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RESEARCH PAPER

Optimal amounts of coconut oil in diets improve the growth, antioxidant capacity and lipid metabolism of large yellow croaker (*Larimichthys crocea*)

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Abstract
The purpose of this study was to investigate the effect of different dietary coconut oil (CO) levels on growth, antioxidant capacity and lipid metabolism of juvenile large yellow croaker (*Larimichthys crocea*). Five iso-nitrogen (45% crude protein) and iso-lipid (13% crude lipid) experimental diets were prepared by replacing 0% (the control), 25%, 50%, 75% and 100% fish oil with coconut oil. The results showed that dietary CO had no significant effect on survival rate (SR, $P > 0.05$). However, the specific growth rate was increased significantly when compared with the control group when fish were fed the diet with 50% CO ($P < 0.05$). The saturated fatty acids were increased significantly with increasing dietary CO in the liver and muscle, whereas the content of n-3 PUFA was decreased significantly ($P < 0.05$). The highest activities of glutathione peroxidase and superoxide dismutase in the liver were recorded in fish-fed diet with 50% CO; conversely, the content of malondialdehyde was significantly decreased ($P < 0.05$). The mRNA expression of peroxisome proliferator-activated receptor α, carnitine palmitoyl transferase 1 and acyl-CoA oxidase reached the highest levels in fish-fed diet with 50% CO. To some extent, this indicated that the rapid oxidation reaction of fatty acids to provide energy may be the reason for the rapid growth of large yellow croaker. In conclusion, fish-fed diet with 50% CO increased the growth rate and antioxidant capacity. Therefore, the optimal replacement level of CO to FO in the diet should be 50%.

Keywords Coconut oil · Growth performance · Antioxidant capacity · Lipid metabolism · *Larimichthys crocea*

Introduction

With the continuous expansion of aquaculture and the decline of fish oil (FO) production, the search for suitable FO substitutes has become the focus of attention (FAO 2012; Tacon and Metian 2008). Coconut oil (CO) is regarded as cheaper, more sustainable, and is readily available than the FO. Unlike most oils, CO enriches with middle chain fatty acids (MCFAs), with lauric acid (C12:0) representing 40–50% of the total fatty acids (Figueiredo et al. 2011; Fontagne et al. 2000). For this reason, CO is the most stable oil and its resistance to oxidative rancidity means that it will not be damaged by warmer temperatures (Alice et al. 2006). CO is still an emerging oil with more beneficial effects, and it has a tremendous potential with its cost, sustainability and availability. This makes it a good choice for FO replacement as compared to other vegetable oils (VO) (Apraku et al. 2017; Nordrum et al. 2003; Tseng and Lin 2020).

Many experiments have been conducted to study the effect of CO in diets. In mammals, it has been proven that coconut oil (CO) may be better absorbed and utilized in infant pigs and sheep as compared to other lipids (James et al. 2002; Li et al. 1990; Machmüller et al. 2003). Also, studies in fish have shown that dietary FO partially replaced by CO may significantly increase the growth performance of orange-spotted grouper (Tseng and Lin 2020) and African

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49 catfish (Aderolu and Akinremi 2009). Furthermore, some
50 studies with carp (Fontagne et al. 2000), rainbow trout (Ball-
51 estrazzi et al. 2006) and Atlantic salmon (Røsjø et al. 2000)
52 found that dietary CO is well absorbed without significant
53 adverse effects on the growth performance. However, Craig
54 and Gatlin (1997) reported that red drum fed diets with a
55 high proportion of CO showed significantly reduced growth
56 rate and increased liver lipid deposition. Similar conclu-
57 sions were reached with Russian sturgeon; the growth was
58 decreased when fed high CO diets (Li et al. 2017). This
59 may be due to the different species of fish and the propor-
60 tion of dietary CO in diets. There have been some studies
61 concerning the application of coconut oil in diets for aquatic
62 animals. However, the role of CO in whole or in part replac-
63 ing FO in promoting growth, antioxidant capacity and lipid
AQ3 metabolism capacity needs further study. Therefore, it is
65 important to explore the appropriate level of CO to replace
66 FO in diets (Tables 1 and 2).

67 Large yellow croaker (*Larimichthys crocea*) is one of the
68 most productive mariculture fish in China. Studies on the
69 substitution of VO for FO in the diet of large yellow croaker
70 involve mainly consideration of soybean oil, palm oil and
71 rapeseed oil. The possibility of substituting CO for FO in
72 diets has not been reported (Du et al. 2017; Li et al. 2019a, b;
73 Tan et al. 2016). Consequently, the purpose of this study was
74 to investigate the effect of different dietary coconut oil (CO)
75 levels on growth, antioxidant capacity and lipid metabolism
76 of juvenile large yellow croaker. Moreover, the work aimed
77 to provide the theoretical basis for the application of CO in
78 feed.

79 Materials and methods

80 Experimental diets

81 Five iso-nitrogen (45% crude protein) and iso-lipid (13%
82 crude lipid) experimental diets were prepared by replac-
83 ing 0% (the control), 25%, 50%, 75% and 100% fish oil
84 with coconut oil. All ingredients were separately crushed
85 in advance, using 0.18 mm sieves. The less used ingredi-
86 ents were mixed thoroughly one by one, then added to other
87 ingredients one at a time and mixed thoroughly in a mixer.
88 The oil was then added with the optimal amount of distilled
89 water and mixed thoroughly. This was passed through a
90 0.25 mm sieve and thoroughly mixed. Distilled water (300 g/
91 kg) was added to produce a stiff dough. After transferring
92 to a granulator, distilled water (300 g/kg) was added with
93 stirring in the opposite direction to make a dough. This was
94 extruded into 4 mm particles. All diets were dried in a venti-
95 lated oven at 40 ℃ until attaining a moisture level below 5%
96 and stored at −20 ℃ in opaque bags. Procedures for making

Table 1 Formulation and proximate analysis of the experimental diet for large yellow croaker (% dry matter)

Ingredients	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
Fish meal	33.00	33.00	33.00	33.00	33.00
Soybean meal	24.00	24.00	24.00	24.00	24.00
Wheat starch	23.75	23.75	23.75	23.75	23.75
Wheat gluten meal	5.00	5.00	5.00	5.00	5.00
Fish oil	8.00	6.00	4.00	2.00	0.00
Coconut oil	0.00	2.00	4.00	6.00	8.00
Lecithin	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Mineral premix	1.00	1.00	1.00	1.00	1.00
Mold inhibitor	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.1	0.1	0.1	0.1	0.1
Attractant	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Ingredient					
Crude protein (%DM)	45.37	45.56	45.37	45.51	45.41
Crude lipid (%DM)	13.40	13.35	13.36	13.41	13.24

[1]All those ingredients were supplied by Great Seven Biotechnology Co., Ltd, China

[2]Vitamin premix (mg/kg or g/kg diet): thiamine, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vit. B12, 0.1 mg; vit. K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg-α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; wheat middling, 14.012 g

[3]Mineral premix (mg/kg or g/kg diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; Ca $(H_2PO_4)2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; Zeolite, 15.447 g

[4]Mold inhibitor: sodium propionate

[5]Attractant: the mixture of 50% glycine acid and 50% betaine by weight

97 diets and their storage was in accordance with specific pro-
98 cedures described by Ai et al. (2008).

99 Experimental procedures

100 Before the start of the study, the fish were acclimatized to a
101 floating sea of 4 m × 4 m × 4 m in size for 2 weeks. Then, 60
102 fish of similar size (12.00 ± 0.20 g) were randomly distrib-
103 uted among 15 sea cages (1 m × 1 m × 1.5 m in size). Each
104 diet was fed to the fish in three cages. The fish were fed
105 twice daily at 5:00 and 17:00 for 10 weeks. After the end of
106 the study, all the fish were starved for 24 h, and the fish in
107 each cage were anesthetized and weighed. Environmental
108 conditions (temperature. 24.3–29.1 ℃, salinity 26.2–28.7‰,
109 oxygen level 6.2–7.4 mg/L) were good for acclimation.

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Table 2 Fatty acid profiles of the experimental diets (% total fatty acids)

Ingredients	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
C8:0	0.00	1.15	2.15	3.29	4.27
C10:0	0.00	1.01	1.89	2.85	3.81
C12:0	0.43	7.52	14.01	21.37	28.11
C14:0	5.75	7.31	8.56	9.67	11.04
C16:0	19.28	17.18	15.73	13.52	12.10
C18:0	4.19	3.81	3.64	3.48	3.27
∑SFA[a]	29.64	35.83	41.95	48.04	54.52
C16:1n-7	4.02	3.32	2.52	1.79	1.03
C18:1n-9	12.32	11.02	9.69	8.51	7.20
∑MUFA[b]	16.34	14.34	12.20	10.30	8.22
C18:2n-6	10.36	10.75	11.22	11.60	11.96
C20:4n-6	0.61	0.52	0.41	0.34	0.22
∑n-6 PUFA[c]	10.97	11.26	11.63	11.93	12.18
C18:3n-3	1.84	1.70	1.59	1.48	1.37
C20:5n-3	5.68	4.90	4.22	3.48	2.68
C22:6n-3	8.19	7.17	6.13	5.15	3.99
∑n-3 PUFA[d]	15.71	13.78	11.94	10.12	8.03

[a]SFA: saturated fatty acids
[b]MUFA: mono-unsaturated fatty acids
[c]n-6 PUFA: n-6 poly-unsaturated fatty acids
[d]n-3 PUFA: n-3 poly-unsaturated fatty acids

Sample collection and analysis

The fish were sampled 24 h after starvation. Eugenol (1:10,000) was used for anaesthesia; the weight and number of fish in each cage were weighed and counted, and three fish were randomly taken and stored in − 20 ℃ for experimental analysis. The fish livers were weighed, and the hepatosomatic index (HSI) was calculated. The livers from 12 fish (groups of three were mixed in a single tube) were immediately placed into the labelled cryopreservation tube in liquid nitrogen and stored at − 80 ℃ before analysis.

The sample was in a ventilated 105 ℃ drying oven to dry the sample to constant weight (AOAC 2003). The crude protein (whole body protein), crude lipid (whole body lipid) and the ash of the sample were determined after Ai et al. (2008). The fatty acid composition was analyzed using the procedures described by Metcalfe et al. (1966). Fatty acid methyl esters were identified and quantified by an HP6890 gas chromatograph (Agilent Technologies, Santa Clara, California, USA) with a capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector.

Antioxidant and lipid metabolism index

Malondialdehyde (MDA), total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were measured via the test kit produced by Nanjing Jiancheng Bioengineering Institute (China). All procedures were carried out in strict accordance with the instructions.

RNA extraction and real-time quantitative PCR

The samples were ground in liquid nitrogen, and ~ 100 mg samples were taken and fully reacted in 1 ml RNAiso TM Plus from Takara (Japan) for total RNA extraction. RNA concentration and purity were determined by ultraviolet colorimetry (Nanodrop Thermo Fisher Scientific, USA), and RNA quality was determined by 1.5% agarose gel electrophoresis. The total RNA concentration was diluted to 500 ng/μL, and reverse transcription was performed immediately with the kit from Takara (Japan).

The real-time quantitative PCR procedure was performed in a total volume of 20 μL. The technique was programmed as follows. 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 15 s, and 72 °C for 10 s. The specificity of the product was determined by melting point curve analysis. The primer sequences (sterol-regulatory element binding protein-1 (*srebp-1*), fatty acid synthase (*fas*), stearoyl-coenzyme A desaturase (*scd-1*), peroxisome proliferator-activated receptor α (*ppar- α*), cetyl-CoA carboxylase (*acc*), adipose triglyceride lipase (*atgl*), acyl-CoA oxidase (*aco*), carnitine palmitoyltransferase1 (*cpt-1*) and *β-actin*) were calculated according to Cui et al. (2009) (Table 3).

Calculations and statistical analysis

Growth and somatic indices were calculated according to the following:

$$\text{Weight gain rate(WGR, \%)} = \left(w_t - w_o\right) \times 100/w_o.$$

$$\text{Specific growth rate(SGR, \%/day)} = \left\{\left(\text{Ln}\left(w_t\right) - \ln\left(w_o\right)\right)/\text{duration (70 days)}\right\} \times 100.$$

$$\text{Survival rate(SR, \%)} = 100 \times (\text{FN/IN}).$$

$$\text{Hepatosomatic index(HSI, \%)} = \text{weight of liver/weight of fish} \times 100.$$

$$\text{Feed conversion ratio(FCR)} = F/\left(w_t - w_o\right).$$

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Table 3 Primer pair sequences for real-time PCR

Target genes	Forward (5′–3′)	Reverse (5′–3′)	References
srebp-1	TCTCCTTGCAGTCTGAGCCAAC	TCAGCCCTTGGATATGAGCCT	Cai et al. (2016)
scd-1	AAAGGACGCAAGCTGGAACT	CTGGGACGAAGTACGACACC	Cai et al. (2016)
fas	CAGCCACAGTGAGGTCATCC	TGAGGACATTGAGCCAGACAC	Yan et al. (2015)
acc	TGTCGGAGGAGAACTCGGAAG	GCCTTAGATTCTGCACGGGG	Yan et al. (2015)
ppar-α	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC	Yan et al. (2015)
cpt-1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	Yan et al. (2015)
atgl	CCATGCATCCGTCCTTCAACC	GAGATCCCTAACCGCCCACT	Yan et al. (2015)
aco	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	Yan et al. (2015)
β-actin	CTACGAGGGTTATGCCCTGCC	TGAAGGAGTAACCGCGCTCTGT	Yan et al. (2015)

srebp-1 sterol-regulatory element binding protein-1, *fas* fatty acid synthase, *scd-1* stearoyl-coenzyme A desaturase, *acc* acetyl-CoA carboxylase, *ppar-α* peroxisome proliferator-activated receptor α, *cpt1* carnitine palmitoyl transferase 1, *atgl* adipose triglyceride lipase, *aco* acyl-CoA oxidase

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174 Protein efficiency ratio(PER) $= (w_t - w_o)/P$.
175

176 w_t—final weight of fish, w_o—initial weight of fish, FN—
177 final number, IN—initial number of fish in each cage, *F*—
178 feed intake (g), *P*—amount of protein in the diet (%).
179 An one-way ANOVA was used for data statistics (SPSS
180 25.0). Tukey's test was used for significance comparison
181 between groups. $P<0.05$ indicates a significant difference.
182 The data are expressed as means SEM. The data with the
183 same superscript show no significant difference ($P<0.05$).

184 # Results

185 ## Survival and growth performance

186 There was no significant difference in survival rate (SR)
187 among dietary treatments ($P>0.05$). With increasing die-
188 tary CO, the specific growth rate (SGR) increased first and
189 then decreased. In fish that were fed the diet with 50% CO,
190 the SGR was significantly higher than the control group
AQ4 ($P<0.05$). The hepatosomatic index (HSI) was increased
192 by increasing dietary CO. Thus, fish-fed diets containing
193 75% and 100% CO had significantly higher HSI than the
194 control group ($P<0.05$) (Table 4).

Body composition 195

No significant differences were observed among dietary 196
treatments in terms of the moisture, ash, crude protein, crude 197
lipid and muscle lipid ($P>0.05$). When compared with the 198
control group, the liver lipid was significantly increased in 199
fish-fed diets with 75% and 100% CO ($P<0.05$). Moreo- 200
ver, in fish-fed diet with 100% CO, the liver lipid was the 201
highest. This was significantly higher than the other groups 202
($P<0.05$) (Table 5). 203

The liver and muscle fatty acid composition 204

The liver saturated fatty acids (SFAs) (C10:0, C12:0, C14:0 205
and C18:0) were significantly increased in fish-fed diets 206
with increasing dietary CO when compared with the control 207
group ($P<0.05$). However, the content of C16:0 and mono- 208
unsaturated fatty acids (MUFA) (C16:1n-7 and C18:1n-9) 209
in fish-fed diets with CO were not significantly different 210
among the dietary treatments ($P>0.05$). The liver content 211
of C20:4n-6 showed a decreasing trend with increasing die- 212
tary CO, but there was not any significant difference among 213
dietary treatments ($P>0.05$). The liver content of C18:3n-3, 214
C20:5n-3 and C22:6n-3 was significantly decreased with 215

Table 4 Survival and growth performance of large yellow croaker-fed diets with graded levels of coconut oil

Index	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
Survival rate (%)	81.67 ± 2.11	83.33 ± 3.41	82.78 ± 3.09	84.44 ± 4.03	86.11 ± 2.88
Initial body weight (g)	11.92 ± 0.23	12.04 ± 0.29	11.99 ± 0.14	12.02 ± 0.05	12.01 ± 0.20
Final body weight (g)	59.28 ± 2.11^{a}	63.12 ± 1.98^{a}	70.75 ± 3.44^{b}	65.5 ± 0.89^{ab}	61.12 ± 1.66^{a}
Specific growth rate (%/day)	2.32 ± 0.12^{a}	2.37 ± 0.11^{a}	2.54 ± 0.17^{b}	2.42 ± 0.09^{ab}	2.35 ± 0.06^{a}
Hepato-somatic index (%)	1.25 ± 0.12^{a}	1.21 ± 0.10^{a}	1.2 ± 0.09^{a}	1.41 ± 0.13^{b}	1.55 ± 0.14^{c}

The data are expressed as means SEM. The data with the same superscript show no significant difference ($P>0.05$). SEM, standard error of means. ($n=3$)

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Table 5 Body composition (dry weight %) of large yellow croaker-fed diets with graded levels of coconut oil

Index	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
Moisture (%)	73.25 ± 1.66	73.31 ± 0.99	73.82 ± 2.01	73.76 ± 2.33	73.45 ± 1.33
Ash (% d.w.)	13.02 ± 0.33	13.18 ± 0.28	13.2 ± 0.19	13.08 ± 0.23	13.19 ± 0.26
Crude protein (% d.w.)	60.59 ± 0.64	59.86 ± 0.55	61.49 ± 0.19	61.84 ± 0.77	60.36 ± 1.12
Crude lipid (% d.w.)	30.34 ± 0.33	30.39 ± 0.31	28.58 ± 0.55	28.44 ± 0.62	29.30 ± 0.58
Liver lipid content (% d.w.)	59.60 ± 1.30^{a}	58.22 ± 1.51^{a}	61.25 ± 1.28^{ab}	64.77 ± 1.52^{b}	69.85 ± 0.57^{c}
Muscle lipid content (% d.w.)	24.38 ± 0.61	24.91 ± 0.97	24.7 ± 0.53	23.77 ± 0.53	23.69 ± 0.44

The data are expressed as means SEM. The data with the same superscript show no significant difference ($P > 0.05$)

SEM standard error of means. ($n = 3$)

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216 increasing dietary CO, and the ratio of n-3 PUFA/n-6 PUFA was also significantly decreased ($P < 0.05$).

218 Similarly to liver, the muscle SFAs (C10:0, C12:0, C14:0 and C18:0) were significantly increased with increasing dietary CO ($P < 0.05$). However, the muscle content of C16:1n-7 was gradually decreased with increasing dietary CO. When the dietary CO was higher than 50%, the muscle content was significantly higher than the control group ($P < 0.05$). Meanwhile, the trend of MUFA and C20:4n-6 was the same as the C16:1n-7. However, the n-6 PUFA was not significantly different among dietary treatments in the muscle ($P > 0.05$). When dietary CO was higher than 50%, the muscle n-3 PUFA (C18:3n-3, C20:5n-3 and C22:6n-3) were decreased significantly than the control group ($P < 0.05$). Also, the ratio of n-3 PUFA / n-6 PUFA was decreased significantly with increasing dietary CO ($P < 0.05$) (Tables 6 and 7). 232

Oxidation and antioxidant parameters in the liver and intestine 233 234

235 With increasing dietary CO, the activity of glutathione peroxidase (GSH-Px) in the liver increased initially and then decreased. The peak was reached when dietary CO was 50%, 237

Table 6 Fatty acid composition (% total fatty acids) in the liver of large yellow croaker-fed diets with graded levels of coconut oil

Index	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
C8	0	0	0	0	0
C10	0	0.03 ± 0.01^{a}	0.10 ± 0.01^{b}	0.20 ± 0.02^{b}	0.27 ± 0.02^{c}
C12	0.28 ± 0.03^{a}	1.54 ± 0.12^{b}	3.19 ± 0.15^{c}	5.74 ± 0.55^{d}	7.13 ± 0.65^{d}
C14	3.30 ± 0.08^{a}	4.23 ± 0.31^{a}	5.82 ± 0.12^{b}	8.48 ± 0.45^{c}	9.90 ± 0.22^{d}
C16	19.37 ± 1.56	21.46 ± 0.56	21.97 ± 0.61	21.17 ± 0.91	21.82 ± 0.89
C18	5.63 ± 0.28^{a}	7.73 ± 0.21^{b}	7.83 ± 0.31^{b}	8.49 ± 0.39^{b}	10.12 ± 0.22^{c}
∑SFA	28.58 ± 1.59^{a}	34.98 ± 1.09^{ab}	38.92 ± 0.98^{bc}	44.08 ± 2.33^{cd}	49.24 ± 1.43^{d}
C16:1n-7	7.21 ± 0.42	6.77 ± 0.32	6.80 ± 0.47	6.03 ± 0.18	6.39 ± 0.11
C18:1n-9	16.85 ± 1.15	17.87 ± 1.18	16.69 ± 0.42	17.92 ± 0.77	17.31 ± 0.71
∑MUFA	24.06 ± 1.58	24.65 ± 1.49	23.50 ± 0.99	23.95 ± 0.86	23.70 ± 0.83
C18:2n-6	11.75 ± 0.63	11.65 ± 0.61	11.21 ± 1.13	11.90 ± 0.25	10.29 ± 0.26
C20:4n-6	0.71 ± 0.02^{cd}	0.66 ± 0.05^{c}	0.59 ± 0.01^{bc}	0.51 ± 0.02^{b}	0.34 ± 0.03^{a}
∑n-6 PUFA	12.46 ± 0.62	12.31 ± 0.15	11.80 ± 1.23	12.41 ± 0.25	10.62 ± 0.27
C18:3n-3	1.94 ± 0.07^{c}	1.67 ± 0.02^{b}	1.55 ± 0.02^{b}	1.54 ± 0.03^{b}	1.12 ± 0.01^{a}
C20:5n-3	3.89 ± 0.14^{e}	3.38 ± 0.03^{d}	2.69 ± 0.05^{c}	2.31 ± 0.03^{b}	1.22 ± 0.02^{a}
C22:6n-3	5.60 ± 0.32^{d}	4.55 ± 0.10^{c}	3.17 ± 0.10^{b}	2.36 ± 0.07^{b}	1.15 ± 0.08^{a}
∑n-3 PUFA	11.43 ± 0.35^{e}	9.60 ± 0.15^{d}	7.41 ± 0.16^{c}	6.21 ± 0.07^{b}	3.48 ± 0.11^{a}
n-3/n-6 PUFA	0.92 ± 0.06^{d}	0.78 ± 0.01^{cd}	0.63 ± 0.05^{bc}	0.50 ± 0.02^{ab}	0.33 ± 0.01^{a}

The data are expressed as means SEM. The data with the same superscript show no significant difference ($P > 0.05$)

SEM standard error of means. ($n = 3$), *SFA* saturated fatty acids, *MUFA* mono-unsaturated fatty acids, *n-6 PUFA* n-6 poly-unsaturated fatty acids, *n-3 PUFA* n-3 poly-unsaturated fatty acids

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Table 7 Fatty acid composition (% total fatty acids) in the muscle of large yellow croaker-fed diets with graded levels of coconut oil

Index	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
C8	0	0	0	0	0
C10	0	0.15 ± 0.02[a]	0.33 ± 0.03[b]	0.52 ± 0.07[cd]	0.73 ± 0.12[d]
C12	0.60 ± 0.01[a]	3.50 ± 0.21[b]	6.53 ± 0.53[c]	9.95 ± 0.76[d]	12.43 ± 1.02[d]
C14	3.87 ± 0.10[a]	5.26 ± 0.11[b]	7.01 ± 0.24[c]	8.40 ± 0.07[d]	9.87 ± 0.52[d]
C16	19.94 ± 0.32	19.63 ± 0.18	19.33 ± 0.52	18.98 ± 0.53	18.74 ± 0.14
C18	4.02 ± 0.16[a]	4.34 ± 0.24[ab]	4.63 ± 0.14[ab]	4.98 ± 0.15[bc]	5.31 ± 0.17[c]
∑SFA	28.24 ± 0.39[a]	32.74 ± 0.56[b]	37.49 ± 1.18[c]	42.31 ± 0.80[d]	46.45 ± 1.47[e]
C16:1n-7	4.62 ± 0.05[d]	4.22 ± 0.02 [cd]	3.77 ± 0.15[bc]	3.4 ± 0.14[ab]	2.99 ± 0.23[a]
C18:1n-9	14.5 ± 0.33	14.24 ± 0.03	13.99 ± 0.48	13.88 ± 0.37	13.66 ± 0.34
∑MUFA	19.12 ± 0.28[c]	18.46 ± 0.04[bc]	17.77 ± 0.62[ab]	17.28 ± 0.50[ab]	16.65 ± 0.50[a]
C18:2n-6	11.7 ± 0.41	11.41 ± 0.10	11.64 ± 0.26	11.55 ± 0.55	11.67 ± 0.32
C20:4n-6	0.76 ± 0.01[c]	0.66 ± 0.01[bc]	0.55 ± 0.02[b]	0.44 ± 0.04[a]	0.34 ± 0.05[a]
∑n-6 PUFA	12.46 ± 0.42	12.08 ± 0.09	12.19 ± 0.27	11.78 ± 0.59	12.02 ± 0.35
C18:3n-3	1.57 ± 0.04[b]	1.51 ± 0.01[b]	1.33 ± 0.04[a]	1.25 ± 0.08[a]	1.21 ± 0.06[a]
C20:5n-3	4.25 ± 0.18[d]	3.72 ± 0.27[cd]	3.2 ± 0.02[bc]	2.72 ± 0.19[ab]	2.48 ± 0.26[a]
C22:6n-3	8.28 ± 0.28[d]	7.37 ± 0.21[cd]	6.37 ± 0.20[bc]	5.19 ± 0.53[ab]	4.63 ± 0.56[a]
∑n-3 PUFA	14.1 ± 0.49[d]	12.59 ± 0.29[cd]	10.9 ± 0.17[bc]	9.17 ± 0.79[ab]	8.32 ± 0.88[a]
n-3/n-6 PUFA	1.31 ± 0.01[c]	1.04 ± 0.08[b]	0.89 ± 0.02[b]	0.76 ± 0.03[a]	0.69 ± 0.06[a]

The data are expressed as means SEM. The data with the same superscript show no significant difference ($P > 0.05$)

SEM standard error of means. ($n = 3$), *SFA* saturated fatty acids, *MUFA* mono-unsaturated fatty acids, *n-6 PUFA* n-6 poly-unsaturated fatty acids, *n-3 PUFA* n-3 poly-unsaturated fatty acids

238 which was significantly higher than other groups ($P < 0.05$). 239 The liver content of malondialdehyde (MDA) was decreased 240 significantly in fish-fed diets with CO; the minimum was in 241 fish receiving food with 50% CO ($P < 0.05$). Furthermore, 242 dietary CO had no significant effect on the liver enzyme 243 activity of catalase (CAT), superoxide dismutase (SOD) and 244 total antioxidant capacity (T-AOC) ($P > 0.05$). However, the 245 activities of CAT, GSH-Px and SOD in the intestine were 246 significantly higher in fish-fed diets with CO when compared 247 with the control group ($P < 0.05$). Dietary CO did not sig-248 nificantly affect the T-AOC and the content of MDA in the 249 intestine ($P > 0.05$) (Table 8).

Table 8 Antioxidant capacity in the liver and intestine of large yellow croaker-fed diets with graded levels of coconut oil

Index	Coconut oil replacement level/%				
	0%	25%	50%	75%	100%
Liver					
CAT (U/mgprot)	20.79 ± 0.78	21.55 ± 0.67	20.80 ± 0.37	22.07 ± 0.31	23.04 ± 0.99
GSH-PX (U/mgprot)	113.08 ± 8.06[a]	120.38 ± 4.42[ab]	166.19 ± 4.36[d]	143.67 ± 2.87[c]	139.46 ± 1.19[bc]
MDA (nmol/mgprot)	18.72 ± 0.27[b]	17.77 ± 0.91[ab]	15.03 ± 0.28[a]	19.37 ± 0.49[b]	24.46 ± 0.98[c]
SOD (U/mgprot)	140.64 ± 6.72[a]	154.62 ± 6.87[ab]	163.28 ± 5.92[b]	143.10 ± 8.73[a]	144.26 ± 4.97[a]
T-AOC (U/mgprot)	1.76 ± 0.10	1.74 ± 0.09	1.93 ± 0.11	1.94 ± 0.07	1.86 ± 0.06
Intestine					
CAT (U/mgprot)	7.70 ± 0.31[a]	11.42 ± 0.83[b]	17.15 ± 1.21[c]	17.14 ± 0.95[c]	18.77 ± 0.91[c]
GSH-PX (U/mgprot)	62.08 ± 5.72[a]	93.38 ± 3.94[b]	128.35 ± 2.91[c]	123.00 ± 3.56[c]	113.09 ± 2.38[c]
MDA (nmol/mgprot)	8.83 ± 1.10	9.01 ± 0.86	8.68 ± 0.66	9.9 ± 1.31	9.99 ± 1.29
SOD (U/mgprot)	96.27 ± 3.69[a]	117.11 ± 2.82[b]	122.57 ± 3.14[b]	136.46 ± 3.43[c]	127.71 ± 1.29[bc]
T-AOC (U/mgprot)	1.80 ± 0.05	1.98 ± 0.05	1.94 ± 0.06	1.85 ± 0.05	2.01 ± 0.06

The data are expressed as means SEM. The data with the same superscript show no significant difference ($P > 0.05$)

SEM standard error of means. ($n = 3$)

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Expression of genes related to lipid metabolism

Expression of genes associated with lipid synthesis

The mRNA expression of *srebp-1* decreased gradually with increasing dietary CO, but there was not any significant difference among dietary treatments ($P > 0.05$). In fish-fed diets with 75–100% CO, the mRNA expression of *acc* and *scd-1* were decreased significantly compared with other groups ($P < 0.05$). The mRNA expression of *fas* was significantly higher in fish-fed diets with 75% and 100% CO than other groups ($P < 0.05$), especially peaking in the 100% CO group (Fig. 1).

Expression of genes associated with lipid β-oxidation

The mRNA expression of *cpt-1*, *aco* and *ppar-α* increased first and then decreased with increasing dietary CO. The peak was reached in the 50% CO group, which was significantly higher than the control group ($P < 0.05$). The mRNA expression of *atgl* in fish-fed diets with CO were increased significantly when compared with the control group, peaking in fish-fed diet containing 100% CO ($P < 0.05$) (Fig. 2).

Discussion

In the present study, the growth of juvenile large yellow croaker was increased significantly in fish-fed diet containing 50% CO. Tseng and Lin (2017) demonstrated that 30 g/kg CO could increase significantly the growth performance of orange-spotted grouper, which is related to the increase of MCFAs content in diets. When compared with long-chain fatty acids (LCFAs), MCFAs are metabolized more rapidly and completely (Villarino et al. 2007). In addition,

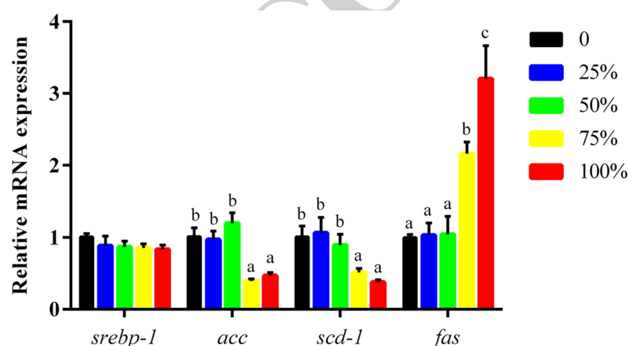

Fig. 1 Effects of dietary CO on the relative expression of sterol regulatory element binding protein-1 (*srebp-1*), stearoyl coenzyme A desaturase (*scd-1*), and fatty acid synthase (*fas*), cetyl-CoA carboxylase (*acc*) in the liver of large yellow croaker. The data are expressed as means SEM. The data with the same superscript show no significant difference ($P > 0.05$). SEM, standard error of means. ($n = 3$)

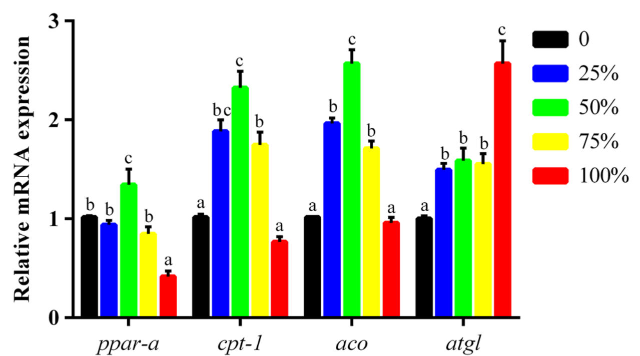

Fig. 2 Effects of dietary CO on the relative expression of peroxisome proliferator-activated receptor α (*ppar-α*), carnitine palmitoyl transferase 1 (*cpt-1*), adipose triglyceride lipase (*atgl*) and acyl-CoA oxidase (*aco*) in the liver of large yellow croaker. The data are expressed as means SEM. The data with the same superscript show no significant difference ($P > 0.05$). SEM, standard error of means. ($n = 3$)

MCFAs are easier to be digested and absorbed (Villarino et al. 2007). Furthermore, MCFAs contribute to the absorption and retention of calcium, magnesium and amino acids (Kadota et al. 2015). Therefore, the optimal MCFAs content in diets may be the main reason to promote growth. However, with increasing dietary CO, namely in fish receiving the diet with 100% CO, a sharp decline was observed in the growth performance. This may be caused by the decrease in diets of essential fatty acid (EFA), which carnivorous fish lack the capacity to synthesize de novo by themselves (Kanazawa 1979; Watanabe 1982). The previous studies demonstrated that when dietary FO was totally replaced by VO, the reason for the decline in growth of marine fish was mostly related to the deficiency of EFA (Turchini et al. 2009; Zuo et al. 2012). Therefore, in order to meet their growth, immunity as well as the normal reproduction and survival of offspring, marine fish must obtain EFA from diets. Turchini et al. (2009) demonstrated that if dietary EFA requirements are met, a significant portion (60–75%) of dietary FO are able to be substituted with alternative lipid sources without significantly affecting the growth performance. Therefore, in this study, growth was not inhibited significantly in fish-fed diet with 100% CO when compared with the control group probably because the dietary EFA met the requirement.

Many studies showed that lipid metabolism of fish was influenced significantly with different dietary FAs composition (Caballero et al. 2002; López et al. 2009; Xu et al. 2011). In this study, with increasing dietary CO, the SFA content in the liver and muscle were increased significantly as the SFA content in diets. Besides, with the decrease in DHA and EPA in diets, the content of DHA and EPA in the liver and muscle was decreased significantly. However, the rate of decrease was lower than that in diets, indicating that DHA and EPA tended to accumulate in the body to maintain the normal physiological function of the fish (Torstensen

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et al. 2004; Trushenski et al. 2010; Zuo et al. 2014). In this study, the HSI and liver lipid content were increased significantly in fish-fed diet with 100% CO compared with the control group. This was consistent with the results in sunshine bass (Nematipour and Gatlin III 1993) and polka dot grouper (Smith et al. 2005). Previous studies have found that fish-fed diets with too much SFAs could make the liver n-3/n-6 PUFA imbalance, resulting in abnormal deposition of fish liver lipid, which is due to excessive SFA. This could affect the metabolism of fatty acids (Leaver et al. 2006; Wang et al. 2016). Therefore, the imbalance of dietary n-3/n-6 PUFA and the low content of n-3 PUFA after the replacement of FO with a high proportion of CO may be one of the main reasons for liver lipid deposition (Zuo et al. 2012).

Compared with SFAs, PUFAs are more vulnerable to free radicals, and are more prone to peroxides (Ferreri and Chatgilialoglu 2009; Wood 1999). Lipid peroxidation may lead to impaired cell membrane function and inactivation of endogenous antioxidant enzymes (Peng et al. 2015; Winzer et al. 2000). The activities of CAT, SOD, and GSH-Px play key roles in the balance of oxidation and antioxidation in fish (Mourente et al. 2007; Ozkan et al. 2013). MDA is the final decomposition product of lipid peroxidation induced by free radicals, which is an important index to measure the degree of liver damage (Martínez et al. 2003). In this study, fish-fed diet with 50% CO showed the highest activities of SOD and GSH-Px, and had the lowest content of MDA in the liver. This suggests that SOD and GSH-Px may contribute to the suppression of MDA. Moreover, the activities of CAT, SOD and GSH-Px in the intestine were significantly higher when compared with fish-fed diets with CO. The results were in accordance with the previous studies in black seabream (Jin et al. 2017) and Russian sturgeon (Li et al. 2017), which demonstrated that fish-fed diets with CO could improve the antioxidant capacity.

The composition of fatty acids in the diet has a great influence on lipid metabolism, especially in lipid synthesis and β-oxidation. Fatty acid synthase (*fas*) plays a key role in lipid synthesis in the liver and is a rate-limiting enzyme for fatty acid synthesis (Smith et al. 2003). In this study, fish-fed diets with high levels of CO showed significantly higher mRNA expression of *fas*. Previous studies have shown that dietary polyunsaturated fatty acids (PUFAs) have an inhibitory effect on *fas* (Blake and Clarke 1990; Kralova et al. 2008). When a high proportion of CO replaced FO, the content of PUFAs in the diet decreased significantly while the SFAs increased significantly, thus reducing the inhibitory effect on *fas* in the liver (Bjermo et al. 2012). Conversely, β-oxidation is an important method of lipid metabolism in fish (Torgeir 1988). *ppar-α* exerts a key role in energy metabolism, which is mainly expressed in the liver. Some important enzymes of fatty acid β-oxidation, such as *aco* and *cpt-1,* were regulated by *ppar-α* (Kersten 2014; Souza-Mello 2015). In this study, fish-fed diet with 100% CO showed the lowest mRNA expression of *ppar-α*, *aco* and *cpt-1*. A previous study using Nile tilapia demonstrated that low expression of *ppar-α* could decrease lipid degradation, which was similar to that of mammals (Ning et al. 2016). Hence, it was concluded that the main reasons for the increase in lipid content in the liver may be attributed to the inhibition of *ppar-α*, *aco*, *cpt-1* and the activation of *fas*, which accelerates the lipid accumulation process in the liver. Dietary CO at an appropriate level has been claimed to have numerous beneficial health effects. Moreover, the utilization efficiency of MUFA is also very efficient when compared with PUFA AQ6 (Villarino et al. 2007). Therefore, fish-fed diet with 50% CO could facilitate the conversion of fatty acids into energy, and thus promote growth performance.

Conclusion

In summary, the results of this study showed that dietary 50% CO could promote the growth performance of large yellow croaker. The beneficial effects could be attributed to the optimal dietary FAs composition and the increased activities of antioxidant enzymes in the liver and intestine.

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Author contributions QA and TD designed the experiments. TD, NX, YL, QL and ZY participated in breeding experiments and the production of feed. TD, NX, YL, DX, XJ and ZY collected the samples. TD performed the experiments and analyzed the data. TD wrote the paper. QA and KM directed and supervised the experiment. The final manuscript was approved by all the authors.

Compliance with ethical standards

Conflict of interest All the authors declare that there are no conflicts of interest.

Animal and human rights statement All applicable international, national and institutional guidelines for the care and use of animals were followed by the authors.

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