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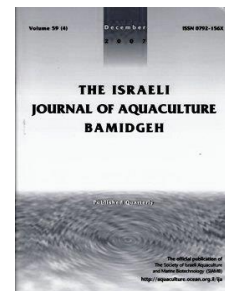
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## Growth Performance, Lipid Deposition and Serum Biochemistry in Golden Pompano *Trachinotus Ovatus* (Linnaeus, 1758) Fed Diets with Various Fish Oil Substitutes

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**Keywords:** *Trachinotus ovatus*; growth performance; lipid sources; fatty acid composition; serum biochemistry

### Abstract

In this study, 640 golden pompano (mean initial weight of 82.85±2.36 g) were randomly allocated into 32 floating net cages. Eight experimental diets involving various lipid sources were given. They were: fish oil (FO), krill oil (KO), soybean oil (SO), corn oil (CO), 1:1 FO-SO, 1:1 FO-CO, 1:1 KO-SO and 1:1 KO-CO. The results showed that the specific growth rate in the SO, CO, 1:1 FO-SO and 1:1 KO-SO groups was significantly lower than in the FO group ( $P<0.05$ ), and the 1:1 KO-CO had lower feed conversion ratio (FCR) among all the diets. The muscle fatty acid compositions were closely correlated with the fatty acid composition of the diets. Moreover, the serum MDA content of golden pompano fed the other seven diets was significantly lower than that of the fish fed the FO diet ( $P<0.05$ ) except for the 1:1KO-CO diet, with the lowest MDA content, which occurred in KO diet. Accordingly, we concluded that the FO, KO, 1:1 FO-SO and 1:1 KO-CO diets resulted in improvements in growth performance in golden pompano. Moreover, KO diet regulated some physiological and biochemical indicators. This study could provide a basis for the response-based selection of optimal lipid sources.

## Introduction

Golden pompano (*Trachinotus ovatus*) is a carnivorous fish, which preys mainly on zooplankton and small crustaceans, shellfish, and fish (Tan et al., 2017). This fish, which is widely distributed in China, Japan, Australia and other countries (Yang, 2006), is considered a highly valuable marine food fish due to its white and tasty meat. It is suitable for cage culture (Ma et al., 2016), and has become one of the most important marine culture species in the Asia-Pacific region (Tan et al., 2016).

Lipids are one of three major nutrients in organisms and play an important role in providing energy, essential fatty acids, and phospholipids. They also promote growth, health, and reproduction of fish. The structural components of membranes are carriers of fat-soluble vitamins, and precursors of eicosanoids, hormones, and vitamin D (Higgs and Dong, 2000). Based on the available knowledge, fish can synthesize omega-7 (n-7) and omega-9 (n-9) while other species cannot synthesize 18:2n-6 and 18:3n-3 fatty acids. Moreover, marine fish cannot biosynthesize the first three fatty acids, from n-3 to n-6 polyunsaturated fatty acid (PUFA); therefore, these are considered essential fatty acids for marine fish. Fish oil (FO) is rich in PUFA, particularly in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). As such it has become the major lipid source for marine fish (Tocher, 2003). However, due to rising demands and increasing prices of FO (FAO, 2012), more and more research on fish oil substitutes have been carried out. In recent years, stable prices and sustainable production have made vegetable oils (VOs) and other animal oils more promising alternatives to FO (Mourente and Bell, 2006; Sun et al., 2011; Trullàs et al., 2016).

Several fish studies on fish have demonstrated that different dietary lipid sources affect growth (Song et al., 2010), fatty acid composition, serum biochemistry, and other factors (Nayak et al., 2017; Yıldız et al., 2018). Some studies had evaluated the effects of other lipid sources to replace FO in several fish species. For example, in large yellow croaker (*Larimichthys crocea*), FO and soybean oil (SO) were found to be more suitable for growth performance (Qiu et al., 2017). In Shortfin Corvina (*Cynoscion parvipinnis*), based on growth performance, 50% FO replaced by SO had no adverse effects (González-Félix et al., 2016). In black seabream (*Acanthopagrus schlegelii*), dietary perilla oil (PO) and SO promoted growth and regulated some physiological and biochemical indicators (Jin et al., 2017). There are a few studies regarding fish oil substitutes on golden pompano. A study in golden pompano concluded that FO could not be completely replaced by SO and lard oil (LO) (Liu et al., 2018). In contrast, Florida Pompano could accept all diets which supplemented fish oil with SFA soybean oil, MUFA soybean oil, C<sub>18</sub>PUFA soybean oil, palm oil and poultry fat oil (Rombenso et al., 2016). However, there are few studies on the replacement of fish oil with mixed oils of vegetable oil and animal oil. This deserves further study.

In the present study, we selected FO, krill oil (KO), SO and corn oil (CO) as the main animal and vegetable oils, and we blended two animal oils with two vegetable oils (1:1 FO-SO, 1:1 FO-CO, 1:1 KO-SO and 1:1 KO-CO) to balance the fatty acid profiles. The aim of this study is to further elucidate the effect of different lipid sources on growth, fatty acid composition, and serum biochemistry in golden pompano. The results might be useful to explore a potential lipid source that could be used as a substitute for fish oil. This study may also provide a theoretical basis for more efficient and healthy feeds for golden pompano.

## Materials and Methods

### Experimental layout

Eight diets that contained different lipid sources were formulated (Table 1). Imported fish meal, chicken meal, pork meal and low-gluten flour were used as the main protein sources. Four main oils, including FO, KO, SO, and CO and blending of two animal oils with two vegetable oils (1:1 FO-SO, 1:1 FO-CO, 1:1 KO-SO and 1:1 KO-CO), were used as the main lipid sources. Apart from the different lipid sources, all the formulated diets had the same ingredients. These ingredients were mixed with lipid and water and then forced through a pelletizer (G-500, South China University of Technology, Guangzhou, China) into 2.5-mm pellet size and air-dried to approximately 10% moisture. The diets were kept in sealed bags at -20°C until use. The fatty acid composition of the experimental diets is shown in Table 2.

**Table 1** Ingredients and proximate composition (g/kg) of the experimental diets.

Ingredient	Diets							
	FO	KO	SO	CO	1:1FO-SO	1:1FO-CO	1:1KO-SO	1:1KO-CO
Fish meal	250	250	250	250	250	250	250	250
Chicken meal	100	100	100	100	100	100	100	100
Pork meal	50	50	50	50	50	50	50	50
Soybean meal	200	200	200	200	200	200	200	200
Corn protein flour	70	70	70	70	70	70	70	70
Low-gluten flour	200	200	200	200	200	200	200	200
Calcium dihydrogen phosphate	16	16	16	16	16	16	16	16
Choline chloride	2	2	2	2	2	2	2	2
Lutein	2	2	2	2	2	2	2	2
Compound premix <sup>1</sup>	30	30	30	30	30	30	30	30
FO <sup>2</sup>	80	0	0	0	0	0	0	0
KO <sup>3</sup>	0	80	0	0	0	0	0	0
SO <sup>4</sup>	0	0	80	0	0	0	0	0
CO <sup>5</sup>	0	0	0	80	0	0	0	0
1:1 FO-SO	0	0	0	0	80	0	0	0
1:1 FO-CO	0	0	0	0	0	80	0	0
1:1 KO-SO	0	0	0	0	0	0	80	0
1:1 KO-CO	0	0	0	0	0	0	0	80
Proximate analysis								
Crude protein	429	432	446	443	451	446	463	463
Crude lipid	125	110	117	120	116	120	112	108
Ash	105	104	101	106	99	96	94	105
Moisture	50	83	7.3	57	72	75	55	51

<sup>1</sup> Compound premix are provided by Guangzhou Nutriera Biotechnology Co., Ltd.

Compound premix provides the following (mg kg<sup>-1</sup> diet): vitamin A (375000 IU) 119.81 mg, vitamin D<sub>3</sub> (77000IU) 1.925 mg, vitamin E 3000 mg, vitamin K<sub>3</sub> 930 mg, vitamin B<sub>1</sub> 600 mg, vitamin B<sub>2</sub> 600 mg, vitamin B<sub>6</sub> 600 mg, vitamin B<sub>12</sub> 4.0 mg, vitamin C 10500 mg, D-calcium 400 mg, nicotinamide 4500 mg, folic acid 185mg, D-Biotin 7.5 mg, inositol 4500 mg, Zn 1750 mg, Mn 1100 mg, Cu 410 mg, Fe 1300, Co 60 mg, I<sub>2</sub> 50 mg, Se 15 mg.

<sup>2</sup> FO, fish oil; <sup>3</sup> KO, krill oil; <sup>4</sup> SO, soybean oil; <sup>5</sup> CO, corn oil.

**Table 2** Fatty acid composition (g/kg) of the experimental diets with different lipid sources<sup>1</sup>.

Fatty acid	Dietary lipid sources							
	FO	KO	SO	CO	1:1FO-SO	1:1FO-CO	1:1KO-SO	1:1KO-CO
C14:0	48.5	23.6	8.9	9.5	25.9	26.8	15.4	15.5
C15:0	3.3	1.7	0.8	0.8	2.5	2.5	1.2	1.2
C16:0	149.7	128.9	112.2	114.1	144.6	148.8	118.9	124.3
C18:0	37.2	30.1	36	26	42.5	38.3	33.5	29.7
C20:0	3.6	1.8	3.7	3.5	4.0	4.3	2.8	3.1
C22:0	1.8	1.3	3.2	1.6	2.8	2.0	2.2	1.5
ΣSFAs <sup>2</sup>	244.1	187.4	164.8	155.5	222.3	222.7	174	175.3
C16:1n7	49	29.7	12.2	13.8	24.7	26.8	19.6	19.8
C18:1n9c	79.4	96	151.1	172.7	114.3	133.6	121	140.4
C24:1n9	2.2	2.5	1.1	1.3	1.7	1.9	1.7	1.7
ΣMUFAs <sup>3</sup>	130.6	128.2	164.4	187.8	140.7	162.3	142.3	161.9
C18:3n3	5.6	9.0	30.3	6.1	17.4	6.3	17.4	7.5
C20:5n3	69.8	83	12.7	15.2	29.7	32.2	41	42.4
C22:6n3	50.5	61.3	15.3	15.6	31.4	33.7	34.1	34.6
Σn-3PUFA <sup>4</sup>	125.9	153.3	58.3	36.9	78.5	72.2	92.5	84.5
C18:2n6c	46.7	62.4	262.3	267.3	153.5	161	148.2	165.4
C18:3n6	1.7	1.3	0.4	0.4	0.8	0.9	0.7	0.8
C20:3n6	1.5	1.0	0.5	0.5	0.8	0.8	0.7	0.7
C20:4n6	8.9	6.9	2.5	2.6	5.3	5.6	4.5	4.5
Σn-6PUFA <sup>5</sup>	58.8	71.6	265.7	270.8	160.4	168.3	154.1	171.4
n-3/n-6 <sup>6</sup>	2.14	2.14	0.22	0.14	0.49	0.43	0.60	0.49
Total PUFA <sup>7</sup>	184.7	224.9	324	307.7	238.9	240.5	246.6	255.9

<sup>1</sup> Some fatty acids, of which the contents are minor, trace amount or not detected, such as 4:0, 6:0, 8:0, 10:0, 11:0, 13:0, 24:0, 15:1n5, 17:1n7, 18:1n9t, 18:1n6t, were not listed in the table. nd, not detected.

<sup>2</sup> SFA, saturated fatty acids.

<sup>3</sup> MUFA, monounsaturated fatty acids.

<sup>4</sup> n-3 PUFA, omega 3 polyunsaturated fatty acids.

<sup>5</sup> n-6 PUFA, omega 6 polyunsaturated fatty acids.

<sup>6</sup> n3/n6 PUFA, omega 3 polyunsaturated fatty acids: omega 6 polyunsaturated fatty acids.

<sup>7</sup> Total PUFA, the sum omega 3 polyunsaturated fatty acids and omega 6 polyunsaturated fatty acids.

### *Experimental conditions and sample collection*

Disease-free golden pompano were obtained from the Tropical Fisheries Research and Development Center, South China Fisheries Research Institute, Chinese Academy of Fishery Sciences (Xincun, Hainan, China). The feeding trial was conducted at Xincun port, Hainan Province, China. The mean body weight of experimental fish was  $82.85 \pm 2.36$  g. The golden pompano were randomly sorted into 32 floating net cages (1 m  $\times$  1 m  $\times$  1.5 m) with 20 fish per cage. Eight groups were included in this experiment, and each group had four replicates. The fish were hand-fed the experimental diets to apparent satiation twice daily (7:00 am and 4:00 pm) for 8 weeks. During the 8-week period, salinity was maintained to 35‰, sea water temperature was maintained at 27–31°C, pH was maintained approximately 7.5–8.2, and the dissolved oxygen was maintained at a level higher than 5 mg/L.

At the end of the experimental feeding period, the fish were counted to assess their survival ratio and individually weighed to determine their growth. Hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF) were determined from six individual fish per cage by after dissecting and collecting viscera and liver and expressing ratios as a percent of body weight. In addition, six fish were randomly selected from each cage to analyze the proximate composition and fatty acid composition of muscle and then stored at -20°C for subsequent analysis. Blood samples were obtained from the caudal vein of six fish from each cage and used for enzymatic activity assays and hematological analysis. All experiments in this study were approved by the Animal Care and Use Committee of South China Sea Fisheries Research Institute, Chinese Academy of fishery Sciences (no.SCSFRI96-253) and performed according to the regulations and guidelines established by this committee.

### *Proximate and fatty acid composition analysis*

Moisture content was determined by drying the samples to a constant weight at 105°C. The ash content was determined using a muffle furnace at 550°C for 8 h. Crude protein content was determined by Kjeldahl method and estimated by multiplying nitrogen by 6.25, and crude lipid was determined using a Soxhlet extraction method. The fatty acid profile of diets and fish tissues were analyzed as described by (Zuo et al., 2012) with few modifications. The freeze-dried samples (~120mg of muscle) were added to a 20 mL volumetric tube with a screw on cover. Then 3 mL potassium hydroxide methanol (1 N) was added and heated in a water bath at 72°C for 20 min. After cooling, 3 mL HCL-methanol (2 N) was added and the mixture was heated at 72°C in a water bath for another 20 min. Previous tests had been conducted to ensure that all fatty acids can be esterified following the above-described procedures. Finally, 1 mL hexane was added to the mixture above, and the mixture shaken vigorously for 1 min, and then allowed to separate into two layers. Fatty acid methyl esters were separated and measured by GC-MS (Agilent technologies 7890B -5977A, USA).

### *Biochemical index of serum analysis*

Glucose (GLU), total protein (TP), cholesterol (CHOL), triglyceride (TG), levels were analyzed using a Mindray BS-420 automatic biochemistry instrument (Shenzhen Mindray Biological Medical Electronics Co., Ltd, China). The activities of malondialdehyde (MDA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD) and lysozyme (LYZM) in serum were assayed with commercial enzyme kits (Zhongsheng Beikong Bio-technology and Science Inc, Beijing Sin-Uk Institute of Biological Technology, Beijing, China).

### *Calculations and statistical analysis*

The following variables were calculated:

Survival rate (SR, %) =  $100 \times (\text{final number of fish}) / (\text{initial number of fish})$ ;

Specific growth rate (SGR, %/day) =  $100 \times (\ln \text{ final individual weight} - \ln \text{ initial individual weight}) / \text{number of days}$ ;

Feed conversion ratio (FCR, %) =  $100 \times \text{dry diet feed (g)} / \text{wet weight gain (g)}$ ;

Condition factor (CF, g/cm<sup>3</sup>) =  $100 \times (\text{body weight, g}) / (\text{body length, cm})^3$ ;

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

Viscerosomatic index (VSI, %) =  $100 \times (\text{viscera weight, g}) / (\text{whole body weight, g})$ .

Data were expressed as the mean  $\pm$  standard errors of mean (SEMs). All statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, USA). Mean values were compared using one-way analysis of variance (ANOVA) followed by the Tukey's multiple range tests. The significance level used was 95% ( $P < 0.05$ ).

## Results

### Growth performance

The effects of different lipid sources on the survival, growth, and somatic indices of golden pompano are presented in Table 3. As shown, fish fed the SO, CO, 1:1 FO-SO and 1:1 KO-SO diets had higher SGR than those fed the FO diet ( $P < 0.05$ ), but the KO, 1:1FO-CO, 1:1 KO-CO groups showed no significant difference from the FO groups ( $P > 0.05$ ). The highest and lowest FCR was found in the SO and 1:1 KO-CO group, respectively. Fish fed the KO diet had a higher HSI than those fed the CO and 1:1 KO-SO diets ( $P < 0.05$ ), but there were not significant differences in fish fed the FO diet than the other seven diets ( $P > 0.05$ ). In addition, VSI and CF were not influenced by the lipid diets ( $P > 0.05$ ). The SR of golden pompano was over 96%, and no significant differences were detected among all the diets ( $P > 0.05$ ).

**Table 3** Effect of dietary lipid sources on survival, growth and somatic indices in golden pompano (*Trachinotus ovatus*) for 8 weeks.

Index	Dietary lipid sources							
	FO	KO	SO	CO	1:1FO-SO	1:1FO-CO	1:1KO-SO	1:1KO-CO
SR <sup>1</sup>	100	97.5 $\pm$ 2.5	96.25 $\pm$ 2.39	97.5 $\pm$ 1.44	100	98.75 $\pm$ 1.25	100	98.75 $\pm$ 1.25
SGR <sup>2</sup>	1.69 $\pm$ 0.02 <sup>a</sup>	1.58 $\pm$ 0.02 <sup>ab</sup>	1.54 $\pm$ 0.03 <sup>b</sup>	1.52 $\pm$ 0.04 <sup>b</sup>	1.54 $\pm$ 0.06 <sup>b</sup>	1.59 $\pm$ 0.02 <sup>ab</sup>	1.55 $\pm$ 0.02 <sup>b</sup>	1.58 $\pm$ 0.04 <sup>ab</sup>
FCR <sup>3</sup>	1.91 $\pm$ 0.05 <sup>ab</sup>	1.88 $\pm$ 0.1 <sup>ab</sup>	2.1 $\pm$ 0.13 <sup>a</sup>	2.04 $\pm$ 0.05 <sup>ab</sup>	1.95 $\pm$ 0.04 <sup>ab</sup>	2.03 $\pm$ 0.07 <sup>ab</sup>	1.95 $\pm$ 0.05 <sup>ab</sup>	1.62 $\pm$ 0.23 <sup>b</sup>
CF <sup>4</sup>	4.15 $\pm$ 0.06	5.28 $\pm$ 0.27	5.08 $\pm$ 0.68	4.42 $\pm$ 1.07	4.55 $\pm$ 0.38	4.36 $\pm$ 0.34	5.39 $\pm$ 0.32	4.11 $\pm$ 0.32
HSI <sup>5</sup>	1.07 $\pm$ 0.02 <sup>ab</sup>	0.97 $\pm$ 0.04 <sup>b</sup>	1 $\pm$ 0.03 <sup>ab</sup>	1.2 $\pm$ 0.08 <sup>a</sup>	1.09 $\pm$ 0.09 <sup>ab</sup>	1.05 $\pm$ 0.05 <sup>ab</sup>	0.99 $\pm$ 0.02 <sup>b</sup>	1.08 $\pm$ 0.07 <sup>ab</sup>
VSI <sup>6</sup>	5.52 $\pm$ 0.08	5.31 $\pm$ 0.11	5.35 $\pm$ 0.14	5.55 $\pm$ 0.24	5.44 $\pm$ 0.2	5.5 $\pm$ 0.1	5.47 $\pm$ 0.18	5.51 $\pm$ 0.19

Mean values (n=4) within values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ( $P > 0.05$ ).

<sup>1</sup>SR, Survival rate.

<sup>2</sup>SGR, Specific growth rate.

<sup>3</sup>FCR, Feed conversion ratio.

<sup>4</sup>CF, Condition factor.

<sup>5</sup>HSI, Hepatosomatic index.

<sup>6</sup>VSI, Viscerosomatic index.

### Proximate and fatty acid composition in muscle

The effects of different lipid sources on proximate composition in the muscle are presented in Table 4. As shown, the dietary lipid sources significantly affected the moisture, ash, crude protein, and crude lipid content ( $P < 0.05$ ). Fish fed the 1:1 KO-SO and 1:1 KO-CO diets had significantly higher moisture content in their muscles than those fed the FO diets ( $P < 0.05$ ). The muscle ash content of the fish fed the 1:1 FO-SO diet was significantly higher than in those fed the diets containing FO and KO diets ( $P < 0.05$ ). The crude protein content in the muscle of fish fed the KO, CO and 1:1 FO-SO diets was also significantly higher than those of the fish fed the FO diet ( $P < 0.05$ ), and no significant difference was found between other four diets and FO diet ( $P > 0.05$ ). Fish fed the FO diet had lowest crude protein values in their muscle among all diets. In addition, fish fed the FO diet showed the maximum value of crude lipid content in the muscle, being significantly higher than fish fed the diets with four blend oils ( $P < 0.05$ ).

**Table 4** Effect of dietary lipid sources on proximate composition (g/kg) in muscle in golden pompano (*Trachinotus ovatus*) for 8 weeks.

Index	Dietary lipid sources							
	FO	KO	SO	CO	1:1FO-SO	1:1FO-CO	1:1KO-SO	1:1KO-CO
Moisture	727.3 $\pm$ 0.7 <sup>bc</sup>	722.3 $\pm$ 3.9 <sup>c</sup>	731.3 $\pm$ 2.8 <sup>abc</sup>	723.7 $\pm$ 4.6 <sup>c</sup>	729.7 $\pm$ 0.3 <sup>abc</sup>	735.7 $\pm$ 1.9 <sup>ab</sup>	737.3 $\pm$ 0.9 <sup>a</sup>	737.7 $\pm$ 4.3 <sup>a</sup>
Ash	14 $\pm$ 0 <sup>bc</sup>	13.8 $\pm$ 0.3 <sup>c</sup>	14.3 $\pm$ 0.5 <sup>abc</sup>	14.8 $\pm$ 0.5 <sup>abc</sup>	15.3 $\pm$ 0.3 <sup>a</sup>	14.3 $\pm$ 0.3 <sup>abc</sup>	15 $\pm$ 0 <sup>ab</sup>	15 $\pm$ 0.4 <sup>ab</sup>
Crude protein	206.3 $\pm$ 0.3 <sup>b</sup>	214.7 $\pm$ 2.2 <sup>a</sup>	208 $\pm$ 0.6 <sup>b</sup>	215 $\pm$ 3.1 <sup>a</sup>	216 $\pm$ 0.6 <sup>a</sup>	208.7 $\pm$ 1.8 <sup>b</sup>	209 $\pm$ 0 <sup>b</sup>	207.3 $\pm$ 0.7 <sup>b</sup>
Crude lipid	51.7 $\pm$ 0.9 <sup>a</sup>	47.3 $\pm$ 5.7 <sup>ab</sup>	42 $\pm$ 4 <sup>abc</sup>	45.3 $\pm$ 5.8 <sup>abc</sup>	37 $\pm$ 1.0 <sup>bc</sup>	38.3 $\pm$ 0.9 <sup>bc</sup>	35.7 $\pm$ 1.3 <sup>c</sup>	40 $\pm$ 2.1 <sup>bc</sup>

Mean values (n=4) within values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ( $P > 0.05$ ).

The effects of different lipid sources on the proportion of fatty acids in muscle are presented in Table 5. Golden pompano fed the 1:1 KO-CO diet had significantly higher levels of MUFAs than those fed the 1:1 FO-SO diet ( $P<0.05$ ), and no differences were detected between the other six groups. Different lipid sources had a significant effect on n-3 PUFA ( $P<0.05$ ), and the highest and lowest n-3 PUFA proportions were found in the fish fed the KO and CO diets, respectively. Fish fed the FO diet had significantly lower n-6 PUFA proportion and n-3/n-6 ratios than other diets ( $P<0.05$ ), with the highest n-6 PUFA proportion and n-3/n-6 ratios in muscle both occurred in the fish fed the CO diet. Total PUFA of fish fed the FO diet was lower than in other diets ( $P<0.05$ ). The highest total PUFA was found in fish fed the SO and CO diets.

**Table 5** Effect of dietary lipid sources on fatty acid (% total fatty acids) in muscle in golden pompano (*Trachinotus ovatus*) for 8 weeks.

Dietary lipid sources								
Fatty acid	FO	KO	SO	CO	1:1FO-SO	1:1FO-CO	1:1KO-SO	1:1KO-CO
C12:0	0.06±0.002 <sup>a</sup>	0.05±0.001 <sup>b</sup>	0.03±0.001 <sup>e</sup>	0.03±0.001 <sup>e</sup>	0.04±0.001 <sup>c</sup>	0.04±0.002 <sup>c</sup>	0.04±0.002 <sup>c</sup>	0.03±0.001 <sup>d</sup>
C14:0	5.48±0.091 <sup>a</sup>	3.22±0.075 <sup>b</sup>	1.83±0.023 <sup>e</sup>	1.59±0.02 <sup>f</sup>	3.11±0.051 <sup>b</sup>	3.15±0.026 <sup>b</sup>	2.33±0.029 <sup>c</sup>	2.15±0.066 <sup>d</sup>
C15:0	0.48±0.012 <sup>a</sup>	0.32±0.012 <sup>c</sup>	0.21±0.007 <sup>e</sup>	0.19±0.004 <sup>e</sup>	0.37±0.017 <sup>b</sup>	0.37±0.007 <sup>b</sup>	0.25±0.01 <sup>d</sup>	0.25±0.006 <sup>d</sup>
C16:0	29.48±0.32 <sup>a</sup>	27.83±0.36 <sup>b</sup>	22.28±0.21 <sup>d</sup>	22.38±0.17 <sup>d</sup>	25.18±0.47 <sup>c</sup>	25.43±0.18 <sup>c</sup>	25.05±0.38 <sup>c</sup>	25.05±0.35 <sup>c</sup>
C17:0	0.49±0.02 <sup>a</sup>	0.33±0.02 <sup>c</sup>	0.23±0.01 <sup>de</sup>	0.21±0.01 <sup>e</sup>	0.38±0.02 <sup>b</sup>	0.38±0.02 <sup>bc</sup>	0.27±0.01 <sup>d</sup>	0.24±0.01 <sup>de</sup>
C18:0	6.28±0.09 <sup>a</sup>	6.18±0.09 <sup>ab</sup>	5.19±0.12 <sup>e</sup>	4.68±0.12 <sup>f</sup>	6.06±0.15 <sup>abc</sup>	5.72±0.09 <sup>cd</sup>	5.91±0.15 <sup>bc</sup>	5.48±0.06 <sup>de</sup>
C20:0	0.43±0.02 <sup>a</sup>	0.34±0.02 <sup>ab</sup>	0.34±0.03 <sup>ab</sup>	0.33±0.02 <sup>ab</sup>	0.39±0.05 <sup>ab</sup>	0.41±0.04 <sup>ab</sup>	0.31±0.05 <sup>ab</sup>	0.29±0.05 <sup>b</sup>
C21:0	0.042±0.02	0.037±0.01	0.057±0.03	0.022±0.01	0.022±0.01	0.023±0.01	0.025±0.01	0.024±0.01
C22:0	0.4±0.08	0.32±0.06	0.37±0.08	0.3±0.08	0.4±0.08	0.32±0.06	0.37±0.08	0.3±0.08
C23:0	0.1±0.01 <sup>ab</sup>	0.08±0.01 <sup>b</sup>	0.1±0 <sup>ab</sup>	0.08±0.01 <sup>b</sup>	0.12±0.02 <sup>a</sup>	0.1±0.01 <sup>ab</sup>	0.1±0.01 <sup>ab</sup>	0.09±0.02 <sup>ab</sup>
ΣSFAs <sup>1</sup>	43.16±0.36 <sup>a</sup>	38.71±0.34 <sup>b</sup>	30.64±0.2 <sup>e</sup>	29.79±0.36 <sup>e</sup>	36.04±0.59 <sup>c</sup>	35.89±0.28 <sup>c</sup>	34.64±0.51 <sup>d</sup>	33.93±0.4 <sup>d</sup>
C14:1n5	0.05±0.001	0.04±0.002	0.02±0	0.02±0.001	0.03±0.001	0.03±0.001	0.14±0.111	0.03±0.001
C16:1n7	6.96±0.05 <sup>a</sup>	4.81±0.11 <sup>b</sup>	2.76±0.08 <sup>e</sup>	2.5±0.07 <sup>e</sup>	3.93±0.14 <sup>c</sup>	3.98±0.08 <sup>c</sup>	3.42±0.09 <sup>d</sup>	3.26±0.09 <sup>d</sup>
C18:1n9c	21.38±0.23 <sup>e</sup>	22.63±0.38 <sup>de</sup>	25.1±0.56 <sup>abc</sup>	26.75±0.19 <sup>a</sup>	23.85±0.84 <sup>cd</sup>	24.38±0.65 <sup>bcd</sup>	24.93±0.75 <sup>abc</sup>	26.15±0.72 <sup>ab</sup>
C22:1n9	0.42±0.06 <sup>ab</sup>	0.56±0.09 <sup>a</sup>	0.39±0.06 <sup>ab</sup>	0.39±0.05 <sup>ab</sup>	0.39±0.05 <sup>ab</sup>	0.37±0.06 <sup>b</sup>	0.49±0.04 <sup>ab</sup>	0.49±0.04 <sup>ab</sup>
C24:1n9	0.64±0.07 <sup>b</sup>	0.84±0.04 <sup>a</sup>	0.55±0.06 <sup>b</sup>	0.56±0.04 <sup>b</sup>	0.61±0.07 <sup>b</sup>	0.64±0.04 <sup>b</sup>	0.68±0.06 <sup>ab</sup>	0.63±0.1 <sup>b</sup>
ΣMUFAs <sup>2</sup>	29.43±0.32 <sup>ab</sup>	28.88±0.46 <sup>ab</sup>	28.82±0.46 <sup>ab</sup>	30.22±0.17 <sup>ab</sup>	28.79±0.7 <sup>b</sup>	29.38±0.51 <sup>ab</sup>	29.62±0.75 <sup>ab</sup>	30.55±0.57 <sup>a</sup>
C18:3n3	0.79±0.01 <sup>e</sup>	1.15±0.05 <sup>c</sup>	2.49±0.04 <sup>a</sup>	0.72±0.02 <sup>e</sup>	1.85±0.01 <sup>b</sup>	0.79±0.02 <sup>e</sup>	1.87±0.06 <sup>b</sup>	0.99±0.06 <sup>d</sup>
C20:3n3	0.16±0.01 <sup>d</sup>	0.23±0.02 <sup>bcd</sup>	0.45±0.04 <sup>a</sup>	0.16±0.02 <sup>d</sup>	0.32±0.04 <sup>b</sup>	0.16±0.02 <sup>d</sup>	0.3±0.04 <sup>bc</sup>	0.21±0.05 <sup>cd</sup>
C20:5n3	4.04±0.04 <sup>b</sup>	4.72±0.14 <sup>a</sup>	0.85±0.02 <sup>f</sup>	0.7±0.02 <sup>f</sup>	1.48±0.09 <sup>e</sup>	1.63±0.05 <sup>de</sup>	1.98±0.07 <sup>c</sup>	1.8±0.14 <sup>cd</sup>
C22:6n3	10.38±0.11 <sup>b</sup>	12.15±0.36 <sup>a</sup>	5.44±0.26 <sup>d</sup>	5.18±0.34 <sup>d</sup>	7.06±0.83 <sup>c</sup>	7.81±0.13 <sup>c</sup>	7.98±0.3 <sup>c</sup>	7.65±0.18 <sup>c</sup>
Σn-3PUFA <sup>3</sup>	15.36±0.13 <sup>b</sup>	18.25±0.44 <sup>a</sup>	9.22±0.25 <sup>e</sup>	6.75±0.34 <sup>f</sup>	10.71±0.9 <sup>d</sup>	10.39±0.16 <sup>de</sup>	12.13±0.35 <sup>c</sup>	10.65±0.19 <sup>d</sup>
C18:2n6c	8.63±0.16 <sup>f</sup>	10.79±0.38 <sup>e</sup>	27.2±0.23 <sup>b</sup>	28.73±0.21 <sup>a</sup>	20.75±0.23 <sup>cd</sup>	20.53±0.21 <sup>cd</sup>	20±0.38 <sup>d</sup>	21.03±0.17 <sup>c</sup>
C18:3n6	0.17±0.04 <sup>ab</sup>	0.21±0.03 <sup>a</sup>	0.11±0.02 <sup>ab</sup>	0.09±0.02 <sup>b</sup>	0.17±0.04 <sup>ab</sup>	0.16±0.04 <sup>ab</sup>	0.14±0.04 <sup>ab</sup>	0.14±0.04 <sup>ab</sup>
C20:3n6	0.29±0.03	0.24±0.02	0.21±0.04	0.18±0.02	0.23±0.03	0.22±0.03	0.21±0.03	0.22±0.05
C20:4n6	1.05±0.04 <sup>a</sup>	0.95±0.05 <sup>a</sup>	0.46±0.03 <sup>c</sup>	0.46±0.02 <sup>c</sup>	0.71±0.07 <sup>b</sup>	0.73±0.05 <sup>b</sup>	0.67±0.03 <sup>b</sup>	0.63±0.03 <sup>b</sup>
C22:2n6	0.12±0.01 <sup>c</sup>	0.14±0.01 <sup>c</sup>	0.36±0.03 <sup>a</sup>	0.38±0.03 <sup>a</sup>	0.26±0.03 <sup>b</sup>	0.24±0.02 <sup>b</sup>	0.23±0.03 <sup>b</sup>	0.25±0.05 <sup>b</sup>
Σn-6PUFA <sup>4</sup>	10.29±0.03 <sup>f</sup>	12.27±0.31 <sup>e</sup>	28.29±0.27 <sup>b</sup>	29.81±0.21 <sup>a</sup>	22.07±0.25 <sup>c</sup>	21.84±0.2 <sup>de</sup>	21.22±0.32 <sup>c</sup>	22.23±0.2 <sup>d</sup>
n-3/n-6 <sup>5</sup>	1.49±0.01 <sup>a</sup>	1.49±0.06 <sup>a</sup>	0.33±0.01 <sup>d</sup>	0.23±0.01 <sup>e</sup>	0.48±0.04 <sup>c</sup>	0.48±0.01 <sup>c</sup>	0.57±0.01 <sup>b</sup>	0.48±0.01 <sup>c</sup>
Total PUFA <sup>6</sup>	25.64±0.13 <sup>d</sup>	30.52±0.4 <sup>c</sup>	37.51±0.48 <sup>a</sup>	36.56±0.44 <sup>a</sup>	32.78±1.06 <sup>b</sup>	32.23±0.28 <sup>b</sup>	33.35±0.64 <sup>b</sup>	32.88±0.19 <sup>b</sup>



Mean values (n=4) within values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ( $P > 0.05$ ).

<sup>1</sup>SFA, saturated fatty acids.

<sup>2</sup>MUFA, monounsaturated fatty acids.

<sup>3</sup>n-3 PUFA, omega 3 polyunsaturated fatty acids.

<sup>4</sup>n-6 PUFA, omega 6 polyunsaturated fatty acids.

<sup>5</sup>n3/n6 PUFA, omega 3 polyunsaturated fatty acids: omega 6 polyunsaturated fatty acids.

<sup>6</sup>Total PUFA, the sum omega 3 polyunsaturated fatty acids and omega 6 polyunsaturated fatty acids.

### Analysis of serum biochemistry index

The effects of different lipid sources on serum biochemistry are presented in Table 6. The GLU, TP, AST, LYZM, and SOD values showed no significant differences among the diets ( $P > 0.05$ ). The serum CHOL level of the golden pompano fed the 1:1 KO-CO diet was significantly higher than that of the fish fed the FO diet ( $P < 0.05$ ), and no significant differences were detected among the other treatments. The serum TG content of the fish fed the CO diet was significantly higher than that of the fish fed the other seven diets ( $P < 0.05$ ). Moreover, the serum MDA content of golden pompanos fed the other seven diets was significantly lower than the fish fed the FO diet ( $P < 0.05$ ) however in the 1:1KO-CO diet, the lowest MDA content occurred in KO diet. Alanine content of aminotransferase in the 1:1 FO-CO group was significantly lower than in the FO group ( $P < 0.05$ ), and the highest ALT content was found in the FO group.

**Table 6** Effect of dietary lipid sources on the biochemistry index of serum biochemistry in golden

Index	Dietary lipid sources							
	FO	KO	SO	CO	1:1FO-SO	1:1FO-CO	1:1KO-SO	1:1KO-CO
GLU <sup>1</sup> (mmol/L)	12.2±0.59	12.35±0.44	12.26±0.97	12.33±0.63	10.6±0.87	10.29±0.79	9.61±1.44	11.06±1.05
TP <sup>2</sup> (g/L)	45.09±1.37	42.77±0.56	42.9±1.18	43.02±0.74	41.96±0.51	42.8±1.37	42.7±1.55	44.9±1.67
CHOL <sup>3</sup> (mmol/L)	4.65±0.28 <sup>b</sup>	4.89±0.08 <sup>ab</sup>	5.03±0.08 <sup>ab</sup>	4.96±0.1 <sup>ab</sup>	4.92±0.22 <sup>ab</sup>	5.02±0.23 <sup>ab</sup>	5.25±0.21 <sup>ab</sup>	5.36±0.28 <sup>a</sup>
TG <sup>4</sup> (mmol/L)	2.56±0.21 <sup>b</sup>	2.43±0.3 <sup>b</sup>	2.28±0.24 <sup>b</sup>	3.42±0.43 <sup>a</sup>	2.34±0.13 <sup>b</sup>	2.19±0.03 <sup>b</sup>	2.07±0.23 <sup>b</sup>	2.43±0.09 <sup>b</sup>
MDA <sup>5</sup> (nmol/ml)	20.14±0.65 <sup>a</sup>	14.72±1.42 <sup>c</sup>	17.25±0.68 <sup>b</sup>	16.88±0.74 <sup>b</sup>	17.81±0.33 <sup>b</sup>	17.77±0.29 <sup>b</sup>	17.24±0.26 <sup>b</sup>	18.48±0.49 <sup>ab</sup>
AST <sup>6</sup> (U/L)	153.23±11.86	147.1±5.02	157.95±22.01	167.28±30.35	139.08±27.36	120.68±17.29	138.98±5.75	138.53±6.15
ALT <sup>7</sup> (U/L)	26.83±1.52 <sup>a</sup>	21.45±0.52 <sup>ab</sup>	23.05±3.2 <sup>ab</sup>	24.13±4.29 <sup>ab</sup>	22.28±5.42 <sup>ab</sup>	16.6±1.99 <sup>b</sup>	23±1.27 <sup>ab</sup>	21.45±1.74 <sup>ab</sup>
LYZM <sup>8</sup> (mg/L)	2.63±0.24	2.99±0.54	2.82±0.81	2.74±0.53	2.08±0.09	2.42±0.35	2.77±0.49	2.53±0.27
SOD <sup>9</sup> (U/ml)	99.21±8.49	100.13±5.7	93.15±8.23	100.29±10.14	92.48±7.71	90.86±11.06	90.11±7.67	100.91±10.37

Mean values (n=4) within values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ( $P > 0.05$ ).

<sup>1</sup>GLU, Glucose.

<sup>2</sup>TP, total protein.

<sup>3</sup>CHOL, cholesterol.

<sup>4</sup>TG, triglyceride.

<sup>5</sup>MDA, malondialdehyde.

<sup>6</sup>AST, aspartate aminotransferase.

<sup>7</sup>ALT, alanine aminotransferase.

<sup>8</sup>LYZM, lysozyme.

<sup>9</sup>SOD, superoxide dismutase.

### Discussion

FO has long been regarded as a high-quality lipid source because of its abundance of unsaturated fatty acids, particularly n-3 PUFA such as EPA and DHA (Tocher, 2003; Tur et al., 2012). Marine fish have limited ability to synthesize essential fatty acids (EFAs), so they need to obtain a sufficient amount of these compounds from their food to meet their normal growth requirements. This study showed that golden pompano fed the FO and KO diets showed better growth performance than fish fed a single lipid source, and fish fed the 1:1 FO-CO and 1:1 KO-CO diets showed better growth performance than the fish fed the mixed sources. FO and KO had a higher amount of n-3 PUFA, n-3/n-6, EPA and DHA (Table 6); thus, FO and KO are optimal in fulfilling the EFA requirements of golden



pompano. Several studies have demonstrated that KO was more effective in increasing n-3PUFA than FO (Ramprasath et al., 2013). There are between 125 and 750 million metric tons of krill in the Antarctic (according to the Food and Agriculture Organization of the United Nations; <http://www.fao.org/fishery/species/3393/en>), and KO is a rich source of n-3 PUFA (Ulven et al., 2011). KO plays a key role in the alleviation of hepatic steatosis and has antioxidant and antihyperlipidaemic effects (Tore et al., 2012) due to its abundance of natural antioxidants such as astaxanthin (Zhou et al., 2017). SO and CO have an abundance of n-6 and n-9 PUFAs, which are capable of being bioconverted into PUFA by freshwater fish species (Yildiz, et al., 2018), and this might explain why VOs can improve the growth performance of freshwater but not marine fish. Among the blended oils tested in this study, 1:1 FO-CO and 1:1 KO-CO resulted in greater improvement in growth performance, similar to the findings obtained in the Gibel carp (*Carassius auratus gibelio*) fed the 1:1 FO-CO diet (Chen et al., 2011). Fish fed the CO diet showed the higher HSI, whereas those fed the KO and 1:1 KO-SO diets showed lower HSI. High HSI is one of the main symptoms of fatty liver. One study reported that feeding a low n-3 PUFA diet will cause changes in the liver's neutral fat content and increase liver fat content significantly (Bell et al., 2001). HSI indicates the utilization of fat components in feed or greater supply of fat components. In other words, it reflects the deposition of fat in the liver. In this study, KO and CO showed the highest and lowest n-3 PUFA contents, respectively, and HSI was negatively correlated with n-3PUFA content. If n-3 PUFA content is low, the ability of the liver to participate in fat metabolism is decreased and a large amount of fat absorbed from the diet is deposited in the liver cells, which significantly increases the HSI (Feng et al., 2004).

Muscle composition was found to be directly affected by feed composition, and this study showed that different lipid sources had significant effects on proximate composition in muscle. Fish fed the KO diet showed higher protein content, which might indicate that higher PUFA levels could promote protein deposition in muscle (Bell, et al., 2001). In the present study, fish fed the FO diet had higher muscle lipid content. Similar results were previously found in Large Yellow Croaker *Larmichthys crocea*, (Qiu, et al., 2017). However, a study reported that crude lipid content in muscle are not significantly influenced by the dietary lipid sources in large yellow croaker (Wang et al., 2012). This discrepancy may attribute to different lipid sources, fatty acid composition, and feeding strategy. Previous studies have demonstrated that saturated fatty acids (SFAs) are easier to deposit in tissues than monounsaturated fatty acids (MUFAs) and PUFAs (Clarke et al., 1990). Indeed, in this study, the highest SFA content was observed in the FO diet, whereas the 1:1 KO-SO diet contained the lowest SFA content.

In this study, the muscle fatty acid composition of golden pompano generally reflected the dietary fatty acid composition and this is in agreement with other studies in other fish species (Du et al., 2008; Li et al., 2016; Sun et al., 2011). The n-3 PUFA content in the muscle of the fish fed the KO diet was significantly higher than that of the fish fed the FO diet. Similarly, fish fed the KO diet showed the highest EPA and DHA content in muscle, consistent with research reports (Ramprasath, et al., 2013). DHA and EPA are vital PUFAs, which are essential fatty acids for most marine carnivorous fish, and too low or high amounts of DHA in feed will affect normal growth and development of fish. These PUFAs are known to exert a variety of health benefits, including hypotriglyceridaemic and anti-inflammatory effects, in addition to anticancer (Park et al., 2013), anti-depression (Busolo et al., 2018), anti- cardiovascular disease (Ruxton et al., 2004), and anti-arthritis effects (Miles and Calder, 2012). According to the composition of fatty acid, golden pompano fed the KO diet showed improved growth performance compared with the fish fed the FO diet.

Serum indicators could reflect the physiological metabolic state of fish, which is closely related to their nutritional status. Changes in serum markers can elucidate the mechanism underlying nutrient metabolism changes in the body. No significant differences in GLU, TP, AST, LYZM and SOD were found in this study. The transport of fat in fish mainly depends on serum; thus, the blood lipid levels could reflect the body lipid metabolism (Hiraoka et al., 1979). In the present study, the lowest serum CHOL content was observed in fish fed the FO diet. Higher levels of EPA and DHA in fish oil may promote secretion of high-density lipoprotein, thereby speeding up the clearance of cholesterol in the plasma.

Fatty acids can influence susceptibility of tissue lipids to peroxidative damage. Higher n-3PUFA levels can elevate the unsaturation index of lipids in fish tissues and make them more prone to free radical attack. Fish tissues and commercial diets supplemented with marine fish oils containing high levels of PUFAs are highly susceptible to peroxidative damage (Welker and Congleton, 2003). Therefore, in this study, fish fed the FO diet had higher MDA and ALT which is in accordance with results obtained in black seabream (*Acanthopagrus schlegelii*) (Jin, et al., 2017) and *Carassius auratus gibelio* (Zhang et al., 2012). The present study indicated that KO diets cause damage to fish and exert a certain degree of stress reaction in the fish body. Nevertheless, fish fed the KO diet had lower MDA content among all the diets. This may be related to KO's natural antioxidants (Zhou, et al., 2017). This finding indicated that the presence of KO in the diet could improve the normal functions of golden pompano, and the relationship between KO and the stress reaction would be a meaningful future topic of research.

In conclusion, KO and FO as single lipid sources appeared to perform better in terms of promoting growth performance, and 1:1 KO-CO as mixture lipid sources also promoted better growth, while SO and CO did not seem to be suitable as lipid sources in golden pompano. Meanwhile, KO could regulate some physiological and biochemical indicators. The present study also indicated that different lipid sources had significant effects on fatty acid composition, serum biochemistry, and lipid metabolism, with a mixture of oils promoting these aspects. Further intensive research is required.

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