

1 **Title**

2 Long-chain polyunsaturated fatty acid metabolism in carnivorous marine teleosts: insight into the
3 profile of endogenous biosynthesis in golden pompano *Trachinotus ovatus*

4
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19 **Keywords:**

20 *Trachinotus ovatus*, ratio of ALA/LA, growth performance, biosynthetic ability of LC-PUFA

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This is the peer reviewed version of the following article: Wang, S, Wang, M, Zhang, H, et al. Long-chain polyunsaturated fatty acid metabolism in carnivorous marine teleosts: Insight into the profile of endogenous biosynthesis in golden pompano *Trachinotus ovatus*. *Aquaculture Research* 2020; 51: 623–635, which has been published in final form at <https://doi.org/10.1111/are.14410>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for self-archiving.

26 **Abbreviations**

- 27 ALA, α -linolenic acid (18:3n-3)
- 28 ARA, arachidonic acid (20:4n-6)
- 29 BHT, butylated hydroxytoluene
- 30 DHA, docosahexaenoic acid (22:6n-3)
- 31 DPA, docosapentaenoic acid (22:5n-3)
- 32 EFA, essential fatty acids
- 33 Elovl, elongase of very long-chain fatty acids
- 34 EPA, eicosapentaenoic acid (20:5n-3)
- 35 Fad, fatty acyl desaturase
- 36 FCR, feed conversion ratio
- 37 HSI, hepatosomatic index
- 38 LA, linoleic acid (18:2n-6)
- 39 LC-PUFA, long-chain polyunsaturated fatty acids
- 40 MUFA, Monounsaturated fatty acids
- 41 NAMBS, Nan Ao Marine Biology Station
- 42 PUFA, polyunsaturated fatty acids
- 43 SFA, saturated fatty acids
- 44 SR, survival rate
- 45 SGR, specific growth rate
- 46 WGR, weight gain rate
- 47
- 48

49 **Abstract**

50 Golden pompano *Trachinotus ovatus* is an important farmed carnivorous marine teleost. Although
51 some enzymes for long-chain polyunsaturated fatty acids (LC-PUFA) biosynthesis have been
52 identified, the ability of *T. ovatus* for endogenous biosynthesis is unknown. Here, we evaluated *in*
53 *vivo* LC-PUFA synthesis in a 56-day culture experiment using six diets (D1-D6) formulated with
54 linseed and soybean oils to produce dietary linolenic/linoleic acid (ALA/LA) ratios ranging from
55 0.14 to 2.20. The control diet (D0) used fish oil as lipid source. The results showed that, compared
56 with the corresponding indices of fish fed D0, the weight gain rate and specific growth rate, as well
57 as the contents of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in tissues (liver,
58 muscle, brain and eye) of D1-D6 groups were significantly lower ($P < 0.05$). These data suggested
59 that *T. ovatus* could not synthesize LC-PUFA from C₁₈ PUFA or such ability was very low. However,
60 tissue levels of 20:4n-3 in fish fed diets D1-D6 were higher than that of D0 fish ($P < 0.05$), and
61 positively correlated with dietary ALA/LA ratio, while levels of EPA showed no difference among
62 the D1-D6 groups. These results indicated that $\Delta 5$ desaturation, required for the conversion of
63 20:4n-3 to EPA, may be lacking or very low, suggesting incomplete LC-PUFA biosynthesis ability
64 in *T. ovatus*.
65

66 **Introduction**

67 Long-chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic (ARA; 20:4n-6),
68 eicosapentaenoic (EPA; 20:5n-3), and docosahexaenoic (DHA; 22:6n-3) acids are important
69 structural components of cell membranes (Marsh, 2008) and act as eicosanoid precursors (Villalta
70 *et al.*, 2008), as well as playing important roles in maintaining normal growth and metabolism
71 (Sargent *et al.*, 2002). Fish oil is the main source of dietary LC-PUFA for farmed fish, with around
72 75 % of the total global supply of fish oil used in aquaculture (Tocher, 2015). However, the scarcity
73 of fish oil resources makes it impossible to further increase the yield, which therefore impacts the
74 development of aquaculture activities (Naylor *et al.*, 2000; Tacon and Metian, 2009). For this reason,
75 terrestrial vegetable oils have been considered as the most likely alternatives, because of the low
76 cost, global availability and stable supply (Nasopoulou and Zaetakis, 2012). However, the
77 polyunsaturated fatty acids (PUFA) in vegetable oils are predominantly linoleic (LA, 18:2n-6) and
78 α -linolenic (ALA, 18:3n-3) acids, while the fatty acids that perform vital physiological functions in
79 fish are EPA, ARA and DHA, which are abundant fish oil, are not present (Sargent *et al.*, 2002).
80 Freshwater fish and salmonid species generally possess the capacity to synthesize LC-PUFA from
81 ALA and LA, while marine fish other than *Siganus canaliculatus* (Li *et al.*, 2008) are assumed to
82 lack this ability because of one or more of the key enzymes involved in the LC-PUFA biosynthesis
83 pathway are absent and, thus, LC-PUFA are required in their diets (Bell *et al.*, 1999; Sargent *et al.*,
84 2002; Regost *et al.*, 2003). Therefore, the lack of LC-PUFA in vegetable oil places restrictions in
85 their application in feed for marine fish. Consequently, there is a need to clarify the mechanisms
86 underpinning the low LC-PUFA biosynthetic capacity of marine fish in order to develop methods
87 for increasing such capability.

88 Fatty acyl desaturase (Fads2) and elongase (Elovl) enzymes are involved in the biosynthesis
89 of LC-PUFA but, due to competition between n-3 and n-6 PUFA substrates, the conversion of
90 ALA to EPA and DHA can be influenced by the dietary levels of LA and vice versa (Tocher and
91 Glencross, 2015). Thus, an optimum dietary balance of ALA/LA is important for the biosynthesis
92 of LC-PUFA. Many studies have shown that the dietary ALA/LA ratio also influenced fatty acid
93 deposition and metabolism in fish (Thanuthong *et al.*, 2011; Tian *et al.*, 2016; Chen *et al.*, 2017).
94 Studies in two marine herbivorous fish (*Siganus canaliculatus* and *Scatophagus argus*) specifically
95 showed that an appropriate dietary ALA/LA ratio could also improve the expression level of key

96 enzymes involved in the biosynthesis of LC-PUFA and the content of LC-PUFA in tissues (Xie *et al.*, 2014; 2015; 2016; 2018).
97

98 Golden pompano, *Trachinotus ovatus* is a carnivorous marine fish that prey mainly on
99 zooplankton and fish (Tan *et al.*, 2016). Due to its fast growth rate, high disease resistance, and high
100 flesh quality, *T. ovatus* has developed rapidly along the southern coast of China (Lin *et al.*, 2011). In
101 2015, domestic aquaculture production exceeded 180,000 tons (Yang, 2015). Recently, the impact
102 of dietary lipid source on growth performance, body composition and lipid metabolism was
103 investigated in juvenile, *T. ovatus* (Liu *et al.*, 2018). However, the precise nutritional requirements
104 of *T. ovatus* remain largely unknown (Li *et al.*, 2019). While two enzymes that might be involved
105 in the biosynthesis of LC-PUFA have been cloned in *T. ovatus*, including an Elovl5 (Zhu *et al.*, 2018)
106 and a Fads2-like desaturase (Han *et al.*, 2015), their precise functions have not been identified. Very
107 recently, a new desaturase was found in *T. ovatus*, which might possess $\Delta 4$ desaturase and potential
108 $\Delta 5/8$ desaturase activity (Zhu *et al.*, 2019). Thus, potential molecular components of the LC-PUFA
109 biosynthetic pathway are being reported in *T. ovatus*, but the actual activity of the pathway *in vivo*
110 requires further study. The aim of the present study was therefore to investigate the endogenous
111 capability of *T. ovatus* for LC-PUFA biosynthesis and further to determine effect of dietary ALA/LA
112 ratio on LC-PUFA biosynthesis and accumulation in key tissues.

113

114 **Material and methods**

115 *Experimental diets*

116 Formulations and proximate compositions of the experimental diets are presented in Table 1.
117 Seven iso-nitrogenous (50.0 %) and iso-lipidic (12.0 %) experimental diets were formulated, with
118 fish oil (rich in LC-PUFA) used as lipid source in the control diet (D0), while soybean oil and linseed
119 oil (both devoid of LC-PUFA) were used as lipid sources for the other six diets (D1-D6) in blends
120 to produce five ratios of ALA to LA of around 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5, respectively. The
121 principal fatty acid compositions of the diets are detailed in Table 2.

122 All the dry ingredients were finely ground and sieved with a 60-mesh sieve, then thoroughly
123 mixed with their respective oil mixtures. An appropriate amount of water was added to produce stiff
124 doughs that were then passed through a meat grinder with the appropriate diameter diet to prepare
125 pellets. Pellets were air dried and sieved into proper pellet sizes. All experimental diets were stored

126 at -20 °C until use.

127

128 *Experimental fish and feeding trial*

129 All procedures performed on fish were in accordance with the National Institutes of Health
130 guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and
131 approved by the Institutional Animal Care and Use Committee of Shantou University (Guangdong,
132 China). The feeding experiment was conducted at an experimental floating sea cage site at Nan Ao
133 Marine Biology Station (NAMBS) of Shantou University, Southern China. Approximately 1000
134 juvenile *T. ovatus* of the same genetic background were obtained from a private breeding facility in
135 Raoping, Guangdong province, China. Prior to the commencement of the feeding trial, all fish were
136 fed on the mixed diets (D1-D6) for 2 weeks to acclimatize the fish to the experimental conditions
137 and deplete their lipid reserves in a large floating sea cage (2 m x 2 m x 3 m).

138 After acclimation, similar-sized fish (average initial body weight 8.32 ± 0.02 g) were randomly
139 distributed into 21 floating sea cages at 25 fish per cage (1 m x 1 m x 1.5 m) in triplicates per dietary
140 treatment. The fish were fed the experimental diets twice a day (at 07:00 and 17:00) to apparent
141 satiation for 56 days, with the amount of feed provided recorded daily. Water temperature, salinity
142 and dissolved oxygen were measured daily, with temperature ranging from 19.96 to 29.63 °C,
143 salinity from 35 to 37 ‰, and dissolved oxygen at about 7 mg.L⁻¹ for the duration of the trial. Any
144 dead fish were weighed and used to calculate feed conversion rate (FCR).

145

146 *Evaluation of growth performance and sample collection*

147 At the end of the feeding trial, all fish were fasted for 24 h prior to final sampling. Fish were
148 anesthetized by 0.01% 2-phenoxyethanol. Survival rate (SR) was calculated and growth
149 performance evaluated by weight gain rate (WGR) and special growth rate (SGR). Four fish were
150 randomly collected from each replicate cage (12 fish per treatment) and frozen at -20 °C for
151 subsequent determination of whole body composition. The liver of the sampled fish was excised
152 and weighed to determine hepatosomatic index (HSI). The liver, muscle, brain and eyes of these
153 six fish were sampled, pooled into 1.5 ml tubes (RNAase-Free, Axygen, USA) and then stored at -
154 80 °C for fatty acid composition determination or RNA extraction.

155

156 *Chemical analysis*

157 *Proximate composition*

158 The nutrient composition (moisture, crude protein, crude lipid and ash) of the experimental
159 diets and whole-body of juvenile *T. ovatus* samples were measured according to AOAC (1995) as
160 described in detail previously (Li *et al.*, 2005, 2008; Xie *et al.*, 2014). Briefly, moisture was
161 determined by drying samples in an oven at 105 °C to constant weight. Crude protein (N * 6.25)
162 content was determined using an auto-Kjeldahl System (Kjeltec™8400; FOSS, Denmark). Crude
163 lipid was measured by petroleum ether (B.P. 40-60 °C for 3 h) extraction using the Soxhlet method
164 (SZF-06A; Xinjia Yiqi CO., LTD, China). For ash content, samples were incinerated in a muffle
165 furnace (CWF1100; Carbolite, Germany) at 550 °C for 12 h.

166

167 *Fatty acids analysis*

168 Total lipid in feeds and tissues of *T. ovatus* were extracted with chloroform/methanol (2:1, v/v)
169 containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, and fatty acid methyl esters
170 prepared by transesterification with boron trifluoride diethyl etherate (ca. 48 %, Acros Organics,
171 Waltham, MA, USA) as described previously (Li *et al.*, 2005, 2008). The fatty acid composition of
172 feeds, liver, muscle, brain and eyes were determined using gas chromatograph (GC-2010; Shimadzu,
173 Kyoto, Japan) with GC parameters as described in detail previously (Xie *et al.*, 2014).

174

175 *Gene expression analysis by real-time quantitative RT-PCR (qRT-PCR)*

176 Total RNA was extracted from liver, brain and eyes using BioFast Simply P Total RNA
177 Extraction Kit (BioFlux, Japan). The quantity of isolated RNA was determined using NanoDrop
178 2000 spectrophotometer (NanoDrop Technologies, USA) and the quality of total RNA was assessed
179 by electrophoresis in 1 % agarose gel. Reverse transcription was performed using the FastKing
180 gDNA Dispelling RT SuperMix (TIANGEN Biotech Co., Ltd., Beijing, China) including a genomic
181 DNA elimination reaction. The mRNA levels of fatty acyl desaturase (*fads2*-like) (Han *et al.*, 2015)
182 and elongase5 (*elovl5*) (Zhu *et al.*, 2018) as well as the housekeeping β -actin (Tan *et al.*, 2016) in
183 tissues were determined by real-time PCR using specific primers designed with Primer 5 Software
184 (Table 3). The PCR was carried out on a Lightcycler 480 system (Roche, Basel, Switzerland) in a
185 final volume of 10 μ l containing 5 μ l SYBR Green Supermix (Biorad, Hercules, CA, USA), 0.5 μ l

186 each primer, 3 µl ddH₂O and 1 µl cDNA. The PCR program consisted of an initial DNA denaturation
187 at 94 °C for 5 min, followed by 45 cycles at 95 °C for 10 s, annealing 60 °C for 20 s, and with a
188 final extension step at 95 °C for 5 s, 65 °C for 1 min, and 40 °C for 10 s. The relative mRNA levels
189 were normalized with β-actin. Normalized gene expression of group D0 was set to 1, and the other
190 dietary groups D1-D6 of different ratios of ALA/LA were expressed relative to the D0 (FO) group.
191 The optimized comparative Ct ($2^{-\Delta\Delta C_t}$) method method was used to evaluate gene expression levels.

192

193 *Statistical analyses*

194 All data are presented as mean ± SEM (standard error of mean). Comparisons amongst
195 treatments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using
196 SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The level of significant difference was set at P
197 < 0.05.

198

199 **Results**

200 *Growth performance*

201 The growth performance of fish at the end of the 8-week (56 days) feeding trial is shown in
202 Table 4. Growth performance indices including WGR and SGR of fish fed diets D1-D6 were
203 significantly lower than those of fish fed diet D0, while there was no significant differences among
204 groups D1-D6. The FCR and HSI of groups D1-D6 were significantly higher than the D0 group. SR
205 in the fish fed D0 was 100 %, and was lower in fish fed diets D1-D6, with lowest SR of 66% in fish
206 fed D2, and SR of 92 % in D5 and D6 groups.

207

208 *Proximate composition*

209 The biochemical compositions of whole body of juvenile *T. ovatus* fed the experimental diets
210 with different dietary ALA/LA ratios are shown in Table 5. Proportions of protein, lipid and ash did
211 not differ significantly among the dietary groups, although whole body of fish fed diet D0 showed
212 the lowest moisture content that was significantly difference from that of fish fed diet D1.

213

214 *Tissue fatty acid composition*

215 The fatty acid compositions of liver is shown in Table 6. The contents of ARA, EPA, and DHA

216 in D1-D6 groups were significantly lower than the D0 group, which essentially reflected the dietary
217 fatty acid profiles. However, the contents of 18:4n-3, 20:4n-3, 18:3n-6 and 20:3n-6 in fish fed diets
218 D1-D6 were significantly higher than in fish fed D0. The levels of 18:3n-3 and 20:4n-3 increased
219 with increasing dietary ALA/LA ratio, while 18:2n-6 and 20:3n-6 displayed the opposite pattern.

220 The fatty acid compositions of muscle, brain and eyes showed the same trends as that described
221 above for liver. Thus, the proportions of ARA, EPA and DHA in groups D1-D6 were lower than in
222 the D0 group, while levels of 18:3n-3, 20:4n-3, 18:2n-6 and 20:3n-6 varied with the dietary ALA/LA
223 ratio and were significantly higher in fish fed diets D1-D6 compared to the D0 group (Tables 7-9).

224 Notably, the relative levels of DHA in the brain and eyes were higher than those in muscle
225 and liver in fish fed all the diets. The proportions of EPA in all tissues were relatively low and similar
226 in fish fed diets D1-D6. The ratio of DHA and EPA was higher in brain and eyes compared to liver
227 and muscle, and also higher in fish fed diets D1-D6 than in fish fed diet D0.

228

229 *Levels of fads2 and elovl5 gene expression in liver, brain and eyes*

230 The mRNA levels of *fads2*-like desaturase in liver were affected by the ratio of dietary
231 ALA/LA, with the highest levels found in fish fed diets D5 and D6, both of which were higher than
232 the expression levels in fish fed the other diets including diet D0 (Fig. 1). The lowest *fads2*-like
233 mRNA level was found in fish fed diet D2 group, which was even lower than in fish fed diet D0.
234 The mRNA levels of *fads2*-like in brain were significantly higher in fish fed diets D1-D6 compared
235 to fish fed diet D0, but there was no significant differences among D1-D6 groups. However, the
236 expression level of *fads2*-like in eyes displayed no differences between any dietary groups (Fig. 1).

237 The expression levels of *elovl5* in liver and eyes were not different among the experimental
238 groups (Fig. 2). The mRNA levels of *elovl5* in brain increased with increased dietary ratio of
239 ALA/LA among fish fed diets D1-D6, with the levels of *elovl5* in the D2-D6 groups significantly
240 higher than in the D0 group.

241

242 **Discussion**

243 The biosynthesis of LC-PUFA is a process that involves consecutive desaturation and
244 elongation steps of C₁₈ PUFA substrates, ALA or LA, catalyzed by desaturase and elongase enzymes,
245 respectively (Cook, 1996; Bell and Tocher, 2009). The synthesis of ARA is accomplished by $\Delta 6$ Fad

246 desaturation of LA to 18:3n-6, which is elongated by Elov15 (elongase) to 20:3n-6 and then
247 desaturated by $\Delta 5$ Fad to ARA. Similarly, synthesis of EPA from ALA uses the same enzymes, $\Delta 6$
248 Fad, Elov15 and $\Delta 5$ Fad, to desaturate ALA to 18:4n-3, which is further elongated to 20:4n-3 and
249 then desaturated to EPA. However, DHA synthesis requires 2-4 additional steps with at least one or
250 more desaturase and elongase enzymes involved (Sprecher, 2000). In the present study, the
251 proportions of pathway intermediates, i.e., 18:3n-6, 18:4n-3, 20:3n-6 and 20:4n-3, in liver, muscle,
252 brain and eyes of fish fed diets D1-D6 were significantly higher than in fish fed diet D0. Notably,
253 the percentages of 20:4n-3 increased with increased dietary ALA/LA ratio, while the proportions of
254 20:3n-6 showed the opposite trend. These data suggest that *T. ovatus* has the ability to desaturate
255 LA and ALA to 18:3n-6 or 18:4n-3, respectively, followed by elongation to 20:3n-6 and 20:4n-3,
256 respectively, which requires the activities of $\Delta 6$ Fad and Elov15 enzymes. However, the proportions
257 of ARA and EPA were lower in fish fed diets D1-D6 than in fish fed D0. This strongly suggests that
258 *T. ovatus* lacks the $\Delta 5$ desaturation activity required to convert 20:3n-6 and 20:4n-3 to ARA and
259 EPA, respectively, similar to many/most other marine teleost fish species (Leaver *et al.*, 2008;
260 Tocher *et al.*, 2010). Therefore, *T. ovatus* possess $\Delta 6$ Fads2 and Elov15 activities, consistent with
261 the fact that cDNAs of these genes have been cloned in many marine fish species (Seiliez *et al.*, Xie
262 *et al.*, 2014, 2016, Zheng *et al.*, 2009, Monroig *et al.*, 2012), whereas it lacks a $\Delta 5$ Fad, a deficiency
263 that has little consequence in the LC-PUFA-rich marine ecosystem (Tocher, 2010). This is consistent
264 with juvenile *T. ovatus* lacking the ability to biosynthesize LC-PUFA, specifically EPA, ARA and
265 DHA, from ALA or LA and, thus, require dietary LC-PUFA to meet their EFA requirements.
266 Consistent with this, no differences were observed in the growth performance among juvenile *T.*
267 *ovatus* fed diets D1-D6, although growth in these groups was significantly lower than in fish fed
268 diet D0. Long-chain PUFA are essential for the normal growth and survival of teleosts (Bell *et al.*,
269 1986; Lee, 2001) and, hence, the absence of dietary EFA from fish diets can result in reduced growth,
270 increased mortality and other pathologies (Sargent *et al.*, 2002; Glencross *et al.*, 2010). Similarly, in
271 the present study fish, fish fed diets D1-D6 showed lower SGR and survival than fish fed D0.
272 Overall the data suggest that juvenile *T. ovatus* were not capable of endogenously producing the key
273 EFA, ARA, EPA and DHA when fed diets rich in ALA and LA. Therefore, while they express $\Delta 6$
274 Fads2 and Elov15 activities and, therefore, some ability to convert ALA and LA to 20:4n-3 and
275 20:3n-6, respectively, a deficiency in $\Delta 5$ desaturase activity means *T. ovatus* lacked the capability

276 for the endogenous biosynthesis of EPA and DHA, and thus LC-PUFA (e.g. FO) should be included
277 in diets formulated for aquaculture.

278 While the fatty acid composition analysis showed that *T. ovatus* did not have a complete LC-
279 PUFA biosynthesis pathway, the high proportions of DHA and high ratios of DHA/EPA found in
280 brain and eyes of fish fed diets D1-D6, which were higher than in fish fed the control diet D0,
281 suggested that *T. ovatus* may have the capability of converting EPA to DHA. DHA plays important
282 roles in neural tissues, however, most marine fish such as cod, cobia, and Asian sea bass, lack the
283 capability to synthesize DHA from C18 PUFA. Tocher (2010) speculated that the retention of $\Delta 6$
284 Fad and Elov15 activities in marine fish may be related to the need to maintain DHA levels in critical
285 neural tissues (brains and eyes) via endogenous production from EPA. Therefore, the high expression
286 of $\Delta 6$ Fads in the brain and eye of *T. ovatus* may help to maintain membrane DHA levels in neural
287 tissues at times of high demand. If the DHA found in brain and eyes was of dietary origin, then the
288 level should be higher in fish fed diet D0, and there should be no difference in DHA contents among
289 the groups D1-D6. In fact, the ratio of DHA/EPA was different among the groups of D1-D6,
290 consistent with the EPA levels. This suggested that at least a portion of the DHA in brain and eyes
291 was derived from endogenous metabolism.

292 The expression of *fads2*-like mRNA levels in liver was affected by the dietary ratio of ALA/LA.
293 The expression of *fads2*-like mRNA was the highest when the dietary ratio of ALA/LA was 1.92
294 (group D5), which was consistent with other studies in fish that showed dietary ALA/LA ratio
295 influenced the expression of *fads2*. For example, $\Delta 6$ fad expression was highest in fish fed diets
296 with ALA/LA ratios of 1.93 and 1.72 in *Siganus canaliculatus* (Xie *et al.*, 2014) and *Scatophagus*
297 *Argus* (Xie *et al.*, 2015), respectively. In contrast, the mRNA level of *elov15* in liver showed no
298 difference among groups D1-D6, which was different from other studies (Mohd-Yusof *et al.*, 2010;
299 Monroig *et al.*, 2013; Wang *et al.*, 2014). However, the expressions of both *fads2*-like and *elov15*
300 were significantly up-regulated in brain when fish oil (D0) was replaced by mixed vegetable oil
301 (D1-D6), which suggested that the both key enzymes were involved in DHA biosynthesis in the
302 brain.

303 The *fads2*-like and *elov15* sequences investigated in the present study were those reported in
304 previous studies although the function of *fads2*-like has not been characterized (Han *et al.*, 2015;
305 Zhu *et al.*, 2018). Very recently, a further Fad of *T. ovatus* has been reported and shown to have

306 mainly $\Delta 4$ desaturation activity and possibly residual $\Delta 5$ and $\Delta 8$ activities, but no $\Delta 6$ Fad activity
307 (Zhu et al., 2019). This Fad was expressed mainly in brain, followed by eyes and liver, suggesting
308 that it could be involved in DHA biosynthesis in brain and eyes. Furthermore, as this Fad did not
309 have $\Delta 6$ activity, it may suggest that the *fads2*-like in the present study would have $\Delta 6$ desaturation
310 activity, consistent with *T. ovatus* having the ability to convert ALA and LA to 20:4n-3 and 20:3n-
311 6, respectively.

312 Nutritional factors can affect the activities of key enzymes involved in LC-PUFA biosynthesis
313 through the *in vivo* regulation of these genes. Many studies have reported in both of freshwater and
314 marine fish species that reducing dietary levels of LC-PUFA by replacing fish oil with vegetable oil
315 in feeds resulted in higher expression levels of some desaturase and elongase genes (Zheng *et al.*,
316 2005b; Izquierdo *et al.*, 2008 Seiliez *et al.*, 2003; Liu *et al.*, 2018). However, it has been reported
317 that the expression of $\Delta 6$ Fad in liver was lower with the replacement of dietary fish oil by rapeseed
318 oil in European sea bass (Mourente *et al.*, 2002). In Atlantic cod, liver and intestinal $\Delta 6$ Fad
319 expression and activity showed no significant difference with fed diets containing either vegetable
320 or fish oil (Tocher *et al.*, 2006). In the current study, the expression level of *fads2*-like in liver of
321 fish fed diet D2 (ALA/LA ratio of 0.5) was significantly lower than in fish fed diet D0 (FO group),
322 while higher levels of ALA and higher ALA/LA ratios resulted in expression of $\Delta 6$ *fads2* being
323 higher in liver of fish fed diets D5 and D6 than in fish fed D0. This effect on the expression of $\Delta 6$
324 *fads2* might be due to the precise interaction between the different levels and ratios of ALA and LA
325 in the experimental diets. On the other hand, the expression level of *fads2*-like in brain of fish fed
326 diets D1-D6 groups was markedly higher than in fish fed D0 (FO group), whereas there was no
327 effect of dietary ratio of ALA/LA. As the mention above, endogenous DHA biosynthesis in brain of
328 *T. ovatus* may be via the direct activity of the $\Delta 4$ Fad or via the “Sprecher shunt” pathway if the
329 Fads2-like desaturase is able to desaturate 24:5n-3. However, the activity of the Fads2-like
330 desaturase and the specific regulatory mechanisms of Fads2-like in brain requires further study.

331 With the rapid development of aquaculture, balancing the increasing demand and supply of FO
332 is one of the most serious constrains that could impact the continued growth of farming activities.
333 Vegetable oils, potentially rich in ALA and LA, could be the most suitable alternatives (Nasopoulou
334 and Zeatakis, 2012). While replacement of FO with vegetable oil has been successful for some
335 omnivorous fishes, it is difficult to meet the LC-PUFA requirement of many carnivorous marine fish

336 (Tocher, 2010; Turchini *et al.*, 2009), due to limited information on the biosynthesis ability of LC-
337 PUFA in these species. In the present study, we showed regulation of fatty acid desaturase and
338 elongase genes by dietary ALA/LA ratio, revealing that juvenile *T. ovatus* has some ability to
339 convert ALA and LA to 20:4n-3 and 20:3n-6, respectively, but does not have a complete LC-PUFA
340 biosynthetic pathway, likely lacking biologically significant $\Delta 5$ desaturase activity.

341 In conclusion, based on growth performance, tissue fatty acid compositions and the expression
342 of key enzymes involved in the biosynthesis of LC-PUFA, the current results suggested that juvenile
343 *T. ovatus* possessed the ability to convert 18:3n-3 or 18:2n-6 to 20:4n-3 and 20:3n-6, respectively.
344 It might also have some ability to synthesize DHA from EPA in brain and eyes. However, *T. ovatus*
345 lacked a complete LC-PUFA biosynthetic pathway. Thus, EFAs, especially EPA, DHA and ARA,
346 are required in diets of *T. ovatus* to maintain normal growth and survival.

347

348 **Acknowledgments**

349 This work was financially supported by the China Agriculture Research System (CARS-47),
350 Guangdong MEPP Fund (GDOE NO. 2019A30), National Key R&D Program of China
351 (2018YFD0900400), National Natural Science Foundation of China (No. 31873040 and No.
352 31702357), Guangdong Agriculture Research System (2019KJ150) and Natural Science
353 Foundation of Guangdong Province (2018A030313910).

354

355 **Author's contribution and conflict of interest**

356 Wang, S., Wang, M., Tocher, D.R. and Li, Y. wrote the manuscript. Li, Y. and Wang, M.,
357 designed the experiments. Zhang, H. and You, C. provided experimental supporting, Yan, X. and
358 Guo, H. performed the growth experiment. Chen, C performed the fatty acid composition analysis.
359 The authors declare that they have no conflict of interest.

360

361 **Data availability statement**

362 The authors confirm that the data supporting the results in the paper are included in the tables
363 and figures in the paper, and not archived in a public repository with the legal requirements.

364

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519 <https://doi.org/10.3390/ijms20010023>

520 Table 1 Ingredients, formulations and proximate compositions of the experimental diets.

521

Ingredient (g/kg of dry weight)	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
Casein	410.0	410.0	410.0	410.0	410.0	410.0	410.0
Fermented soybean meal	210.0	210.0	210.0	210.0	210.0	210.0	210.0
Cassava starch	110.0	110.0	110.0	110.0	110.0	110.0	110.0
α -Starch	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Fish oil	90.0	/	/	/	/	/	/
Soybean oil	/	90.0	64.4	41.9	17.5	4.5	/
Linseed oil	/	/	25.6	48.1	72.5	85.5	90.0
Lecithin	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Choline chloride	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Lysine	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Monocalcium phosphate	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Lutein	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix ^a	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Mineral premix ^b	20.0	20.0	20.0	20.0	20.0	20.0	20.0
CMC ^c	68.0	68.0	68.0	68.0	68.0	68.0	68.0
<i>Proximate composition (% dry weight)</i>							
Moisture	14.2	13.7	13.6	14.0	13.7	14.3	14.1
Crude protein	50.2	50.7	50.9	50.3	50.0	51.0	50.5
Crude lipid	12.0	12.4	12.2	12.2	12.7	12.5	12.6
Ash	4.6	4.7	4.7	4.6	4.6	5.1	5.0

522 ^a Vitamin premix (/kg premix): VA, 1100000IU; VD3, 320000IU; VB12, 8mg ; VK3, 1000mg; VB1,
 523 1500mg; VB2, 2800mg; calcium pantothenate, 2000mg ;nicotinamide, 7800mg ;folic acid, 400mg ;
 524 inositol, 12800mg; VB6:1000mg.

525 ^b Mineral premix (/kg premix): were purchased from Guangdong Guangdong feed group of China.

526 ^c CMC: carboxy methyl cellulose

527

528 Table 2
 529 Fatty acid compositions (% total fatty acids) of the experimental diets for golden pompano,
 530 *Trachinotus ovatus*.

Fatty acid	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
14:0	5.56	0.65	0.65	0.67	0.64	0.66	0.66
16:0	21.83	12.31	11.25	10.26	9.27	8.70	8.50
18:0	5.42	4.97	4.74	4.69	4.63	4.61	4.57
22:0	1.55	nd	nd	nd	nd	nd	nd
16:1n-7	4.96	0.24	0.43	0.30	0.24	0.21	0.21
18:1n-9	19.43	20.50	19.75	19.04	18.24	17.77	17.47
18:3n-3 (ALA)	6.80	6.99	17.22	26.82	36.99	43.06	45.35
18:4n-3	0.31	nd	nd	nd	nd	nd	nd
20:4n-3	0.33	nd	nd	nd	nd	nd	nd
20:5n-3 (EPA)	7.88	nd	nd	nd	nd	nd	nd
22:5n-3 (DPA)	1.54	nd	nd	nd	nd	nd	nd
22:6n-3 (DHA)	9.17	nd	nd	nd	nd	nd	nd
18:2n-6 (LA)	12.36	50.55	43.00	34.88	27.11	23.33	20.63
18:3n-6	0.35	nd	nd	nd	nd	nd	nd
20:3n-6	0.43	nd	nd	nd	nd	nd	nd
20:4n-6 (ARA)	2.31	nd	nd	nd	nd	nd	nd
Σ SFA	34.35	17.95	16.64	15.62	14.54	13.97	13.73
Σ MUFA	26.60	21.23	20.56	19.66	18.70	18.17	17.87
Σ n-3 PUFA	24.07	7.45	17.53	27.13	37.26	43.30	45.57
Σ n-6 PUFA	15.08	50.55	43.00	34.88	27.11	22.33	20.63
n-3/n-6 PUFA	1.60	0.15	0.40	0.78	1.37	1.94	2.21
ALA/LA	0.55	0.14	0.40	0.77	1.36	1.92	2.20

531 nd: not detected (< 0.01).

532 MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

533

534

535 Table 3
536 Nucleotide sequences of the primers used to assay gene expression by real-time polymerase chain
537 reaction

Target gene	Forward/Reverse (5' to 3')	Reference/GenBank
<i>fads2</i> -like	F: CATCACCTTCGTCAGGTTTCT	KP295471
	R: TTAACCAGTCCCGGTGTTTC	
<i>elovl5</i>	F: CCACGCTACCATGCTGAATA	KY860144
	R: ATGAGAGGCCGTAGTAGGAATA	
β -actin	F: TACGAGCTGCCTGACGGACA	Tan et al., 2017
	R: GGCTGTGATCTCCTTCTGC	

538

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541

542 Table 4
 543 Growth performance, feed utilization efficiency and survival rate of juvenile golden pompano fed
 544 different diets for 8 weeks¹.

	Dietary treatments						
	D0	D1 (0.0)	D2 (0.5)	D3 (1.0)	D4 (1.5)	D5 (2.0)	D6 (2.5)
Initial weight (g)	8.40±0.00	8.27±0.07	8.27±0.07	8.27±0.07	8.27±0.07	8.40±0.00	8.40±0.00
Final weight (g)	46.57±3.19 ^b	29.69±1.32 ^a	30.65±1.10 ^a	30.27±2.77 ^a	32.78±0.75 ^a	31.35±1.28 ^a	31.12±0.94 ^a
WGR (%) ²	416.60±6.44 ^b	256.00±9.22 ^a	269.04±18.52 ^a	266.32±34.17 ^a	296.68±11.97 ^a	273.19±15.26 ^a	270.47±11.11 ^a
SGR (% day ⁻¹) ³	3.05±0.12 ^b	2.28±0.04 ^a	2.33±0.09 ^a	2.30±0.17 ^a	2.46±0.05 ^a	2.35±0.07 ^a	2.34±0.05 ^a
FCR ⁴	1.19±0.09 ^a	2.18±0.14 ^b	2.41±0.10 ^b	2.54±0.22 ^b	2.11±0.07 ^b	2.09±0.19 ^b	2.08±0.05 ^b
HSI (%) ⁵	1.81±0.14 ^a	4.28±0.31 ^{bc}	4.77±0.21 ^c	3.94±0.41 ^{bc}	3.52±0.16 ^{bc}	3.37±0.20 ^b	3.64±0.18 ^{bc}
SR (%) ⁶	100.00 ^c	89.33±2.67 ^{bc}	66.00±2.00 ^a	70.67±3.52 ^a	73.33±4.81 ^{ab}	92.00±4.00 ^c	92.00±2.31 ^c

545 ¹ Values (mean ± SEM of 6 samples from three replicate groups) with different superscript letters within a row are
 546 significantly different ($P < 0.05$)

547 ² Weight gain rate (WGR, %) = $100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$;

548 ³ Specific growth rate (SGR, % day⁻¹) = $100 \times [\text{Ln}(\text{final weight}) - \text{Ln}(\text{initial weight})] / \text{days}$;

549 ⁴ Feed conversion rate (FCR) = feed intake (dry matter)/fish wet weight gain (g);

550 ⁵ Hepatosomatic index (HSI, %) = $100 \times \text{liver weight} / \text{body weight}$;

551 ⁶ Survival rate (SR, %) = $100 \times \text{survived fish number} / \text{total fish number}$.

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555 Table 5

556 Proximate compositions (% dry weight) of whole body of golden pompano fed different diets for 8

557 weeks¹.

558

	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
Moisture (% wet weight)	67.96±1.07 ^a	72.75±0.60 ^b	68.83±1.68 ^{ab}	70.44±1.23 ^{ab}	71.31±0.47 ^{ab}	69.67±0.45 ^{ab}	70.21±0.85 ^{ab}
Protein (%)	34.07±1.29	27.01±0.26	30.84±3.03	32.97±1.73	28.88±1.77	30.38±1.47	29.64±2.22
Lipid (%)	53.56±1.70	54.77±0.92	51.76±0.60	52.25±0.68	53.62±0.98	55.47±0.57	54.82±0.85
Ash (%)	11.36±0.08	11.97±0.53	12.00±0.42	11.62±0.72	11.95±0.10	12.71±0.04	12.68±0.20

559 ¹ Values (mean ± SEM of 6 samples from three replicate groups) with different superscript letters within a row are

560 significantly different ($P < 0.05$)

561

562

563 Table 6
 564 The fatty acid composition (% total fatty acids) of liver from juvenile golden pompano fed with
 565 diets containing different ratios of ALA/LA¹.

Fatty acid	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
14:0	1.61±0.10 ^b	1.05±0.02 ^a	0.92±0.04 ^a	1.05±0.06 ^a	1.02±0.04 ^a	1.04±0.04 ^a	1.05±0.05 ^a
16:0	25.07±0.52 ^d	19.96±0.42 ^{bc}	16.95±0.60 ^a	18.72±0.21 ^{ab}	20.77±0.20 ^c	18.49±0.44 ^{ab}	21.58±0.39 ^c
18:0	7.28±0.34 ^b	4.51±0.05 ^a	4.46±0.17 ^a	4.32±0.22 ^a	4.96±0.15 ^a	4.37±0.16 ^a	4.33±0.30 ^a
∑SFA	34.97±0.72 ^d	26.07±0.44 ^{bc}	23.05±0.77 ^a	24.94±0.41 ^{ab}	27.65±0.22 ^c	24.96±0.55 ^{ab}	27.96±0.44 ^c
16:1	3.37±0.09 ^b	1.38±0.06 ^a	1.34±0.03 ^a	1.41±0.08 ^a	1.48±0.10 ^a	1.52±0.13 ^a	1.56±0.10 ^a
18:1	35.12±1.37 ^d	33.06±0.88 ^{cd}	27.88±0.19 ^a	28.02±0.27 ^a	31.22±0.17 ^{bc}	29.93±0.29 ^{ab}	33.79±0.32 ^{cd}
∑MUFA	38.49±1.35 ^d	34.44±0.92 ^{bc}	29.22±0.21 ^a	29.43±0.30 ^a	32.70±0.24 ^{bc}	31.45±0.39 ^{ab}	35.36±0.36 ^{cd}
18:3n-3(ALA)	4.52±0.13 ^a	4.26±0.04 ^a	6.71±0.27 ^b	8.83±0.22 ^c	11.77±0.25 ^d	15.51±0.32 ^f	13.49±0.18 ^e
18:4n-3	0.14±0.01 ^a	0.31±0.02 ^b	0.32±0.01 ^b	0.31±0.00 ^b	0.33±0.01 ^b	0.33±0.01 ^b	0.34±0.02 ^b
20:4n-3	0.92±0.20 ^a	1.67±0.04 ^b	2.50±0.07 ^b	3.56±0.09 ^c	5.04±0.14 ^d	6.24±0.20 ^e	6.39±0.34 ^e
20:5n-3(EPA)	0.78±0.05 ^b	0.56±0.02 ^a	0.54±0.02 ^a	0.58±0.03 ^a	0.58±0.02 ^a	0.57±0.04 ^a	0.53±0.02 ^a
22:5n-3(DPA)	0.93±0.10	nd	Nd	nd	nd	nd	nd
22:6n-3(DHA)	5.04±0.16 ^b	0.29±0.01 ^a	0.40±0.01 ^a	0.50±0.01 ^a	0.43±0.02 ^a	0.49±0.03 ^a	0.41±0.02 ^a
∑n-3PUFA	14.74±0.50 ^c	7.06±0.07 ^a	10.59±0.26 ^b	13.95±0.21 ^c	18.41±0.33 ^d	23.61±0.38 ^f	21.42±0.26 ^e
18:2n-6(LA)	6.62±0.17 ^a	25.42±1.17 ^d	29.60±0.72 ^e	24.47±0.33 ^d	15.94±0.19 ^c	15.10±0.37 ^c	10.98±0.32 ^b
18:3n-6	0.22±0.01 ^a	0.45±0.01 ^b	0.45±0.03 ^b	0.43±0.01 ^b	0.44±0.01 ^b	0.42±0.01 ^b	0.45±0.02 ^b
20:3n-6	1.09±0.02 ^a	4.18±0.11 ^f	3.32±0.14 ^e	2.71±0.11 ^d	1.90±0.05 ^c	1.75±0.08 ^{bc}	1.49±0.08 ^b
20:4n-6(ARA)	0.45±0.09 ^b	0.11±0.00 ^a	0.12±0.00 ^a	0.12±0.00 ^a	0.14±0.00 ^a	0.12±0.00 ^a	0.11±0.00 ^a
∑n-6PUFA	8.16±0.17 ^a	29.71±1.27 ^d	33.04±0.71 ^e	27.31±0.30 ^d	17.98±0.20 ^c	16.97±0.39 ^c	12.59±0.30 ^b
∑PUFA	22.90±0.61 ^a	36.77±1.28 ^b	43.67±0.87 ^c	41.26±0.38 ^c	36.39±0.26 ^b	40.59±0.74 ^c	34.01±0.48 ^b
n-3/n-6PUFA	1.81	0.24	0.32	0.51	1.02	1.39	1.70
ALA/LA	0.68	0.17	0.23	0.36	0.74	1.08	1.23
DHA/EPA	6.46±0.18 ^c	0.52±0.03 ^a	0.74±0.05 ^{ab}	0.86±0.05 ^b	0.74±0.03 ^{ab}	0.86±0.07 ^b	0.77±0.02 ^{ab}

566 ¹ Values (mean ± SEM of 6 samples from three replicate groups) with different superscript letters within a row are
 567 significantly different ($P < 0.05$)

568 nd: not detected

569

570 Table 7
 571 The fatty acid composition (% total fatty acids) of muscle from juvenile golden pompano fed with
 572 diets containing different ratios of ALA/LA¹.

Fatty acid	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
14:0	4.51±0.05 ^b	1.12±0.02 ^a	1.12±0.01 ^a	1.14±0.04 ^a	1.11±0.03 ^a	1.07±0.04 ^a	1.19±0.04 ^a
16:0	22.78±0.14 ^c	16.92±0.32 ^b	16.34±0.59 ^{ab}	15.99±0.34 ^{ab}	15.04±0.34 ^a	15.25±0.35 ^a	15.84±0.46 ^{ab}
18:0	5.73±0.07 ^b	4.49±0.08 ^a	4.40±0.08 ^a	4.45±0.10 ^a	4.45±0.19 ^a	4.29±0.10 ^a	4.18±0.11 ^a
∑SFA	33.33±0.14 ^d	24.07±0.35 ^c	23.39±0.62 ^{bc}	23.01±0.26 ^{abc}	22.14±0.23 ^{ab}	21.60±0.38 ^a	22.19±0.97 ^{ab}
16:1	4.99±0.05 ^b	1.15±0.06 ^a	1.15±0.06 ^a	1.51±0.05 ^a	1.13±0.07 ^a	1.16±0.07 ^a	1.33±0.07 ^a
18:1	26.05±0.33	23.56±0.52	23.36±0.82	22.47±0.63	22.38±0.62	22.10±0.59	22.69±0.55
∑MUFA	31.54±0.37 ^b	25.04±0.56 ^{ab}	24.10±0.63 ^a	23.88±0.68 ^a	24.29±0.49 ^a	23.43±0.63 ^a	24.22±0.60 ^a
18:3n-3(ALA)	5.44±0.06 ^a	4.53±0.10 ^a	8.96±0.43 ^b	14.01±0.76 ^c	18.90±0.27 ^d	23.99±0.88 ^e	24.20±1.02 ^e
18:4n-3	0.27±0.02 ^a	0.46±0.01 ^b	0.45±0.01 ^b	0.46±0.01 ^b	0.47±0.01 ^{bc}	0.50±0.03 ^c	0.47±0.01 ^{bc}
20:4n-3	1.14±0.02 ^a	0.92±0.02 ^a	1.38±0.24 ^a	2.74±0.08 ^b	3.59±0.06 ^c	4.31±0.21 ^d	4.51±0.19 ^d
20:5n-3(EPA)	3.09±0.03 ^c	0.31±0.02 ^a	0.31±0.02 ^a	0.42±0.04 ^{ab}	0.45±0.02 ^b	0.35±0.03 ^{ab}	0.39±0.03 ^{ab}
22:5n-3(DPA)	2.55±0.05 ^b	0.25±0.01 ^a	0.26±0.02 ^a	0.26±0.01 ^a	0.27±0.01 ^a	0.23±0.01 ^a	0.29±0.02 ^a
22:6n-3(DHA)	10.72±0.20 ^b	1.32±0.11 ^a	1.35±0.14 ^a	1.76±0.18 ^a	1.86±0.13 ^a	1.38±0.06 ^a	1.43±0.16 ^a
∑n-3PUFA	22.39±0.27 ^c	7.08±0.09 ^a	12.67±0.11 ^b	18.93±0.23 ^c	24.81±0.23 ^d	30.03±0.44 ^e	30.54±0.44 ^e
18:2n-6(LA)	10.11±0.08 ^a	34.92±0.61 ^e	33.62±1.19 ^e	26.52±0.48 ^d	21.82±0.32 ^c	18.53±0.27 ^b	16.79±0.45 ^b
18:3n-6	0.18±0.02 ^a	0.40±0.02 ^b	0.41±0.02 ^b	0.42±0.01 ^{bc}	0.42±0.01 ^{bc}	0.45±0.03 ^c	0.43±0.02 ^{bc}
20:3n-6	0.98±0.01 ^a	3.25±0.15 ^d	2.93±0.20 ^d	2.07±0.25 ^c	1.63±0.04 ^b	1.23±0.06 ^{ab}	1.13±0.06 ^a
20:4n-6(ARA)	0.62±0.02 ^b	0.21±0.02 ^a	0.19±0.01 ^a	0.22±0.02 ^a	0.25±0.02 ^a	0.19±0.02 ^a	0.20±0.01 ^a
∑n-6PUFA	11.71±0.10 ^a	38.70±0.64 ^f	35.95±0.82 ^e	29.11±0.49 ^d	23.96±0.38 ^c	20.02±0.31 ^b	18.16±0.48 ^b
∑PUFA	32.1±0.27 ^a	45.78±0.61 ^b	48.02±0.82 ^{bc}	48.04±0.59 ^{bc}	48.77±0.47 ^c	50.05±0.72 ^c	48.70±0.81 ^c
n-3/n-6PUFA	1.53	0.18	0.38	0.65	1.03	1.5	1.68
ALA/LA	0.54	0.13	0.27	0.53	0.87	1.29	1.44
DHA/EPA	3.47±0.05	4.26±0.18	4.35±0.25	4.19±0.20	4.13±0.28	3.94±0.15	3.67±0.27

573 ¹ Values (mean ± SEM of 6 samples from three replicate groups) with different superscript letters within a row are
 574 significantly different ($P < 0.05$)

575

576 Table 8
 577 The fatty acid composition (% total fatty acids) of brain from juvenile golden pompano fed with
 578 diets containing different ratios of ALA/LA¹.

Fatty acid	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
14:0	0.97±0.14 ^b	0.44±0.02 ^a	0.46±0.08 ^a	0.51±0.03 ^a	0.44±0.03 ^a	0.40±0.02 ^a	0.48±0.03 ^a
16:0	18.55±0.40 ^b	16.69±0.64 ^a	16.03±0.10 ^a	16.39±0.12 ^a	16.15±0.19 ^a	16.11±0.05 ^a	16.54±0.32 ^a
18:0	12.97±0.44 ^b	11.85±0.22 ^{ab}	11.43±0.61 ^{ab}	11.15±0.23 ^a	11.95±0.23 ^{ab}	12.57±0.40 ^{ab}	12.44±0.40 ^{ab}
∑SFA	32.84±0.30 ^b	29.53±0.77 ^a	28.44±0.52 ^a	28.54±0.30 ^a	29.06±0.27 ^a	29.54±0.43 ^a	29.85±0.50 ^a
14:1	1.96±0.11	2.00±0.12	1.97±0.24	1.60±0.09	1.84±0.19	2.30±0.15	2.24±0.12
15:1	1.22±0.07 ^{ab}	1.13±0.06 ^{ab}	1.10±0.12 ^{ab}	0.93±0.04 ^a	1.05±0.09 ^{ab}	1.39±0.08 ^b	1.32±0.11 ^b
16:1	2.20±0.16 ^b	1.36±0.04 ^a	1.30±0.03 ^a	1.37±0.03 ^a	1.45±0.04 ^a	1.41±0.03 ^a	1.46±0.06 ^a
18:1	21.68±0.38 ^a	22.76±0.17 ^{ab}	22.57±0.13 ^{ab}	22.54±0.26 ^{ab}	22.96±0.30 ^b	22.93±0.33 ^{ab}	23.29±0.29 ^b
24:1n-9	0.87±0.04 ^a	1.34±0.06 ^b	1.42±0.16 ^b	1.26±0.02 ^b	1.51±0.03 ^b	1.33±0.03 ^b	1.28±0.05 ^b
∑MUFA	27.93±0.43	28.58±0.39	28.35±0.46	27.70±0.40	28.80±0.50	29.35±0.59	29.58±0.40
18:3n-3(ALA)	1.47±0.16 ^a	1.84±0.23 ^a	3.53±0.53 ^{ab}	5.72±0.38 ^{bc}	5.51±0.30 ^{bc}	6.91±0.84 ^c	7.00±0.68 ^c
18:4n-3	0.16±0.01 ^a	0.53±0.02 ^b	0.54±0.01 ^b	0.53±0.03 ^b	0.57±0.01 ^b	0.54±0.02 ^b	0.48±0.02 ^b
20:4n-3	0.45±0.04 ^a	0.85±0.06 ^a	1.49±0.04 ^b	2.02±0.03 ^c	2.25±0.10 ^{cd}	2.49±0.18 ^d	2.60±0.13 ^d
20:5n-3(EPA)	3.83±0.03 ^b	1.98±0.10 ^a	1.92±0.19 ^a	1.69±0.05 ^a	2.02±0.10 ^a	1.80±0.14 ^a	1.68±0.08 ^a
22:5n-3(DPA)	2.46±0.11 ^b	0.91±0.03 ^a	1.01±0.03 ^a	0.97±0.04 ^a	1.12±0.017 ^a	1.12±0.08 ^a	1.10±0.03 ^a
22:6n-3(DHA)	23.10±1.00 ^b	15.01±0.46 ^a	14.71±0.64 ^a	14.48±0.40 ^a	15.86±0.35 ^a	15.37±0.75 ^a	15.89±0.97 ^a
∑n-3PUFA	31.30±0.78 ^f	20.59±0.35 ^a	22.65±0.43 ^b	24.87±0.20 ^c	26.75±0.21 ^d	27.70±0.54 ^e	28.26±0.33 ^e
18:2n-6(LA)	3.73±0.39 ^a	13.83±0.59 ^c	13.63±1.52 ^c	12.31±0.57 ^c	8.62±0.42 ^b	7.85±0.64 ^b	7.39±0.50 ^b
18:3n-6	0.16±0.01 ^a	0.40±0.02 ^b	0.43±0.03 ^b	0.46±0.03 ^b	0.41±0.01 ^b	0.41±0.02 ^b	0.37±0.01 ^b
20:3n-6	0.40±0.02 ^a	1.97±0.14 ^d	1.68±0.08 ^{cd}	1.52±0.10 ^c	1.05±0.23 ^b	0.90±0.06 ^b	0.79±0.04 ^{ab}
20:4n-6(ARA)	2.46±0.11 ^b	0.91±0.03 ^a	1.01±0.03 ^a	0.97±0.04 ^a	1.12±0.02 ^a	1.12±0.08 ^a	1.10±0.03 ^a
∑n-6PUFA	6.13±0.43 ^a	18.71±0.84 ^c	17.94±1.42 ^c	16.48±0.59 ^c	12.30±0.41 ^b	11.33±0.65 ^b	10.50±0.92 ^b
PUFA	37.43±0.39 ^a	39.30±0.76 ^{ab}	40.59±1.13 ^{ab}	41.36±0.6 ^b	39.05±0.52 ^{ab}	39.03±0.92 ^{ab}	38.76±0.27 ^{ab}
n-3/n-6PUFA	5.11	1.10	1.26	1.51	2.17	2.44	2.69
ALA/LA	0.39	0.13	0.26	0.46	0.64	0.88	0.95
DHA/EPA	6.03±0.21 ^a	7.58±0.54 ^{ab}	7.66±0.29 ^{ab}	8.57±0.20 ^b	7.85±0.28 ^{ab}	8.54±0.52 ^b	9.46±0.77 ^b

579 ¹ Values (mean ± SEM of 6 samples from three replicate groups) with different superscript letters within a row are
 580 significantly different ($P < 0.05$)

581

582 Table 9

583 The fatty acid composition (% total fatty acids) of eyes from juvenile golden pompano fed with
584 diets containing different ratios of ALA/LA¹.

Fatty acid	Dietary treatments						
	D0	D1	D2	D3	D4	D5	D6
14:0	3.06±0.17 ^b	0.91±0.04 ^a	0.99±0.01 ^a	0.97±0.08 ^a	0.92±0.01 ^a	0.85±0.05 ^a	0.94±0.03 ^a
16:0	20.37±0.32 ^c	16.04±0.36 ^b	15.27±0.12 ^{ab}	15.43±0.24 ^{ab}	14.81±0.56 ^{ab}	14.69±0.20 ^{ab}	14.31±0.19 ^a
18:0	6.67±0.41	5.36±0.25	5.18±0.15	6.23±0.87	5.64±0.35	5.68±0.70	6.01±0.40
∑SFA	30.11±0.10 ^b	22.31±0.42 ^a	21.44±0.17 ^a	22.63±0.95 ^a	21.37±0.90 ^a	21.22±0.82 ^a	21.26±0.49 ^a
16:1	4.06±0.15 ^b	1.28±0.07 ^a	1.28±0.06 ^a	1.21±0.07 ^a	1.18±0.06 ^a	1.21±0.11 ^a	1.41±0.12 ^a
18:1	22.68±0.54 ^{ab}	24.52±0.71 ^b	23.30±0.36 ^{ab}	21.96±1.11 ^{ab}	22.38±0.24 ^{ab}	22.95±0.53 ^{ab}	21.68±0.46 ^a
∑MUFA	27.37±0.45 ^b	26.17±0.80 ^{ab}	25.08±0.41 ^{ab}	23.70±1.19 ^a	24.22±0.30 ^a	24.78±0.43 ^{ab}	23.83±0.58 ^a
18:3n-3(ALA)	4.82±0.39 ^a	5.12±0.20 ^a	9.62±0.18 ^b	13.90±0.31 ^c	19.21±0.83 ^d	22.77±0.17 ^e	22.46±0.19 ^e
18:4n-3	0.26±0.01 ^a	0.45±0.01 ^b	0.46±0.00 ^b	0.45±0.02 ^b	0.45±0.02 ^b	0.46±0.02 ^b	0.46±0.01 ^b
20:4n-3	1.29±0.10 ^a	1.36±0.05 ^a	2.32±0.10 ^b	3.03±0.09 ^b	4.23±0.34 ^c	4.09±0.34 ^c	4.50±0.19 ^c
20:5n-3(EPA)	2.90±0.21 ^b	0.43±0.03 ^a	0.50±0.05 ^a	0.46±0.04 ^a	0.35±0.05 ^a	0.52±0.07 ^a	0.43±0.04 ^a
22:5n-3(DPA)	2.99±0.17 ^b	0.37±0.02 ^a	0.52±0.03 ^a	0.56±0.07 ^a	0.52±0.05 ^a	0.48±0.11 ^a	0.62±0.05 ^a
22:6n-3(DHA)	17.63±1.53 ^b	4.41±1.07 ^a	4.98±0.45 ^a	6.08±1.04 ^a	6.57±0.73 ^a	4.61±0.19 ^a	7.65±0.28 ^a
∑n-3PUFA	29.64±0.79 ^d	11.69±0.84 ^a	17.93±0.33 ^b	24.05±0.74 ^c	30.88±0.42 ^{de}	32.47±0.27 ^e	35.67±0.31 ^f
18:2n-6(LA)	9.56±0.38 ^a	34.85±0.84 ^f	31.48±0.39 ^e	25.67±0.63 ^d	20.75±0.79 ^c	19.00±0.49 ^{bc}	16.63±0.77 ^b
18:3n-6	0.22±0.01 ^a	0.43±0.00 ^b	0.42±0.01 ^b	0.45±0.04 ^b	0.45±0.03 ^b	0.41±0.02 ^b	0.44±0.02 ^b
20:3n-6	0.97±0.03 ^a	3.48±0.18 ^c	2.64±0.09 ^d	2.14±0.09 ^c	1.57±0.09 ^b	1.23±0.08 ^{ab}	1.17±0.04 ^{ab}
20:4n-6(ARA)	1.27±0.09 ^b	0.41±0.05 ^a	0.36±0.03 ^a	0.63±0.12 ^a	0.41±0.12 ^a	0.49±0.11 ^a	0.65±0.06 ^a
∑n-6PUFA	12.89±0.26 ^a	39.82±0.92 ^f	35.55±0.27 ^e	29.62±0.39 ^d	23.53±0.70 ^c	21.53±0.42 ^{bc}	19.25±0.76 ^b
∑PUFA	42.53±0.53 ^a	51.51±0.98 ^b	53.48±0.43 ^b	53.67±0.66 ^b	54.41±1.01 ^b	54.00±0.60 ^b	54.91±0.53 ^b
n-3/n-6PUFA	2.30	0.29	0.50	0.81	1.31	1.51	1.85
ALA/LA	0.50	0.15	0.31	0.54	0.93	1.20	1.35
DHA/EPA	6.08±0.32 ^a	10.26±1.23 ^{ab}	9.96±0.48 ^{ab}	13.22±1.57 ^{bc}	18.77±0.39 ^c	8.87±1.27 ^{bc}	17.79±2.28 ^{bc}

585 ¹ Values (mean ± SEM of 6 samples from three replicate groups) with different superscript letters within a row are
586 significantly different ($P < 0.05$)

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588

589 **Figure Legends**

590 Fig. 1. Relative mRNA expression levels of *fads2*-like genes in liver, brain and eyes of
591 golden pompano fed the experimental diets with different dietary ALA/LA ratio for 8
592 weeks

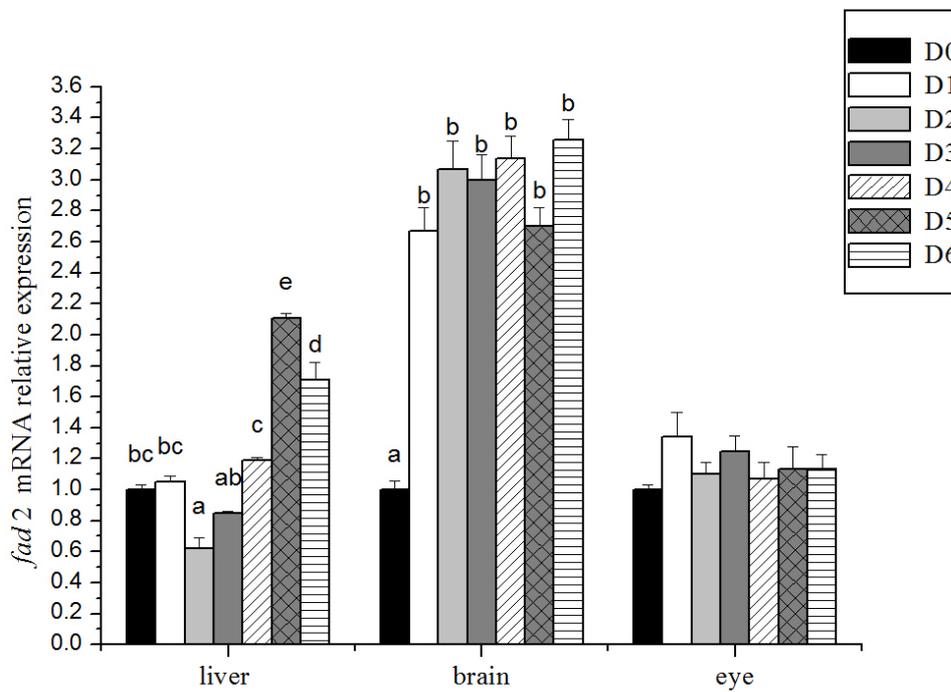
593

594 Fig. 2. Relative mRNA expression levels of *elov5* genes in liver, brain and eyes of
595 golden pompano fed the experimental diets with different dietary ALA/LA ratio for 8
596 weeks

597

598 **Figures**

599 **Fig. 1.**



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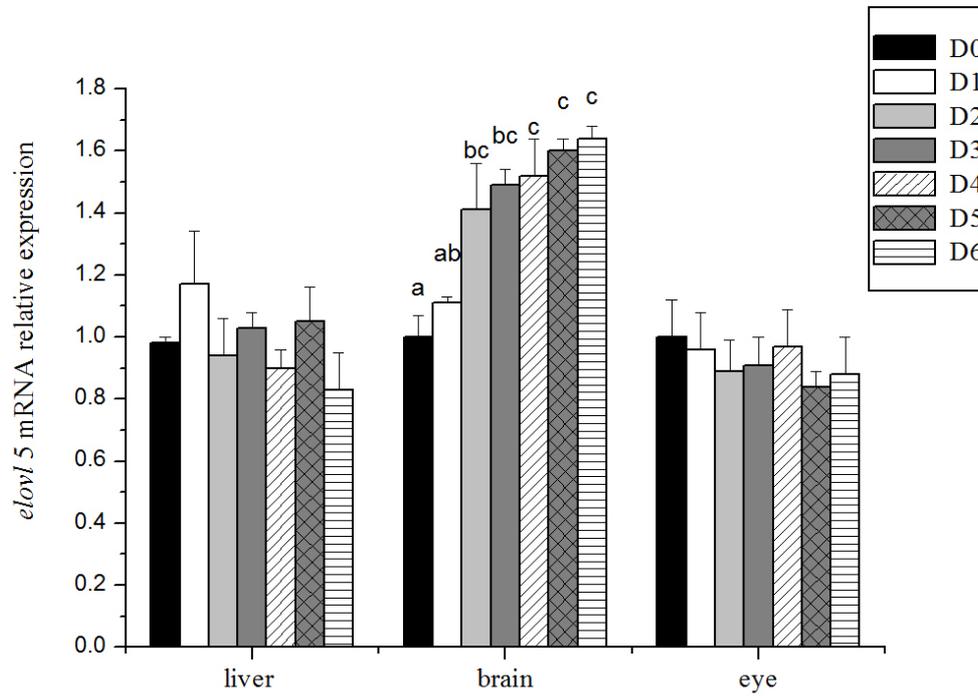
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608 **Fig. 2.**



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610