDNA ends (RACE) using the SMART er® RACE 5′/3′ Kit (Clontech). According to the instructions of the kit, the first-strand cDNA was synthesized and then diluted to 1:10 by Tricine-EDTA buffer for PCR. The total volume of the initial Outer 3′ RACE PCR reaction was 50 μL, which contained 25 μL of 2 × Gflex PCR Buffer, 5 μL of 10 × Universal Primer UPM, 2.5 μL of the first-strand cDNA as template, 1.0 μL of Tks Gflex DNA Polymerase, 1.0 μL of 10 μM Outer primer FAD6-F2, and 15.5 μL of sterile deionized water. The total volume of the Inner 3′ RACE PCR reaction was 50 μL, which contained 1.0 μL of Outer PCR, 25 μL of 2 × Gflex PCR Buffer, 1.0 μL of Tks Gflex DNA Polymerase, 1.0 μL of 10 μM UPS, 1.0 μL of 10 μM Outer primer FAD6-F3, and 21.0 μL of Sterile deionized water. For 5′ RACE, FAD6-R2 and FAD6-R3 (Table 2) were used for the Outer and Inner primers, respectively. The initial 3′ and 5′ RACE PCR amplification conditions were as follows: 1 cycle at 94 °C for 1 min; 30 cycles at 98 °C for 10 s, 55 °C for 15 s and 68 °C for 1 min. The PCR products were purified with the Takara Mini BESTA garose Gel DNA Extraction Kit Ver. 4.0 (Takara, China). The purified PCR products were ligated

with pMD20 (Takara, China) and inserted into competent *Escherichia coli* cells. The cloned cDNA products were sequenced by TaKaRa Biotechnology Co., Ltd.

Sequence analysis.

The cloned sequences were verified by similarity BLASTn search programs (http://blast.ncbi.nlm.nih.gov/). The open reading frame (ORF) was determined using the ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and the amino acid sequence was translated from this coding region using the sequence manipulation suite (SMS) tool (http://www.bio-soft.net/sms/index.html). Prediction of the molecular mass and the theoretical isoelectric point (pI) of the Δ6-desaturase-like protein was calculated by ProtParam (http://web.expasy.org/protparam/). The deduced amino acid of Δ6-desaturase-like gene RNA was analyzed by multiple sequence alignment using a Clustal W program and the phylogenetic tree was generated by the neighbor joining (NJ) method using 1000 bootstrap replicates with MEGA 7.0 software.

Proximate composition and fatty acid analysis.

The biochemical composition of diets was analyzed using the AOAC standard method (AOAC, 1995). The diets were dried in an oven at 105 °C until a constant weight was reached for dry matter analysis. The crude protein (N × 6.25) and lipid contents were analyzed by the Kjeldahl and Folch (1957) method. Muscle and liver samples were freeze-dried for 48 h prior to the analyses of the fatty acid composition of tissues. Fatty acid methyl esters (FAME) were prepared by methanol-benzene-acetyl chloride according to Sukhija and Palmquist (1988); then, the FAME were analyzed via Agilent 6890 gas chromatography (Agilent, USA).

Real-time quantitative PCR (RT-qPCR).

The expression of the Δ6-desaturase gene in different tissues was conducted by using real-time fluorescent quantitative PCR (RT-qPCR) (SYBR GreenII) on a StepOne Plus Real-Time PCR system. The reference gene was β-actin (GenBank accession no: KT995162.1), which showed no changes in the β-actin gene expression in our investigations. The extracted and reverse transcribed total RNA from each sample used the same quantities of total RNA (1000 ng). Primers, FAD6-F4, and FAD6-R4 (Table 3) were designed with primer 3.0 according to the cloned Δ6-desaturase cDNA. The final volume of the Real-time qPCR amplification reaction was 20 μL, which contained 10 μL of 2 × SYBR Premix Ex TaqTMII (TaKaRa), 0.4 μL of rox reference dye, 2.0 μL of diluted cDNA template, 0.8 μL of forward primer, 0.8 μL of reverse primer, and 6.0 μL sterile deionized water. Relative quantification was conducted using the StepOne Plus Real-Time system. PCR was conducted with the following conditions: 95 °C for 7 min; 40 cycles at 95 °C for 15 s, 60 °C for 1 min; followed by a melting curve at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. All samples were run in triplicate. The relative Δ6-desaturase gene expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis.

The statistical significance of the data was analyzed by one-way analysis of variance (ANOVA), and Tukey's multiple comparison test was used to compare the different diet treatment groups. $P < 0.05$ was considered to indicate a statistically significant difference. Statistical analysis was conducted with SPSS 17.0 software.

Results

Cloning and characterization of full length Δ6-desaturase-like cDNA.

The full-length Δ6-desaturase-like cDNA was isolated from the liver tissues of the Manchurian trout. The resulting cDNA sequence of 2448 bp (GenBank accession no: MH587164) comprised a 1365 bp ORF, a 114 bp 5'- untranslated region (UTR), and a 969 bp 3'-UTR. The deduced Δ6-desaturase protein sequence contained 454 amino acids with a molecular weight of 52.33 kDa and a theoretical isoelectric point of 8.97. A putative polyadenylation signal (ATTAAA) was found at 2419, which is 12 nucleotides upstream of the poly A tail (Fig. 1). Sequence analysis showed three histidine-rich motifs, HXXXH (residues 190 to 194), HXXHH (residues 227 to 231), and QXXHH (residues 392 to 396; Fig. 2). A predicted cytochrome b5 domain, signified by "HPGG" (at aa positions 63), was also observed (Fig. 2).

Homology analysis.

The identities of Δ6-desaturase-like gene amino acid sequence of the Manchurian trout and other fish were studied via multiple sequence alignment (Fig. 2). The predicted Δ6-desaturase-like cDNA sequences had a variable identity (71-96%) with the Δ6-desaturase genes from other fish, as shown using the tools on BLASTX (Fig. 2). The Manchurian trout Δ6-desaturase-like has about a 96% homology with the Atlantic salmon Δ6-desaturase. The neighbor-joining phylogenic tree for Δ6-desaturase and Δ5-desaturase between selected fish species was determined via MEGA 7.0 (shown in Fig. 3). The Manchurian trout and Atlantic salmon formed two monophyletic sub-branches, and clustered with both the Rainbow trout and the Masu salmon (*Oncorhynchus masou*).

Tissue distribution of Δ6-desaturase-like mRNA in the Manchurian Trout.

The tissue distribution of Δ6-desaturase-like mRNA expression was studied via RT-qPCR (Fig. 4). The Δ6-desaturase-like gene mRNA expression level was highest in the liver, which was significantly higher than in other tissues ($P < 0.05$). The second highest Δ6-desaturase-like mRNA expression level were found in the brain, which was higher than that of other tissues except for the liver ($P < 0.05$). No significant differences in Δ6-desaturase-like mRNA expression were found among heart, stomach, ovary, intestine, gill, spleen, muscle, and eye ($P > 0.05$).

The fatty acid compositions of muscle and liver samples.

The fatty acid compositions of muscle and liver tissue in the Manchurian trout are shown in Tables 4 and 5. The SFA composition in the FO diet was significantly lower than in both LO and SO diets ($P < 0.05$); however, the difference between LO and SO was not significant ($P > 0.05$). The MUFA composition in the LO diet was significantly higher than in FO and SO diets ($P < 0.05$). The n-6 PUFA composition in the SO diet was significantly higher than in both FO and LO diets ($P < 0.05$). The LA (18:2n-6) composition in the SO diet was significantly higher than in both FO and SO diets ($P < 0.05$). The DHA and EPA compositions in FO were significantly higher than in both LO and SO diets ($P < 0.05$). The ALA (18:3n-3) composition in the LO diet was significantly higher than in both FO and SO diets ($P < 0.05$). The ARA (20:4n-6) composition in the SO diet was significantly higher than in the LO diet ($P < 0.05$).

The Δ6-desaturase-like mRNA expression in response to different oil sources.

The effects of different oil sources in the feed on the expression of Δ6-desaturase-like gene mRNA were determined in liver tissue (Fig. 5). The obtained expression levels were significantly higher in fish fed with LO and SO diets than in fish fed with FO diet ($P < 0.05$). However, there were no significant differences between fish that were fed with LO and SO diets ($P > 0.05$). These results suggest that the levels Δ6-desaturase-like gene expression in the liver of Manchurian trout were up-regulated in response to the amount of LNA and LA in the diet.

Discussion

The Δ6-desaturase gene plays an important role in the fatty acid metabolism. Until today, many Δ6-desaturase genes have been cloned in fishes (Seliez et al. 2001; Ren et al. 2013; Huang et al. 2017). For the first time, this study provides the sequence and expression patterns of a fatty acyl Δ6-desaturase-like gene of the Manchurian trout. The predicted amino acid of the Δ6-desaturase-like gene in the Manchurian trout showed typical characteristics of Δ5- and Δ6-desaturase enzymes, comprising a cytochrome b5-like superfamily domain and three histidine-rich regions (HXXXH, HXXHH, and QXXHH) (Monroig et al. 2010; Vagner and Santigosa 2010). As a heme-binding electron donor, the cytochrome b5 domain plays an important role in desaturation reactions involved in HUFA biosynthesis (Vagner and Santigosa 2010). Phylogenetic analysis showed that the Δ6-desaturase-like gene of the Manchurian trout has high homology to other salmonids such as the Atlantic salmon, the Rainbow trout, and the Masu salmon. The results indicate similar characteristics and function of the Δ6-desaturase in these cold-water fishes.

Δ6-desaturase-like mRNA expression was mainly found in the liver and brain, with low levels found in other tissues. This is similar to previous findings for both the Rainbow

trout and the Gilthead sea bream (Seliez et al. 2001), the Common carp (Ren et al. 2013), the Zebra fish (Hastings et al. 2001), and Chu's croaker (Huang et al. 2017). The high expression levels of Δ6-desaturase-like mRNA in these tissues may be because the liver is the most active site of PUFA biosynthesis, and is important for the high level of DHA in nerve tissue (Tocher et al. 2006; Zheng et al. 2009; Ren et al. 2013).

As has been previously reported, the fatty acid composition of the feed affects the fatty acid composition of fish (Menoyo et al., 2005). Similar results were obtained in this experiment. The fatty acid composition of the muscle and liver tissue of the Manchurian trout were modified by different oil sources in their feed. Addition of vegetable oils (FO and SO) decreased the DHA and EPA composition of the liver and muscle tissue, which is consistent with previously published results (Menoyo et al. 2005; Turchini and Francis 2009; Turchini et al. 2011; Nayak et al. 2017). In this experiment, the composition of ALA and LA in muscle and liver tissues was lower than that of the feed, indicating that ALA and LA were expended during the β-Oxidation processes (Turchini and Francis 2009). The 20:4n-6 composition of muscle and liver in the SO diet group was higher than that in feed (not detected), which indicates that LA was converted to 20:4n-6. The DHA composition of muscle in LO was higher than that in feed, which indicates that ALA was converted to DHA in the muscle, suggesting that the Manchurian trout may be able to synthesize n-3 LC-PUFA through desaturation and elongation, as has been reported for the rainbow trout (Turchini and Francis 2009).

The study of Δ6-desaturase-like gene mRNA expression regulation on LC-PUFA biosynthetic pathways in the Manchurian trout provide more information about the regulation of these enzymes in cold-water fish. In these, it is generally believed that freshwater fish have the ability to convert C18 PUFA to C20 and C22 PUFA (Sprecher 2000; Tanomman et al. 2013). Previous studies showed that the Δ6-desaturase expression level in fresh-water fish increased when additional fish oil in the diet was replaced by vegetable oil (Zheng et al. 2005; Tocher et al. 2006). In this experiment, the expression of Δ6-desaturase-like gene mRNA in the liver increased significantly when LO and SO replaced the FO in the diet. In both the Atlantic salmon and the Rainbow trout, similar results were obtained (Seiliez et al. 2001). Δ6-desaturase is the rate-limiting enzyme involved in LC-PUFAs biosynthesis from 18:3n-3 and 18:2n-6. This study demonstrated that the high levels of DHA and EPA in the diet (FO) inhibited the Δ6-desaturase-like expression, which agrees with the results of previous studies (Zheng et al. 2005; Vagner and Santigosa 2010).

This is the first report of the sequence of the Δ6-desaturase-like gene and its expression patterns in the Manchurian trout. Clearly, the high sequence similarity between the Manchurian trout sequence and sequences of other salmonids suggests that this gene likely has the same function as a rate limiting enzyme in LC-PUFA biosynthesis. LO and SO in the feed up-regulate the Δ6-desaturase-like gene expression compared to FO in the diet. In addition, the Manchurian trout may have the ability to synthesize long-chain unsaturated fatty acids (LC-PUFAs) from ALA and LA. However, further studies are required to elucidate the ALA/LA ratio and molecular mechanisms of Δ6-desaturase involved in PUFA metabolism.

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Table 1. Ingredient and composition of experimental diets.

	FO	*SO*	*LO*
Defatted fishmeal [1]	44	44	44
Soybean meal	22	22	22
Gelatin	2	2	2
Wheat flour	10.45	10.45	10.45
Fish oil	16	0	0
Linseed oil	0	0	16
Sunflower oil	0	16	0
Soy lecithin	2	2	2
Vitamin premix [2]	1.5	1.5	1.5
Mineral premix [3]	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5
Ethoxy quin	0.05	0.05	0.05
Proximate composition			
Crude protein	39.45	39.84	39.50
Crude lipid	18.36	18.31	18.39
Ash	11.29	11.20	11.16

[1] Fishmeal was defatted with alcohol.
[2] One kilogram of vitamin mix contained the following: VB_1 25 mg; VB_2 45 mg; VB_6 20 mg; VB_{12} 0.l mg; VK_3, l0 mg; inositol 800 mg; VB_3 60 mg; niacin acid 200 mg; folic acid 20 mg; biotin 1.20 mg; VA 32 mg; VD_3 5 mg; VC 2150 mg; ethoxy quin 150 mg; wheat middling 16.51g.

[3] One kilogram of mineral mix contained the following: KI 80 mg; NaF 200 mg; $FeSO_4 \cdot H_2O$ 8000 mg; $CoCl_2 \cdot 6H_2O$ (1%) 5000 mg; $ZnSO_4 \cdot H_2O$ 5000 mg; $CuSO_4 \cdot 5H2O$ 1000 mg; $MnSO_4 \cdot H_2O$ 6000 mg; $Ca(H_2PO_4)_2 \cdot H_2O$ 750000 mg; $MgSO_4 \cdot 7H_2O$ 120000 mg; NaCl 1000 mg; zoelite powder 94270 mg.

Table 2. Fatty acid composition (% total fatty acids) of experimental diets.

Fatty acid	*FO*	*SO*	*LO*
C14:0	7.30	1.30	1.30
C15:0	1.00	0.10	0.12
C18:0	3.80	1.70	2.64
C16:0	22.10	10.30	8.50
∑SFA	34.20	13.40	12.56
C16:1	6.60	1.30	1.80
C18:1	15.40	23.90	20.00
C20:1n-9	2.50	0.20	3.74
C22:1n-9	3.50	0.50	6.60
∑MUFA	28.00	25.90	32.14
C18:2n-6(LA)	7.10	53.90	18.90
∑n-6PUFA	7.10	53.90	18.90
C18:3n-3(ALA)	2.20	0.70	29.50
C20:3n-3	1.10	0.20	0.40
C20:5n-3(EPA)	10.70	2.20	2.60
C22:6n-3(DHA)	16.60	3.60	3.90
∑n-3PUFA	30.60	6.70	36.40

SFA: saturated fatty acids.
MUFA: mono-unsaturated fatty acids.
n-6 PUFA: n-6 poly-unsaturated fatty acids.
n-3 PUFA: n-3 poly-unsaturated fatty acids.

Table 3. Primers used for PCR in the Manchurian trout.

Primer	*Sequence (5'-3')*	*Usage*
FAD6-F1	CGACMAGTGGTTGGTCATCG	RT-PCR
FAD6-R1	TTCAGTGACCYGACAACATC	RT-PCR
FAD6-F2	TCGCTTCTTCTGCTGTTACT	3'RACE
FAD6-F3	ACTGGCTCACCATGCAGTTG	3'RACE
FAD6-R2	ATGGTCCTGGCTGGGCTCTG	5'RACE
FAD6-R3	GCAACGGCTTCAGAAACTTC	5'RACE
FAD6-F4	GCATTCCATCCCAATCCTAA	qRT-PCR
FAD6-R4	CCCAGGTAGAGGCTGAAGAA	qRT-PCR
β-actin -F	ACTGGGACGACATGGAGAAG	qRT-PCR
β-actin -R	GAGGCGTACAGGGACAACAC	qRT-PCR

Table 4. Main fatty acid composition (% total fatty acids) of the dorsal muscle of Manchurian trout fed with different experimental diets.

Fatty acid	*Initial*	*FO*	*SO*	*LO*
C14:0	3.98	3.48±0.07^{b}	0.72±0.32^{a}	1.30±0.02^{a}
C16:0	22.21	19.01±0.57^{b}	12.89±0.56^{a}	13.46±0.59^{a}
C18:0	8.73	4.03±0.11^{a}	3.79±0.22^{a}	4.19±0.12^{a}
C24:0	1.13	1.71±0.07^{b}	0.91±0.06^{a}	1.04±0.07^{a}
∑SFA*	38.84	30.46±0.33^{b}	20.09±0.98^{a}	21.58±0.43^{a}
C16:1	3.62	3.79±0.15^{b}	1.08±0.05^{a}	1.43±0.06^{a}
C18:1n-9	23.57	12.33±0.40^{a}	16.62±0.59^{b}	16.49±0.61^{b}
C20:1	1.86	1.62±0.06^{b}	0.38±0.02^{a}	2.34±0.07^{c}
C22:1n-9	0.48	0.63±0.03^{b}	0.09±0.01^{a}	2.68±0.18^{c}
C24:1	1.09	0.98±0.06^{b}	0.53±0.01^{a}	0.97±0.06^{b}
∑MUFA	30.62	19.35±0.10^{a}	18.71±0.69^{a}	23.90±0.87^{b}
C18:2n-6 (LA)	14.21	7.99±0.30^{a}	38.28±0.74^{c}	14.31±0.55^{b}
C20:4n-6	1.23	0.99±0.08^{ab}	1.05±0.07^{b}	0.76±0.06^{a}
∑n-6PUFA*	15.72	9.32±0.22^{a}	40.88±0.67^{c}	15.60±0.50^{b}
C18:3n-3(ALA)	1.86	4.51±0.29^{b}	1.36±0.10^{a}	15.89±0.32^{c}
C20:5n-3(EPA)	1.73	6.78±0.29^{c}	2.33±0.11^{a}	3.41±0.12^{b}
C22:6n-3(DHA)	11.18	29.36±0.56^{c}	15.80±0.55^{a}	19.03±0.59^{b}
∑n-3PUFA*	14.82	40.86±0.56^{b}	19.55±0.77^{a}	38.77±1.03^{b}

Values are means ± SE from three treatments of fish with four fish per treatment. Different superscript letters in the same row indicate significant differences ($P < 0.05$).
* Including minor components not shown.

Table 5. Main fatty acid composition (% total fatty acids) of the liver of Manchurian trout fed different experimental diets.

Fatty acid	*Initial*	*FO*	*SO*	*LO*
C14:0	3.09	2.81±0.10^{c}	1.02±0.08^{a}	1.54±0.18^{b}
C16:0	32.18	30.05±0.57^{b}	22.71±0.34^{a}	20.83±0.47^{a}
C17:0	0.69	1.22±0.06^{b}	0.35±0.00^{a}	0.46±0.01^{a}
C18:0	11.10	6.67±0.05^{a}	7.33±0.03^{b}	6.56±0.06^{a}
∑SFA*	49.22	43.36±0.77^{b}	33.73±0.45^{a}	31.52±0.61^{a}
C18:1n-9	28.76	9.98±0.07^{a}	14.57±0.31^{b}	18.63±0.32^{c}
C16:1	2.54	2.34±0.05^{c}	0.86±0.04^{a}	1.45±0.05^{b}
C20:1	1.41	1.12±0.06^{b}	0.32±0.02^{a}	2.32±0.06^{c}
C22:1n-9	0.61	0.27±0.02^{a}	0.42±0.03^{a}	1.36±0.05^{b}
C24:1	2.87	3.06±0.11^{c}	1.08±0.06^{a}	1.84±0.04^{b}
∑MUFA	36.19	16.78± 0.31^{a}	17.24±0.46^{a}	25.47±0.48^{b}
C18:2n-6 (LA)	8.28	3.71±0.07^{a}	25.41±0.59^{c}	11.43±0.12^{b}
C20:4n-6	0.27	3.14±0.07^{b}	3.36±0.09^{b}	1.67±0.07^{a}
∑n-6PUFA*	8.77	7.02±0.14^{a}	30.59±0.68^{c}	13.44±0.19^{b}
C18:3n-3(ALA)	2.49	1.29±0.06^{a}	0.95±0.29^{a}	11.87±0.12^{b}
C20:5n-3(EPA)	0.80	3.95±0.10^{c}	1.29±0.06^{a}	2.33±0.12^{b}
C22:6n-3(DHA)	2.47	27.53±0.62^{b}	16.15±0.55^{a}	14.85±0.53^{a}
∑n-3PUFA*	5.82	32.90±0.79^{c}	18.41±0.41^{a}	29.59±0.77^{b}

Values are means ± SE from three treatments of fish with four fish per treatment. Different superscript letters in the same row indicate significant differences ($P < 0.05$).
* Including some minor components not shown.

Fig. 1. cDNA and predicted amino acid sequences of the Δ6-desaturase-like gene in the Manchurian trout. The single-letter amino acid code is shown above each corresponding codon. The translated region and the untranslated region are indicated by uppercase and lowercase letters, respectively. ATG and TAA indicate the start codon and stop codon, respectively (black boxes). The putative polyadenylation signal ATTAAA is double-underlined. Three underlined regions indicate the predicted conserved regions, which are known histidine-rich motifs. GenBank Accession Number: MH587164.

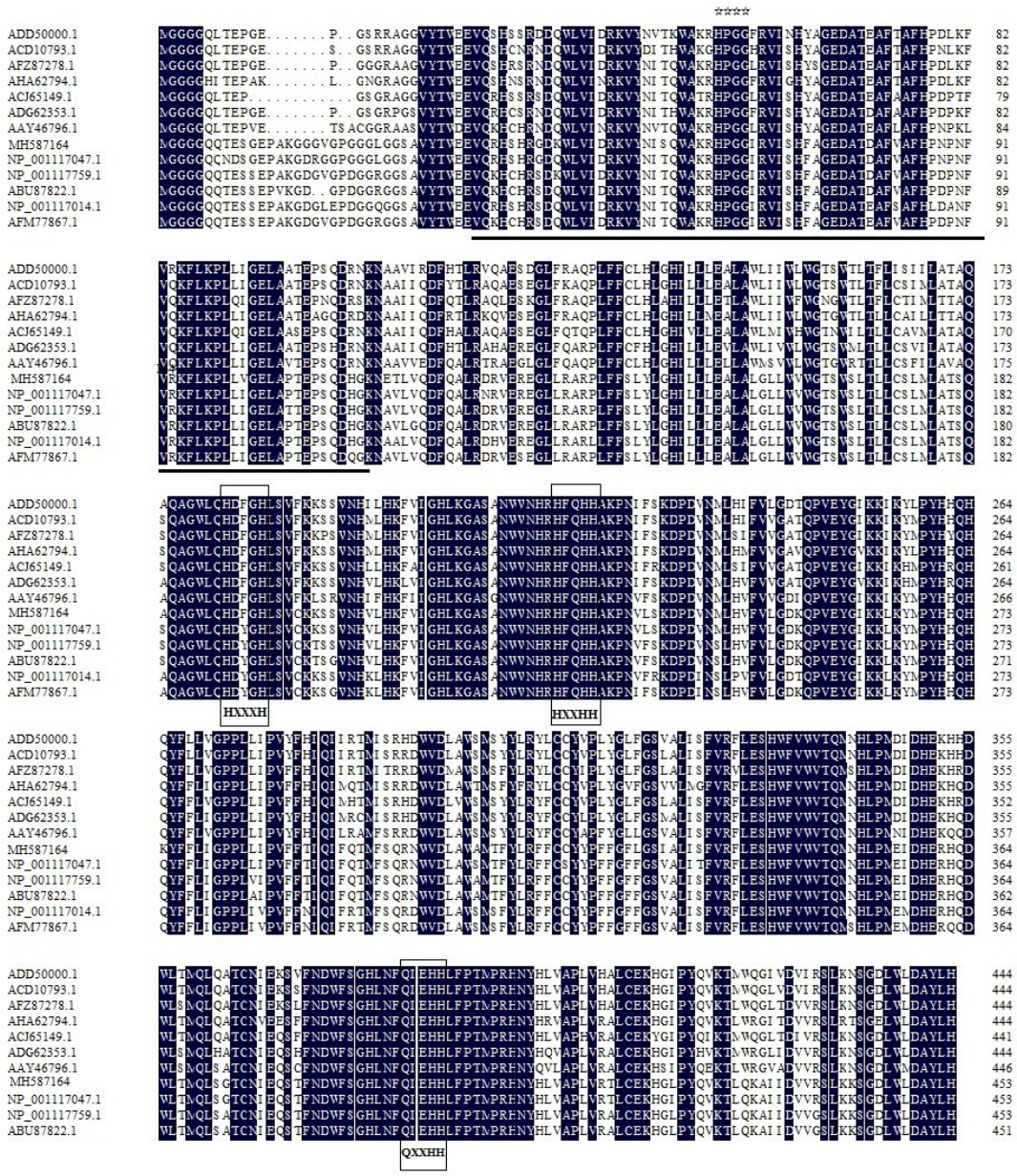

Fig. 2. Multiple alignment of predicted desaturase sequence of the Manchurian trout with corresponding sequences from other fish species. Identical residues (100%) are shaded in dark grey. The predicted cytochrome *b5* domain is underlined, and the heme-binding motif HPGG is marked with asterisks above the sequence. The histidine boxes (HXXXH, HXXHH, and QXXHH) are shown as black boxes. The following fish with Δ5- and Δ6-desaturase sequences (reported in GenBank) were included: *Brachymystax lenok* (MH587164), *Salmo salar* (NP_001117047.1), *Oncorhynchus mykiss* (NP_001117759.1), *Salmo salar* (NP_001117014.1), *Oncorhynchus masou* (ABU87822.1), *Oncorhynchus mykiss* (AFM77867.1), *Thunnus maccoyii* (ADG62353.1), *Sparus aurata* (ADD50000.1), *Dicentrarchus labrax* (ACD10793.1), *Scatophagus argus* (AHA62794.1), *Rachycentron canadum* (ACJ65149.1), *Lateolabrax japonicas* (AFZ87278.1), and *Gadus morhua* (AAY46796.1).

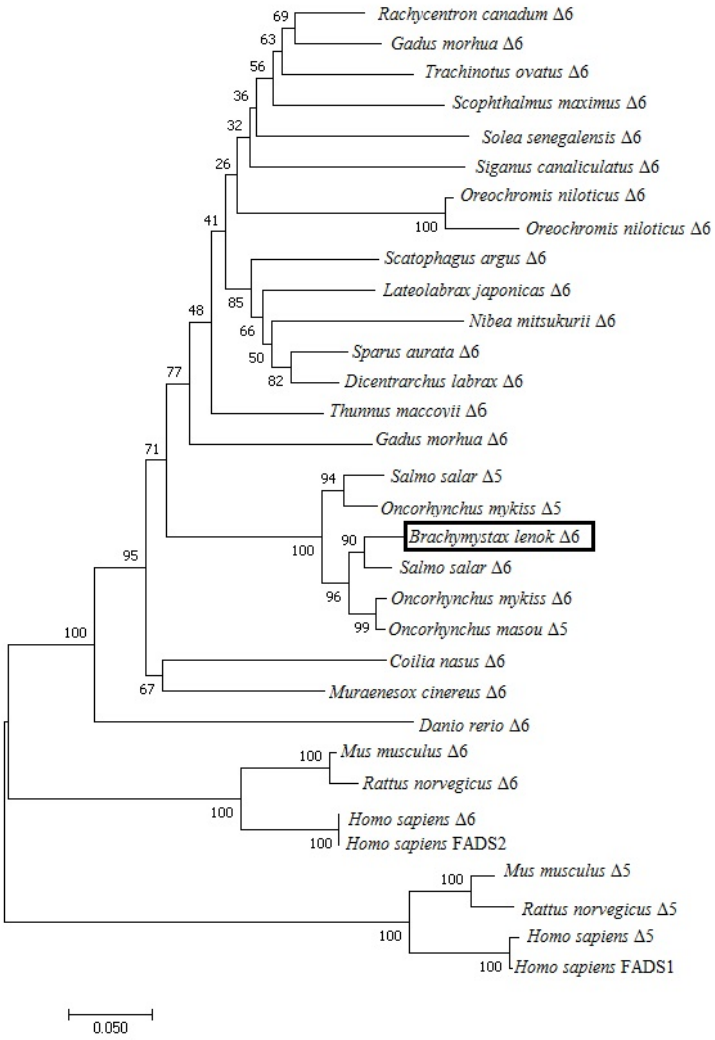

Fig. 3. Phylogenetic tree based on different fish species comparing the predicted Δ6-desaturase-like amino acid sequence of Manchurian trout with other Δ5-, Δ6-Fad, FADS1, and FADS2 sequences. The tree was constructed by MEGA 7.0 using the Neighbor-joining method (bootstrap value 1000 times). The Δ6-desaturase of the Manchurian trout is indicated with a black box. The following species with Δ5-, Δ6-Fad, FADS1, and FADS2 sequences (reported in GenBank) were included: *Brachymystax lenok* Δ6 (MH587164), *Salmo salar* Δ6 (NP_001117047.1), *Oncorhynchus mykiss* Δ6 (NP_001117759.1), *Salmo salar* Δ5 (NP_001117014.1), *Oncorhynchus masou* Δ5 (ABU87822.1), *Oncorhynchus mykiss* Δ5 (AFM77867.1), *Thunnus maccoyii* Δ6 (ADG62353.1), *Sparus aurata* Δ6 (ADD50000.1), *Dicentrarchus labrax* Δ6 (ACD10793.1), *Scatophagus argus* Δ6 (AHA62794.1), *Rachycentron canadum* Δ6 (ACJ65149.1), *Lateolabrax japonicas* Δ6 (AFZ87278.1), *Gadus morhua* Δ6 (AAY46796.1), *Lates calcarifer* Δ6 (ACS91458.1), *Coilia nasus* Δ6 (AHA82393.1), *Nibea mitsukurii* Δ6 (ACX54437.1), *Trachinotus ovatus* Δ6 (AKQ44348.1), *Oreochromis niloticus* Δ6 (AGV52807.1), *Solea senegalensis* Δ6 (AEQ92868.1), *Scophthalmus maximus* Δ6 (AAS49163.1), *Muraenesox cinereus* Δ6 (AEV57604.1), *Siganus canaliculatus* Δ6 (ABR12315.2), *Oreochromis niloticus* Δ6 (NP_001266552.1), *Mus musculus* Δ6 (AAD20017.1), *Rattus norvegicus* Δ6 (BAA75496.1), *Homo sapiens* Δ6 (AAD20018.1), *Mus musculus* Δ5 (BAB69894.1), *Rattus norvegicus* Δ5 (AAG35068.1), *Homo sapiens* Δ5 (AAF29378.1), *Danio rerio* Δ6 (AAG25710.1), *Homo sapiens* ADS2 (NP_004256), and *Homo sapiens* FADS1 (NP_037534).

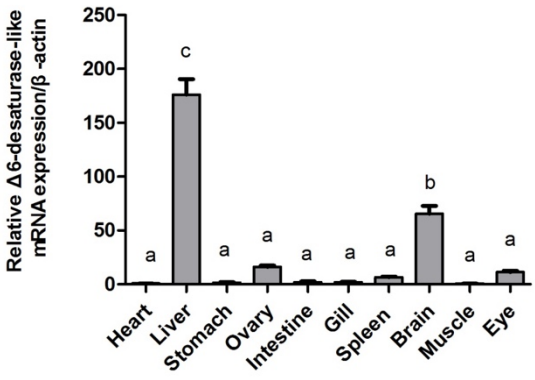

Fig. 4. Relative Δ6-desaturase-like mRNA expression levels normalized to β-actin levels in different tissues of the Manchurian trout compared to control tissue (heart). Values are means ± SE (n = 6). Different letters on the bars indicate that the expression levels in these tissues are statistically significantly different at $P < 0.05$.

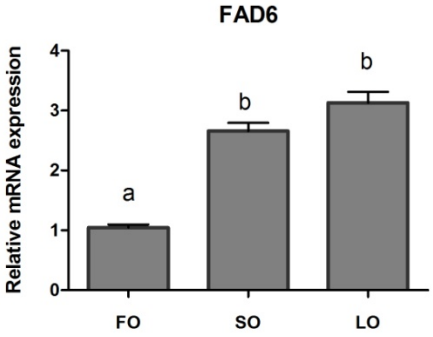

Fig. 5. Effect of different oil sources in diets on the Δ6-desaturase-like mRNA expression normalized to β-actin in the liver of Manchurian trout compared to FO diet. Values are means ± SE from three treatments of fish (n = 3) with four fish per treatment. Different letters on the bars indicate statistical significance ($P < 0.05$).