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1 **Title**

2 Total Replacement of Dietary Fish Oil with a Blend of Vegetable Oils in the Marine
3 Herbivorous Teleost *Siganus canaliculatus*

4

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Abstract

32 To investigate the feasibility of total replacement of dietary fish oil with vegetable oils
33 (VO) and the optimal dietary polyunsaturated fatty acid (PUFA) level in the marine
34 herbivorous teleost *Siganus canaliculatus*, six isonitrogenous (32 %) and isolipidic
35 (8 %) diets were formulated. Control diet (FO) used fish oil as lipid source, whereas
36 diets VO1-VO5 contained various blends of palm, soybean, rapeseed and linseed oils,
37 in which the dietary PUFA levels were 42.0 %, 38.2 %, 33.8 %, 29.9 % and 27.1 %,
38 respectively. After *S. canaliculatus* juveniles were fed with the diets for 9 weeks, their
39 growth performance exhibited no significant difference among the dietary groups. The
40 tissue fatty acid profiles in liver and fillet generally reflected the dietary fatty acid
41 compositions, and showed no significant difference among the VO dietary groups.
42 The results suggested that dietary fish oil can be replaced completely by VO without
43 affecting their growth performance. Concerning the effects of the dietary FA profile
44 on the survival rate, HSI and VSI, and PUFA composition in fillets, diets VO1 and
45 VO2 were more favorable compared with diets VO3–VO5. Considering the
46 availability and cost of the VOs, diet VO2 was recommended for practical use in *S.*
47 *canaliculatus*.

48

49 **Keywords:** *Siganus canaliculatus*; dietary PUFA level; lipid selectivity; growth
50 performance; fatty acid composition.

51

52 **Introduction**

53 With the increasing demand for seafood products, world aquaculture production is
54 estimated to reach approximately 85 million tons in 2022, although annual production
55 growth is projected to average 2.5 % in 2013–2022 compared to 6.1 % in 2003–2012
56 (FAO 2014). The FAO has estimated that the high cost of fishmeal, fish oil (FO), and
57 other feed ingredients is one of the main causes of this slower growth. As global
58 demand is higher than the supply, the cost of fishmeal is expected to increase by 6 %
59 and that of FO by 23 % in 2022 compared with that in 2013 (FAO 2014). This
60 situation has led researchers in fish nutrition and feeds to develop alternative lipid
61 sources to dietary FO in recent years.

62 Due to their ready availability and relatively stable cost (Turchini *et al.* 2003, Francis
63 *et al.* 2006), vegetable oils (VOs) have been evaluated as FO substitutes either alone
64 or as blends formulated to replicate the fatty acid composition present in FO in terms
65 of the proportion of total saturated fatty acids (SFA), monounsaturated fatty acids
66 (MUFA), and polyunsaturated fatty acid (PUFA) (Torstensen *et al.* 2005, Francis *et al.*
67 2007a). Furthermore, available data have indicated that, provided the requirement for
68 essential fatty acids is met, a significant portion of dietary FO can be replaced by
69 alternative lipid sources without significantly affecting growth performance, feed
70 efficiency, and feed intake in most finfish species studied (Turchini *et al.* 2009). For
71 instance, the replacement of FO by corn oil did not affect the growth performance of
72 brown trout (*Salmo trutta*) (Arzel *et al.* 1994). Similarly, the partial substitution of FO
73 by different VO or animal fats had no significant effect on the growth performance of
74 brown trout (Turchini *et al.* 2003). In two populations of Arctic charr (*Salvelinus*
75 *alpinus*), the replacement of FO by echium oil had no effect on the growth, feed
76 efficiency, and muscle and liver lipid contents (Tocher *et al.* 2006). In addition, the
77 replacement of FO by different linseed and coconut oil blends in the diets of Arctic
78 charr did not affect their growth performance or negatively affect the oxidative status
79 of the flesh or plasma (Olsen and Henderson 1997). In Atlantic salmon (*Salmo salar*),
80 changing the dietary fatty acid composition by replacing FO with a VO blend during
81 both freshwater and seawater stages did not markedly alter body lipid stores (Nanton
82 *et al.* 2007). Therefore, existing data indicated the feasibility of the substitution of
83 dietary FO by appropriate VOs in feeds for farmed fish.

84 The terrestrial VO alternatives to FO do not contain the required and essential

85 long-chain PUFA (LC-PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3),
86 docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6).
87 Therefore, although alternative VOs can be used without any apparent detrimental
88 effects on fish performance, the n-3 LC-PUFA concentration in final fish fillets is
89 reduced (Sargent *et al.* 2002). In recent years, increasing research has been conducted
90 to mitigate this effect of dietary VO in modifying fatty acid compositions of farmed
91 fish. In addition, this research has contributed greatly to the advancement of our
92 knowledge of fish lipid metabolism; however, a complete solution remains to be
93 found (Turchini *et al.* 2009). If fish have all the necessary enzymes such as $\Delta 6$ fatty
94 acid desaturase (fad), $\Delta 5$ fad, Elovl5 elongase, and/or $\Delta 4$ fad, they can biosynthesize
95 LC-PUFA through a pathway involving a series of desaturation and elongation of
96 α -linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6). However, most
97 marine fishes are unable to produce LC-PUFA because of apparent deficiencies in one
98 or more steps (enzymes) of the biosynthetic pathway. Moreover, almost all FO
99 substitution studies in marine fishes have been conducted in carnivorous species but
100 rarely in herbivorous or omnivorous species.

101 The rabbitfish *Siganus canaliculatus* is an herbivorous marine teleost, feeding on
102 algae and seagrass. *S. canaliculatus* is a commercially valuable species widespread
103 along the Indo-West Pacific coast and has become one of the most harvested species
104 in southeastern Asia, including along the coast of southeast China. It is also the
105 subject of aquaculture activity with the development of a suitable formulated diet a
106 necessity for the emerging culture industry. However, information regarding optimal
107 lipid sources and PUFA requirements of rabbitfish is scant. In our recent studies, we
108 reported that *S. canaliculatus* may have the ability to convert LA and ALA into
109 LC-PUFA in both brackish water (10 ppt) and seawater (32 ppt) (Li *et al.* 2008) and
110 that it exhibits activities for elongation and $\Delta 6$, $\Delta 5$, and $\Delta 4$ fatty acid desaturation (Li
111 *et al.* 2010, Monroig *et al.* 2012). Our preliminary research results revealed that
112 soybean oil (SO) can replace up to 67% or 45% of total dietary FO for *S.*
113 *canaliculatus* without negatively compromising the growth performance or nutritional
114 quality of fish (Xu *et al.* 2012). The expression of key genes involved LC-PUFA
115 biosynthesis was also affected by the dietary LA:ALA ratio, with ratios of 0.52 or
116 2.13 showing better growth performance and LC-PUFA biosynthesis in rabbitfish (Liu,
117 2011). These findings suggested that FO can be partially or completely replaced by
118 VO in feeds for rabbitfish.

119 The present study aimed to determine the optimal lipid sources and dietary PUFA
120 contents for *S. canaliculatus* by using a combination of palm, soybean, rapeseed, and
121 linseed oils as replacements for FO. The results of this study provide a scientific basis
122 for developing highly effective and low-cost formulated feeds for rabbitfish by using
123 different VO sources, and increase our knowledge regarding FO replacement in
124 marine herbivorous fishes.

125

126 **Materials and methods**

127 **Experimental diets**

128 Using fishmeal and soybean meal as protein sources and FO, palm, soybean,
129 rapeseed and linseed oils as lipid sources, six formulated diets were prepared with
130 approximately equal contents of total protein (32 %), lipid (8 %), but with varying
131 lipid sources and PUFA concentrations. In the control diet, FO was used as the lipid
132 source, and the proportion of PUFA in the FO diet was 35.8% of total fatty acids.
133 Diets VO1–VO5 contained a blend of palm, soybean, rapeseed and linseed oils as
134 lipid sources with ratios of ALA:LA of 0.39, 0.39, 0.37, 0.40 and 0.37, respectively,
135 and PUFA levels of 42.0 %, 38.2 %, 33.8 %, 29.9 %, and 27.1 % of total fatty acids,
136 respectively. The feed ingredients and diet proximate compositions are listed in
137 Table 1. The ingredients were thoroughly mixed and moist pellets (Φ 4 mm) were
138 manufactured using an extruder. After air drying at room temperature, the feeds
139 were stored at -20 °C prior to feeding.

140 **Experimental fish and feeding conditions**

141 *S. canaliculatus* juveniles (approximately 12 g wet weight and sex visually
142 indistinguishable) were captured from the coast near Nan Ao Marine Biology Station
143 (NAMBS) of Shantou University, South China. Prior to the experiment, the fish were
144 acclimated to laboratory conditions and fed an equal mixture of the six experimental
145 diets for 2 weeks.

146 A 9-week growth experiment using the experimental diets was conducted from
147 October to December in an aquarium system at NAMBS. Each dietary group had
148 three replicates and thus a total of twenty-one cylindrical tanks (220 L) were used.
149 Fish of approximately equal size were pooled in a plastic bucket and 18 fish
150 individually weighed and randomly allocated to each tank after anesthetizing with
151 0.01 % 2-phenoxyethanol (Sigma-Aldrich, USA) (Table 2). During the experimental
152 period, half of the aquarium water was changed twice a day (morning and evening).
153 Oxygen saturation was maintained through aeration, and temperature was maintained
154 at 20 ± 3 °C. Photoperiod was set at 12 h light and 12 h dark. The fish were fed to
155 satiation three times a day (around 8:00, 12:00, and 16:00), and the diet weight fed
156 was recorded daily for each tank. Fecal matter was removed using an auto-discharge
157 device in the culture system every day.

158 **Evaluation of growth performance and sample collection**

159 The fish were weighed at the beginning and end of the experiment. At the end of the
160 experiment, six fish from each dietary group were sampled after anesthetizing in 0.01 %
161 2-phenoxyethanol to measure body weight, length, and liver and viscera weights.
162 Growth performance was evaluated by measuring weight gain (WG), specific growth
163 rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). These
164 parameters as well as condition factor (CF), hepatosomatic index (HSI), and
165 viscerosomatic index (VSI) were calculated using the following formulae:

$$166 \quad \text{WG (\%)} = 100 \times (\text{Wf} - \text{Wi}) / \text{Wi}$$

$$167 \quad \text{SGR (\%)} = 100 \times (\ln \text{Wf} - \ln \text{Wi}) / \text{d}$$

$$168 \quad \text{FCR} = \text{Fd} / \text{WG}$$

$$169 \quad \text{PER} = \text{WG} / \text{Fp}$$

$$170 \quad \text{CF} = 100 \times [(\text{body weight, g}) \times (\text{body length, cm})^{-3}]$$

$$171 \quad \text{HSI (\%)} = 100 \times \text{liver weight} \times (\text{body weight})^{-1}$$

$$172 \quad \text{VSI (\%)} = 100 \times \text{viscera weight} \times (\text{body weight})^{-1}$$

173 In these formulae, Wf and Wi were the final and initial body weight, respectively;
174 d was experimental days; and Fd and Fp were the amount of diet and protein
175 consumed by fish, respectively.

176 The livers and fillets were sampled from six fish at the beginning of the
177 experiment and from nine fishes in each dietary group at the end of the experiment
178 after anesthetizing in 0.01 % 2-phenoxyethanol. All samples were immediately frozen
179 in liquid nitrogen and stored at -80°C prior to fatty acid analysis. Six fish from each
180 dietary group were collected for determining the biochemical composition of the
181 whole fish.

182 **Chemical analysis**

183 *Biochemical composition*

184 The methods for determination of biochemical composition were similar to those
185 described previously (Li et al. 2008). Briefly, the protein content of the diets and
186 whole fish samples was calculated by determining the total nitrogen content through

187 the Kjeldahl method. The crude lipid content was measured using the Soxhlet
188 extraction method. The ash content was measured by combusting the samples in a
189 muffle furnace at 550 °C for 6 h. The dry matter was determined by exposing the
190 dietary samples to 105 °C in a dry oven overnight. Triplicate analyses were conducted
191 for each sample.

192 *Lipid extraction and fatty acid analysis*

193 Lipid extraction and fatty acid analysis were performed as described previously (Li *et*
194 *al.* 2005, 2008). In brief, total lipid of liver and muscle tissues was extracted using
195 chloroform and methanol in a 2:1 ratio, and fatty acid methyl esters were prepared by
196 transesterifying the total lipid samples with boron trifluoride etherate (ca. 48 %, Acros
197 Organics, NJ, USA). Fatty acid methyl esters were separated using a gas
198 chromatograph (GC; GC-17A; Shimadzu, Kyoto, Japan) equipped with an auto
199 sampler and a hydrogen-flame ionization detector. Individual fatty acids were
200 identified by comparison with known commercial standards (Sigma, USA) and
201 quantified using the CLASS-GC10 GC workstation (Shimadzu, Kyoto, Japan).

202 **Statistical analysis**

203 Data were expressed as mean \pm S.E.M (n=3). Differences among the dietary groups
204 were analyzed using one-way ANOVA followed by Tukey's multiple comparison.
205 The significance level was set at $P < 0.05$. Statistical analyses were performed using
206 the software package Origin[®], Version 7.0 (USA).

207

208 **Results**

209 **Growth performance of different dietary groups**

210 The growth performance of *S. canaliculatus* fed diets having different PUFA profiles
211 for 9 weeks is shown in Table 2. The total replacement of dietary FO by a
212 combination of palm, soybean, rapeseed and linseed oils showed no negative effect on
213 growth performance. Thus, WG, SGR, FCR, and PER did not differ significantly
214 between the FO and VO diet groups. However, the survival rate exhibited a
215 decreasing trend with reducing proportion of dietary PUFA. In particular, the survival
216 rate in fish fed the VO5 diet (PUFA, 27.2 %) was significantly lower than that in fish
217 fed the VO1 diet (PUFA, 41.6 %) or the FO diet ($P < 0.05$). HSI and VSI were

218 negatively correlated with dietary PUFA contents, and these indexes were
219 significantly higher in fish fed the VO4 diet than in fish fed the FO and VO1 diets.
220 The biochemical composition of the whole fish body including moisture, ash, protein,
221 and total lipid concentrations did not differ significantly among the dietary groups
222 (Table 3).

223 **Fatty acid compositions of liver and fillet**

224 The fatty acid profiles of tissues were markedly influenced by dietary oil sources and
225 PUFA content (Tables 4 and 5), reflecting the fatty acid compositions of the
226 respective diets. The contents of ALA, LA and 18:1n-9 were markedly higher in the
227 fillets of fish fed the diets containing the VO blends than in those of fish fed the FO
228 diet. In contrast, proportions of EPA and DHA were lower in the fillets of fish fed
229 diets containing the VO blend than in those of fish fed the FO diet. The contents of
230 14:0, 16:0, 18:0, and total SFA in the livers of fish fed the VO diets did not differ
231 significantly compared with those in the livers of fish fed the FO diet. In both the liver
232 and fillet, the contents of LA and 18:1n-9 were higher in fish fed the VO diets than in
233 fish fed the FO diet ($P < 0.05$). However, the proportion of ALA was only higher in
234 the fillets, and not liver, of fish fed the VO diets than in fish fed the FO diet ($P < 0.05$).
235 Furthermore, the percentage of ALA was lower in the liver (0.01 % – 0.39 %) but
236 higher in the fillets (0.74 % – 4.34 %). The content of ARA was significantly higher
237 in the liver of fish fed the FO diet than in liver of fish fed the VO diets; however,
238 ARA in the fillet did not significantly differ among the dietary groups. The contents
239 of EPA, 22:5n-3, and DHA were higher in the fillet and those of DHA higher in the
240 liver of fish fed the FO diet than in fish fed the VO diets ($P < 0.05$). The proportion of
241 total PUFA in the liver did not differ significantly among the dietary groups. However,
242 the total PUFA content was highest in the fillet of fish fed the VO1 and VO2 diets (P
243 < 0.05).

244

245 **Discussion**

246 The present study indicated that FO in a practical diet with 8% lipid for *S.*
247 *canaliculatus* can be completely replaced by a combination of VOs (palm, soybean,
248 rapeseed and linseed oils) without marked adverse effects on growth performance in
249 terms of WG, SGR, feed utilization, and PER. These results are in agreement with

250 those of previous studies, which reported that the partial or total replacement of
251 dietary FO by VO did not affect growth performance (Bell *et al.* 2001, Huang *et al.*
252 2007, Peng *et al.* 2008, Xu *et al.* 2012, Mozanzadeh *et al.* 2016). However, the
253 survival rate exhibited a positive trend with dietary PUFA content with the survival
254 rate in fish fed the VO5 diet (PUFA, 27.2 %) being significantly lower than that in
255 fish fed the VO1 diet (PUFA, 41.6 %). This suggested that lower dietary PUFA
256 contents may adversely affect the survival rate of *S. canaliculatus*. However, dietary
257 18:1n-9 content may also influence survival as diets with higher contents of 18:1n-9,
258 such as in VO3, VO4 and VO5, showed lower survival rates. Although, Ferreira *et al.*
259 (2015) also reported a correlation between high dietary 18:1n-9 and low survival in
260 tilapia, *Oreochromis niloticus*, there has been extensive research on the use of
261 18:1n-9-rich vegetable oils in fish feeds without any reports of major effects on
262 survival (Turchini and Mailer, 2011). HSI and VSI were highest in fish fed the VO4
263 diet, and significantly higher than in fish fed the FO and VO1 diets. This is of
264 potential significance as both VSI and HSI directly affect the yield in fish production
265 (Wang *et al.* 2005). One possible explanation for the effects on these indices could be
266 that the digestibility of PUFA is higher than that of MUFA and SFA (Francis *et al.*
267 2007b), and the proportion of PUFA was lower and those of SFA and MUFA higher
268 in the VO4 diet than in the FO diet. Thus, the lipid content was more easily
269 maintained in the liver and viscera of fish fed the VO4 diet. In the present study, the
270 dietary content of PUFA and the replacement of FO by VO did not affect the
271 proximate composition of whole fish. This was in agreement with previous studies in
272 other marine fish species, which reported that the replacement of dietary FO with
273 different concentrations of soybean oil concentrations did not affect the whole body
274 biochemical composition of red seabream, turbot, and *Platichthys stellatus* Pallas
275 (Huang *et al.* 2007, Regost *et al.* 2003, Lee *et al.* 2003).

276 The proportion of dietary PUFA and the replacement of FO by a combination of
277 palm, soybean, rapeseed and linseed oils markedly affected tissue fatty acid
278 compositions in *S. canaliculatus*. The fatty acid profiles in both liver and fillet
279 reflected the dietary fatty acid compositions, which was consistent with the findings
280 of many other studies (Caballero *et al.* 2002, Tocher *et al.* 2003, Torstensen *et al.*
281 2004a,b, 2005, Nanton *et al.* 2007, Stubhaug *et al.* 2007). For example, the
282 proportions of EPA, DHA, and total n-3 PUFA, but not of ARA, were higher in the

283 fillet of fish fed the FO diet than in fillets of fish fed the VO diets. However,
284 compared with the levels of LC-PUFA, 18:1n-9, LA and ALA exhibited the reverse
285 trend. Therefore, the replacement of FO with VO reduced the proportions of EPA,
286 DHA, and total n-3 PUFA in fish and increased the percentages of 18:1n-9, LA and
287 ALA. Similar results have been reported in other marine fish species where studies
288 have reported that replacing dietary FO with VO increased the concentrations of
289 dietary 18:1n-9, LA and ALA and reduced the concentrations of dietary marine n-3
290 fatty acids, EPA, and DHA (Bahurmiz and Ng 2007, Mørkøre *et al.* 2007,
291 Yildirim-Aksoy *et al.* 2007, Du *et al.* 2008, Glencross *et al.* 2016) resulting in the
292 fatty acid compositions of dietary VO being reflected in the fatty acid compositions of
293 whole fish, organs, and flesh (Tocher *et al.* 2015).

294 In both the liver and fillet, ALA and LA were well retained. The mean
295 percentage of LA in the liver and fillet was 1.8 % – 4.9 % and 3.7 %–14.0 %,
296 respectively. By contrast, the percentage of ALA in the liver was very low (0.15 %–
297 0.39 %). These data suggested that LA was more directly deposited in both the liver
298 and fillet, whereas ALA gets metabolized to a greater extent. A similar result was
299 observed in Murray Cod where ALA appeared to be more catabolized or bioconverted
300 (Francis *et al.* 2009) and LA tended to be directly deposited in fish tissues (Francis *et*
301 *al.* 2009, Trushenski *et al.* 2008). However, a different result was obtained in marine
302 carnivorous fishes such as large yellow croaker, black sea bream, and gilthead sea
303 bream where ALA but not LA contributed to an increase in growth (Zuo *et al.* 2014,
304 Peng *et al.* 2008, Montero *et al.* 2008). This may be because of a difference in
305 endogenous metabolism, that is, the limited dietary ALA content could satisfy the
306 growing demand of herbivorous rabbitfish compared to other marine species. All
307 dietary groups appeared to convert EPA into DHA as the EPA level in tissues was
308 markedly lower than that in the diets and the body lipid content of 22:5n-3 also
309 increased. In addition, Tan *et al.* (2009) reported that significant elongation and
310 desaturation of EPA into DHA was observed in yellow catfish.

311 Although the proportion of total n-3 and n-6 PUFA in the liver differed
312 significantly between fish fed the FO diet and fish fed the VO diet, the proportion of
313 total PUFA in the liver did not differ significantly among dietary groups. One possible
314 explanation may be that the progressive reduction in the concentration of n-3 PUFA in
315 the VO diets was offset by an increase in the concentration of n-6 PUFA (Grant *et al.*
316 2008). The proportions of total PUFA in the fillets of fish fed the VO diets showed a

317 positive relationship with the corresponding dietary PUFA concentrations, which was
318 highest in fish fed the VO1 diet and differed significantly among fish fed the VO3 –
319 VO5 diets, except for fish fed the VO2 diet. This indicated that fish fed a diet having a
320 low PUFA concentration may result in a decreased PUFA concentration in the fillet.
321 Notably, ARA content did not significantly differ between the fillet of fish fed the FO
322 and VO diets, which was consistent with our previous study and suggested that the
323 biosynthesis of LC-PUFA in rabbitfish can compensate for the reduced dietary ARA
324 (Li et al. 2008). Therefore, this indicated that rabbitfish can efficiently utilize and
325 store n-6 PUFA.

326 In conclusion, the results of the present study revealed that the complete
327 replacement of dietary FO with a combination of VOs had no negative effects on the
328 growth performance of *S. canaliculatus*. Concerning the effects of the dietary FA
329 profile on the survival rate, HSI and VSI, and total PUFA content in fillets, diets VO1
330 and VO2 were more favorable compared with diets VO3–VO5. Moreover, compared
331 with rapeseed oil, palm oil is more available and has a lower cost. Therefore, the VO2
332 diet is recommended for practical use in *S. canaliculatus* culture.

333

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340

341 **References**

- 342 Arzel, J., Martinez Lopez, F. X., Métailler, R., Stéphan, G., Viau, M., Gandemer, G.
343 & Guillaume, J. 1994. Effect of dietary lipid on growth performance and body
344 composition of brown trout (*Salmo trutta*) reared in seawater. *Aquaculture*, 123,
345 361-375.
- 346 Bahurmiz, O. M. & Ng, W.-K. 2007. Effects of dietary palm oil source on growth,
347 tissue fatty acid composition and nutrient digestibility of red hybrid tilapia,
348 *Oreochromis* sp., raised from stocking to marketable size. *Aquaculture*, 262,

349 382-392.

350 Bell, J. G., McEvoy, J., Tocher, D. R., McGhee, F., Campbell, P. J. & Sargent, J. R.
351 2001. Replacement of Fish Oil with Rapeseed Oil in Diets of Atlantic Salmon
352 (*Salmo salar*) Affects Tissue Lipid Compositions and Hepatocyte Fatty Acid
353 Metabolism. *The Journal of Nutrition*, 131, 1535-1543.

354 Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. & Izquierdo, M.
355 S. 2002. Impact of different dietary lipid sources on growth, lipid digestibility,
356 tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus*
357 *mykiss*. *Aquaculture*, 214, 253-271.

358 Du, Z. Y., Clouet, P., Huang, L. M., Degrace, P., Zheng, W. H., He, J. G., Tian, L. X.
359 & Liu, Y. J. 2008. Utilization of different dietary lipid sources at high level in
360 herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic
361 fatty acid oxidation. *Aquaculture Nutrition*, 14, 77-92.

362 FAO 2014. *The state of world fisheries and aquaculture*, ROME.

363 Ferreira, M. W., Costa, D. V., & Leal, C. A. 2015. Dietary Oil Sources on the Innate
364 Immunity and Resistance of Nile Tilapia, *Oreochromis niloticus*, to
365 *Streptococcus agalactiae* Challenge. *Journal of The World Aquaculture Society*,
366 46(3), 252-262.

367 Francis, D. S., Peters, D. J. & Turchini, G. M. 2009. Apparent in Vivo Δ -6 Desaturase
368 Activity, Efficiency, and Affinity Are Affected by Total Dietary C18 PUFA in
369 the Freshwater Fish Murray Cod. *Journal of Agricultural and Food Chemistry*,
370 57, 4381-4390.

371 Francis, D. S., Turchini, G. M., Jones, P. L. & De Silva, S. S. 2006. Effects of dietary
372 oil source on growth and fillet fatty acid composition of Murray cod,
373 *Maccullochella peelii peelii*. *Aquaculture*, 253, 547-556.

374 Francis, D. S., Turchini, G. M., Jones, P. L. & De Silva, S. S. 2007a. Dietary Lipid
375 Source Modulates in Vivo Fatty Acid Metabolism in the Freshwater Fish,
376 Murray Cod (*Maccullochella peelii peelii*). *Journal of Agricultural and Food*
377 *Chemistry*, 55, 1582-1591.

378 Francis, D. S., Turchini, G. M., Jones, P. L. & De Silva, S. S. 2007b. Effects of fish
379 oil substitution with a mix blend vegetable oil on nutrient digestibility in Murray
380 cod, *Maccullochella peelii peelii*. *Aquaculture*, 269, 447-455.

381 Glencross, B., Blyth, D., Irvin, S., Bourne, N., Campet, M., Boisot, P. & Wade, N. M.
382 2016. An evaluation of the complete replacement of both fishmeal and fish oil in

383 diets for juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, 451, 298-309.

384 Grant, A. A. M., Baker, D., Higgs, D. A., Brauner, C. J., Richards, J. G., Balfry, S. K.
385 & Schulte, P. M. 2008. Effects of dietary canola oil level on growth, fatty acid
386 composition and osmoregulatory ability of juvenile fall chinook salmon
387 (*Oncorhynchus tshawytscha*). *Aquaculture*, 277, 303-312.

388 Huang, S. S. Y., Oo, A. N., Higgs, D. A., Brauner, C. J. & Satoh, S. 2007. Effect of
389 dietary canola oil level on the growth performance and fatty acid composition of
390 juvenile red sea bream, *Pagrus major*. *Aquaculture*, 271, 420-431.

391 Lee, S.-M., Lee, J. H. & Kim, K.-D. 2003. Effect of dietary essential fatty acids on
392 growth, body composition and blood chemistry of juvenile starry flounder
393 (*Platichthys stellatus*). *Aquaculture*, 225, 269-281.

394 Li, Y.-y., Chen, W.-z., Sun, Z.-w., Chen, J.-h. & Wu, K.-g. 2005. Effects of n-3
395 HUFA content in broodstock diet on spawning performance and fatty acid
396 composition of eggs and larvae in *Plectorhynchus cinctus*. *Aquaculture*, 245,
397 263-272.

398 Li, Y.-y., Hu, C.-b., Zheng, Y.-j., Xia, X.-a., Xu, W.-j., Wang, S.-q., Chen, W.-z., Sun,
399 Z.-w. & Huang, J.-h. 2008. The effects of dietary fatty acids on liver fatty acid
400 composition and $\Delta 6$ -desaturase expression differ with ambient salinities in
401 *Siganus canaliculatus*. *Comparative Biochemistry and Physiology Part B:*
402 *Biochemistry and Molecular Biology*, 151, 183-190.

403 Li, Y., Monroig, Ó., Zhang, L., Wang, S., Zheng, X., Dick, J. R., You, C. & Tocher,
404 D. R. 2010. Vertebrate fatty acyl desaturase with $\Delta 4$ activity. *Proceedings of the*
405 *National Academy of Sciences*, 107, 16840-16845.

406 Liu, X.-b, 2011. Research on PUFA requirement of rabbitfish, *Siganus canaliculatus*.
407 Master Degree These, Shantou University.

408 Mørkøre, T., Netteberg, C., Johnsson, L. & Pickova, J. 2007. Impact of dietary oil
409 source on product quality of farmed Atlantic cod, *Gadus morhua*. *Aquaculture*,
410 267, 236-247.

411 Monroig, Ó., Wang, S., Zhang, L., You, C., Tocher, D. R. & Li, Y. 2012. Elongation
412 of long-chain fatty acids in rabbitfish *Siganus canaliculatus*: Cloning, functional
413 characterisation and tissue distribution of Elovl5- and Elovl4-like elongases.
414 *Aquaculture*, 350-353, 63-70.

415 Montero, D., Grasso, V., Izquierdo, M. S., Ganga, R., Real, F., Tort, L., Caballero, M.
416 J. & Acosta, F. 2008. Total substitution of fish oil by vegetable oils in gilthead

417 sea bream (*Sparus aurata*) diets: Effects on hepatic Mx expression and some
418 immune parameters. *Fish & Shellfish Immunology*, 24, 147-155.

419 Mozanzadeh, M. T., Agh, N., Yavari, V., Marammazi, J. G., Mohammadian, T. &
420 Gisbert, E. 2016. Partial or total replacement of dietary fish oil with alternative
421 lipid sources in silvery-black porgy (*Sparidentex hasta*). *Aquaculture*, 451,
422 232-240.

423 Nanton, D. A., Vegusdal, A., Rørå, A. M. B., Ruyter, B., Baeverfjord, G. &
424 Torstensen, B. E. 2007. Muscle lipid storage pattern, composition, and adipocyte
425 distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and
426 vegetable oil. *Aquaculture*, 265, 230-243.

427 Olsen, R. E. & Henderson, R. J. 1997. Muscle fatty acid composition and oxidative
428 stress indices of Arctic charr, *Salvelinus alpinus* (L.), in relation to dietary
429 polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition*, 3,
430 227-238.

431 Peng, S., Chen, L., Qin, J. G., Hou, J., Yu, N., Long, Z., Ye, J. & Sun, X. 2008.
432 Effects of replacement of dietary fish oil by soybean oil on growth performance
433 and liver biochemical composition in juvenile black seabream, *Acanthopagrus*
434 *schlegeli*. *Aquaculture*, 276, 154-161.

435 Regost, C., Arzel, J., Robin, J., Rosenlund, G. & Kaushik, S. J. 2003. Total
436 replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot
437 (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid
438 metabolism. *Aquaculture*, 217, 465-482.

439 Sargent, J. R., Tocher, D. R. & J.Gordon, B. 2002. The lipids. Pages 181-257 in J. E.
440 Halver & R. W. Hardy editors. *Fish Nutrition*. Elsevier (Academic Press), San
441 Diego.

442 Stubhaug, I., Lie, Ø. & Torstensen, B. E. 2007. Fatty acid productive value and
443 β -oxidation capacity in Atlantic salmon (*Salmo salar* L.) fed on different lipid
444 sources along the whole growth period. *Aquaculture Nutrition*, 13, 145-155.

445 Tan, X.-y., Luo, Z., Xie, P. & Liu, X.-j. 2009. Effect of dietary linolenic acid/linoleic
446 acid ratio on growth performance, hepatic fatty acid profiles and intermediary
447 metabolism of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture*, 296,
448 96-101.

449 Tocher, D. R., Agaba, M. K., Hastings, N. & Teale, A. J. 2003. Biochemical and
450 molecular studies of the polyunsaturated fatty acid desaturation pathway in fish.

451 Pages 211-228 in H. I. Browman & A. B. Skiftesvik editors. The Big Fish Bang:
452 Proceedings of the 26th Annual Larval Fish Conference. Institute of Marine
453 Research (IMR) / Fishlarvae.com, Bergen.

454 Tocher, D. R., Dick, J. R., MacGlaughlin, P. & Bell, J. G. 2006. Effect of diets
455 enriched in $\Delta 6$ desaturated fatty acids (18:3n-6 and 18:4n-3), on growth, fatty
456 acid composition and highly unsaturated fatty acid synthesis in two populations
457 of Arctic charr (*Salvelinus alpinus* L.). Comparative Biochemistry and
458 Physiology Part B: Biochemistry and Molecular Biology, 144, 245-253.

459 Torstensen, B. E., Bell, J. G., Rosenlund, G., Henderson, R. J., Graff, I. E., Tocher, D.
460 R., Lie, Ø. & Sargent, J. R. 2005. Tailoring of Atlantic Salmon (*Salmo salar* L.)
461 Flesh Lipid Composition and Sensory Quality by Replacing Fish Oil with a
462 Vegetable Oil Blend. Journal of Agricultural and Food Chemistry, 53,
463 10166-10178.

464 Torstensen, B. E., Frøyland, L. & Lie, Ø. 2004a. Replacing dietary fish oil with
465 increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon
466 (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme
467 activities. Aquaculture Nutrition, 10, 175-192.

468 Torstensen, B. E., Frøyland, L., Ørnstrud, R. & Lie, Ø. 2004b. Tailoring of a
469 cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*)
470 fed vegetable oils. Food Chemistry, 87, 567-580.

471 Tocher, D. R. & Glencross, B. D. 2015. Lipids and Fatty Acids. Pages 47-94. Dietary
472 Nutrients, Additives, and Fish Health. John Wiley & Sons, Inc.

473 Trushenski, J., Lewis, H. & Kohler, C. 2008. Fatty Acid Profile of Sunshine Bass: II.
474 Profile Change Differs Among Fillet Lipid Classes. Lipids, 43, 643-653.

475 Turchini, G.M. & Mailer, R.J. 2011. Rapeseed (canola) oil and other monounsaturated
476 fatty acid-rich vegetable oils. In: Fish Oil Replacement and Alternative Lipid
477 Sources in Aquaculture Feeds. (Turchini, G.M., Ng, W.-K. and Tocher, D.R.,
478 Eds), pp.161-208. Taylor & Francis, CRC Press, Boca Raton.

479 Turchini, G. M., Mentasti, T., Frøyland, L., Orban, E., Caprino, F., Moretti, V. M. &
480 Valfré, F. 2003. Effects of alternative dietary lipid sources on performance,
481 tissue chemical composition, mitochondrial fatty acid oxidation capabilities and
482 sensory characteristics in brown trout (*Salmo trutta* L.). Aquaculture, 225,
483 251-267.

484 Turchini, G. M., Torstensen, B. E. & Ng, W.-K. 2009. Fish oil replacement in finfish

485 nutrition. *Reviews in Aquaculture*, 1, 10-57.

486 Wang, J.-T., Liu, Y.-J., Tian, L.-X., Mai, K.-S., Du, Z.-Y., Wang, Y. & Yang, H.-J.
487 2005. Effect of dietary lipid level on growth performance, lipid deposition,
488 hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*). *Aquaculture*, 249,
489 439-447.

490 Xu, S., Wang, S., Zhang, L., You, C. & Li, Y. 2012. Effects of replacement of dietary
491 fish oil with soybean oil on growth performance and tissue fatty acid
492 composition in marine herbivorous teleost *Siganus canaliculatus*. *Aquaculture*
493 *Research*, 43, 1276-1286.

494 Yildirim-Aksoy, M., Lim, C., Davis, D. A., Shelby, R. & Klesius, P. H. 2007.
495 Influence of Dietary Lipid Sources on the Growth Performance, Immune
496 Response and Resistance of Nile Tilapia, *Oreochromis niloticus*, to
497 *Streptococcus iniae* Challenge. *Journal of Applied Aquaculture*, 19, 29-49.

498 Zuo, R., Mai, K., Xu, W., Turchini, G. M. & Ai, Q. 2014. Dietary ALA, But not LNA,
499 Increase Growth, Reduce Inflammatory Processes, and Increase Anti-Oxidant
500 Capacity in the Marine Finfish *Larimichthys crocea*. *Lipids*, 50, 149-163.

501

Table 1
Ingredients and composition of experimental diets for *Siganus canaliculatus*

	Diets					
	FO	VO1	VO2	VO3	VO4	VO5
Ingredients (g/100 g diet)						
Fish meal	33	33	33	33	33	33
Soybean meal	22	22	22	22	22	22
α -Starch	5	5	5	5	5	5
Starch	20.9	20.9	20.9	20.9	20.9	20.9
Cellulose	9	9	9	9	9	9
Mineral Mixture ^a	2	2	2	2	2	2
Vitamin Mixture ^b	1	1	1	1	1	1
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5
L-Methionine	0.5	0.5	0.5	0.5	0.5	0.5
Choline	0.08	0.08	0.08	0.08	0.08	0.08
Vitamin C	0.02	0.02	0.02	0.02	0.02	0.02
Fish oil	6					
Palm oil		1	2	1.5	3	4
Rapeseed oil		2	1	3	2	1
Soybean oil		2	2	1	0.5	0.5
Linseed oil		1	1	0.5	0.5	0.5
Proximate composition (% , dry matter basis)						
Dry matter	89.65	90.13	90.04	91.65	91.23	89.32
Crude protein	33.01	32.84	31.98	32.04	31.94	32.55
Crude lipid	8.33	8.16	8.13	8.32	8.45	8.39
Ash content	9.97	9.46	10.05	10.66	10.73	9.89
Main fatty acids (% area)						
14:0	5.60	1.54	1.74	1.68	1.86	1.79
16:0	22.80	16.30	20.10	17.54	22.66	26.66
16:1	5.76	1.86	1.86	1.83	1.88	1.94
18:0	4.84	4.60	4.67	4.45	4.47	4.60
18:1n-9	21.38	30.78	29.31	37.82	36.74	35.00
18:2n-6	7.60	23.24	20.89	17.52	14.83	13.64
18:3n-3	1.73	9.07	8.06	6.51	5.95	5.06
20:1	0.31	0.97	0.91	0.35	0.07	0.94
20:3n-3	0.01	0.06	0.33	0.37	0.37	0.19
20:4n-6	1.15	0.98	0.81	0.88	0.91	0.80
22:1n-9	0.75	0.01	0.01	0.29	0.20	0.23
20:5n-3	10.23	3.69	3.36	3.54	3.28	3.12
22:5n-3	1.59	0.59	0.71	0.61	0.80	0.62
22:6n-3	15.06	4.97	5.06	5.38	4.97	4.50
Σ saturates	33.23	22.44	26.51	23.67	28.99	33.05
Σ monoenes	28.20	33.62	32.09	40.30	38.89	38.11
Σ n-3 PUFA	28.62	18.38	17.52	16.41	15.37	13.49
Σ n-6 PUFA	8.75	24.22	21.7	18.4	15.74	14.44
n-3/n-6	3.27	0.76	0.81	0.89	0.98	0.93
Σ PUFA	35.77	41.95	38.18	33.83	29.94	27.12

a The amounts of following ingredients per kg of premix were as follows: iron, 10 g; zinc, 3.2 g; manganese, 3 g; cobalt, 52 mg; iodine, 65 mg; and selenium, 15 mg.

b The amounts of following vitamins per kg of premix were as follows: A, 1×10^6 IU; D₃, 3×10^5 IU; E, 5,000 IU; K₃, 1,040 mg; B₁, 1,500 mg; B₂, 2,400 mg; B₆, 1,200 mg; B₁₂, 5 mg; nicotinic acid, 8,000 mg; D-calcium pantothenate, 3,200 mg; folic acid, 400 mg; biotin, 10 mg; inositol, 12,000 mg; and C-monophospholipid, 16,000mg.

Table 2
Growth performance of *Siganus canaliculatus* fed the experimental diets for 9 weeks*

Growth index	Dietary groups					
	FO	VO1	VO2	VO3	VO4	VO5
Initial weight (g)	12.04 ± 0.06	11.98 ± 0.08	11.87 ± 0.17	11.88 ± 0.02	11.91 ± 0.04	12.08 ± 0.12
Final weight (g)	44.75 ± 0.67	41.55 ± 2.02	39.56 ± 0.51	39.96 ± 0.51	37.59 ± 1.98	38.31 ± 0.16
Weight gain (%)	271.66 ± 5.42	246.80 ± 17.84	233.48 ± 6.26	236.31 ± 11.31	231.99 ± 10.48	216.03 ± 3.77
Specific growth rate (%)	2.08 ± 0.02	1.97 ± 0.08	1.91 ± 0.03	1.92 ± 0.05	1.82 ± 0.09	1.83 ± 0.01
Feed conversion ratio	1.31 ± 0.11	1.33 ± 0.05	1.41 ± 0.05	1.32 ± 0.02	1.30 ± 0.02	1.33 ± 0.02
Protein efficiency ratio	2.65 ± 0.06	2.55 ± 0.08	2.57 ± 0.08	2.61 ± 0.01	2.59 ± 0.06	2.62 ± 0.04
Survival	98.15 ± 1.85 ^a	98.15 ± 1.85 ^a	90.74 ± 3.70 ^{ab}	87.03 ± 3.70 ^{ab}	88.89 ± 3.21 ^{ab}	83.33 ± 3.21 ^b
Hepatosomatic index (%)	2.46 ± 0.09 ^b	2.67 ± 0.10 ^b	2.82 ± 0.10 ^{ab}	2.90 ± 0.14 ^{ab}	3.61 ± 0.23 ^a	3.13 ± 0.16 ^{ab}
Viscerosomatic index(%)	14.20 ± 0.38 ^b	15.09 ± 0.44 ^b	16.22 ± 0.26 ^{ab}	15.36 ± 0.48 ^{ab}	17.63 ± 1.02 ^a	14.48 ± 0.26 ^b

*Values (mean ± SEM of three replicates) in each row with different superscript letters were significantly different ($P < 0.05$).

Table 3
Biochemical composition of whole body of *Siganus canaliculatus* fed the experimental diets for 9 weeks*

Composition (%)	Dietary groups				
	FO	VO1	VO2	VO3	VO5
Moisture	73.69 ± 0.54	71.94 ± 1.81	67.12 ± 1.33	70.02 ± 2.15	73.99 ± 0.35
Crude protein	15.59 ± 0.33	15.71 ± 0.32	15.94 ± 0.90	15.92 ± 0.60	16.15 ± 0.21
Crude lipid	8.18 ± 0.18	8.31 ± 0.20	8.58 ± 0.27	8.61 ± 0.21	8.63 ± 0.17
Crude Ash	3.43 ± 0.16	3.62 ± 0.21	3.31 ± 0.12	3.53 ± 0.33	3.85 ± 0.25

*Values are mean ± SEM of three replicates in each row.

Table 4
Main fatty acids in the liver of *Siganus canaliculatus* fed the experimental diets for 9 weeks*

Main fatty acids (% area)	Dietary groups					
	FO	VO1	VO2	VO3	VO4	VO5
12:0	0.48 ± 0.01 ^b	0.59 ± 0.01 ^{ab}	0.64 ± 0.03 ^{ab}	0.69 ± 0.05 ^{ab}	0.74 ± 0.01 ^a	0.62 ± 0.08 ^{ab}
14:0	2.15 ± 0.10	2.77 ± 0.14	2.18 ± 0.10	2.34 ± 0.12	2.47 ± 0.10	2.15 ± 0.10
16:0	37.57 ± 0.79	33.48 ± 0.32	34.59 ± 1.76	35.23 ± 0.87	35.32 ± 0.55	33.56 ± 0.49
16:1	15.24 ± 0.49 ^a	10.99 ± 0.30 ^b	11.41 ± 0.66 ^{ab}	12.00 ± 0.01 ^{ab}	11.88 ± 0.20 ^{ab}	12.83 ± 0.05 ^{ab}
18:0	6.05 ± 0.18	7.03 ± 0.75	6.96 ± 0.18	5.96 ± 0.42	5.90 ± 0.02	5.90 ± 0.11
18:1n-9	25.53 ± 0.08 ^b	28.79 ± 0.39 ^{ab}	29.06 ± 0.75 ^{ab}	30.39 ± 1.21 ^{ab}	29.47 ± 0.57 ^{ab}	31.73 ± 1.37 ^a
18:2n-6	1.82 ± 0.02 ^b	4.57 ± 0.20 ^a	4.74 ± 0.53 ^a	4.60 ± 0.20 ^a	4.42 ± 0.40 ^a	4.02 ± 0.19 ^a
18:3n-6	0.18 ± 0.01 ^b	0.85 ± 0.05 ^a	0.97 ± 0.11 ^a	0.82 ± 0.04 ^a	0.73 ± 0.03 ^a	0.72 ± 0.06 ^a
18:3n-3	0.01 ± 0.02 ^b	0.39 ± 0.06 ^a	0.36 ± 0.09 ^a	0.35 ± 0.07 ^a	0.26 ± 0.01 ^a	0.27 ± 0.09 ^a
20:3n-6	0.22 ± 0.01 ^b	0.98 ± 0.02 ^{ab}	1.13 ± 0.15 ^{ab}	0.97 ± 0.01 ^{ab}	0.87 ± 0.09 ^{ab}	0.88 ± 0.41 ^{ab}
20:3n-3	0.54 ± 0.08	0.72 ± 0.08	0.80 ± 0.07	0.71 ± 0.01	0.76 ± 0.10	0.54 ± 0.12
20:4n-6	2.15 ± 0.03 ^a	1.12 ± 0.07 ^b	1.08 ± 0.21 ^b	0.98 ± 0.06 ^b	1.10 ± 0.07 ^b	1.09 ± 0.08 ^b
20:5n-3	0.34 ± 0.02	0.14 ± 0.03	0.17 ± 0.03	0.17 ± 0.03	0.13 ± 0.02	0.12 ± 0.01
22:5n-3	0.94 ± 0.02	0.38 ± 0.02	0.46 ± 0.02	0.42 ± 0.05	0.41 ± 0.01	0.45 ± 0.06
22:6n-3	5.31 ± 0.17 ^a	2.55 ± 0.07 ^{bc}	3.22 ± 0.31 ^b	2.91 ± 0.11 ^{bc}	3.07 ± 0.32 ^{bc}	2.67 ± 0.08 ^c
∑SFA	46.24 ± 0.87	43.87 ± 0.91	44.36 ± 1.87	44.22 ± 1.46	44.44 ± 0.51	42.23 ± 0.40
∑MUFA	41.92 ± 0.54	40.91 ± 0.03	41.55 ± 0.30	42.63 ± 1.23	42.44 ± 0.70	45.90 ± 1.55
∑n-6 PUFA	4.36 ± 0.02 ^b	7.52 ± 0.32 ^a	7.91 ± 0.99 ^a	7.36 ± 0.11 ^{ab}	7.11 ± 0.53 ^{ab}	6.71 ± 0.36 ^{ab}
∑n-3 PUFA	6.67 ± 0.18 ^a	3.45 ± 0.13 ^b	4.21 ± 0.45 ^b	3.83 ± 0.21 ^b	3.87 ± 0.34 ^b	3.51 ± 0.24 ^b
n-3/n-6	1.53 ± 0.03 ^a	0.46 ± 0.02 ^b	0.53 ± 0.01 ^b	0.52 ± 0.02 ^b	0.55 ± 0.01 ^b	0.52 ± 0.01 ^b
∑PUFA	11.03 ± 0.20	10.97 ± 0.46	12.12 ± 1.44	11.20 ± 0.87	10.98 ± 0.87	10.22 ± 0.60

*Values (mean ± SEM of three replicates) in each row with different superscript letters were significantly different ($P < 0.05$).

Table 5
Main fatty acids in the fillet of *S. canaliculatus* fed the experimental diets for 9 weeks*

Main fatty acids (% area)	Dietary groups					
	FO	VO1	VO2	VO3	VO4	VO5
12:0	0.33 ± 0.08	0.33 ± 0.03	0.33 ± 0.03	0.33 ± 0.02	0.37 ± 0.03	0.33 ± 0.01
14:0	4.57 ± 0.68 ^a	1.96 ± 0.12 ^b	1.93 ± 0.01 ^b	1.96 ± 0.14 ^b	1.93 ± 0.14 ^b	1.83 ± 0.13 ^b
16:0	27.75 ± 0.18	22.97 ± 0.55	25.90 ± 1.66	26.31 ± 1.16	25.66 ± 0.39	25.18 ± 0.84
16:1	10.75 ± 0.15 ^a	6.12 ± 0.17 ^b	6.25 ± 0.15 ^b	6.88 ± 0.81 ^b	6.81 ± 0.51 ^b	6.76 ± 0.29 ^b
18:0	4.45 ± 0.58	4.53 ± 0.05	4.78 ± 0.10	4.20 ± 0.01	4.70 ± 0.38	4.37 ± 0.24
18:1n-9	19.54 ± 1.12 ^d	31.07 ± 0.01 ^{abc}	28.18 ± 0.41 ^c	32.18 ± 0.47 ^{ab}	33.83 ± 0.40 ^a	32.97 ± 0.41 ^{ab}
18:2n-6	3.67 ± 0.05 ^d	13.96 ± 0.69 ^a	12.50 ± 0.53 ^{ab}	10.63 ± 0.37 ^{bc}	9.32 ± 0.16 ^c	9.56 ± 0.15 ^c
18:3n-6	0.20 ± 0.01	0.74 ± 0.10	0.73 ± 0.18	0.60 ± 0.07	0.60 ± 0.07	0.64 ± 0.04
18:3n-3	0.74 ± 0.10 ^c	4.34 ± 0.19 ^a	3.72 ± 0.19 ^a	2.78 ± 0.11 ^b	2.58 ± 0.14 ^b	2.26 ± 0.07 ^b
20:3n-6	0.24 ± 0.02 ^b	0.86 ± 0.06 ^a	0.77 ± 0.04 ^a	0.75 ± 0.02 ^a	0.70 ± 0.03 ^a	0.75 ± 0.07 ^a
20:3n-3	0.88 ± 0.06	0.77 ± 0.04	0.71 ± 0.19	0.55 ± 0.04	0.55 ± 0.07	0.49 ± 0.01
20:4n-6	1.46 ± 0.06	1.43 ± 0.13	1.46 ± 0.08	1.28 ± 0.01	1.21 ± 0.06	1.17 ± 0.10
20:5n-3	2.53 ± 0.14 ^a	0.66 ± 0.03 ^b	0.92 ± 0.17 ^b	0.69 ± 0.07 ^b	0.79 ± 0.01 ^b	0.70 ± 0.02 ^b
22:5n-3	3.71 ± 0.23 ^a	1.76 ± 0.07 ^b	2.19 ± 0.39 ^b	1.74 ± 0.07 ^b	1.67 ± 0.16 ^b	1.82 ± 0.09 ^b
22:6n-3	12.33 ± 0.49 ^a	5.68 ± 0.19 ^b	5.79 ± 0.76 ^b	5.19 ± 0.08 ^b	5.18 ± 0.27 ^b	5.73 ± 0.18 ^b
∑SFA	36.77 ± 0.29 ^a	29.44 ± 0.70 ^b	32.61 ± 1.55 ^{ab}	32.46 ± 1.27 ^{ab}	32.29 ± 0.91 ^{ab}	31.38 ± 1.21 ^{ab}
∑MUFA	30.82 ± 0.61 ^c	37.54 ± 0.08 ^a	35.05 ± 0.54 ^b	39.63 ± 1.28 ^a	41.21 ± 0.91 ^a	40.30 ± 0.66 ^a
∑n-6PUFA	5.56 ± 0.02 ^f	16.99 ± 0.40 ^a	15.45 ± 0.40 ^b	13.26 ± 0.29 ^c	11.71 ± 0.03 ^d	12.12 ± 0.16 ^d
∑n-3PUFA	19.31 ± 0.86 ^a	12.44 ± 0.48 ^b	12.62 ± 1.51 ^b	10.39 ± 0.33 ^{bc}	10.21 ± 0.28 ^{bc}	10.50 ± 0.35 ^{bc}
n-3/n-6	3.47 ± 0.16 ^a	0.73 ± 0.01 ^b	0.81 ± 0.08 ^b	0.78 ± 0.01 ^b	0.87 ± 0.03 ^b	0.87 ± 0.02 ^b
∑PUFA	24.87 ± 0.84 ^{bc}	29.43 ± 0.88 ^a	28.06 ± 1.89 ^{ab}	23.65 ± 0.61 ^{bc}	21.92 ± 0.26 ^c	22.62 ± 0.51 ^{bc}

*Values (mean ± SEM of three replicates) in each row with different superscript letters were significantly different ($P < 0.05$).