

## Molecular Cloning and Expression Analysis of the Full-Length cDNA of the Acetyl-CoA Carboxylase Gene in Grass Carp (*Ctenopharyngodon idella*) Postprint

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### Abstract

This study employed reverse transcription PCR (RT-PCR) and rapid amplification of cDNA ends (RACE) techniques to clone the full-length cDNA of acetyl-CoA carboxylase (ACC2) gene from grass carp, and used real-time fluorescent quantitative PCR to investigate ACC2 gene expression in various tissues including hepatopancreas, spleen, brain, foregut, midgut, hindgut, kidney, muscle, heart, and mesenteric fat. Additionally, the expression changes of ACC2 mRNA in hepatopancreas and muscle of grass carp fed different lipid source diets for 12 weeks, as well as in hepatopancreas at 3, 6, 12, and 24 h after refeeding following starvation, were examined. The results showed that the full-length cDNA of grass carp ACC2 gene was 7,533 bp, containing a 7,149 bp open reading frame encoding 2,382 amino acids. The calculated molecular mass of ACC2 protein was 268.34 ku, with an isoelectric point of 6.13. Alternative splicing was observed in grass carp ACC2 gene, generating another isoform containing 8 fewer amino acids than the 268.34 ku ACC2 protein. ACC2 gene was expressed in all examined tissues, with the highest relative expression level of ACC2 mRNA in muscle (29.13) and the lowest in hepatopancreas (1.90). The relative expression level of ACC2 mRNA in muscle showed no significant difference from that in brain, foregut, and heart ( $P > 0.05$ ), but was significantly higher than in other tissues ( $P < 0.05$ ). Feeding different lipid source diets had no significant effect on the relative expression level of ACC2 mRNA in hepatopancreas and muscle of grass carp ( $P > 0.05$ ). After refeeding following starvation, the relative expression level of ACC2 mRNA in grass carp hepatopancreas peaked at 12 h (6.17), then decreased significantly, reaching only 2.84 at 24 h. This study successfully cloned the full-length cDNA of grass carp ACC2 gene, whose main functional sites, ATP-binding site and biotin-binding site, were basically

conserved compared with other vertebrates. Grass carp ACC2 gene was mainly expressed in tissues with active lipid catabolism such as muscle; feeding different lipid source diets had no significant effect on the relative expression level of ACC2 mRNA in grass carp hepatopancreas; after refeeding following starvation, the relative expression level of ACC2 mRNA in hepatopancreas was highest at 12 h post-feeding.

## Full Text

### Molecular Cloning and Expression Analysis of Full-Length cDNA of Acetyl-CoA Carboxylase Gene from Grass Carp (*Ctenopharyngodon idella*)

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#### Abstract

This study employed reverse transcription PCR (RT-PCR) and rapid amplification of cDNA ends (RACE) techniques to clone the full-length cDNA of acetyl-CoA carboxylase (ACC2) from grass carp. Real-time quantitative PCR was used to investigate ACC2 gene expression across various tissues including hepatopancreas, spleen, brain, anterior intestine, mid-intestine, posterior intestine, kidney, muscle, heart, and mesenteric adipose tissue. Additionally, we examined ACC2 mRNA expression changes in hepatopancreas and muscle of grass carp fed diets with different lipid sources for 12 weeks, as well as in hepatopancreas at 3, 6, 12, and 24 hours after starvation and refeeding.

The results revealed that the full-length ACC2 cDNA was 7,533 bp, containing a 7,149 bp open reading frame encoding 2,382 amino acids. The calculated molecular weight of ACC2 protein was 268.34 ku with an isoelectric point of 6.13. Alternative splicing was identified in grass carp ACC2 gene, generating another isoform lacking eight amino acids compared to the 268.34 ku ACC2 protein. ACC2 gene was expressed in all examined tissues, with the highest relative mRNA expression level detected in muscle (29.13) and the lowest in hepatopancreas (1.90). Muscle ACC2 mRNA expression showed no significant difference from brain, anterior intestine, and heart ( $P>0.05$ ), but was significantly higher than other tissues ( $P<0.05$ ). Different dietary lipid sources had no significant effect on ACC2 mRNA expression in hepatopancreas and muscle ( $P>0.05$ ). Following starvation and refeeding, hepatopancreatic ACC2 mRNA expression peaked at 12 hours (6.17) and then decreased markedly to 2.84 at 24 hours.

In conclusion, we successfully cloned the full-length cDNA of grass carp ACC2 gene. The main functional sites, including ATP-binding and biotin-binding sites,

are essentially conserved compared with other vertebrates. Grass carp ACC2 gene is predominantly expressed in tissues with active lipolysis such as muscle. Dietary lipid source does not significantly affect ACC2 mRNA expression in hepatopancreas, and the highest expression level occurs at 12 hours after refeeding following starvation.

**Keywords:** grass carp; acetyl-CoA carboxylase ; molecular cloning; gene expression

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## Introduction

Acetyl-CoA carboxylase (ACC) is the rate-limiting enzyme in fatty acid synthesis. Using biotin as a cofactor, it catalyzes the conversion of acetyl-CoA to malonyl-CoA (MA), providing substrates for fatty acid synthesis [1-2]. In prokaryotes, ACC comprises three subunits encoded by separate genes: biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP), and carboxyltransferase (CT). In eukaryotes, a single gene encodes all three functional domains [3]. Two ACC isoforms exist: ACC1 (or ACC ) and ACC2 (or ACC ), encoded by ACACA and ACACB genes, respectively. These isoforms exhibit distinct tissue distribution patterns—ACC1 is predominantly expressed in lipogenic tissues such as liver, adipose tissue, and mammary gland to supply substrates for fatty acid synthesis, whereas ACC2 is mainly expressed in tissues with active fatty acid oxidation such as muscle and heart, where it regulates carnitine palmitoyltransferase-I (CPT-I) activity [4].

In homeothermic animals, full-length cDNAs of ACC1 have been cloned from rat [5] and cattle [6], while human ACC2 cDNA has also been characterized. Human ACC2 gene contains a 7,449 bp open reading frame encoding 2,483 amino acids and is located on chromosome 12q23 [7]. ACC2 shares 75% amino acid sequence similarity with ACC1, with the main difference residing in the N-terminus—mammalian ACC2 contains approximately 140 additional amino acids that target it to the mitochondrial outer membrane [8-9]. Research on fish ACC genes remains limited, though full-length cDNA of grass carp ACC1 has been previously cloned [10].

Grass carp (*Ctenopharyngodon idella*), belonging to Cypriniformes and Cyprinidae, represents an important aquaculture species in China due to its rapid growth, delicious meat, wide feed availability, and economic benefits. However, intensive aquaculture has led to excessive hepatic lipid accumulation and associated metabolic disorders in fish. With rapid advances in molecular biology, investigating nutritional regulation mechanisms at the molecular level has become a new trend in nutrition research. This study aimed to clone the full-length cDNA of grass carp ACC2 gene, analyze its tissue expression patterns, and examine ACC2 mRNA expression changes in muscle and hepatopancreas of grass carp fed different lipid sources, as well as in hepatopancreas after starvation and refeeding. These investigations will help

elucidate the role of ACC2 in lipid metabolism regulation, provide references for optimizing grass carp feed formulation, and offer theoretical support for addressing nutritional fatty liver disease in farmed fish.

## Materials and Methods

**1.1.1 Experimental Fish** Experimental fish were purchased from Huandun Fishery in Ganyu District, Lianyungang City, with specifications of (56.4±1.8) g/fish.

**1.1.2 Experimental Primers** The primers used in this study are listed in Table 1. Three pairs of ACC2 real-time quantitative PCR primers were designed. For candidate reference genes, two pairs of primers were designed for each of  $\beta$ -actin (ACTB), eEF1A, glyceraldehyde-3-phosphate dehydrogenase (GAPDH)1, GAPDH2, RPL13A, and TUBB2. Primers with amplification efficiency between 0.90 and 1.05 were selected as final quantitative primers.

**Table 1 Primer sequences and expected product sizes for the experiment**

Primer names	Sequence (5' →3' )	Expected product size
<b>Reverse transcription PCR primers</b>		
ACC2-F1	AGTTTGTCACCTCGCTTTGGA	
ACC2-R1	CACTCACATAGCCCACCATT	
ACC2-F2	AATGGTGGGCTATGTGAGT	
ACC2-R2	GCAGAGGGTGACCTGAGTA	
ACC2-F3	ACCAGGAACCGTATTAGAG	
ACC2-R3	TAAGGCAGCCATACAAAC	
ACC2-F4	ACCGTAGTGGAGTTAGAGGC	
ACC2-R4	GAGCAGCACCCAAGTAGAG	
ACC2-F5	AAGACMGAATYTACMGWAACCT	
ACC2-R5	CGACTRCCRTAKCGCATC	
ACC2-F6	ACCCTTCAAAGATAGAGGAGTC	
ACC2-R6	GCRGGTGTAGTCYTGAGGHG	
ACC2-F7	TTACGGGCATCTGAGTTA	
ACC2-R7	ACTTVAGCAYCTGRTCRTA	
ACC2-F8	ACAGTTGAGGTTGCCATTC	
ACC2-R8	CYRCTTKGTTATTRTCCC	
<b>3' and 5' RACE primers</b>		
Oligo(dT)16AP	CTGATCTAGAGGTACCGGATCC(T)16	
Race3-R	CTGATCTAGAGGTACCGGATCC	
AC-F1	GGAGCGTAAAGACTTGGAG	
AC-F2	GTTTGCGGCGACTGCTGTT	
AC-R1	TCGCTAGTCCCTCGTCCACATC	

Primer names	Sequence (5' →3' )	Expected product size
AC-R2	TCATCTCAACATTGGCATAG	
<b>RT-qPCR primers</b>		
qACC2-F3	TCTACTTGGGTGCTGCTCG	
qACC2-R3	TGATGAGGTCAGAATGGCG	
qeEF1A-F3	CGCCAGTGTTGCCTTCGT	
qeEF1A-R3	CGCTCAATCTTCCATCCCTT	
qACTB-F3	GATGATGAAATTGCCGCACTG	
qACTB-R3	ACCGACCATGACGCCCTGATGT	
qGAPDH1-F1	CCAAGTGTCAGGACGAACAGAGG	
qGAPDH1-R1	TGCGACCGAATCCGTTGATACC	
qGAPDH2-F2	CAAGGCTGTGGGCAAAGTCATTCC	
qGAPDH2-R2	GAGGCGGCAGGTGAGGTCAAC	
qRPL13A-F	CTTCTGGAGGACAGTAAGAGGTAT	
qRPL13A-R	GGAGGAGGGATGCCATCAAAGAC	

**1.1.3 Total RNA Extraction and First-Strand cDNA Synthesis** Six grass carp were dissected alive 6 hours after feeding. Tissues including hepatopancreas, spleen, kidney, anterior intestine, mid-intestine, posterior intestine, mesenteric adipose tissue, brain, white muscle, and heart were rapidly isolated and ground in liquid nitrogen. Total RNA from each tissue was extracted using the RNeasy Lipid Tissue Mini Kit (QIAGEN) following the manufacturer's protocol. RNA integrity was assessed by 1% agarose gel electrophoresis, and RNA concentration was determined using a nucleic acid-protein quantifier. First-strand cDNA was synthesized using the QIAGEN reverse transcription kit with Oligo(dT)16AP as primer, following the recommended procedures for DNA contamination removal and reverse transcription.

**1.1.4 ACC2 Gene cDNA Cloning** Based on zebrafish (*Danio rerio*) (XM\_009301377) and previously amplified grass carp sequences, four pairs of degenerate primers and four pairs of specific primers were designed to amplify eight core fragments of ACC2 gene. The 25  $\mu$ L PCR reaction system contained: 12.5  $\mu$ L Sapphire Amp Fast PCR Master Mix (TaKaRa), 1  $\mu$ L mixed first-strand cDNA from hepatopancreas and heart, 0.5  $\mu$ L each of forward and reverse primers, and 10.5  $\mu$ L double-distilled water (ddH<sub>2</sub>O). Amplification conditions were: 94°C denaturation for 40 s, 52°C annealing for 30 s, and 72°C extension for 1 min, for 35 cycles, with initial denaturation at 95°C for 3 min and final extension at 72°C for 7 min. Products were detected by 1% agarose gel electrophoresis, gel-purified, cloned, and sequenced.

**1.1.5 3' and 5' RACE of Grass Carp ACC2 Gene** Based on the core sequences of ACC2 gene, 3' and 5' RACE specific primers were designed (Table 1). The 3' and 5' ends were cloned following the method described by Cheng et al. [10].

**1.1.6 Sequence Analysis** The core sequences and 3' and 5' end sequences were assembled using SeqMan software from the DNASTar 7.1 package to obtain the full-length ACC2 gene. Signal peptides were predicted using <http://www.cbs.dtu.dk/services/SignalP/>, and protein domains were analyzed through <http://smart.embl-heidelberg.de/>.

**1.2 Tissue Expression of Grass Carp ACC2 Gene** Experimental fish were purchased from Huandun Fishery in Ganyu District, Lianyungang City, with specifications of  $(56.4 \pm 1.8)$  g/fish. Six grass carp were dissected alive, and tissues including hepatopancreas, spleen, kidney, anterior intestine, mid-intestine, posterior intestine, mesenteric adipose tissue, brain, white muscle, and heart were rapidly isolated and ground in liquid nitrogen. Total RNA from each tissue was extracted and first-strand cDNA was synthesized using random primers following the recommended procedures for DNA contamination removal and reverse transcription. SYBR Green RT-qPCR was performed on a Step One Plus PCR instrument (ABI) using the QuantiNova SYBR Green PCR Kit (QIAGEN). Each sample was run in triplicate. Candidate reference genes included ACTB, eEF1A, GAPDH1, GAPDH2, RPL13A, and TUBB2. Reference genes were selected using the geNorm algorithm in GenEx 6.0.1 software for quantitative analysis of grass carp ACC2 gene tissue expression.

**1.3.1 Experimental Diets** Three experimental groups were established with different lipid sources: 5% fish oil (fish oil group), 5% lard oil (lard oil group), and 5% soybean oil (soybean oil group). Other dietary components were identical among groups (Table 2). Main ingredients including fish meal, fish oil, and premix were provided by Yuetai Group. All ingredients were weighed, mixed thoroughly for 10 min, and 500 mL water per kg of ingredients was added. After kneading for 15 min, pellets were produced using a portable meat grinder, dried in an oven at 55°C for 3 h, cooled at room temperature for 1 h, sealed in bags, labeled, and stored for later use.

**Table 2 Composition and nutrient levels of experimental diets (DM basis)**

Items	Fish oil	Lard oil	Soybean oil
<b>Ingredients</b>			
Imported fish meal			
Soybean meal			
Cottonseed meal			
Blood powder			
Wheat middling			
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>			
L-Lys			
DL-Met			
Choline			

Items	Fish oil	Lard oil	Soybean oil
Fish oil	5%		
Lard oil		5%	
Soybean oil			5%
Premix <sup>1</sup>			
<b>Total</b>			
<b>Nutrient levels<sup>2</sup></b>			
Crude protein			
Crude fat			

<sup>1</sup>The premix provided the following per kg of diets: VA 5,000 IU, VD 2,000 IU, VK 5 mg, VE 50 mg, VB1 8 mg, VB2 10 mg, VB12 0.03 mg, VB6 8 mg, pantothenic acid 30 mg, nicotinic acid 30 mg, folic acid 3 mg, biotin 0.4 mg, VC 180 mg, inositol 100 mg, Mg 300 mg, Cu 4 mg, Fe 170 mg, Zn 150 mg, Mn 22 mg, I 1 mg, Co 0.25 mg, Se 0.4 mg.

<sup>2</sup>Calculated values.

**1.3.2 Feeding Management** The feeding trial was conducted in nine tanks (240 L each) of a single-cycle controlled experimental aquaculture system. A total of 180 grass carp were purchased from Huandun Fishery in Ganyu District, Lianyungang City, with specifications of (56.4±1.8) g/fish. Fish were randomly divided into three groups with three replicates per group (20 fish per tank). The culture period lasted 12 weeks at 25-28°C. After stocking, fish were fed commercial diets for one week to acclimate them to surface feeding, after which experimental diets were gradually introduced. During the trial, fish were fed four times daily at 08:00, 11:00, 14:00, and 17:00. Initial daily feeding rate was set at 2% of body weight (25 g/tank) and adjusted based on feed consumption. Daily feed intake and feeding behavior were recorded, with feeding amount gradually increased to 35 g/tank.

**1.3.3 Effects of Different Dietary Lipid Sources on ACC2 Gene Expression** After the feeding trial, remaining fish were counted and weighed individually. Three fish per tank (nine per group) were dissected to collect viscera for viscerosomatic index calculation. Hepatopancreas and muscle were collected for total RNA extraction and ACC2 gene expression analysis.

**1.3.4 Effects of Starvation and Refeeding on ACC2 Gene Expression** After the feeding trial, fish were fasted for 24 h and then refed. At 3, 6, 12, and 24 h post-refeeding, three fish per tank (nine per group) were dissected to collect hepatopancreas for total RNA extraction and ACC2 gene expression analysis.

**1.3.5 Calculation Formulas** Weight gain rate (%) = 100 × (final mean body weight - initial mean body weight) / initial mean body weight

Feed coefficient = total feed intake per tank / total weight gain per tank

Viscerosomatic index (%) =  $100 \times \text{visceral weight} / \text{final body weight}$

**1.3.6 Data Analysis** Reference genes were selected using the geNorm algorithm in GenEx 6.0.1 software. Relative ACC2 gene expression was calculated using the  $2^{-\Delta\Delta Ct}$  method and expressed as mean  $\pm$  standard deviation. Statistical analysis was performed using SPSS 17.0 software. All data passed normality and homogeneity of variance tests. Inter-group differences were analyzed by one-way ANOVA, with multiple comparisons conducted using LSD test.  $P < 0.05$  was considered statistically significant.

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## Results

### 2.1 Molecular Characteristics of Grass Carp ACC2 Full-Length cDNA

The full-length cDNA of grass carp ACC2 gene was 7,533 bp, containing a 7,149 bp open reading frame encoding 2,382 amino acids. The calculated molecular weight of ACC2 protein was 268.34 ku with an isoelectric point of 6.13, designated as ACC -1 (GenBank accession number: MF611923). Additionally, alternative splicing generated another isoform of grass carp ACC2 (267.53 ku), designated as ACC -2 (GenBank accession number: MF611924), which lacked eight amino acids compared to ACC -1.

### 2.2 Tissue Expression of Grass Carp ACC2 Gene

Using RPL13A and eEF1A as reference genes, RT-qPCR analysis revealed that ACC2 gene was expressed in all ten examined tissues. The highest relative mRNA expression was detected in muscle (29.13), followed by brain (19.45), anterior intestine (15.33), and heart (14.40), while the lowest expression was found in hepatopancreas (1.90). Muscle ACC2 mRNA expression showed no significant difference from brain, anterior intestine, and heart ( $P > 0.05$ ), but was significantly higher than other tissues ( $P < 0.05$ , Figure 1 [Figure 1: see original paper]).

*Value columns with different letters indicate significant difference ( $P < 0.05$ ). The same applies below.*

### Figure 1 Expression analysis of ACC2 gene in different tissues of grass carp

### 2.3 Effects of Different Dietary Lipid Sources on Growth Performance

Growth performance was measured after the feeding trial to calculate weight gain rate, feed coefficient, and viscerosomatic index. As shown in Table 3, weight gain rates in fish oil and soybean oil groups were 139.6% and 138.8%, respectively, significantly higher than that in lard oil group (128.8%), indicating that fish oil and soybean oil are suitable lipid sources for grass carp, while lard oil was less effective. Feed coefficients in fish oil and soybean oil groups were 1.14

and 1.17, respectively, significantly lower than that in lard oil group ( $P < 0.05$ ), suggesting that lard oil is not suitable as a sole lipid source for grass carp.

**Table 3 Effects of different dietary lipid sources on growth indexes of grass carp**

	Initial Groups weight/g	Final weight/g	Visceral somatic index/%	Weight gain rate/%	Feed coefficient
Fish oil	56.40±1.80	135.10±0.10 <sup>a</sup>	8.74±0.57	139.60±0.10 <sup>a</sup>	1.14±0.01 <sup>a</sup>
Lard oil	56.40±1.80	129.00±0.70 <sup>b</sup>	8.22±0.17	128.80±1.20 <sup>b</sup>	1.24±0.01 <sup>b</sup>
Soybean oil	56.40±1.80	133.00±1.00 <sup>a</sup>	8.89±0.50	138.80±1.70 <sup>a</sup>	1.17±0.02 <sup>a</sup>

*In the same column, values with different letter superscripts indicate significant difference ( $P < 0.05$ ).*

**2.4 Effects of Different Dietary Lipid Sources and Starvation/Refeeding on ACC2 Gene Expression** Six reference genes were analyzed with Ct values and amplification efficiencies from different lipid source groups and starvation/refeeding groups using software analysis. RPL13A and GAPDH2 were automatically selected as reference genes for hepatopancreas and muscle expression in different lipid source groups, while ACTB and GAPDH1 were selected for starvation/refeeding expression analysis. The results showed that different dietary lipid sources had no significant effect on ACC2 mRNA expression in hepatopancreas and muscle ( $P > 0.05$ , Figure 2 [Figure 2: see original paper]). After starvation and refeeding, hepatopancreatic ACC2 mRNA expression increased significantly at 12 h post-feeding ( $P < 0.05$ ), reaching a peak value of 6.17, then decreased significantly ( $P < 0.05$ ) to 2.84 at 24 h (Figure 3 [Figure 3: see original paper]).

**Figure 2 Relative expression level of ACC2 mRNA in hepatopancreas and muscle of grass carp fed diets with different lipid sources**

**Figure 3 Relative expression level of ACC2 mRNA in hepatopancreas of grass carp after starvation and refeeding**

## Discussion

**3.1 Structure of Grass Carp ACC2 Protein** Analysis of functional domains in grass carp ACC2 amino acid sequence is shown in Figure 4 [Figure 4: see original paper]. Grass carp ACC2 is encoded by a single gene containing three domains: BC, BCCP, and CT, which constitute the three subunits

of ACC2. The glycine-rich region (GGGGKG) is considered the ATP-binding domain [11]. The BC domain of grass carp ACC2 contains an ATP-binding site at Gly371-376, which is identical to those in grass carp ACC1 [10], half-smooth tongue sole [12], and humans [7,13], indicating high conservation of this sequence. The conserved Met-Lys-Met sequence is recognized as the biotin-binding site [14]. The BCCP domain of grass carp ACC2 contains two tetrapeptide sequences, Val-Met-Lys-Met and Arg-Met-Lys-Met, located at amino acid residues 840-843 and 1698-1701, respectively. Studies have shown that biotin upregulates ACC2 gene expression in hypothalamus, and excess biotin administration suppresses food intake in mice [15].

*BC: biotin carboxylase; BCCP: biotin carboxyl carrier protein; CT: carboxyl-transferase; ACC2 central region: ACC2 central region.*

#### **Figure 4 Three domains of ACC2 protein in grass carp**

**3.2 Tissue Expression of Grass Carp ACC2 Gene** Grass carp ACC2 gene was expressed in all ten examined tissues, with the highest expression in muscle and the lowest in hepatopancreas. Muscle expression was 15.3-fold higher than hepatopancreas expression (Figure 1 [Figure 1: see original paper]). Abu-Elheiga et al. [7] reported that ACC2 gene is highly expressed in human heart and skeletal muscle. Similarly, studies have shown that rat ACC2 is predominantly expressed in oxidative tissues such as skeletal muscle and heart [4]. These findings are consistent with our results, indicating that grass carp ACC2 gene, like that of other mammals, is mainly expressed in muscle. Since the product of ACC2, malonyl-CoA, is a potent inhibitor of CPT-I, ACC2-deficient mammals exhibit significantly reduced malonyl-CoA levels in heart and skeletal muscle. Other studies have confirmed that ACC2 activity determines malonyl-CoA levels in heart, further demonstrating that ACC2 gene regulates fatty acid oxidation in skeletal muscle and heart [16-17].

#### **3.3 Effects of Different Dietary Lipid Sources on Growth Performance**

In this study, no significant differences in viscerosomatic index were observed among groups, possibly due to low dietary lipid levels. Weight gain rates in fish oil and soybean oil groups were significantly higher than in lard oil group, while feed coefficient was significantly higher in lard oil group compared to fish oil and soybean oil groups. These results indicate that fish oil and soybean oil are suitable lipid sources for grass carp with no significant difference between them, whereas lard oil is not suitable as a sole lipid source. Zhang et al. [18] found that bullfrogs fed diets containing fish oil, soybean oil, and palm oil showed high weight gain rates with no significant differences among these groups, but all were significantly higher than those fed poultry fat and lard oil. Chen et al. [19] fed red tilapia fry diets containing 4% lard oil, 4% soybean oil, 4% soybean oil-fish oil mixture, and 4% fish oil for 60 days, observing that final body weight and weight gain rate in soybean oil and soybean oil+fish oil groups were significantly higher than in lard oil group, which had the highest feed coefficient. Soybean

oil group showed the best growth performance with no significant differences in hepatosomatic index among groups, suggesting soybean oil is a good lipid source for red tilapia fry. Cheng et al. [20] used soybean oil and lard oil as lipid sources for grass carp and found that lard oil group had the highest lipid content in hepatopancreas and muscle and most mesenteric fat accumulation, with hepatopancreas morphology showing fatty liver tendency, while soybean oil group showed normal hepatopancreas morphology, indicating soybean oil is superior to lard oil for grass carp. Liu et al. [21] fed grass carp fry diets containing fish liver oil, soybean oil, rapeseed oil, lard oil, and mixed oil, finding that mixed oil and fish liver oil groups showed the best growth performance with highest relative weight gain rate and lowest feed coefficient, followed by soybean oil and lard oil groups. These findings are generally consistent with our results.

**3.4 Effects of Different Dietary Lipid Sources and Starvation/Refeeding on ACC2 Gene Expression** In this study, grass carp were fed diets containing fish oil, soybean oil, and lard oil for 12 weeks to investigate ACC2 expression changes in hepatopancreas and muscle. No significant differences in ACC2 mRNA expression were observed in hepatopancreas or muscle among groups (Figure 2 [Figure 2: see original paper]), suggesting that ACC2 expression in grass carp is relatively stable and not affected by dietary lipid type. Limited research has examined ACC2 mRNA expression changes in fish fed different lipid sources. Olson et al. [22] found that ACC2 gene knockout in mouse skeletal muscle had no significant effect on body weight, food intake, or body composition, and that total malonyl-CoA content and fatty acid oxidation rate in skeletal muscle also remained unchanged, indicating metabolic compensation and minimal impact of ACC2 deficiency on energy balance. Studies on male rats revealed no direct correlation between skeletal muscle fatty acid oxidation and ACC2 gene phosphorylation [23]. Torstensen et al. [24] fed Atlantic salmon diets containing capelin oil, palm oil, sunflower oil, and mixed oil (capelin oil:sunflower oil=1:1), finding no significant effects on fatty acid -oxidation in muscle and liver or on salmon growth, indirectly supporting our finding that different lipid sources do not significantly affect ACC2 expression in grass carp muscle. Our previous study also found that different dietary lipid sources had no significant effect on ACC1 gene expression in grass carp hepatopancreas [10].

In this study, hepatopancreatic ACC2 mRNA expression increased significantly at 12 h after starvation and refeeding, then decreased significantly at 24 h (Figure 3 [Figure 3: see original paper]). Oh et al. [25] reported that ACC2 mRNA expression in rat liver increased significantly at 12 h after feeding and remained elevated at 24 h post-refeeding, suggesting transcriptional regulation of ACC2 gene expression. These results partially differ from our findings, possibly due to species differences. Ryu et al. [26] reported that ACC1 mRNA expression in mouse liver increased significantly after starvation and refeeding with high-fat diet. Other studies also found that ACC1 and fatty acid synthase gene expres-

sion in chicken liver increased significantly 2 h after starvation and refeeding, while CPT-I mRNA expression decreased significantly [27]. In contrast, grass carp hepatopancreatic ACC2 mRNA expression showed no significant difference during 0-6 h after refeeding, suggesting that fatty acid synthesis dominates during this period in grass carp hepatopancreas.

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## Conclusion

1. The full-length cDNA of ACC2 gene was successfully cloned from mixed hepatopancreas and heart tissues of grass carp. The main functional sites, including ATP-binding and biotin-binding sites, are essentially conserved compared with other vertebrates.
2. Grass carp ACC2 gene is predominantly expressed in tissues with active lipolysis such as muscle.
3. Dietary lipid source does not significantly affect ACC2 mRNA expression in grass carp hepatopancreas.
4. After starvation and refeeding, the highest ACC2 mRNA expression level in hepatopancreas occurs at 12 h post-feeding.

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