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# Characterization, mRNA expression and regulation of Δ6 fatty acyl desaturase (FADS2) by dietary n − 3 long chain polyunsaturated fatty acid (LC-PUFA) levels in grouper larvae (*Epinephelus coioides*)

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ABSTRACT

N − 3 long chain polyunsaturated fatty acids (LC-PUFAs) are essential for marine fish to maintain normal growth and physiological functions, although the ability of LC-PUFA biosynthesis is limited. FADS2 was chosen to investigate its characterization, mRNA expression and regulation by dietary n − 3 LC-PUFA in the present study due to its being considered as the rate-limiting enzyme involved in LC-PUFA biosynthetic pathway. The FADS2 of grouper, when expressed in yeast, was shown to desaturate 18:3n − 3 and 18:2n − 6, indicating that it coded for a Δ6 desaturase enzyme. Lower desaturation of 20:3n − 3 and 20:2n − 6 indicated trace Δ8 activity. Following 4 weeks of feeding trial, the FADS2 mRNA expressions of grouper larvae that were fed diets with different LC-PUFA levels were measured and results showed that the expression of FADS2 increased significantly with dietary LC-PUFA from 0.52% to 0.94% and then decreased significantly ($P < 0.05$). The different growth performance and fatty acid composition of grouper among five treatment larvae also demonstrated that though the ability to synthesize LC-PUFA in grouper larvae was limited. These results showed that the FADS2 of grouper also displayed Δ8 desaturase ability when expressed in yeast, and dietary n − 3 LC-PUFA could regulate the biosynthesis of LC-PUFA through influencing the expression of FADS2.



## 1. Introduction

N − 3 long chain polyunsaturated fatty acids (LC-PUFAs) have been termed essential fatty acids (EFA) for marine fish, which are necessary for its normal development and functioning of nervous system and sensory organs (Benitez-Santana et al., 2007; Ishizaki et al., 2001). Meanwhile, n − 3 LC-PUFAs also play crucial role in structural components of cell membranes (Marsh, 2008), eicosanoids precursors (Bell et al., 2006; Tocher, 2003; Villalta et al., 2008), and regulator of gene expression (Calder, 2012; Deckelbaum et al., 2006). However, previous studies have shown that most marine fish, unlike freshwater species, need exogenous n − 3 LC-PUFAs to maintain normal growth, survival and physiological functions (Izquierdo et al., 1989; Kanazawa et al., 1979; Lee and Cho, 2009), which means that marine fish species have limited capacity to synthesize n − 3 LC-PUFAs de novo from their 18-carbon precursor fatty acids (Kanazawa et al., 1979; Tocher, 2003). N − 3 LC-PUFAs are also beneficial for human health, which could promote the development of neuronal tissues and protect against cardiovascular, immune and inflammatory condition (Calder, 2007; Salem et al., 2001). However, fish are the main source of LC-PUFAs for humans. Therefore, large amounts of research have drawn much attention on expression and activities of enzymes involved in LC-PUFA biosynthetic pathway.

The LC-PUFA biosynthetic pathway in fish was traditionally considered to proceed through consecutive desaturation and elongation reactions to convert C18 polyunsaturated fatty acids (PUFAs) linolenic acid (LNA, 18:3n − 3) and linoleic acid (LA, 18:2n − 6) to LC-PUFA, including docosahexenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) (Monroig et al., 2011). The Δ6 fatty acyl desaturase (FADS2) has been widely studied in fish since it was considered as the rate-limiting enzyme involved in LC-PUFA biosynthetic pathway (Hastings et al., 2004; Mohd-Yusof et al., 2010; Santigosa et al., 2011; Tocher et al., 2006; Zheng et al., 2009a). The enzyme is responsible for the step to convert 18:3n − 3 and 18:2n − 6 to 18:4n − 3 and 18:3n − 6, respectively. Meanwhile, it also involved in the "Sprecher shunt" pathway to produce DHA through EPA (Monroig et al., 2011; Sprecher, 2000). Monroig et al. (2011) found that FADS2 also displayed higher Δ8 activity than freshwater/diadromous species, which demonstrated a possible alternative "elongation–Δ8 desaturation–Δ5 desaturation" pathway. Monroig et al. (2011) also found that FADS2 activities of some marine fish are comparable with freshwater/diadromous species in yeast expression system, and this may indicate that the activity of FADS2 was enough for LC-PUFA biosynthesis of those marine fish species. However, to our knowledge no information is available on this assumption. Although the functional characterization and regulation of

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FADS2 have been widely studied on freshwater/seawater fish, no study has been conducted to determine the functional characterization and nutritional regulation of orange spotted grouper FADS2. One purpose of this study was to determine the activity of grouper Δ6 desaturase and whether the grouper putative FADS2 has the Δ8 desaturase ability. The nutritional regulation of FADS2 was also investigated to better understand the mechanism of LC-PUFA biosynthesis.

Orange spotted grouper, *Epinephelus coioides*, is a popular fish cultured in Southeast Asia and is a good candidate for intensive aquaculture of the region due to its fast growth, efficient feed conversion and high market value (Millamena, 2002). Lin et al. (2007) have reported that fish oil could be fully substituted by vegetable oils in fish meal-based diets for juvenile grouper without negatively affecting growth performance and feed utilization, which supposed that grouper may need a low level of n−3 LC-PUFAs or this LC-PUFA could be biosynthesized from ALA (18:3n−3) to some extent. However, as far as we know, no information was available on n−3 LC-PUFAs requirement and its biosynthetic ability of grouper. Compared with juvenile, fish larvae are much more sensitive to deficiency of dietary n−3 LC-PUFAs due to its important functions to maintain normal development and quick growth (Benitez-Santana et al., 2007; Bransden et al., 2005; Izquierdo, 1996). This difference makes fish larvae become a potential ideal model to study the ability of LC-PUFA biosynthesis, especially for some species with low n−3 LC-PUFAs requirement. Thus the present study was designed to investigate characterization, mRNA expression and regulation of FADS2 by dietary n−3 LC-PUFA levels in grouper larvae.

## 2. Materials and methods

### 2.1. Grouper

Grouper larvae were bought from a local fish rearing farm in Yandun, Hainan, China. The body mass of grouper used for cloning FADS2 was 10.0 g and the initial body weight of grouper larvae used for n−3 LC-PUFA level study was 70 ± 2 mg (29DAH).

### 2.2. Total RNA extraction and reverse transcription

Total RNA was extracted from grouper liver using Trizol Reagent (Takara, Japan) followed by quality measurement on a 1.2% denaturing agarose gel and yield determination on NanoDrop® ND-1000 (Wilmington, DE). The RNA was treated with RNA-Free DNase (Takara, Japan) to remove DNA contaminant and reversely transcribed to cDNA by PrimeScript™ RT reagent Kit (Takara, Japan) following the instructions provided by the manufacturer.

### 2.3. Functional characterization of grouper FADS2 by heterologous expression in Saccharomyces cerevisiae

PCR fragment corresponding to the open reading frame (ORF) of FADS2 was amplified from grouper liver cDNA using High Fidelity PrimeScript® RT-PCR Kit with primers containing restriction sites (underlined in Table 1). The DNA fragment containing the grouper FADS2 was digested with corresponding restriction endonucleases (Takara, Japan) and ligated into a similar restricted yeast episomal plasmid PYES2 (Invitrogen, UK). Then the purified plasmids containing FADS2 ORF were transformed to *S. cerevisiae* competent cells using S.c. EasyComp Transformation Kit (Invitrogen, UK). Transformation and selection of yeast with recombinant plasmids (pYES2–FADS2), and yeast culture were conducted as described in detail previously (Agaba et al., 2004; Li et al., 2013; Tocher, 2003). A single colony of transgenic yeast was grown in *S. cerevisiae* minimal medium$^{-uracil}$ supplemented with one of the following FA substrates: 18:3n−3 (0.5 mM), 18:2n−6 (0.5 mM), 20:3n−3 (0.75 mM), 20:2n−6 (0.75 mM), 20:4n−3 (0.75 mM), 20:3n−6 (0.75 mM), 22:5n−3 (1.0 mM) and 22:4n−6 (1.0 mM). The final concentrations of substrate FA were as described by Monroig et al. (2010). All the FA substrates (>98 ~ 99% pure) were purchased from Cayman Chemical Co. and chemicals used to prepare the *S. cerevisiae* minimal medium$^{-uracil}$ were from Sigma Chemical Co. Ltd. After 2 days' culture, yeast was harvested and washed twice by 5 mL ice-cold HBSS (Invitrogen, UK) and freeze dried for 24 h for further analyses. Yeast transformed with PYES2 containing no insert was grown under the same conditions as a control treatment.

### 2.4. Fatty acid analysis of cultured yeast

Fatty acid methyl ester (FAME) was prepared by the incubation of dried yeast samples with 1 N KOH–Methanol and then 2 N HCl–Methanol. After incubation, FAME was extracted by adding 1 mL hexane containing 0.01% BHT as antioxidant. FAMEs were separated and quantified using HP6890 gas chromatograph equipment with a fused silica capillary column (007-CW) and a flame ionization detector. The procedures for analysis of the fatty acid profiles were based on the method described by Metcalfe et al. (1966) with some modification (Ai et al., 2008). The column temperature was programmed to rise from 150 °C up to 200 °C at a rate of 15 °C min$^{-1}$, from 200 °C to 250 °C at a rate of 2 °C min$^{-1}$. Injector and detector temperature were 250 °C, respectively. Proportions of substrate FA converted to elongated FA product were calculated as [product area / (product area + substrate area)] * 100. The identities of fatty acid peaks and their double bond positions were performed by GCMS–QP2010Ultura (Shimadzu, Japan) as described previously (Agaba et al., 2004; Hastings et al., 2001).

### 2.5. n−3 LC-PUFA level study

Triplicate groups of grouper larvae (29DAH, 70 ± 2 mg) were fed to apparent satiation six times daily (07:00, 09:00, 11:00, 13:00, 15:00 and 17:00) for 4 weeks with five isoproteic (58% crude protein) and isolipidic (16% crude lipid) diets containing graded levels of n−3 LC-PUFA (0.52, 0.94, 1.57, 1.97 and 2.43%) (Tables 2 & 3). Prior to harvest, the fish were fasted for 24 h and weighted. Specific growth rate (SGR, percent per day) was calculated as 100 × [ln(final weight) − ln(initial weight)] / days. Visceral mass from five fish in each cage were pooled into 1.5 mL tube (RNase-Free, Axygen, USA), frozen in liquid nitrogen and then stored at −80 °C for later analysis of FADS2 expression. The remaining fish of each tank were pooled into 5 mL tube, frozen in liquid nitrogen and then stored at −80 °C for the assay of body composition and fatty acid composition.

**Table 1**
Details of primer pairs (restriction sites for HindIII and Xho I underlined) used for the cloning of grouper Δ6 ORF in pYES2, and qPCR primers.

| Aim | Target gene | Primer | Primer sequence (5′–3′) | Reference[a] |
|---|---|---|---|---|
| ORF cloning | Δ6 FAD | H3FAF | CCCAAGCTTGGGAGGATGGGAGGTGGAGGCCA | GenBank: EU715405 |
| | | X1FAR | CCGCTCGAGCGGTCATTTATGGAGATATGCGT | |
| qPCR | Δ6 FAD | FAqF | CTCATCATTTGGGTCTGGG | GenBank: EU715405 |
| | | FAqR | GAAGATGTTGGGTTTAGCG | |
| | β-actin | ACqF | TACGAGCTGCCTGACGGACA | GenBank: AY510710 |
| | | ACqR | GGCTGTGATCTCCTTCTGCA | |

[a] GenBank (http://www.ncbi.nlm.nih.gov/).

**Table 2**
Formulation and proximate analysis of the experimental diets (% dry weight).

| Ingredients (%) | Dietary n−3 LC-PUFA contents (% dry weight) | | | | |
|---|---|---|---|---|---|
| | 0.52 | 0.94 | 1.57 | 1.97 | 2.43 |
| Casein[a] | 13 | 13 | 13 | 13 | 13 |
| Defatted white fish meal[a] | 35 | 35 | 35 | 35 | 35 |
| Defatted krill meal[a] | 10 | 10 | 10 | 10 | 10 |
| Squid meal[a] | 5 | 5 | 5 | 5 | 5 |
| Hydrolyzed fish meal[a] | 8 | 8 | 8 | 8 | 8 |
| LT-Yeast[a] | 2 | 2 | 2 | 2 | 2 |
| Alginate sodium | 2 | 2 | 2 | 2 | 2 |
| α-starch | 5 | 5 | 5 | 5 | 5 |
| Vitamin premix[b] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Mineral premix[c] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Attractant[d] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Ethoxyquin | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Choline chloride | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| DHA enriched oil[e] | 0.33 | 1.45 | 2.56 | 3.66 | 4.8 |
| EPA enriched oil[f] | 0.02 | 0.66 | 1.31 | 1.95 | 2.58 |
| Palmitin[g] | 9.75 | 7.99 | 6.23 | 4.49 | 2.72 |
| ARA enrich oil[h] | 1 | 1 | 1 | 1 | 1 |
| Soy lecithin | 4 | 4 | 4 | 4 | 4 |
| Mold inhibitor[i] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Proximate analysis (n = 3) | | | | | |
| Crude protein (%) | 57.98 | 57.21 | 57.15 | 57.20 | 57.71 |
| Crude lipid (%) | 15.09 | 15.24 | 15.11 | 15.46 | 15.75 |
| Ash (%) | 16.08 | 16.11 | 15.29 | 15.30 | 15.19 |

[a] Casein: crude protein 87.91% dry matter, crude lipid 1.69% dry matter; defatted fish meal: crude protein 73.36% dry matter, crude lipid 1.52% dry matter; defatted krill meal: crude protein 71.80% dry matter, crude lipid 2.93% dry matter; squid meal: crude protein 61.72% dry matter, crude lipid 3.16% dry matter; hydrolyzed fish meal: crude protein 77.10% dry matter, crude lipid 4.60% dry matter.
[b] Vitamin premix (IU or g $kg^{-1}$ vitamin premix): retinal palmitate, 3,000,000 IU; cholecalciferol, 1,200,000 IU; DL-α-tocopherol acetate, 40.0 g; menadione, 8.0 g; thiamin-HCl, 5.0 g; riboflavin, 5.0 g; D-calcium pantothenate, 16.0 g; pyridoxine-HCl, 4.0 g; meso-inositol, 200.0 g; D-biotin, 8.0 g; folic acid, 1.5 g; para-aminobenzoic acid, 5.0 g; niacin, 20.0 g; cyanocobalamin, 0.01 g; ascorbyl polyphosphate (contained 25% ascorbic acid), 100.0 g.
[c] Mineral premix (g $kg^{-1}$): $Ca(H_2PO_4)_2 \cdot H_2O$, 675.0; $CoSO_4 \cdot 4H_2O$, 0.15; $CuSO_4 \cdot 5H_2O$, 5.0; $FeSO_4 \cdot 7H_2O$, 50.0; KCl, 50.0; KI, 0.1; $MgSO_4 \cdot 2H_2O$, 101.7; $MnSO_4 \cdot 4H_2O$, 18.0; NaCl, 80.0; $Na_2SeO_3 \cdot H_2O$, 0.05; $ZnSO_4 \cdot 7H_2O$, 20.0.
[d] Attractant (g 100 $g^{-1}$): betaine, 50; glycine,15; alanine, 10; argine, 10; taurine, 10; inosine-5′-monophosphoric acid, 5.
[e] DHA enriched oil: DHA content, 40.64% of TFA; in the form of DHA-methylester; Jiangsu Tiankai Biotechnology Co., Ltd., China.
[f] EPA enriched oil: EPA content, 46.41% of TFA; DHA content, 23.66% of TFA; both in the form of triglyceride; Hebei Haiyuan Health biological Science and Technology Co., Ltd., China.
[g] Palmitin: palmitic acid content, 98.7% of TFA, in the form of methylester; Shanghai Zhixin Chemical Co., Ltd., China.
[h] ARA enriched oil: ARA content, 53.69% of TFA, in the form of ARA-methylester; JIANGSU TIANKAI Biotechnology Co., Ltd., China.
[i] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

**Table 3**
Fatty acid composition of the experimental diets (% total fatty acids).

| Fatty acid | Dietary n−3 LC-PUFA contents (% dry weight) | | | | |
|---|---|---|---|---|---|
| | 0.52 | 0.94 | 1.57 | 1.97 | 2.43 |
| 14:0 | 1.17 | 1.51 | 2.08 | 2.35 | 2.72 |
| 16:0 | 69.64 | 59.20 | 47.90 | 44.71 | 34.05 |
| 18:0 | 1.50 | 1.81 | 1.98 | 1.91 | 2.10 |
| 20:0 | 0.73 | 0.85 | 1.05 | 1.09 | 1.21 |
| ∑SFA[a] | 73.04 | 63.37 | 53.01 | 50.06 | 40.08 |
| 18:1 | 6.23 | 6.47 | 6.87 | 6.63 | 6.96 |
| ∑MUFA[b] | 6.23 | 6.47 | 6.87 | 6.63 | 6.96 |
| 18:2n−6 | 8.36 | 8.95 | 10.76 | 9.00 | 9.25 |
| 18:3n−6 | 0.38 | 0.27 | 0.45 | 0.34 | 0.40 |
| 20:4n−6 | 2.81 | 3.20 | 3.48 | 3.41 | 3.77 |
| ∑n−6 PUFA[c] | 11.55 | 12.42 | 14.69 | 12.75 | 13.42 |
| 18:3n−3 | 1.01 | 1.08 | 1.41 | 1.02 | 1.06 |
| 18:4n−3 | 0.29 | 0.32 | 0.44 | 0.37 | 0.43 |
| 20:5n−3 | 1.48 | 3.11 | 5.34 | 6.71 | 8.56 |
| 22:6n−3 | 2.92 | 6.09 | 11.04 | 13.78 | 18.22 |
| ∑n−3 PUFA[d] | 5.70 | 10.59 | 18.23 | 21.88 | 28.27 |
| n−3/n−6 PUFA | 0.48 | 0.85 | 1.25 | 1.73 | 2.14 |
| n−3 LC-PUFA[e] | 4.40 | 9.20 | 16.38 | 20.49 | 26.78 |
| DHA/EPA[f] | 1.98 | 1.96 | 2.07 | 2.05 | 2.13 |
| EPA/ARA[g] | 0.53 | 0.97 | 1.53 | 1.97 | 2.27 |

[a] SFAs: saturated fatty acids.
[b] MUFAs: mono-unsaturated fatty acids.
[c] n−6 PUFAs: n−6 polyunsaturated fatty acids.
[d] n−3 PUFAs: n−3 polyunsaturated fatty acids.
[e] n−3 LC-PUFAs: n−3 long chain polyunsaturated fatty acids.
[f] DHA/EPA: 22:6n−3/20:5n−3.
[g] EPA/ARA:20:5n−3/20:4n−6

### 2.6. Fatty acid analysis of tissue sample

Four fish of each replicate tank, which were stored at −80 °C, were dissected to separate the visceral tissues and muscle on glass plate maintained at 0 °C. Fatty acid methyl esters (FAMEs) from fatty acids of freeze-dried diets and fish tissue samples were prepared and quantified using HP6890 gas chromatograph following the method described above.

### 2.7. Real-time quantitative PCR analysis of FADS2 expression

Real-time RT-PCR was carried out in a quantitative thermal cycler (Mastercyclerepprealplex, Eppendorf, Germany). The amplification was performed in a total volume of 25 μL containing 2× SYBR® Premix Ex Taq™ (Perfect Real Time) (Takara, Japan), 0.5 μL of each primer (10 μmol $L^{-1}$), and 1 μL of cDNA mix. The real-time RT-PCR program was as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 57 °C for 10 s, and 72 °C for 20 s. The real-time RT-PCR primer pairs for Δ6 desaturase and β-actin (Table 1) were designed by Primer Premier 5.0 based on the published nucleotide sequences. At the end of each PCR reaction, melting curve analysis of amplification products was carried out to confirm that a single PCR product was present in these reactions. Standard curves were made with five different dilutions (in triplicate) of the cDNA samples and amplification efficiency was analyzed according to the following equation $E = 10^{(-1/Slope)} - 1$. The expression levels of the target genes were calculated followed the $2^{-\Delta\Delta t}$ method described by Yao et al. (2009).

### 2.8. Statistical analysis

The results were given as means ± S.E.M. (standard error of the mean). Data from each treatment were subjected to one-way analysis of variance (ANOVA) and correlation analysis where appropriate using SPSS 19.0 for Windows. Tukey's multiple range test was chosen as a multiple comparison test and the significance level of 5% was used.

## 3. Results

### 3.1. Functional characterization of grouper fatty acyl desaturase

The putative FADS2 of grouper was functionally characterized by determining the fatty acid profiles of transformed *S. cerevisiae* with either empty PYES2 vector (control) or the vector containing FADS2 ORF inserts and grown in the presence of potential fatty acid substrates for Δ6 Fad (18:3n−3 and 18:2n−6), Δ5 Fad (20:4n−3 and 20:3n−6), Δ4 Fad (22:5n−3 and 22:4n−6) and Δ8 Fad (20:3n−3 and 20:2n−6). Fatty acid composition of the yeast transformed with only PYES2 shows four main fatty acids, namely 16:0, 16:1n−7; 18:0 and 18:1n−9 (Hastings et al., 2001). When yeast transformed with grouper desaturase ORF was cultured in the presence of Δ6 Fad substrates, two additional peaks were observed in the fatty acid profiles of *S. cerevisiae*, corresponding to the exogenously added fatty acids, 18:3n−3 and 18:2n−6, and

**Table 4**
Activity of grouper putative fatty acyl desaturase in the yeast expression system[a].

| Fatty acid substrate | Product | Conversion (%) | Activity |
|---|---|---|---|
| 18:3n−3 | 18:4n−3 | 9.78 | Δ6 |
| 18:2n−6 | 18:3n−6 | 4.4 | Δ6 |
| 20:3n−3 | 20:4n−3 | 3.67 | Δ8 |
| 20:2n−6 | 20:3n−6 | 1.64 | Δ8 |
| 20:4n−3 | 20:5n−3 | ND | Δ5 |
| 20:3n−6 | 20:4n−6 | ND | Δ5 |
| 22:5n−3 | 22:6n−3 | ND | Δ4 |
| 22:4n−6 | 22:5n−6 | ND | Δ4 |

[a] ND: non-detected.

their desaturated products, 18:4n−3 and 18:3n−6, respectively. The GC traces of desaturase transformed yeast in the presence of Δ8 Fad substrates showed large peaks for the exogenously added fatty acids, 20:3n−3 and 20:2n−6, but only traces of desaturated products, 20:4n−3 and 20:4n−6, respectively. Meanwhile, no additional peaks were observed in the fatty acid profiles of *S. cerevisiae*, when the transformed yeast was cultured in the presence of potential fatty acid substrates for Δ5 and Δ4 Fad. However, in this heterologous assay system, the grouper desaturase showed a preference for n−3 fatty acid substrates, with approximately 9.78% of 18:3n−3 converted to 18:4n−3 and only 4.40% of 18:2n−6 to 18:3n−6 (Table 4).

### 3.2. Effects of n−3 LC-PUFA level on growth performance and fatty acid composition

The specific growth rate (SGR) increased significantly ($P < 0.05$) with dietary n−3 LC-PUFA from 0.52% to 1.57% and leveled off thereafter (Table 5). The fatty acid composition of carcass, muscle and visceral mass has been presented in Tables 6, 7 and 8, respectively. Saturated fatty acid (SFA) content of carcass, muscle and visceral mass decreased significantly ($P < 0.05$), while the content of DHA and EPA increased markedly with increasing dietary n−3 LC-PUFA ($P < 0.05$). Moreover, 16:1 occurred in all fish larvae fed experiment diets, although with only trace amount. Meanwhile, the concentration of 18:3n−6 (desaturated product of FADS2) in grouper larvae was comparable with that of diets. N−6 PUFA and n−3 LC-PUFA had a similar variation trend with SFA, while ARA/EPA had an inverse trend with SFA.

### 3.3. Effects of n-3 LC-PUFA level on FADS2 expression

Relative mRNA expression of Δ6 desaturase in visceral mass of grouper larvae was significantly affected by dietary n−3 LC-PUFA ($P < 0.05$). The expression of Δ6 desaturase increased significantly with dietary n−3 LC-PUFA from 0.52% to 0.94% and then decreased significantly ($P < 0.05$) (Fig. 1).

## 4. Discussion

FADS2, which catalyzes the first desaturation step, plays a crucial role in the LC-PUFA biosynthetic pathway and thus was commonly used to indicate the synthesis capacity in fish (Tocher et al., 2006). Functional characterizations of FADS2 were typically conducted by heterologous expression in *S. cerevisiae* and most of teleost FADS2 are also typically Δ6 desaturase (Mohd-Yusof et al., 2010; Santigosa et al., 2011; Tocher et al., 2006; Zheng et al., 2009a). However, Fads2-like cDNAs encoding desaturases with Δ5 (Hastings et al., 2004), Δ4 (Li et al., 2010; Morais et al., 2012), and bifunctional Δ6/Δ5 (Hastings et al., 2001; Morais et al., 2012) activities have been also identified. In the present study, the Δ6 desaturase was shown to desaturate the C20 PUFA substrates, 20:3n−3 and 20:2n−6, to 20:4n−3 and 20:3n−6, respectively, which indicated that FADS2 also showed Δ8 desaturase activity. The dual Δ6/Δ8 activities have also been found in some marine fish species, such as rabbitfish, cobia, Atlantic cod, gilthead seabream and turbot (Monroig et al., 2011). The Elvol5 of this grouper could elongate 18:3n−3 and 18:2n−6 (unpublished results), which confirms that potential "elongation–Δ8 desaturation–Δ5 desaturation" pathway is alternative to the classic pathway in LC-PUFA biosynthetic pathway of grouper. The confirmed alternative pathway made grouper an ideal model to study LC-PUFA biosynthetic pathway. However, the efficiency of FADS2 towards Δ6 and Δ8 is lower than diadromous (Atlantic salmon), freshwater (Rainbow trout and Zebrafish), and marine (Rabbitfish, Cobia, Atlantic cod, Gilthead seabream and Turbot) fish described by Monroig et al. (2011), but higher than the meager (Monroig et al., 2013). The lower efficiency of FADS2 indicated the opinion that FADS2 may be another rate-limiting enzyme in the LC-PUFA biosynthetic pathway of grouper besides lacking of Δ5 fatty acyl desaturase.

As dietary n−3 LC-PUFA increased from 0.94% to 1.57%, the SGR increased significantly and thereafter leveled off. The changing trend of SGR may indicate that 1.57% n−3 LC-PUFA could meet the minimum requirement of grouper larvae and activities of enzymes involved in LC-PUFA biosynthetic pathways cannot meet the requirement for LC-PUFA of grouper larvae. The n−3 LC-PUFA requirement of the grouper larvae was comparable to the gilthead seabream larvae (Rodriguez et al., 1998), but higher than the common carp larvae (Radunzneto et al., 1996) and striped bass larvae (Webster and Lovell, 1990). The higher requirement for n−3 LC-PUFA of grouper larvae may be due to the lower efficiency and activity of Δ6 desaturase and other enzymes involved in LC-PUFA biosynthetic pathway. This was similar with some marine species of which the limited capacity of LC-PUFA biosynthesis has been well demonstrated (Almaida-Pagán et al., 2007; Mourente et al., 2005; Vagner and Santigosa, 2010; Xu et al., 2014).

The fatty acid composition of grouper larvae was also significantly affected by dietary n−3 LC-PUFA. The concentration of DHA and EPA in fish larvae decreased significantly with decreasing dietary n−3 LC-PUFA, which may also indicate that the capacity to synthesize LC-PUFA in grouper was limited. The content of n−3 LC-PUFA in grouper larvae of the first two treatments was much higher than that of the diets and the concentration of 16:0 had a reverse trend, which confirmed that LC-PUFA was conserved preferentially at the expense of saturated fatty acid and mono-unsaturated fatty acid (Falk-Petersen et al., 1989; Rainuzzo et al., 1994). The ratio of DHA to EPA is higher in the carcass of grouper larvae than in the diet, which may indicate a priority utilization of EPA than DHA. This may be caused by a higher peroxisomal beta-oxidation of EPA (Madsen et al., 1998) or the conversion of EPA through "Sprecher shunt" (Sprecher, 2000). The content of 18:3n−6, desaturated products of Δ6 desaturase, in grouper larvae

**Table 5**
Growth response and survival of larval grouper fed diets with graded levels of n−3 LC-PUFA.[a]

| Growth response | Dietary n−3 LC-PUFA contents (% dry weight) | | | | |
|---|---|---|---|---|---|
| | 0.52 | 0.94 | 1.57 | 1.97 | 2.43 |
| Initial body weight (g) | 0.071 ± 0.002 | 0.071 ± 0.002 | 0.071 ± 0.002 | 0.071 ± 0.002 | 0.071 ± 0.002 |
| Final body weight (g) | 1.36 ± 0.02[a] | 1.44 ± 0.06[a] | 1.72 ± 0.05[b] | 1.79 ± 0.08[b] | 1.69 ± 0.03[b] |
| SGR ($\%d^{-1}$)[1] | 10.89 ± 0.05[a] | 11.08 ± 0.15[a] | 11.76 ± 0.11[b] | 11.89 ± 0.17[b] | 11.68 ± 0.06[b] |

[1]SGR: specific growth rate.
[a] Data with the same superscript letter in the same row are not significantly different as determined by Tukey's test ($P > 0.05$).

**Table 6**
Fatty acid composition (% total fatty acids) in the carcass of larval grouper fed the diets with graded n−3 LC-PUFA.[a]

| Fatty acid | Dietary n−3 LC-PUFA contents (% dry weight) | | | | |
|---|---|---|---|---|---|
| | 0.52 | 0.94 | 1.57 | 1.97 | 2.43 |
| 14:0 | 1.52 ± 0.021[a] | 1.52 ± 0.04[a] | 1.78 ± 0.03[b] | 2.09 ± 0.03[c] | 2.50 ± 0.02[d] |
| 16:0 | 41.48 ± 0.38[d] | 40.19 ± 0.62[d] | 35.43 ± 0.34[c] | 31.31 ± 0.54[b] | 26.85 ± 0.08[a] |
| 18:0 | 6.83 ± 0.44[c] | 6.59 ± 0.09[bc] | 6.32 ± 0.29[bc] | 5.40 ± 0.21[ab] | 4.59 ± 0.11[a] |
| 20:0 | 1.19 ± 0.02[abc] | 1.12 ± 0.01[a] | 1.14 ± 0.02[ab] | 1.23 ± 0.02[c] | 1.20 ± 0.01[bc] |
| ∑SFAs[1] | 51.01 ± 0.63[d] | 49.42 ± 0.66[d] | 44.67 ± 0.34[c] | 40.02 ± 0.42[b] | 35.14 ± 0.17[a] |
| 16:1 | 3.96 ± 0.20[a] | 3.03 ± 0.12[b] | 2.25 ± 0.18[c] | 2.39 ± 0.09[c] | 2.17 ± 0.03[c] |
| 18:1 | 13.58 ± 0.35[c] | 11.29 ± 0.19[b] | 9.22 ± 0.31[a] | 9.44 ± 0.13[a] | 8.67 ± 0.03[a] |
| ∑MUFAs[2] | 17.55 ± 0.40[c] | 14.32 ± 0.31[b] | 11.48 ± 0.44[a] | 11.83 ± 0.16[a] | 10.84 ± 0.06[a] |
| 18:2n−6 | 12.19 ± 0.19[c] | 10.90 ± 0.04[b] | 11.25 ± 0.08[b] | 9.63 ± 0.06[a] | 9.18 ± 0.05[a] |
| 20:4n−6 | 4.49 ± 0.09[c] | 4.13 ± 0.08[b] | 3.84 ± 0.07[ab] | 3.76 ± 0.03[a] | 3.95 ± 0.04[ab] |
| ∑n−6 PUFAs[3] | 16.68 ± 0.15[a] | 15.03 ± 0.04[b] | 15.08 ± 0.02[b] | 13.39 ± 0.05[a] | 13.12 ± 0.09[a] |
| 18:3n−3 | 1.50 ± 0.10[ab] | 1.46 ± 0.05[ab] | 1.61 ± 0.04[b] | 1.34 ± 0.14[ab] | 1.20 ± 0.05[a] |
| 20:5n−3 | 1.83 ± 0.04[a] | 3.17 ± 0.02[b] | 4.58 ± 0.05[c] | 5.82 ± 0.05[d] | 7.03 ± 0.06[e] |
| 22:6n−3 | 4.76 ± 0.23[a] | 8.07 ± 0.24[b] | 11.54 ± 0.25[c] | 14.75 ± 0.12[d] | 17.98 ± 0.05[e] |
| ∑n−3 PUFAs[4] | 8.10 ± 0.30[a] | 12.70 ± 0.30[b] | 17.73 ± 0.18[c] | 21.90 ± 0.06[d] | 26.21 ± 0.06[e] |
| n−3/n−6 PUFAs | 0.49 ± 0.02[a] | 0.85 ± 0.02[b] | 1.18 ± 0.01[c] | 1.64 ± 0.01[d] | 2.00 ± 0.01[e] |
| n−3 LC-PUFAs[5] | 6.60 ± 0.25[a] | 11.24 ± 0.26[b] | 16.12 ± 0.21[c] | 20.56 ± 0.15[d] | 25.02 ± 0.01[e] |
| ARA/EPA[6] | 2.45 ± 0.11[d] | 1.30 ± 0.02[c] | 0.84 ± 0.02[b] | 0.65 ± 0.01[ab] | 0.56 ± 0.00[a] |
| DHA/EPA[7] | 2.60 ± 0.11 | 2.55 ± 0.06 | 2.52 ± 0.08 | 2.54 ± 0.02 | 2.56 ± 0.03 |

[1]SFAs: saturated fatty acids.
[2]MUFAs: mono-unsaturated fatty acids.
[3]n−6 PUFAs: n−6 polyunsaturated fatty acids.
[4]n−3 PUFAs: n−3 polyunsaturated fatty acids.
[5]n−3 LC-PUFAs: n−3 long chain polyunsaturated fatty acids.
[6]ARA/EPA: 20:4n−6/20:5n−3.
[7]DHA/EPA: 22:6n−3/20:5n−3.
[a] Some fatty acids, of which the contents are minor, trace amount or notdetected, such as C22:0, C24:0,C14:1, C20:1n−9, C22:1n−11, C20:2n−6, C20:3n−6, C22:5n−3, were not listed in the table. Different superscript letters in each row show significant differences among dietary treatments by Tukey's test ($P > 0.05$).

was comparable with that of the diets, which may also indicate low activity of FADS2.

Besides the lack of Δ5 FAD, the activity of FADS2 was not sufficient to transform PUFAs to LC-PUFAs to meet the LC-PUFA requirements of grouper larvae through the changing trend of growth and fatty acid composition. Meanwhile, the results of Lin et al. (2007) have indicated that grouper may need a low level of n−3 LC-PUFA or this LC-PUFA could be biosynthesized from ALA (18:3n−3) to some extent, which is in conflict with the result of this study. The differences in results observed may be a result from differences in size and development

**Table 7**
Fatty acid composition (% total fatty acids) in the muscle of larval grouper fed the diets with graded n−3 LC-PUFA.[a]

| Fatty acid | Dietary n−3 LC-PUFA contents (% dry weight) | | | | |
|---|---|---|---|---|---|
| | 0.52 | 0.94 | 1.57 | 1.97 | 2.43 |
| 14:0 | 1.27 ± 0.07[ab] | 1.44 ± 0.02[bc] | 1.15 ± 0.05[a] | 1.66 ± 0.06[c] | 1.40 ± 0.05[ab] |
| 16:0 | 37.05 ± 0.22[a] | 37.65 ± 0.50[a] | 29.70 ± 0.63[b] | 33.58 ± 0.07[c] | 29.11 ± 0.04[b] |
| 18:0 | 6.89 ± 0.01[a] | 7.03 ± 0.06[ab] | 6.89 ± 0.08[a] | 7.23 ± 0.11[b] | 8.21 ± 0.06[c] |
| ∑SFAs[1] | 45.21 ± 0.28[c] | 46.12 ± 0.42[c] | 37.74 ± 0.64[a] | 42.47 ± 0.02[b] | 38.72 ± 0.03[a] |
| 16:1 | 9.31 ± 0.28[c] | 10.34 ± 0.28[c] | 5.94 ± 0.06[a] | 7.15 ± 0.33[b] | 5.66 ± 0.10[a] |
| 18:1 | 20.30 ± 0.20[c] | 16.95 ± 0.40[b] | 13.74 ± 0.16[a] | 13.63 ± 0.45[a] | 13.62 ± 0.16[a] |
| ∑MUFAs[2] | 29.6 ± 0.09[c] | 27.29 ± 0.12[b] | 19.68 ± 0.22[a] | 20.79 ± 0.78[a] | 19.28 ± 0.26[a] |
| 18:2n−6 | 13.29 ± 0.09[e] | 10.81 ± 0.04[d] | 9.57 ± 0.05[c] | 8.22 ± 0.05[b] | 7.58 ± 0.03[a] |
| 20:4n−6 | 6.02 ± 0.10[d] | 5.00 ± 0.08[bc] | 5.27 ± 0.10[c] | 4.31 ± 0.05[a] | 4.73 ± 0.06[b] |
| ∑n−6 PUFAs[3] | 19.31 ± 0.19[c] | 15.81 ± 0.13[b] | 14.84 ± 0.08[a] | 12.53 ± 0.10[a] | 12.31 ± 0.10[a] |
| 18:3n−3 | 0.84 ± 0.01[a] | 0.87 ± 0.01[a] | 0.86 ± 0.01[a] | 0.86 ± 0.01[a] | 1.02 ± 0.03[b] |
| 20:5n−3 | 2.09 ± 0.06[a] | 2.85 ± 0.08[b] | 4.36 ± 0.07[c] | 4.45 ± 0.01[c] | 4.74 ± 0.07[d] |
| 22:6n−3 | 3.78 ± 0.12[a] | 6.11 ± 0.09[b] | 15.18 ± 0.30[c] | 12.67 ± 0.23[d] | 16.39 ± 0.01[e] |
| ∑n−3 PUFAs[4] | 6.71 ± 0.19[a] | 9.83 ± 0.17[b] | 20.40 ± 0.36[c] | 17.98 ± 0.24[d] | 22.14 ± 0.08[e] |
| n−3 LC-PUFAs[5] | 5.87 ± 0.18[a] | 7.96 ± 0.17[b] | 19.54 ± 0.37[c] | 17.02 ± 0.24[d] | 21.13 ± 0.08[e] |
| n−3/n−6 PUFAs | 0.30 ± 0.01[a] | 0.57 ± 0.01[b] | 1.37 ± 0.02[c] | 1.44 ± 0.03[c] | 1.80 ± 0.01[d] |
| ARA/EPA[6] | 2.88 ± 0.04[d] | 1.75 ± 0.02[c] | 1.21 ± 0.01[b] | 0.97 ± 0.01[a] | 1.00 ± 0.00[a] |
| DHA/EPA[7] | 1.81 ± 0.00[a] | 2.14 ± 0.02[b] | 3.48 ± 0.02[d] | 2.85 ± 0.05[c] | 3.46 ± 0.05[d] |

[1]SFAs: saturated fatty acids.
[2]MUFAs: mono-unsaturated fatty acids.
[3]n−6 PUFAs: n−6 polyunsaturated fatty acids.
[4]n−3 PUFAs: n−3 polyunsaturated fatty acids.
[5]n−3 LC-PUFAs: n−3 long chain polyunsaturated fatty acids.
[6]ARA/EPA: 20:4n−6/20:5n−3.
[7]DHA/EPA: 22:6n−3/20:5n−3.
[a] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0,C14:1, C20:1n−9, C22:1n−11, C20:2n−6, C20:3n−6, and C22:5n−3, were not listed in the table. Different superscript letters in each row show significant differences among dietary treatments by Tukey's test ($P > 0.05$).

**Table 8**
Fatty acid composition (% total fatty acids) in the visceral mass of larval grouper fed the diets with graded n−3 LC-UFA.[a]

| Fatty acid | Dietary n−3 LC-PUFA contents (% dry weight) | | | | |
|---|---|---|---|---|---|
| | 0.52 | 0.94 | 1.57 | 1.97 | 2.43 |
| 14:0 | 1.62 ± 0.03$^{a}$ | 1.65 ± 0.02$^{a}$ | 1.90 ± 0.04$^{b}$ | 2.25 ± 0.05$^{c}$ | 2.59 ± 0.02$^{d}$ |
| 16:0 | 43.60 ± 0.40$^{d}$ | 42.88 ± 0.37$^{d}$ | 37.30 ± 0.26$^{c}$ | 32.75 ± 0.44$^{b}$ | 26.17 ± 0.24$^{a}$ |
| 18:0 | 7.79 ± 0.10$^{b}$ | 7.64 ± 0.47$^{b}$ | 6.07 ± 0.21$^{a}$ | 5.35 ± 0.14$^{a}$ | 4.70 ± 0.27$^{a}$ |
| 20:0 | 1.26 ± 0.03$^{ab}$ | 1.16 ± 0.02$^{a}$ | 1.15 ± 0.02$^{a}$ | 1.24 ± 0.02$^{ab}$ | 1.37 ± 0.06$^{b}$ |
| ∑SFAs[1] | 54.28 ± 0.31$^{b}$ | 53.33 ± 0.77$^{b}$ | 46.42 ± 0.19$^{a}$ | 41.60 ± 0.42$^{a}$ | 34.83 ± 0.56$^{a}$ |
| 16:1 | 3.21 ± 0.04$^{b}$ | 1.95 ± 0.31$^{a}$ | 1.99 ± 0.15$^{a}$ | 1.82 ± 0.16$^{a}$ | 1.81 ± 0.11$^{a}$ |
| 18:1 | 12.16 ± 0.27$^{d}$ | 9.84 ± 0.05$^{cd}$ | 7.90 ± 0.11$^{bc}$ | 7.78 ± 0.39$^{bc}$ | 7.24 ± 0.29$^{a}$ |
| ∑MUFAs[2] | 15.36 ± 0.23$^{b}$ | 11.79 ± 0.15$^{a}$ | 9.88 ± 0.26$^{a}$ | 9.60 ± 0.55$^{a}$ | 9.05 ± 0.40$^{a}$ |
| 18:2n−6 | 12.93 ± 0.07$^{d}$ | 11.68 ± 0.15$^{c}$ | 11.84 ± 0.06$^{c}$ | 10.15 ± 0.04$^{b}$ | 9.50 ± 0.01$^{a}$ |
| 20:4n−6 | 4.04 ± 0.08$^{b}$ | 3.89 ± 0.08$^{ab}$ | 3.69 ± 0.10$^{ab}$ | 3.51 ± 0.17$^{a}$ | 3.98 ± 0.02$^{ab}$ |
| ∑n−6 PUFAs[3] | 16.97 ± 0.09$^{c}$ | 15.57 ± 0.16$^{b}$ | 15.53 ± 0.07$^{b}$ | 13.66 ± 0.19$^{a}$ | 13.48 ± 0.01$^{a}$ |
| 18:3n−3 | 1.83 ± 0.03$^{b}$ | 1.63 ± 0.01$^{ab}$ | 1.85 ± 0.05$^{b}$ | 1.43 ± 0.07$^{a}$ | 1.56 ± 0.14$^{ab}$ |
| 20:5n−3 | 1.80 ± 0.02$^{a}$ | 3.41 ± 0.04$^{b}$ | 4.93 ± 0.04$^{c}$ | 6.42 ± 0.09$^{d}$ | 7.83 ± 0.16$^{e}$ |
| 22:6n−3 | 3.58 ± 0.01$^{a}$ | 7.11 ± 0.08$^{b}$ | 10.76 ± 0.10$^{c}$ | 14.51 ± 0.12$^{d}$ | 18.02 ± 0.14$^{e}$ |
| ∑n−3 PUFAs[4] | 7.22 ± 0.03$^{a}$ | 12.15 ± 0.12$^{b}$ | 17.54 ± 0.14$^{c}$ | 22.36 ± 0.13$^{d}$ | 27.40 ± 0.35$^{e}$ |
| n−3/n−6 PUFAs | 0.43 ± 0.00$^{a}$ | 0.78 ± 0.01$^{b}$ | 1.13 ± 0.01$^{c}$ | 1.64 ± 0.01$^{d}$ | 2.03 ± 0.03$^{e}$ |
| n−3 LC−PUFAs[5] | 5.39 ± 0.01$^{a}$ | 10.52 ± 0.12$^{b}$ | 15.69 ± 0.09$^{c}$ | 20.93 ± 0.20$^{d}$ | 25.85 ± 0.30$^{e}$ |
| ARA/EPA[6] | 2.24 ± 0.06$^{d}$ | 1.14 ± 0.02$^{c}$ | 0.75 ± 0.02$^{b}$ | 0.55 ± 0.02$^{a}$ | 0.51 ± 0.01$^{a}$ |
| DHA/EPA[7] | 1.99 ± 0.02$^{a}$ | 2.09 ± 0.01$^{ab}$ | 2.18 ± 0.03$^{bc}$ | 2.26 ± 0.02$^{cd}$ | 2.30 ± 0.03$^{d}$ |

[1]SFAs: saturated fatty acids.
[2]MUFAs: mono-unsaturated fatty acids.
[3]n−6 PUFAs: n−6 polyunsaturated fatty acids.
[4]n−3 PUFAs: n−3 polyunsaturated fatty acids.
[5]n−3 LC-PUFAs: n−3 long chain polyunsaturated fatty acids.
[6]ARA/EPA: 20:4n−6/20:5n−3.
[7]DHA/EPA: 22:6n−3/20:5n−3.
[a] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:1n−9, C22:1n−11, C20:2n−6, C20:3n−6, and C22:5n−3, were not listed in the table. Different superscript letters in each row show significant differences among dietary treatments by Tukey's test ($P > 0.05$).

stage. In the present study, the higher conversion efficiency of the n−3 substrates evaluated compared to n−6 substrate may suggest the potential substitution of fish oil.

Besides the fatty acid composition, the FADS2 gene expression in the liver of grouper in this study was also regulated by dietary n−3 LC-PUFA. The expression and regulation of FADS2 have been widely studied on freshwater fish and seawater fish. Some previous study have shown that the expression of FADS2 was higher in freshwater fish fed vegetable oil than those fed fish oil (Seiliez et al., 2001; Zheng et al., 2004), which can be interpreted as an inhibition by n−3 LC-PUFA. Additionally, similar results have been found in freshwater fish hepatocytes fed vegetable oils compared to fish oil (Bell et al., 2001, 2002; Tocher et al., 2001, 2003, 2004). As for seawater fish, Seiliez et al. (2003) have found that the expression of FADS2 in gilthead seabream liver was higher in fish fed a LC-PUFA-free diet than in fish fed a LC-PUFA-rich diet. Similar results have been found in some other studies (González-Rovira et al., 2009; Vagner et al., 2007a, 2007b). In the present study, the expression of FADS2 decreased significantly with increasing dietary n−3 LC-PUFA except the first treatment ($P < 0.05$), which could also confirm the inhibition of n−3 LC-PUFA for the expression of FADS2. The expression of FADS2 was highest in the treatment fed the diet with 0.94% n−3 LC-PUFA. Meanwhile, no significant differences were found between the concentrations of 18:3n−6 and 18:4n−3 in each treatment, which may also confirm that the higher requirement for n−3 LC-PUFA of grouper may be due to the low activity and expression of FADS2. Unlike the results described above, González-Rovira et al. (2009) demonstrated that dietary olive oil did not significantly improve the expression of Δ6 desaturase compared to fish oil. The conflicting results may be due to the low expression of Δ6 desaturase of this species. Previous studies have also found that the effect of n−3 LC-PUFA on the expression of FADS2 was also related to the development stage of larvae (Morais et al., 2011; Vagner et al., 2009), which may account for why no significant difference was found between the fish fed 0.52% LC-PUFA and 2.43% LC-PUFA in the present study. Although the regulation of FADS2 of fish in response to dietary fatty acids has been widely assessed, the regulatory mechanism was rarely studied. FADS2 has been demonstrated to be regulated by two transcription factors: Sterol Regulatory Element Binding Protein-1 (SREBP-1) and Peroxisome Proliferator-Activated Receptors (PPARs) in mammals (Nakamura and Nara, 2002). It has been demonstrated that dietary n−3 LC-PUFA could reduce FADS2 promoter activity by increasing the methylation rate of the promoter region of this gene, and thus suppress LC-PUFA synthesis in Atlantic Salmon (Zheng et al., 2009b) and Japanese Seabass (Xu et al., 2014). However, the regulation mechanism of transcription factors on FADS2 was still unclear. Further study should be conducted to investigate the regulator mechanism of Δ6 desaturase to interpret the LC-PUFA biosynthetic pathway.

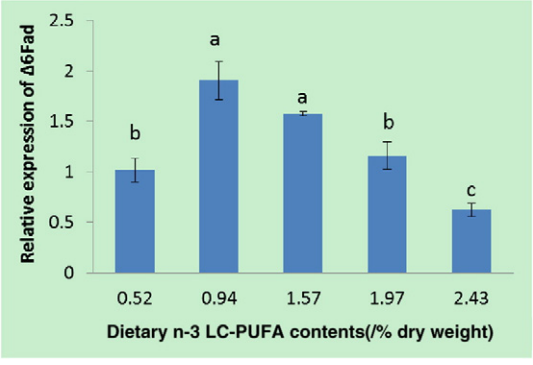


**Fig. 1.** Relative mRNA expression of Δ6 FAD in the visceral mass of grouper larvae fed with graded dietary n−3 LC-PUFA for 4 weeks. Relative mRNA expression was evaluated by real-time quantitative PCR. Values are means ± S.E.M. (n = 3). Bars of the same gene bearing with different letters are significantly different by Tukey's test ($P < 0.05$).

In conclusion, FADS2 of grouper showed activities of Δ6 desaturase and Δ8 desaturase when expressed in yeast, and dietary n−3 LC-PUFA could regulate the biosynthesis of LC-PUFA through influencing the expression of FADS2. FADS2 is the rate-limiting enzyme in the LC-PUFA biosynthetic pathway of grouper due to its low activity.

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