## Title

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


## Authors

Shuqi Wang[1], Xuebing Liu[1], Shude Xu[1], Qingyang Wu[1], Cuihong You[1], Óscar Monroig[2], Douglas R. Tocher[2], Yuanyou Li[1, 3]*

## Addresses

[1] Marine Biology Institute of Shantou University & Guangdong Provincial Key Laboratory of Marine Biotechnology, Shantou, Guangdong, China

[2] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, Scotland, UK

[3] School of Marine Science, South China Agricultural University, Guangzhou, China



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*Correspondence to: Prof. Yuan-you Li, Ph.D.
Marine Biology Institute
Shantou University
Shantou, Guangdong 515063, China
Tel: +86–754–86503157
Fax: +86–754–86500613
E–mail: yyli@stu.edu.cn

# Abstract

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



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

# Introduction

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

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

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

long-chain PUFA (LC-PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6). Therefore, although alternative VOs can be used without any apparent detrimental effects on fish performance, the n-3 LC-PUFA concentration in final fish fillets is reduced (Sargent *et al.* 2002). In recent years, increasing research has been conducted to mitigate this effect of dietary VO in modifying fatty acid compositions of farmed fish. In addition, this research has contributed greatly to the advancement of our knowledge of fish lipid metabolism; however, a complete solution remains to be found (Turchini et al. 2009). If fish have all the necessary enzymes such as Δ6 fatty acid desaturase (fad), Δ5 fad, Elovl5 elongase, and/or Δ4 fad, they can biosynthesize LC-PUFA through a pathway involving a series of desaturation and elongation of a-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6). However, most marine fishes are unable to produce LC-PUFA because of apparent deficiencies in one or more steps (enzymes) of the biosynthetic pathway. Moreover, almost all FO substitution studies in marine fishes have been conducted in carnivorous species but rarely in herbivorous or omnivorous species.

The rabbitfish *Siganus canaliculatus* is an herbivorous marine teleost, feeding on algae and seagrass. *S. canaliculatus* is a commercially valuable species widespread along the Indo-West Pacific coast and has become one of the most harvested species in southeastern Asia, including along the coast of southeast China. It is also the subject of aquaculture activity with the development of a suitable formulated diet a necessity for the emerging culture industry. However, information regarding optimal lipid sources and PUFA requirements of rabbitfish is scant. In our recent studies, we reported that *S. canaliculatus* may have the ability to convert LA and ALA into LC-PUFA in both brackish water (10 ppt) and seawater (32 ppt) (Li *et al.* 2008) and that it exhibits activities for elongation and Δ6, Δ5, and Δ4 fatty acid desaturation (Li *et al.* 2010, Monroig *et al.* 2012). Our preliminary research results revealed that soybean oil (SO) can replace up to 67% or 45% of total dietary FO for *S. canaliculatus* without negatively compromising the growth performance or nutritional quality of fish (Xu *et al.* 2012). The expression of key genes involved LC-PUFA biosynthesis was also affected by the dietary LA:ALA ratio, with ratios of 0.52 or 2.13 showing better growth performance and LC-PUFA biosynthesis in rabbitfish(Liu, 2011). These findings suggested that FO can be partially or completely replaced by VO in feeds for rabbitfish.

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

# Materials and methods

## Experimental diets

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

## Experimental fish and feeding conditions

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

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

## Evaluation of growth performance and sample collection

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

$$WG\ (\%) = 100 \times (Wf - Wi)/Wi$$

$$SGR\ (\%) = 100 \times (\ln Wf - \ln Wi)/d$$

$$FCR = Fd/WG$$

$$PER = WG/Fp$$

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

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

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

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

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

## Chemical analysis

*Biochemical composition*

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

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

*Lipid extraction and fatty acid analysis*

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

## Statistical analysis

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

# Results

## Growth performance of different dietary groups

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

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

## Fatty acid compositions of liver and fillet

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

# Discussion

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

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

The proportion of dietary PUFA and the replacement of FO by a combination of palm, soybean, rapeseed and linseed oils markedly affected tissue fatty acid compositions in *S. canaliculatus*. The fatty acid profiles in both liver and fillet reflected the dietary fatty acid compositions, which was consistent with the findings of many other studies (Caballero *et al.* 2002, Tocher *et al.* 2003, Torstensen *et al.* 2004a,b, 2005, Nanton et al. 2007, Stubhaug *et al.* 2007). For example, the 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

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

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

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**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 |
| ∑saturates | 33.23 | 22.44 | 26.51 | 23.67 | 28.99 | 33.05 |
| ∑monoenes | 28.20 | 33.62 | 32.09 | 40.30 | 38.89 | 38.11 |
| ∑n-3 PUFA | 28.62 | 18.38 | 17.52 | 16.41 | 15.37 | 13.49 |
| ∑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 |
| ∑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, $1 \times 10^6$ IU; $D_3$, $3 \times 10^5$ IU; E, 5,000 IU; $K_3$, 1,040 mg; $B_1$, 1,500 mg; $B_2$, 2,400 mg; $B_6$, 1,200 mg; $B_{12}$, 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-monophopholipid, 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 | VO4 | VO5 |
| Moisture | 73.69 ± 0.54 | 71.94 ± 1.81 | 67.12 ± 1.33 | 70.02 ± 2.15 | 73.99 ± 0.35 | 70.90 ± 0.60 |
| Crude protein | 15.59 ± 0.33 | 15.71 ± 0.32 | 15.94 ± 0.90 | 15.92 ± 0.60 | 16.15 ± 0.21 | 15.91 ± 0.24 |
| Crude lipid | 8.18 ± 0.18 | 8.31 ± 0.20 | 8.58 ± 0.27 | 8.61 ± 0.21 | 8.63 ± 0.17 | 9.10 ± 0.11 |
| Crude Ash | 3.43 ± 0.16 | 3.62 ± 0.21 | 3.31 ± 0.12 | 3.53 ± 0.33 | 3.85 ± 0.25 | 3.76 ± 0.34 |

*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 ($P < 0.05$).