Effects of Replacing Squid Oil with Dietary Vegetable Oils on Growth, Fatty Acid Composition and Expression Levels of Fatty Acyl Δ6 Desaturase mRNAs in Black Ear Catfish, Pangasius larnaudii

Thanathip Lamkom1,*, Kanokwan Sarnsamak1, Kanjana Payooha1 and Sirawut Klinbunga2

ABSTRACT

A 6-month feeding trial on four experimental diets containing squid oil (SO), palm oil (PO), soybean oil (SBO) and sunflower oil (SFO) in juvenile black ear catfish (Pangasius larnaudii) was performed. Fish fed each source of vegetable oils exhibited a higher final weight and ADG (months 4 – 6) than those fed the SO diet (P < 0.05). Final weight gain (FWG) and specific growth rate (SGR) of fish fed PO and SBO were greater than those fed with SO diet (P < 0.05). The EPA/ARA ratio in muscle of fish fed SO and PO was higher than that of fish fed the SFO diet (P < 0.05). In addition, the full-length cDNAs of fatty acyl delta 6 desaturase (Pifadsd6; 1748 bp) were isolated. Its expression levels in fish fed the PO diet were greater than those fed other diets (P < 0.05). Pifadsd6 in fish fed SO was not significantly different from fish fed SBO (P > 0.05) but greater than those fed the SFO diet (P < 0.05). Results of the present study suggest that the diet containing SO may be more appropriately replaced with PO than SBO and SFO in the grow-out diet for P. larnaudii.

Keywords: vegetable oils, fatty acyl delta 6 desaturase mRNAs, Pangasius larnaudii

INTRODUCTION

Source of oil in aquaculture feed relies mainly on fish oil (and squid oil) which contain high n-3 HUFA and essential fatty acids (EFA). The demand for fish oil has consistently increased for farming activities (Barlow, 2000). Nevertheless, the reduction in supply and an increase in prices of fish oil have resulted in efforts to partially or completely substitute with vegetable oils in aquafeeds (Tocher et al., 2001; Bransden et al., 2003; Bahurmiz and Ng, 2007; Izquierdo et al., 2008; Jaya-Ram et al., 2011; Gao et al., 2012; Ren et al., 2012). Pangasiid catfish have been cultured in the Mekong Delta since the 1960s for local consumption (Van Zalinge et al., 2002). Black ear catfish (Pangasius larnaudii) Bocourt, 1866) a member of Family Pangasiidae, is one of main species cultured in Mun River, Ubon Ratchathani Province (Chansri et al., 2002). It is an omnivorous species with very broad natural diets (Termvidchakorn and Hortle, 2013). The harvest size is approximately 1–2 kg with a culture period of 1–2 years.

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Small-scale fish farmers search for feeds which are alternative to commercial feeds containing fish meals and/or fish/squid oils. Unlike marine fishes, freshwater teleosts can effectively use polyunsaturated fatty acid (PUFA) precursor like linoleic (LA) and α-linolenic (ALA) acids for the synthesis of C20/22 highly unsaturated fatty acid (HUFA) (Sargent et al., 2002; Hastings et al., 2005). As a result, several vegetable oils have been partly or completely substituted in fish diets across a variety of freshwater species (Bell et al., 2002; Bransden et al., 2003; Fonseca-Madrigal et al., 2005).

Variable compositions of fatty acid are found from different sources of vegetable oils, for example, palm oil (PO) is rich in saturated fatty acid (SFA) while soybean oil (SBO) and sunflower oil (SFO) are rich in LA but linseed oil is rich in ALA (Zambiasi et al., 2007). The suitable replacement of fish oils depends on the efficiency of fish species that can convert C18 PUFA to C20/22 HUFA (Sargent et al., 2002). The fatty acid profiles of fish liver (reflecting lipid biosynthesis efficiency) and muscle (reflecting the quality of fillets) partially are influenced by the fatty acid composition in diets.

PUFA, particularly LA and ALA, plays an important role as the precursor of HUFA biosynthesis (Hastings et al., 2005). The processes require the activities of fatty acyl desaturase (Fads) and very long-chain fatty acyl elongase (Elov) enzymes. Several studies reported an increase in their transcript levels in freshwater fish (e.g. Danio rerio and Oreochromis niloticus and Cyprinus carpio) fed diets containing vegetable oils (Bell et al., 2001; Tocher et al., 2002b; Zheng et al., 2005; Ren et al., 2012). Effects of various vegetable oils in feed diets on the expression of either enzyme in cultured freshwater fish should be studied.

In this study, the possibility to replace squid oil with different vegetable oils (PO, SBO and SFO) in the grow-out diets of P. larnaudii was investigated. Effects of different formulated diets on the expression levels of fatty acyl delta6 desaturase (PlFadsd6 also called Fads2, and Fads6-like) mRNA were examined using quantitative real-time PCR. In addition, lipid compositions in muscle of the experimental fish were also examined.

**MATERIALS AND METHODS**

**Experimental animal, diet formulation and feeding trial**

SO, PO, SBO, and SFO were purchased from a local market in Ubon Ratchathani. Four diets with the same basal composition (40% protein and gross energy 420.50 Kcal · 100 g⁻¹), but formulated with different sources of oils. SO, PO, SBO, and SFO were subsequently produced. The ingredients were identified by proximate analysis (AOAC, 1997; Table 1). Fingerlings of *P. larnaudii* were obtained from Yasothon Inland Fisheries Research and Development Center, Department of Fisheries, Thailand. Twenty individuals (average body weight = 20.66 ± 3.44 g for overall specimens) were randomly placed into each cage (1x1x1.5 m). Fish were acclimated at the ambient conditions (28 ± 2°C and natural light) for 2 weeks and fed with a commercial diet. A 6 month experiment was carried out in triplicate. Experimental fish were fed twice daily until satiation with four different formulated diets and the body weight was monthly measured. At the end of the experimental period, muscle and liver were dissected out (*N* = 9, 9, 9 and 9 for SO, PO, SBO, and SFO diets, respectively) and the expression level of *PlFadsd6* mRNA was examined using quantitative real-time PCR. Fatty acid profiles were analyzed by gas chromatography (AOAC, 1997). During the experiment, water quality (dissolved oxygen, pH, water temperature, and unionized ammonia) was monitored every two weeks.

Animal care and all experimental procedures were approved by the Animal Experiment Committee, Ubon Ratchathani University (Approval no.21/2552).
Table 1. Formulation (g·100 g⁻¹ diet), proximate analysis (% dry matter basis) and fatty acid composition (% of total fatty acids by weight) of experimental diets

<table>
<thead>
<tr>
<th>Component</th>
<th>SO</th>
<th>PO</th>
<th>SBO</th>
<th>SFO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>44.0</td>
<td>44.0</td>
<td>44.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Rice bran</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Cassava</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Rice mill</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Squid oil</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Palm oil</td>
<td>0</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0</td>
<td>0</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Total ingredients</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Proximate composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>40.34</td>
<td>40.52</td>
<td>40.61</td>
<td>40.32</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>7.87</td>
<td>7.76</td>
<td>7.93</td>
<td>7.93</td>
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<tr>
<td>Fiber (%)</td>
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<td>3.30</td>
<td>2.91</td>
<td>2.79</td>
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<td>Moisture (%)</td>
<td>2.92</td>
<td>2.78</td>
<td>2.67</td>
<td>3.74</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>13.27</td>
<td>13.17</td>
<td>13.01</td>
<td>13.52</td>
</tr>
<tr>
<td><strong>Fatty acid composition</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>22.30</td>
<td>42.00</td>
<td>13.80</td>
<td>10.30</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>37.60</td>
<td>12.10</td>
<td>23.10</td>
<td>14.70</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>32.20</td>
<td>9.41</td>
<td>64.80</td>
<td>70.99</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1.20</td>
<td>9.20</td>
<td>57.20</td>
<td>70.67</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>10.40</td>
<td>0.21</td>
<td>7.50</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Preparation of total RNA and first-strand cDNA

Total RNA was extracted from a piece of liver (approximately 50 mg) of each fish using Trizol following the protocol recommended by the manufacturer (Invitrogen). The concentration of extracted total RNA was spectrophotometrically measured (Sambrook and Russell, 2001). One and a half microgram of DNase I-treated total RNA was reversed-transcribed using an ImProm-II™ Reverse Transcription System (Promega).

Degenerate primer design and PCR

Protein sequences of fatty acyl desaturase (Fads) from zebrafish, *Danio rerio* (accession no. AAG25710), Atlantic salmon, *Salmo salar* (AAL82631), rainbow trout, *Oncorhynchus mykiss* (AAK26745) and gilthead seabream, *Sparus aurata* (AAL17639) were retrieved from GenBank and multiple sequence alignments were performed using Clustal W (Thompson et al., 1994). Degenerate primers for amplification of the partial ORFs of *PlFadsd6* (primers Fad-Degen-F/R, Table 2) were designed. PCR was carried out composing of pre-denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 90 s. The final extension was carried out at 72°C for 7 min. The resulting products were size-fractionated through agarose gels. The amplification fragment was eluted from the gel, cloned into pGEM-T Easy.

Rapid amplification of cDNA end-polymerase chain reaction (RACE-PCR) of *PlFadsd6*

The full-length cDNAs of *PlFadsd6* were isolated using 5'- and 3'-RACE-PCR using
Table 2. Nucleotide sequences of primers used for isolation, characterization and expression analysis of \textit{Plfads6}

<table>
<thead>
<tr>
<th>Application</th>
<th>Primer name</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Plfads6}</td>
<td>Fads-deg-F</td>
<td>5'-AGCAYGACTWCGGYCAYCTGTC-3'</td>
</tr>
<tr>
<td></td>
<td>Fads-deg-R</td>
<td>5'-GTSACCAAHAAAACCGTGG-3'</td>
</tr>
<tr>
<td>5'RACE</td>
<td>Fads-5RACE</td>
<td>5'-GACCAGTGGGGTACATGTTGAGTCTG-3'</td>
</tr>
<tr>
<td>3'RACE</td>
<td>Fads-3RACE</td>
<td>5'-CCAAACGGGATGGGGTGATACCC-3'</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Fads-RT-F</td>
<td>5'-CATGACTTCCGTCATCGTCAG-3'</td>
</tr>
<tr>
<td></td>
<td>Fads-RT-R</td>
<td>5'-GCTGATAGTGGTATAGGGGCTTC-3'</td>
</tr>
<tr>
<td>Quantitative real-time PCR</td>
<td>Fads-qRT-F</td>
<td>5'-CACCTTCAGCACCATGCTAAGCC-3'</td>
</tr>
<tr>
<td></td>
<td>Fads-qRT-R</td>
<td>5'-GCTGATGTTATAGGGCATGTTTC3'</td>
</tr>
<tr>
<td>\textit{\beta-actin}</td>
<td>Actin-RT-F</td>
<td>5'-AGAGAGAAATTGTCCGTACATC-3'</td>
</tr>
<tr>
<td></td>
<td>Actin-RT-R</td>
<td>5'-CTCCGATCCAGACAGATTTTG-3'</td>
</tr>
<tr>
<td>Quantitative real-time PCR</td>
<td>Actin-qRT-F</td>
<td>5'-GGTACACATGTACCCCTGCAAT-3'</td>
</tr>
<tr>
<td></td>
<td>Actin-qRT-R</td>
<td>5'-CTCGGATCCAGACAGATTTTG-3'</td>
</tr>
</tbody>
</table>

a SMART™ RACE cDNA Amplification Kit (Clonetech). RACE-PCR was carried out in a 50 μl reaction mixture containing 1 μl of the template cDNA, 1x Taq buffer, 1.0 mM MgCl₂, 0.2 mM each dNTP, 0.2 mM each primer (see RACE primers in Table 2), 1 unit Taq DNA polymerase. PCR was carried out with the thermal cycling of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 1.5 min. The final extension was carried out at 72°C for 10 min. RACE-PCR products were electrophoresed in a 1.2% agarose gel. Each gel-eluted product was ligated with pGEM-T Easy vector, transformed into \textit{E. coli} JM 109 and sequenced for both directions. Nucleotide sequences of RACE-PCR fragments and that from degenerate PCR were assembled and searched against data in GenBank using BlastX and Blast2X (Altschul et al., 1990; http://ncbi.nlm.nih.gov). The pI value and molecular weight of the deduced proteins were examined using ProtParam (http://www.expasy.org/tools/protparam.html). The functional domains in the deduced proteins were predicted using SMART (http://smart.embl-heidelberg.de).

**Phylogenetic analysis**

The deduced amino acid sequence of \textit{PlFads6} (accession no. AGR45585) was phylogenetically compared with Fads6, Fads5, Fads2 or Fads2-like of \textit{Haplochromis burtoni} (XP_005941747), \textit{Maylandia zebra} (XP_004553069), \textit{Oreochromis niloticus} (XP_005470691 and AGV 52807), \textit{Pundamilia nyererei} (XP_005730157), \textit{Fundulus heteroclitus} (XP_012720846 and XP_012720374), \textit{Solea senegalensis} (accession no. AEQ92868), \textit{Oryzias latipes} (XP_004069637), \textit{Poecilia formosa} (XP_007556957), \textit{Lates calcarifer} (ACY25091), \textit{Scatophagus argus} (AH62794), \textit{Epinephelus cooides} (ACJ26848), \textit{Sparus aurata} (ADD50000), \textit{Denticentrurus labrax} (accession no. ACD10793), \textit{Anguilla japonica} (AHY22375), \textit{Salmo salar} (NP_001165752, XP_014025862 and NP_001117014), \textit{Onchorhyncus masou} (ABU 87822), \textit{Onchorhyncus mykiss} (AFM77867), \textit{Danio rerio} (NP_571720), \textit{Cyprinus carpio} (AlA 19310), \textit{Astyanax mexicanus} (XP_007235183), \textit{Tachysurus fulvi} (AJQ20793), \textit{Clarias macrocephalus} (AGR45589), \textit{Pangasianodon hypophthalmus} (AFN21428), \textit{Xenopus (Silurana) tropicalis} (NP_001120262), \textit{Rattus norvegicus} (AEX15918), \textit{Mus musculus} (NP_062673), \textit{Bos taurus} (NP_001076913) and \textit{Homo sapiens} (NP_004256). Multiple alignments were carried out with Clustal W (Thompson et al., 1994). A bootstrapped neighbor-joining tree bootstrapped 1000 times (Saitou and Nei, 1987) was constructed using MEGA 6.0 (Tamura et al., 2013).
RT-PCR and tissue distribution analysis

The expression of PiFadsd6 (primers Fads-RT-F/R) in various tissues of P. larnaudii (4-month-old females) was analyzed by RT-PCR. β-actin (primers actin-F/R, Table 2) amplified from the same template was included as the positive control. The thermal profiles were 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1.5 min. The final extension was carried out at 72°C for 10 min. The amplicon was electrophoretically analyzed on a 1.5% agarose gel and visualized with a UV transilluminator after ethidium bromide staining (Sambrook and Russell, 2001).

Quantitative real-time PCR

Total RNA was transcribed as above. Standard curves representing 10^2-10^8 copies of recombinant plasmids of PiFadsd6 (primers Fads-qPCR-F/R; Table 2) and the internal control, β-actin (actin-F/R) were constructed. PiFadsd6 and β-actin mRNAs in liver of P. larnaudii were separately amplified in a 10 μl reaction volume containing 100 ng of first strand cDNA template, 0.4 μl of each primer and 5 μl of green master mix. The thermal profile for quantitative real-time PCR was 95°C for 5 min followed by 40 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 30 s. Quantitative real-time PCR of each specimen was carried out in triplicate.

Statistical analysis

The body weight and fatty acid profiles in muscle are presented as mean ± standard error of the mean (or standard deviation). Differences in growth parameters between different groups of samples were analyzed by one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test (R-package). Results were considered significant if P<0.05.

For gene expression analysis, the quantification of PiFadsd6 and β-actin in each sample well was evaluated by reference to the relevant standard curve. The relative expression levels (copy number of PiFadsd6 and that of β-actin) among treatments were statistically tested using ANOVA and Duncan’s new multiple range test (P < 0.05).

RESULTS

Growth performance of Pangasius larnaudii fed with different sources of vegetable oil-supplemented diets

The body weight of P. larnaudii fed different sources of dietary oils was measured monthly (Table 3). The average body weight (ABW) and average daily gain (ADG) between treatments were not significantly different during the first three months of the feeding trial (P < 0.05). After 6 months of feeding, the final weight (FW) and ADG at months 4-6 of the experimental animals fed PO, SBO and SFO diets (320.24 ± 13.74, 322.52 ± 8.49 and 270.53 ± 7.09 g and 1.96 ± 0.71, 1.97 ± 0.44 and 1.47 ± 0.20 g·day⁻¹, respectively) were significantly greater than those of the black ear catfish fed SO (194.42 ± 5.67 g and 0.79 ± 0.06 g·day⁻¹; P < 0.05). However, final weight gain (FWG) and specific growth rate (SGR) of P. larnaudii fed PO (305.64 ± 13.66 g and 1.65 ± 0.02%) and SBO (307.92 ± 8.53 g and 1.68 ± 0.01%) but not SFO (255.57 ± 7.13 g and 1.57 ± 0.01%) diets were significantly greater than those of fish fed the SO diet (179.75 ± 5.63 g and 1.40 ± 0.01%; P < 0.05).

Isolation of PiFadsd6 transcript

The amplification products of 478 bp in length were obtained using degenerate primers for Fads. Nucleotide sequences of this gene segments significantly matched Fads6 of Pangasius hypophthalmus (GenBank accession no. AFN21428.1; E-value = 6e-92). The 5’- and 3’- RACE-PCR of this gene was further carried out and generated 772 bp and 792 bp fragments.
Table 3. Average body weight (± SEM) and growth parameters of *P. larvaludii* fed with different supplementary diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average body weight ± SEM (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SO</td>
</tr>
<tr>
<td>Initial weight</td>
<td>14.67 ± 0.08&lt;sup&gt;ms&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final weight</td>
<td>194.42 ± 5.67&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG (month 1)</td>
<td>25.55 ± 1.17&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG (month 2)</td>
<td>11.01 ± 0.57&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG (month 3)</td>
<td>70.84 ± 1.99&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG (month 4)</td>
<td>26.75 ± 0.53&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG (month 5)</td>
<td>39.17 ± 3.07&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG (month 6)</td>
<td>6.42 ± 7.87&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>FWG (g)</td>
<td>179.75 ± 5.63&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADG (mo 1-3; g·day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.17 ± 0.01&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADG (mo 4-6; g·day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.79 ± 0.06&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>1.40 ± 0.01&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

WG = weight gain; FWG = final weight gain; ADG = average daily gain; SGR = specific growth rate; mo = month

After sequence assembly, the full-length cDNA of *PIFads6* was 1748 bp in length containing an ORF of 1338 bp corresponding to 445 amino acids with 5’ and 3’ UTRs of 88 and 276 bp, respectively. The poly A additional signal (AATAAA) was located between nucleotides 1700-1705 of the characterized transcript (GenBank accession no. KC994461; Figure 1). The deduced *PIFads6* protein contained a cytochrome b5 (Cyt-b5) domain and a heme-binding motif (HPGG) located at amino acid positions 22 – 96 (*E*-value = 1.7e-22) and 54-57 of the deduced *PIFads6* protein. A transmembrane domain was predicted at positions 125-144. A FA desaturase domain was observed at positions 157-419 (*E*-value = 1.1e-36). Three histidine-rich regions (HDFGH, HFQHH, and QIEHH) were found at positions 181-185, 218-222, and 383-387 of the deduced protein, respectively. The deduced amino acid sequence of *PIFads6* showed the greatest similarity to delta-6 desaturase of *Pangasius hypophthalmus* (identity = 95%; *E*-value = 0.0).

**Phylogenetic analysis of *PIFads6***

Large sequence differences between *Fads* of fish, *Xenopus* and mammalian species were observed. In fish, *Fads6* from all species except that of *Salmo salar* and *Fads5* was allocated into different phylogenetic clusters (Figure 2). *Fads* from catfishes clustered together and *PIFads6* showed a close phylogenetic relationship with that of *P. hypophthalmus*. A bootstrapped neighbor-joining tree further indicated that the newly isolated *Fads* in *P. larvaludii* should be recognized as *Fads6*.

**Tissue expression analysis of *PIFads6* and its expression in liver following the feeding trial**

Tissues expression analysis indicated that *PIFads6* was abundantly expressed in brain and liver. No expression of *PIFads6* was found in gill, gut, spleen, pituitary gland, and muscle. Based on a conventional RT-PCR in this study, *PIFads6* mRNA were not found in pituitary gland and muscle (Figure 3A).

The expression level of *PIFads6* in liver of catfish fed the PO diet was greater than those fed other diets (*P < 0.05*). The expression level of *PIFads6* in those fed SO was not significantly different from that in fish fed SBO (*P > 0.05*) but significantly greater than those fed SFO diets (*P < 0.05*; Figure 3B)
Figure 1. The full-length cDNA and deduced amino acids of PiFA<sub>d</sub>6. The putative start (ATG) and stop (TAA) codons are boldfaced and underlined. A poly A additional signal (positions 1700 - 1705) is boldfaced, italicized and underlined. Three histidine boxes are underlined. The predicted Cyt-b<sub>5</sub> (positions 22 - 96) and FA<sub>des</sub>aturase (positions 157 - 419) domains are highlighted. The heme binding domain (HPGG; positions 54 - 57) is italicized and underlined. A transmembrane domain (positions 125 - 144) is illustrated in italics and highlighted.
Figure 2. Phylogenetic tree of deduced amino acid sequences of Pfads6 and Fads from other species. Values at the node represent the percentage of times that the particular node occurred in 1000 trees generated by bootstrapping the original aligned sequences.
Figure 3. (A) Tissue distribution analysis of *PfFadsd6* (A) in various tissues of *P. larnaudii*. β-actin (B) amplified from the same template was included as the reference gene. GI = gills, GU = gut, SP = spleen, BR = brain, PI = pituitary gland, MU = muscle, LI = liver. Lanes M are a 100 bp DNA marker. (B) Histogram showing relative expression levels of *PfFadsd6* in liver of *P. larnaudii* fed various sources of lipids (SO = squid oil; PO = palm oil; SBO = soybean oil; SFO = sunflower oil). The same letters above histograms indicate that the expression levels were not significantly different (*P* > 0.05).

**Fatty acid composition in muscle**

Fatty acid compositions in muscle of *P. larnaudii* fed vegetable oil-containing diets were examined and different fatty acid compositions were compared to those fed the SO diet. The black ear catfish fed the PO-containing diet showed the greatest amounts of palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1cis-9) than those with other treatments (*P* < 0.001). Palmitoleic acid (C16:1) was not found in those fed SBO and SFO diets while ALA (C20:3n-3) was not found in muscle of fish fed the latter diet. The level of LA (18:2 n-6) in the black ear catfish fed the SBO diet was greater than in those fed SO, PO and SFO diets (*P* < 0.01). The EPA (20:5 n-3) levels in *P. larnaudii* fed SFO were significantly lower than those fed other diets (*P* < 0.01). The EPA/ARA ratio and DHA + EPA in those fed SO and PO diets were significantly greater than those fed the SFO diet (*P* < 0.01) while the DHA/EPA ratio was in the opposite direction (Table 4).
Table 4. Fatty acid composition (percent total fatty acids by weight ± SD) of muscle of *P. larnauidii* fed different experimental diets. SO = squid oil; PO = palm oil; SBO = soybean oil; SFO = sunflower oil

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>SO</th>
<th>PO</th>
<th>SBO</th>
<th>SFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid (C14:0)</td>
<td>0.11 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)*</td>
<td>1.33 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stearic acid (C18:0)*</td>
<td>0.25 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.33 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.17 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)*</td>
<td>0.21 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oleic acid (C18:1 cis-9)*</td>
<td>1.40 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.81 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.23 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Linoleic acid (C18:2n6)*</td>
<td>0.35 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.67 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43 ± 0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>ω-Linolenic acid (C18:3n3)*</td>
<td>0.07 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Docosahexaenoic acid (C22:6n3)</td>
<td>0.12 ± 0.03&lt;sup&gt;as&lt;/sup&gt;</td>
<td>0.17 ± 0.04&lt;sup&gt;as&lt;/sup&gt;</td>
<td>0.15 ± 0.03&lt;sup&gt;as&lt;/sup&gt;</td>
<td>0.09 ± 0.05&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4n6)</td>
<td>0.04 ± 0.01&lt;sup&gt;as&lt;/sup&gt;</td>
<td>0.04 ± 0.01&lt;sup&gt;as&lt;/sup&gt;</td>
<td>0.05 ± 0.02&lt;sup&gt;as&lt;/sup&gt;</td>
<td>0.03 ± 0.01&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (C20:5n3)*</td>
<td>0.04 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALA/LA</td>
<td>0.20 ± 0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.30 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.30 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.30 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA + EPA*</td>
<td>0.16 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.21 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHA/EPA*</td>
<td>3.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EPA/ARA*</td>
<td>1.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>a</sup>, **<sup>b</sup>, ***<sup>c</sup> = significance at *P* < 0.05, 0.01 and 0.001, respectively; NS = not significant at *P* < 0.05

**DISCUSSION**

**Better growth performance of *P. larnauidii* fed with diets containing dietary vegetable oils than those fed with SO diet**

Low profitability of catfish industry demands that diet formulation should be improved to reduce feed costs and increase productivity. In this study, a 6-month feeding trial was carried out for evaluation of the total replacement of added SO in the grow-out diet of *P. larnauidii* with PO, SBO or SFO from fingerlings (about 15 g) to marketable size (>300 g). The environmental conditions (dissolved oxygen 4.00 - 6.40 ppm, pH 6.36 - 8.88, water temperature 29.60 - 31.70°C, and unionized ammonia 0.001 - 0.005 ppm) during the feed trial were in the range of standard for aquaculture (Boyd, 1998). The survival rate of experimental animals was 98-100% over a 6 month experimental period.

HUFAs are essential fatty acids for normal growth and survival of teleosts (Tocher *et al.*, 2005a and 2003b). Freshwater teleosts have a capacity to bioconvert LA and ALA into HUFAs (Zheng *et al.*, 2004; Hastings *et al.*, 2005; Tanomman *et al.*, 2013). However, the efficiency for using LA is species-dependent and varied widely. In Nile tilapia (*Oreochromis niloticus*) and tilapia galilaei (*Sarotherodon galilaeus*), a replacement of fish oil with vegetable oil for a 17 week feeding trial was carried out. Growth and FCR of both species fed the SBO-containing diet was better than those with fish oil (*P* < 0.05; Goda *et al.*, 2007).

Pongjanyakul *et al.* (2008) substituted the different ratio of soybean meal in the diet (0, 25, 50, 75, 100 %) in 3 month feeding trial of *P. larnauidii* (23% proteins) and its specific growth rate fed the soybean meal diet was lower than the diet containing fish meal. Accordingly, fish meal which contains the essential fatty acids of fish was included in all experimental diets in our study but more cost-effective diets including 4% PO, SBO and SFO (US$1.0 – 1.5·kg<sup>−1</sup>) was produced to fully replace the SO diet (US$1.8·kg<sup>−1</sup>; Thai Feed Mill Association, 2009).

Asdari *et al.* (2011b) determined the replacement effects of the diet containing fish oil (FO) with SO, crude palm oil (CPO) and linseed oil (LO) on growth performance of *P. hypophthalmus*...
in the 12-week feeding trial. Growth performance of fish fed all vegetable oil-based diets was better than those fed the FO diet. Muscle and liver fatty acid composition for all treatments generally reflected the composition in the diet. Similar results were found in *P. nasutus* juveniles where growth performance of fish fed the CPO diet was significantly higher than fish fed the FO diet (*P* < 0.05; Asdari et al., 2011a). Piedecausa et al. (2007) reported that the n-3/n-6 ratio in tissues of fish fed vegetable oils is greatly reduced. Results from previous studies in *P. nasutus* (Asdari et al., 2011a) and *P. hypophthalmus* (Asdari et al., 2011b) was in agreement with the present study on *P. larnaudii* which suggested that excessive amounts of n-3 fatty acids reduce the overall growth performance of *Pangasius* fish.

During larval development, DHA is required for visual response in gilthead sea bream (Benitez-Santana et al., 2007) and schooling behavior in Pacific threadfin (*Polydactylus sexfilis*) (Masuda et al., 2001). In this study, there was no significance in the growth rate of *P. larnaudii* at the first 3 months. Nevertheless, late feed responses were observed during months 4-6 of the experiment where the black ear catfish fed vegetable oil-based diets exhibited a better growth performance than those fed the SO diet. However, fatty acid composition in muscle revealed a lower amount of EPA (C20:2, n-6) in the feeding SFO diet than other formulated diets. The EPA/ARA ratio in SO and PO was greater than that of SBO and SFO. Based on the muscle lipid composition, the quality of fish from PO and SO diets was not different while those from SBO and SFO diets may be inferior due to the lack of palmitoleic acid (C16:1) with a moderate level of EPA/ARA in the former and the lack of both C16:1 and ALA (C18:3n-3) and a low level of EPA/ARA in the latter. The elevated HUFA in muscle implied that the dietary vegetable oils (i.e. PO and SBO) can be modulated desaturation and elongation activities in *P. larnaudii* as well as in other freshwater fishes (Tocher et al., 2002b, 2003a, 2003b). Nevertheless, the preference of these enzymes to precursor of the HUFA pathway may be different (Zheng et al., 2009). In cobia, the desaturase was more active for LNA, while the elongase preferred both LA and LNA substrates (Zheng et al., 2009).

The diet containing PO as the whole source of lipid supplement apparently modulate higher desaturase activity than LNA-rich (SO) diet. Izquierdo et al. (2008) reported that diets in which fish oil was totally replaced by SBO and rapeseed oils induced a six fold increase of the desaturase mRNA level in *S. aurata*. Similarly, Gao et al. (2012) showed that diet fully replaced of PO could increase a higher DHA concentration in liver than those fed fish oil in *Lateolabrax japonicus* juveniles.

An altered fatty acid profile of the aquaculture species following the replacement of fish/squid oil-containing diets with VO-containing diets is a major concern for health benefits. In the present study, *P. larnaudii* fed PO and SBO diets did not show significant reduction of DHA, EPA, ARA from those fed the SO diet while a lower EPA was observed following the SFO feeding. It is interesting to further investigate about the grow-out diet (i.e. PO) containing a low amount of short chain PUFA but rich in SFA provided a better growth performance of those fed a SO diet.

**Isolation and expression analysis of ** *PlFadsd6* **in** *P. larnaudii* **fed different fatty acid sources**

The full-length cDNAs of *PlFadsd6* were isolated and reported for the first time in *P. larnaudii*. A predicted Cyt-B5 and FA desaturase domains were found in the deduced *PlFadsd6* protein. It functions in heme binding in a diverse range of proteins. In addition, the Cyt-b5 domain is found in proteins that are able to bind to steroids (e.g. progesterone receptor) (Gerdes et al., 1998). The information implied the functional involvement in fatty acid desaturation of the characterized genes.

Phylogenetic analysis indicated that it was closely related with those from other catfishes but distantly related with other teleosts. Tissue distribution analysis illustrated that *PlFadsd6* was expressed only in brain and liver. Similar results were previously reported in *S. solar* (Monroig et al., 2010), *Channa striatus* (Aliyu-Paiko et al., 2012), and cobia (Zheng et al., 2009). This is coincident with the function of liver as the main organ.
responsible for the biosynthesis of HUFA. The high expression of \textit{PfFadsd6} in \textit{P. larnaudii} brain suggested its important function of fatty acid metabolism in brain, which needs to be further clarified.

\textit{PfFadsd6} transcripts were not expressed in pituitary gland and muscle. As a result, fatty acid composition in muscle should reflect the effects of replacing SO with vegetable oil-containing diets. Abundant expression of \textit{PfFadsd6} observed in liver of fish fed PO implied the ability to biosynthesize HUFA from diet substrates of \textit{P. larnaudii}.

The fatty acid compositions in vegetable oils readily affect the HUFA biosynthesis pathway. Increased hepatocyte desaturation and elongation activities in tilapia and zebrafish were reported with vegetable oil-based diets (Tocher \textit{et al.}, 2002a). Similarly, \textit{PfFadsd6} transcripts in the black ear catfish fed PO were higher than other experimental diets (SO, SBO and SFO; \(P < 0.05\)).

Although several studies reported that high LA and ALA contents can up-regulate \textit{Fadsd6} expression (Turchini \textit{et al.}, 2006; Francis \textit{et al.}, 2007; Li \textit{et al.}, 2008), the excess of PUFAs can also inhibit the desaturation of substrates (Bell \textit{et al.}, 1993; Ruyter \textit{et al.}, 2000). Results in this study revealed significant decreases of \textit{PfFadsd6} transcripts in \textit{P. larnaudii} fed SBO (high LA and ALA) and SFO (high LA but low ALA) diets. Nevertheless, up-regulation of these transcripts was observed in the black ear catfish fed the PO diet (moderate level of LA but low level of ALA). The LA/ALA ratios in SO, PO, SBO and SFO were 0.115, 43.81, 7.63 and 220.84, respectively. We speculate that the up-regulation of \textit{PfFadsd6} mRNA may depend on the optimum level of a LA to ALA ratio. The EPA and DHA levels in \textit{P. larnaudii} muscle fed SFO (high LA) were lower than those fed PO (low LA) and SBO (moderate LA). This result was in agreement with the reduction of \textit{PfFadsd6} transcripts in \textit{P. larnaudii} liver. The circumstance suggested the possible inhibition of DHA synthesis in the formulated diet with the excess LA content.

The nutritional regulation of desaturase activities could vary among the developmental stage and capacity of fish to transform these lipids through desaturation and elongation pathways (Tocher \textit{et al.}, 2006). Ren \textit{et al.} (2012) illustrated a decrease of DHA in muscle of \textit{Cyprinus carpio} fed high LA. This may probably due to the detrimental effect to maintain DHA in muscle tissue. Apparently, the diet that contains PO as lipid supplement source significantly induce \textit{PfFadsd6} expression, but not significantly affect on increasing the level of HUFA in muscle compared with the SO control.

The primary mechanism responsible for increased HUFA biosynthesis during limited dietary HUFA intake is through up-regulated expression of desaturase mRNAs (Bell \textit{et al.}, 2003). Reduced transcriptional levels of liver \textit{PfFadsd6} mRNA in \textit{P. larnaudii} fed dietary SBO and SFO diets were found. This was not consonant with several fish species on an increase in both enzymatic and transcriptional activities of liver desaturase with increasing levels of dietary LA and LNA (Tocher \textit{et al.}, 2002b; Zheng \textit{et al.}, 2004; Ling \textit{et al.}, 2006). Apparently, the contradictory results on increased \textit{PfFadsd6} mRNA in black ear fish fed dietary PO is relatively unclear and need further studies. Apparently, the expression of these genes is not solely influenced by dietary levels of n-3 fatty acids alone but could also be positively or negatively regulated by both substrates and products of the HUFA pathway (Zheng \textit{et al.}, 2004).

Considering results from the feeding trial, the growth performance of fish (4-6 months old) fed formulated diets containing vegetable oils was better than fish fed the SO diet. When HUFA profiles in muscle and expression levels of liver \textit{PfFadsd6} mRNA were also taken into the account, the SO was more appropriately replaced with PO than SBO and SFO in the grow-out diet for \textit{P. larnaudii}. However, the replacement effects of diets containing vegetable oils in < 4 month old fish should be confirmed.
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LITERATURE CITED


