Comparative Biochemistry and Physiology, Part B 163 (2012) 184–192

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

Influence of dietary fatty acids on muscle fatty acid composition and expression levels of Δ6 desaturase-like and Elovl5-like elongase in common carp (*Cyprinus carpio* var. Jian)

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ARTICLE INFO

Article history:
Received 19 March 2012
Received in revised form 22 May 2012
Accepted 22 May 2012
Available online 4 June 2012

Keywords:
Common carp (*Cyprinus carpio* var. Jian)
Dietary fatty acids
Δ6 desaturase-like
Elovl5-like elongase
Gene expression

ABSTRACT

The effects of dietary fatty acids on muscle fatty acid composition and liver expression levels of Δ6 desaturase-like and Elovl5-like elongase were investigated in common carp (*Cyprinus carpio* var. Jian). Two Δ6 desaturase-like cDNAs (Fad6-a and Fad6-b) and two Elovl5-like elongase (Elovl5-a and Elovl5-b) cDNAs were cloned. Juvenile carp were fed three semi-purified diets (D1–3) for 6 weeks with different lipid sources: D1, fish oil with high highly unsaturated fatty acids (HUFAs); D2, corn oil with high linoleic acid (LA), but no HUFAs; and D3, linseed oil with high α-linolenic acid (LNA), but no HUFAs. Comparing muscle fatty acids among fish fed D1–3, the content of LA and arachidonic acid (AA) in common carp fed D2 and the content of LNA, EPA and DHA in common carp fed D3 were higher than initial levels ($P<0.05$), respectively. The liver transcript levels of Fad6-a and Elovl5-a in fish fed D2 and D3 were higher than initial levels ($P<0.05$), but Fad6-b and Elovl5-b levels were seldom affected by the diets. The dietary fatty acids affect the muscle fatty acid composition and the liver Fad6-a and Elovl5-a gene expression levels in common carp, and further studies should be undertaken.

1. Introduction

Fish are an important source of n−3 highly unsaturated fatty acids (HUFAs), which have beneficial health effects for humans (Eilander et al., 2007; Ruxton et al., 2007). It is generally thought that freshwater fish have a capacity to convert C_{18} polyunsaturated fatty acid (PUFA), linoleic acid (LA; 18:2n−6) and α-linolenic acid (LNA; 18:3n−3) to $C_{20/22}$ HUFAs, such as arachidonic acid (AA; 20:4n−6), eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3) (Cook, 1996; Buzzi et al., 1997; Glencross, 2009). At the molecular level, the capacity of HUFA biosynthesis was attributed to the levels of desaturase and elongase enzymatic activities, and the capacity of fatty acyl desaturation and elongation varies with species (Tocher et al., 2003). Biosynthesis of HUFAs in fish involves the sequential desaturation and elongation of precursor C_{18} PUFAs, and Δ6-desaturase and Elovl5 elongase are the critical enzymes in the biosynthetic pathway of HUFAs (Tocher et al., 2004; Zheng et al., 2004a). Δ6-Desaturase can catalyze the insertion of double bonds at the Δ6 position in the carbon backbone, and Elovl5 elongase is responsible for the pre-existing chain (Jakobsson et al., 2006). Δ6-Desaturase cDNAs have been cloned from several freshwater fish species (Seiliez et al., 2001, 2003; Zheng et al., 2004b; Hastings et al., 2005), 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). Elovl5 elongase cDNAs have been cloned from several freshwater species, such as zebrafish (*D. rerio*), Nile tilapia (*Oreochromis niloticus*), Atlantic salmon (*S. salar*) and rainbow trout (*Oncorhynchus mykiss*) (Agaba et al., 2004, 2005; Hastings et al., 2005; Zheng et al., 2009). To date, only one Δ6-desaturase was cloned from the common carp (*Cyprinus carpio*) (Hastings et al., 2001), and no Elovl5 elongase has been isolated from the common carp. Common carp (*C. carpio*) are commonly regarded as tetraploid owing to their high chromosome number and DNA content. This has led to the hypothesis that common carp may have more Δ6-desaturases and Elovl5 elongases or a more complicated regulatory mechanism for HUFA biosynthesis than other freshwater fish.

The common carp (*C. carpio* var. Jian) is a freshwater, omnivorous and bottom-living species, and part of their diet includes benthic animals. Common carp in ponds fed formulated feed have unique fatty acid profiles that contain low levels of EPA, DHA and n−3/n−6

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doi:10.1016/j.cbpb.2012.05.016

ratio compared with some other marine fish species and freshwater fish (Ackman, 2002; Guler et al., 2008; Donmez, 2009). It is postulated that the supply of HUFAs from the benthic animals or the formulated feed could possibly impair the HUFA biosynthesis pathway in the common carp. As with all vertebrates, C_{18} PUFAs are essential in the diet of fish, but requirements vary within species (Tocher, 2010). In aquaculture, the fatty acid composition of farmed fish depends on both dietary fatty acid input and the endogenous HUFA biosynthesis capacity. Some fish Δ6-desaturase conversion rates of fatty acids substrates were reported (Hastings et al., 2001; Seiliez et al., 2001; Zheng et al., 2004b, 2005, 2009). The conversion rate of 18:3n−3 to 18:4n−3 was 31.5%, 60.1%, 7.0%, 59.5% and 50.8% in rainbow trout (*O. mykiss*), Atlantic salmon (*S. salar*), common carp (*C. carpio*), trout (*Scophthalmus maximus*) and cobia (*Rachycentron canadum*), respectively. In comparison, the conversion rate of 18:2n−6 to 18:3n−6 was 3.6%, 14.4%, 1.5%, 31.2% and 36.5% in rainbow trout (*O. mykiss*), Atlantic salmon (*S. salar*), common carp (*C. carpio*), trout (*S. maximus*) and cobia (*R. canadum*), respectively. Vegetable oils (VO) are rich in C_{18} PUFAs, but devoid of the n−3HUFAs abundant in fish oil (FO) (Sargent et al., 2002). Feeding fish with VO can have important effects on the HUFA content of the flesh, thus compromising the nutritional value (Bell and Tocher, 2009). The total C_{18} PUFA level in the diet has been demonstrated to not only modulate the activity of bioconversion enzymes, but also to affect the efficiency and affinity of the Δ6-desaturase enzyme (Francis et al., 2009). Our long-term objective is to clarify if the common carp has HUFA biosynthesis capacity and to understand the molecular basis of HUFA biosynthesis and regulation with different C_{18} PUFAs in the diet. We would like to determine if the HUFA biosynthesis pathway can enable efficient and effective use of VO in the common carp while maintaining the nutritional quality of the fish. However, little information on the fatty acid desaturation and elongation in the common carp is available. Therefore, the present study aims to clone common carp (*C. carpio* var. Jian) Δ6 desaturase-like and Elovl5-like elongase genes and examine the effects of dietary fatty acids on fatty acid composition and Δ6 desaturase-like and Elovl5-like elongase gene expression levels in the common carp.

2. Materials and methods

2.1. Diets, animals and experimental design

We prepared three formulated diets (D1–D3) with approximately equal content of total protein but different compositions of essential fatty acids (EFAs) (Table 1). Each diet had casein as a protein source, and FO, corn oil or linseed oil (the latter two are HUFA-free) as the EFAs source. D1 contained 0.56% AA, 4.70% EPA and 6.24% DHA, but D2 and D3 were HUFA-free, with LA and LNA accounting for 52.30% and 0.61%, 18.38% and 45.46% of the total fatty acids, respectively. The diets were prepared every week, packed in separate plastic bags according to the daily ration (2–3% of body weight) and stored at −18 °C. When feeding, the dietary powder was mixed with freshwater and fed as a paste twice daily at 8:00 and 16:00 h. Excess food was offered to ensure that fish were fed to satiation. Uneaten feed and feces were removed by siphoning twice daily prior to feeding.

Common carp juveniles (body mass 45 to 50 g, sex was indistinguishable by eye) were captured from the Yi Xing base of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences. They were randomly distributed into nine glass tanks (20 fish per tank) and randomly assigned to one of three experimental treatments (three replicate tanks per treatment). The glass tanks were connected to a recirculation system with automatic temperature control. The carp were acclimated to laboratory conditions for 2 weeks and then fed the experimental diets for 6 weeks. During the experiment water temperature and pH were monitored daily, and dissolved oxygen and ammonia–N were measured weekly. Water temperature was maintained at 20 °C ± 2 °C, ammonia–N was less than 0.5 mg/L, dissolved oxygen was more than 5 mg/L, and pH varied between 6.5 and 6.7. The photoperiod was set on a 12 h light and 12 h dark cycle.

Table 1
Ingredients and composition of the experimental diets (D1, D2 and D3) for the common carp (*C. carpio* var Jian)[a].

	D1	D 2	D3
Ingredients (g/100 g diet)			
Casein	32	32	32
Gelatin	8	8	8
Dextrin	28	28	28
Cellulose	19	19	19
Fish oil	6	0	0
Corn oil	0	6	0
Linseed oil	0	0	6
Carboxy methyl cellulose	2	2	2
Mineral premix[b]	4	4	4
Vitamin premix[c]	1	1	1
Proximate composition (%)			
Crude protein	35.76	35.44	35.39
Crude lipid	5.86	5.67	5.49
Ash content	6.78	6.89	6.57
Main fatty acids (% total fatty acids)			
18:2n−6 (LA)	2.72	52.30	18.38
20:4n−6 (AA)	0.56	0	0
18:3n−3 (LNA)	0.96	0.61	45.46
20:5n−3 (EPA)	4.70	0	0
22:6n−3 (DHA)	6.24	0	0

[a] All the ingredients and chemicals used were purchased from Sangon and East China Pharmaceuticals Company, Shanghai, China.
[b] Mineral premix consisted of (g kg^{-1} of premix): aluminum chloride, 0.45; cobalt chloride, 0.2; copper sulfate, 2.0; ferrous sulfate, 19.5; potassium iodide, 0.5; potassium chromium sulfate, 0.3; magnesium sulfate, 300.0; manganese sulfate, 7.5; sodium selenite, 0.02; zinc sulfate, 37; cellulose, 632.5.
[c] Vitamin premix consisted of (mg kg^{-1} of premix): thiamin hydrochloride, 10; riboflavin, 20; calcium pantothenate, 40; nicotinic acid, 50; pyridoxine hydrochloride, 10; folic acid, 5; inositol, 400; choline chloride, 2000; menadione, 10; cholecalciferol, 1500 IU; biotin, 1; vitamin B12, 0.02; vitamin A, 3000 IU; vitamin E, 50 IU; vitamin C, 200.

2.2. Sampling

Fish were weighed at the start (Initial Mass) and the end (Final Mass) of the experiment, and fish mortality was recorded daily in each aquarium. At the start of experiment, liver and muscle were collected from six of the water-adapted common carp. After experimentation for 3 days, 14 days and 42 days, three fish from each aquarium were anesthetized in 0.01% 2-phenoxyethanol and killed. Liver and muscle tissues were collected and flash-frozen in liquid nitrogen, and kept at −80 °C for subsequent analysis of fatty acids composition and the tissue-specific expression of Δ6 desaturase-like and Elovl5-like elongase. The fish were fasted for 24 h prior to sampling.

2.3. Cloning of full-length Δ6 desaturase-like and Elovl5-like elongase cDNAs

Total RNA was extracted from 30 to 50 mg of the liver tissue kept at −80 °C, using the Trizol Reagent Kit (Takara, Japan). Internal fragments of the Δ6-desaturase and Elovl5 cDNAs were cloned using reverse-transcription polymerase chain reaction (RT-PCR). First strand cDNA was synthesized using 2.0 μg of total RNA with Reverse Transcriptase M-MLV (Takara). PCR primers to amplify internal fragments of the Δ6-desaturase and Elovl5 elongase cDNAs were designed based on highly conserved regions from the gene of other fish available in the GenBank database and synthesized by Biosune (Shanghai, China). PCR amplifications using primer set A (Table 2) and *Taq* DNA Polymerase (Takara) were performed with an initial denaturation at 94 °C for 2 min; 30 cycles of denaturation step at 94 °C for 30 s, annealing at T_m (annealing temperature according to