British Journal of Nutrition (2021), 125, 732–742 doi:10.1017/S0007114520003244

Effects of dietary terrestrial oils supplemented with L-carnitine on growth, antioxidant capacity, lipid metabolism and inflammation in large yellow croaker (*Larimichthys crocea*)

Xueshan Li[1], Qiang Chen[1], Qiuchi Chen[1], Kangsen Mai[1,2] and Qinghui Ai[1,2]*

[1]*Key Laboratory of Aquaculture Nutrition and Feed, Ministry of Agriculture and Rural Affairs, and Key Laboratory of Mariculture, Ministry of Education, Fisheries College, Ocean University of China, Qingdao, Shandong 266003, People's Republic of China*

[2]*Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao, Shandong 266237, People's Republic of China*

(Submitted 24 February 2020 – Final revision received 10 July 2020 – Accepted 12 August 2020 – First published online 19 August 2020)

Abstract

The present study was conducted to determine the effects of dietary terrestrial oils (TO) supplemented with L-carnitine on growth performance, biochemical and antioxidant response, lipid metabolism and inflammation in large yellow croaker (*Larimichthys crocea*). Three iso-nitrogenous and iso-lipidic experimental diets were formulated with FO (fish oil, the control group), 75 % TO (75 % FO was substituted by the oil mixture with equal amounts of soyabean oil, linseed oil and pork lard) and 75 % TOC (75 % TO supplemented with 800 mg/kg L-carnitine). Compared with the control group, feed efficiency ratio and specific growth rate were significantly increased in fish fed diets with 75 % TO and 75 % TOC. Hepatic lipid content, serum TAG level, LDL-cholesterol level and the mRNA expression of pro-inflammatory genes (*tnfα* and *ifnγ*) were significantly increased in fish fed the diet with 75 % TO compared with the control group. However, the supplementation of 800 mg/kg L-carnitine in the 75 % TO diet repressed hepatic lipid content, serum LDL-cholesterol level and the mRNA expression of *tnfα* and *ifnγ* in fish compared with fish fed the diet with 75 % TO. Total antioxidant capacity, the activity of superoxide dismutase, the mRNA expression of *cpt-I* and the activity of CPT-I were significantly increased in fish fed the diet with 75 % TOC compared with 75 % TO. In conclusion, these results suggested that the supplementation of 800 mg/kg L-carnitine in the diet with TO mixture could increase growth, antioxidant capacity and fatty acid oxidation and decrease the expression of inflammatory genes in large yellow croaker.

Key words: ***Larimichthys crocea*: Terrestrial oils: L-Carnitine: Lipid metabolism: Inflammation**

The rapidly developing aquaculture industry is accompanied by a growing demand for fish oil (FO)[1]. Due to the high price and limited supply of FO, terrestrial oils (TO), which mainly consist of vegetable oil and animal fat, are commonly used to replace FO in aquafeed industry[2]. Many studies have demonstrated that TO can partially replace FO without significantly decreasing growth performance or feed utilisation of European sea bass (*Dicentrarchus labrax* L.)[3], gilt-head sea bream (*Sparus aurata*)[4], Atlantic salmon (*Salmo salar*)[5], turbot (*Scophthalmus maximus* L.)[6] and silvery-black porgy (*Sparidentex hasta*)[7]. However, the decrease in growth and non-specific immunity has also been repeatedly reported in fish when dietary FO is excessively replaced by TO, probably due to the unbalanced dietary fatty acid profile[8,9].

All fish species have an essential demand of both *n*-3 and *n*-6 PUFA[10]. Generally, freshwater and diadromous fish species require linoleic acid (LNA) and *α*-linolenic acid (ALA), whereas marine fish species strictly require long-chain PUFA (LC-PUFA), such as EPA, DHA and arachidonic acid[11,12]. Furthermore, an appropriate ratio of *n*-3:*n*-6 PUFA is important for the growth and health performance of Malabar grouper (*Epinephelus malabaricus*)[13], salmon[14,15] and yellow catfish (*Pelteobagrus fulvidraco*)[16]. In addition to the need for PUFA, fish also need an appropriate amount of SFA and MUFA, which are good dietary energy sources for fish[2,17]. Animal fat contains a high percentage of SFA and MUFA, and it is a cheap alternative lipid resource for aquafeed industry[18]. However, few studies have investigated the effects of a relatively appropriate dietary fatty acid

Abbreviations: *aco*, acyl-CoA oxidase; ALA, α-linolenic acid; *arg-1*, arginase-1; *cd36*, cluster of differentiation 36; *cox-2*, cyclo-oxygenase-2; *cpt-I*, carnitine palmitoyltransferase 1; *fas*, fatty acid synthase; *fatp1*, fatty acid transport protein 1; FO, fish oil; *gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *hl*, hepatic lipase; *ifnγ*, interferon γ; LNA, linoleic acid; *mtp*, microsomal TAG transfer protein; *srebp1*, sterol-regulatory element binding protein 1; T-AOC, total antioxidant capacity; TO, terrestrial oils.

* **Corresponding author:** Professor Qinghui Ai, email qhai@ouc.edu.cn

profile on growth performance, metabolism and health of fish when dietary FO is replaced by a high level of TO.

Large yellow croaker (*Larimichthys crocea*) is an economically important fish in China. When dietary FO is excessively replaced by TO, inflammatory response and abnormal lipid deposition in large yellow croaker often cause huge economic losses[19,20]. Progress is urgently needed to find effective additives to reduce inflammation and abnormal lipid deposition in fish fed the diet with a high level of TO. L-Carnitine plays a crucial role in shuttling long-chain fatty acids into mitochondria[21] and reduces abnormal lipid deposition[22,23]. L-Carnitine can also increase the immunocompetence of several fish species to better cope with biotic and abiotic stresses[24–26]. However, few studies have elucidated the effects of a high level of dietary TO supplemented with L-carnitine on fish. Therefore, the purpose of this study was to determine the effects of a high level of dietary TO supplemented with L-carnitine on growth, lipid deposition and inflammation of large yellow croaker for the wide usage of TO in aquafeed industry.

Methods

Animal ethics

The protocols for animal care and handling were conducted in strict accordance with the Management Rule of Laboratory Animals (Chinese order no. 676 of the State Council, revised 1 March 2017). The present study was approved by the Institutional Animal Care and Use Committee of the Ocean University of China.

Diets formulation

Three iso-nitrogenous (42 % crude protein) and iso-lipidic (12 % crude lipid) experimental diets were formulated, which included FO (the control group), 75 % TO (75 % FO was substituted by TO, dietary lipid source consisted of equal amounts of FO, soyabean oil, linseed oil and pork lard) and 75 % TOC (75 % TO supplemented with 800 mg/kg L-carnitine). 75 % FO was substituted by TO to preserve an appropriate fatty acid profile in the 75 % TO diet. The addition of L-carnitine was based on Sang's[27] study. Ingredients were purchased from Qingdao Great Seven Biotechnology Co. Ltd. Each diet was supplemented with vitamin and mineral premixes, an attractant mixture and a mold inhibitor. Procedures for making diets and storage of experimental diets were in accordance with the protocol described by Ai *et al.*[28]. Ingredients of the three experimental diets (Table 1) and fatty acid profile of oil sources and diets (Table 2) were given in detail.

Fish and experimental procedures

Large yellow croaker juveniles were obtained from Ningde Fufa Fishery Co. Ltd. Before the formal experiment, experimental fish were hand-fed twice daily (05.30 and 17.30 hours) to acclimatise the fish to the diet and environment for 2 weeks. Fish of disease-free and homogenous sizes (initial body weight 15·84 (SEM 0·12) g) were fasted for 24 h. Then, 720 fish were randomly allocated to twelve floating net cages (1·0 m × 1·0 m × 2·0 m).

Table 1. Formulation and chemical proximate analysis of the experimental diets (% dry weight)

	Experimental diets		
Ingredients	FO	75 % TO	75 % TOC
White fishmeal	35	35	35
Soyabean meal	28	28	28
Wheat meal	23·8	23·8	23·72
Soyabean lecithin	1·5	1·5	1·5
Vitamin premix	2	2	2
Mineral premix*	2	2	2
Attractant mixture†	0·1	0·1	0·1
Mold inhibitor‡	0·1	0·1	0·1
Fish oil	7·5	1·875	1·875
Soyabean oil	0	1·875	1·875
Linseed oil	0	1·875	1·875
Pork lard	0	1·875	1·875
L-Carnitine	0	0	0·08
Total	100	100	100
Proximate analysis (DM %, mean values)			
Crude protein	42·29	42·49	42·07
Crude lipid	12·23	12·34	11·98

FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine.
* The mixture of mineral mixture and vitamin mixture was purchased from Qingdao Master Biotechnology Co. Ltd.
† Attractant: mixture of 50 % glycine acid and 50 % betaine by weight.
‡ Mold inhibitor: mixture of 50 % calcium propionic acid and 50 % fumaric acid by weight.

Fish were hand-fed to apparent satiation, twice daily for 70 d. Each diet was randomly allocated to four replicate cages (sixty fish per cage). During the rearing period, the dissolved oxygen content was approximately 7·0 mg/l. Salinity ranged from 25·0 to 32·0 g/l and water temperature from 21·5 to 31·0°C.

Sample collection and analysis of growth data

At the termination of the experiment, fish were starved for 24 h and anaesthetised with MS222 (1:10 000; Sigma). Then, the number and weight of fish were determined for analyses of survival rate and final body weight. The wet weight of the liver, visceral and body and the length of six fish per cage were determined for analyses of morphometric parameters. The muscle and liver samples of the above six fish per cage were collected into 5 ml centrifuge tubes and stored at –80°C for compositional and fatty acid profile analyses. Blood samples of six fish per cage were collected from the caudal vein with a 1 ml syringe and clotted at room temperature to obtain serum samples for biochemical analyses. Liver samples were collected from twelve fish per cage, which contained six fish after serum collection. Samples were immediately frozen in liquid N_2 and stored at –80°C for analyses of antioxidant capacity, enzyme activity and gene expression.

Proximate composition and fatty acid profile analyses

DM was analysed by drying whole fish samples to a constant weight at 105°C. Crude protein and crude lipid contents of ingredients, diets and fish bodies were measured in accordance with the standard methods of the Association of Official Analytical Chemists (AOAC, 1995)[30]. Fish tissue and diet samples were freeze-dried in a lyophilised chamber (Alpha 1-4 LDplus;

British Journal of Nutrition

Table 2. Fatty acid profile of oil sources and the experimental diets (% total fatty acids)* (Mean values)

Fatty acid (% total fatty acids)	Fish oil†	Soyabean oil†	Linseed oil†	Pork lard	Experimental diets		
					FO	75 % TO	75 % TOC
14 : 0	8·77	0·08	0·07	1·23	6·84	2·38	2·31
16 : 0	19·15	10·32	5·39	22·41	22·92	14·63	14·41
18 : 0	4·26	3·95	3·76	15·53	5·61	5·39	5·44
20 : 0	1·18	0·27	0·17	0·83	1·45	0·98	1·28
∑SFA	33·36	14·62	9·39	40·01	36·82	23·38	23·44
16 : 1*n*-7	11·85	0·09	0·10	1·33	8·92	3·54	3·59
18 : 1*n*-9	9·75	26·10	20·45	34·40	13·80	19·17	19·40
∑MUFA	21·60	26·19	20·55	35·73	22·72	22·71	22·99
18 : 2*n*-6	1·54	49·65	15·52	18·37	11·62	23·23	23·65
20 : 4*n*-6	1·30	0·00	0·00	0·24	1·25	0·53	0·59
∑*n*-6 PUFA	2·84	49·65	15·52	18·61	12·87	23·76	24·24
18 : 3*n*-3	0·76	4·92	53·02	0·84	1·50	10·88	10·97
20 : 5*n*-3	12·34	0·00	0·00	0·00	7·22	3·55	3·23
22 : 6*n*-3	7·30	0·00	0·00	0·00	5·36	3·23	3·07
∑*n*-3 PUFA	20·41	4·92	53·02	0·84	14·08	17·66	17·26
n-3:*n*-6 PUFA	7·18	0·10	3·42	0·05	1·09	0·74	0·71
∑*n*-3 LC-PUFA	19·64	0·00	0·00	0·00	12·58	6·77	6·30

FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine; LC-PUFA, long-chain PUFA.
* The contents of some fatty acids are minor and not detected, such as C20 : 0, C22 : 0, C24 : 0, C14 : 1, C20 : 1*n*-9, C22 : 1*n*-11, C20 : 2*n*-6, C18 : 3*n*-6, C20 : 3*n*-6, C18 : 4*n*-3 and C22 : 5*n*-3.
† Fatty acid profile of fish oil, soyabean oil and linseed oil is according to Li *et al.*[29] supplemental data.

Table 3. Sequences of the PCR primers used in this study

Target gene	Forward primers (5′-3′)	Reverse primers (5′-3′)	Accession number
acc1	GACTTGGCGGAATACCTACTGG	GCTTGCTGGATGATCTTTGCTT	XM027273319
aco	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	JX456348
apoB100	AGAGTGTTGTCCAGGATAAAGATGC	CAGGGCTCAGGGTCTCAGTC	KM593126
arg1	AACCACCCGCAGGATTACG	AAACTCACTGGCATCACCTCA	XM019269015
β-Actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	GU584189
cd36	GAGCATGATGGAAAATGGTTCAAAG	CTCCAGAAACTCCCTTTCACCTTAG	KM593122
cox-2	CTGGAAAGGCAACACAAGC	CGGTGAGAGTCAGGGACAT	KP259877
cpt-I	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	JX434612
dgat2	TTCGGTGCTTTCTGCAACTTCG	AAGGATGGGGAAGCGGAAGT	MG013506
elovl4	GGGCTCTTATTGGCTATGCT	TGCTTCTTCCCGTTATCCTC	XM010739301
elovl5	ATCACCTTCCTTCACATCTATCACC	GAGGCACCGAAGTACGAATGG	JQ320377
fad6	TTCGCTTCCTCTGCTGCTATG	CCAGTCACGGTGCTTCTCG	JX434611
fas	CAGCCACAGTGAGGTCATCC	TGAGGACATTGAGCCAGACAC	JX456351
fatp1	CAACCAGCAGGACCCATTACG	CATCCATCACCAGCACATCACC	KM593124
gapdh	GACAACGAGTTCGGATACAGC	CAGTTGATTGGCTTGTTTGG	XM010743420
hl	TCCGTCCATCTATTCATTGACTCTC	GCCACTGTGAACCTTCTTGATATTG	JX456350
ifnγ	TCAGACCTCCGCACCATCA	GCAACCATTGTAACGCCACTTA	KM501500
il-1β	CATAGGGATGGGGACAACGA	AGGGGACGGACACAAGGGTA	KJ459927
il-10	AGTCGGTTACTTTCTGTGGTG	TGTATGACGCAATATGGTCTG	XM010738826
il-6	CGACACACCCACTATTTACAAC	TCCCATTTTCTGAACTGCCTCT	KU140675
mtp	ATGTCCAAAATGTTCTCCATGTCTG	ATGTCAATAGCCAACCCTCCTTG	KP027412
scd1	AAAGGACGCAAGCTGGAACT	CTGGGACGAAGTACGACACC	KP202156
srebp1	TCTCCTTGCAGTCTGAGCCAAC	TCAGCCCTTGGATATGAGCCT	KP342262
tnfα	ACACCTCTCAGCCACAGGAT	CCGTGTCCCACTCCATAGTT	EF165623

acc1, acetyl-CoA carboxylase 1; *aco*, acyl-CoA oxidase; *arg-1*, arginase-1; *cd36*, cluster of differentiation 36; *cox-2*, cyclo-oxygenase-2; *cpt-I*, carnitine palmitoyltransferase 1; *dgat2*, diacylglycerol acyltransferase 2; *elovl4*, long-chain fatty acid protein 4; *elovl5*, elongation of very long-chain fatty acid protein 5; *fad6*, fatty acyl desaturase 6; *fas*, fatty acid synthase; *fatp1*, fatty acid transport protein 1; *gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *hl*, hepatic lipase; *ifnγ*, interferon *γ*; *mtp*, microsomal TAG transfer protein; *scd1*, stearoyl-CoA desaturase 1; *srebp1*, sterol-regulatory element binding protein 1.

Christ) to analyse the moisture content. Lipid contents of freeze-dried liver and muscle samples were determined by chloroform/methanol (v/v, 2:1) following the procedures described by Folch *et al.*[31]. Fatty acid profile of oils and freeze-dried samples was measured according to procedures described by Metcalfe *et al.*[32] with minor modifications[33]. Fatty acid profile was separated and determined by a HP6890 gas chromatograph (Agilent Technologies Inc.). Results were shown as the percentage of each fatty acid to total fatty acid content.

Serum parameters and antioxidant capacity analysis

Contents of serum total cholesterol, total TAG, LDL-cholesterol and HDL-cholesterol and activities of serum alanine

Table 4. Growth and somatic parameters of large yellow croakers (Mean values with their standard errors; *n* 4)

	FO		75 % TO		75 % TOC	
	Mean	SEM	Mean	SEM	Mean	SEM
Initial body weight (g)	15·82	0·25	15·84	0·24	15·85	0·16
Final body weight (g)	35·60^{b}	0·32	38·22^{a}	0·46	37·93^{a}	0·35
Specific growth rate (%/d)*	1·16^{b}	0·01	1·26^{a}	0·03	1·25^{a}	0·00
Survival rate (%)†	87·92	1·97	90·83	0·83	89·17	1·60
Feed intake (%/d)‡	2·13	0·02	2·12	0·02	2·10	0·02
Feed efficiency ratio§	0·52^{b}	0·00	0·56^{a}	0·01	0·56^{a}	0·01
Viscerosomatic index (%)‖	6·92	0·23	7·18	0·21	7·05	0·30
Hepatosomatic index (%)¶	1·98^{b}	0·04	2·14^{a}	0·03	2·07^{a,b}	0·05
Condition factor (%)**	1·02	0·03	1·05	0·02	1·00	0·04

FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine.
^{a,b} Mean values in a row sharing the same superscript letter or absence of letters are not significantly different determined by Tukey's test ($P > 0·05$).
* Specific growth rate (%/d) = (Ln (final body weight) − Ln (initial body weight)) × 100/duration of experimental days.
† Survival rate (%) = 100 × final fish number/initial fish number.
‡ Feed intake (%/d) = 100 × dry feed fed in g/((final body weight + initial body weight)/2)/duration of experimental days.
§ Feed efficiency ratio = wet weight gain in g/dry feed fed in g.
‖ Viscerosomatic index (%) = 100 × visceral wet weight/final body weight.
¶ Hepatosomatic index (%) = 100 × liver wet weight/final body weight.
** Condition factor (%) = 100 × final body weight/(final body length^{3}).

transaminase and aspartate aminotransferase were analysed with matching commercial reagent kits (Mindray Bio Medical Co. Ltd) by an automatic biochemical analyzer (BS180; Mindray). Activities of catalase, superoxide dismutase, total antioxidant capacity (T-AOC) and the content of malondialdehyde in the liver of fish were determined by commercial kits (Nanjing Jiancheng Bioengineering Institute).

Activities of carnitine palmitoyltransferase-I and acyl-CoA oxidase

Mitochondria was extracted, and its integrity was assessed according to procedures described by Suarez & Hochachka[34] and Aprille & Asimakis[35], respectively. CPT-I activity was determined according to the method of Bieber & Fiol[36] with some modifications[37,38]. Briefly, CPT-I activity was determined by the CoA-SH formation in the 5',5'-dithio-bis-(2-nitrobenzoic acid) (Sigma) reaction from palmitoyl-CoA (Sigma) at 412 nm. Results were expressed as 1 μmol of product formed per min per mg of mitochondrial protein at 25°C. Protein concentration of extracted samples was determined according to procedures of Bradford Protein Assay Kit (Beyotime Biotechnology).

Peroxisomal ACO activity was determined by the H_2O_2-dependent oxidation of 2',7'-dichlorofluorescine (Sigma) according to the method of Small *et al.*[39]. The reaction medium consisted of 11 mM potassium phosphate buffer (pH 7·4) (Sangon biotech), 50 μM horseradish peroxidase type II (Sigma), 0·05 mM 2',7'-dichlorofluorescine (Sigma), 40 mM 3-Amino-1,2,4-triazole (Sigma) and 0·02 % Triton-X 100 (Sangon biotech), and the reaction was initiated by the addition of 30 μM palmitoyl-CoA (Sigma). The assay medium contained 10–40 mg of protein in a total volume of 1 ml.

Total RNA extraction, cDNA synthesis and real-time quantitative PCR

Total RNA was extracted from the liver of experimental fish using TRIzol reagent (Takara), and the quality and quantity of RNA were detected by a 1·2 % denaturing agarose gel and a NanoDrop spectrophotometer (Thermo Scientific), respectively. The extracted RNA was reversely transcribed to cDNA by PrimeScript™ RT reagent Kit (Takara). *β-Actin* and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) were used as reference genes to normalise the expression levels of genes[40]. The real-time quantitative PCR primers (Table 3) of candidate genes were designed based on nucleotide sequences of large yellow croaker. The real-time quantitative PCR was performed in a quantitative thermal cycler (Mastercycler ep realplex; Eppendorf). The volume and procedure of real-time quantitative PCR were carried out as described previously[41]. Only one PCR product in these reactions was confirmed by melting curve analysis at the end of each reaction. Standard curves were generated with 4-fold serial dilutions (in triplicate) of cDNA sample, and the amplification efficiency was analysed by the equation: $E = 10^{(-1/\text{slope})} - 1$[42]. The amplification efficiencies of all detected genes ranged between 0·95 and 1·04. The expression levels of all genes were calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$ method) as described by Livak & Schmittgen[43].

Statistical analyses

All data analyses were processed by SPSS 19.0 (IBM). Results were shown as mean values with their standard errors. Statistics were performed to a one-way ANOVA and followed by Tukey's test. Statistics with $P < 0·05$ were considered to be significant.

Results

Survival and growth performance

The survival rate of fish ranged from 87·92 to 90·83 %, but no significant differences were found among dietary treatments ($P > 0·05$). The dietary oil source markedly affected specific growth rate and feed efficiency ratio of fish in the present study, with specific growth rate and feed efficiency ratio significantly

Table 5. Body composition analysis of large yellow croakers (Mean values with their standard errors; *n* 4)

	FO		75 % TO		75 % TOC	
	Mean	SEM	Mean	SEM	Mean	SEM
Whole body (% wet weight)						
Moisture	74·99	0·40	75·06	0·73	75·96	0·80
Lipid	7·16	0·19	6·91	0·37	6·54	0·17
Protein	14·74	0·15	14·86	0·28	14·32	0·17
Liver (% wet weight)						
Moisture	59·64[a]	1·08	53·10[b]	1·11	56·80[a,b]	1·53
Lipid	22·10[b]	0·66	27·13[a]	0·63	23·32[b]	0·94
Muscle (% wet weight)						
Moisture	73·31[b]	0·61	72·53[b]	0·48	74·48[a]	0·64
Lipid	7·95	0·37	8·53	0·34	8·60	0·38

FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine.
[a,b] Mean values in a row sharing the same superscript letter or absence of letters are not significantly different determined by Tukey's test ($P > 0·05$).

increased in fish fed diets with 75 % TO and 75 % TOC compared with the control group ($P < 0·05$). Hepatosomatic index was dramatically increased in fish fed the diet with 75 % TO compared with the control group ($P < 0·05$). When the 75 % TO diet was supplemented with 800 mg/kg L-carnitine, a decreasing trend (from 2·14 to 2·07) was observed in hepatosomatic index of fish compared with fish fed the diet with 75 % TO. There was no significant difference in feed intake, viscerosomatic index or condition factor of fish among dietary treatments ($P > 0·05$) (Table 4).

Body composition analysis

There was no significant difference in the moisture, lipid or protein contents of whole fish body among dietary treatments ($P > 0·05$). Fish fed the diet with 75 % TO had markedly higher lipid content of the liver than the control group ($P < 0·05$). However, the lipid content of the liver was decreased in fish fed the diet with 75 % TOC compared with 75 % TO ($P < 0·05$). No significant difference was found in lipid content of the liver in fish fed the diet with 75 % TOC and the control group. The moisture was markedly decreased in the liver of fish fed the diet with 75 % TO compared with the control group ($P < 0·05$). However, the moisture was dramatically increased in the muscle of fish fed the diet with 75 % TOC compared with 75 % TO and the control group ($P < 0·05$) (Table 5).

Fatty acid profile in the liver

The contents of C14 : 0, C16 : 0, C16 : 1*n*-7, C20 : 4*n*-6 (arachidonic acid), C20 : 5*n*-3 (EPA), C22 : 6*n*-3 (DHA), SFA, *n*-3 LC-PUFA and the ratio of *n*-3:*n*-6 PUFA were dramatically decreased in the liver of fish fed diets with 75 % TO and 75 % TOC compared with the control group ($P < 0·05$). Furthermore, the contents of C18 : 1*n*-9, C18 : 2*n*-6, C18 : 3*n*-3 and *n*-6 PUFA were significantly increased in the liver of fish fed diets with 75 % TO and 75 % TOC compared with the control group ($P < 0·05$). The supplementation of 800 mg/kg L-carnitine in the 75 % TO diet significantly decreased the content of C18 : 3*n*-3, DHA and *n*-3 LC-PUFA in the liver of fish compared with fish fed the diet with 75 % TO (Table 6).

Serum biochemical indexes

The content of TAG was dramatically increased in fish fed the diet with 75 % TO compared with the control group ($P < 0·05$). The content of HDL-cholesterol was significantly decreased in fish fed the diet with 75 % TO compared with the control group ($P < 0·05$). The content of LDL-cholesterol was significantly decreased, but the content of HDL-cholesterol was significantly increased in fish fed the diet with 75 % TOC compared with 75 % TO ($P < 0·05$). No significant difference was found in activities of serum aspartate aminotransferase or alanine transaminase in fish among dietary treatments ($P > 0·05$) (Table 7).

Antioxidant capacity

A decreasing trend (from 1·16 to 1·03) was found in the activity of T-AOC (Fig. 1(C)) in fish fed the diet with 75 % TO compared with the control group, but no significant difference was found between the two treatments ($P > 0·05$). The supplementation of 800 mg/kg L-carnitine in the 75 % TO diet significantly increased the activity of superoxide dismutase (Fig. 1(B)) and T-AOC (Fig. 1(C)) in fish compared with fish fed the diet with 75 % TO ($P < 0·05$). No significant differences were found in the content of malondialdehyde (Fig. 1(A)) or the activity of catalase (Fig. 1(D)) in fish among dietary treatments ($P > 0·05$).

Enzyme activities of CPT-I and ACO

The activity of CPT-I was dramatically increased in fish fed the diet with 75 % TOC compared with fish fed the diet with 75 % TO and the control group ($P < 0·05$). However, no significant difference was found in the activity of ACO among dietary treatments ($P > 0·05$) (Fig. 2).

Expression of genes related to lipid metabolism

The mRNA expression of *diacylglycerol acyltransferase 2* (*dgat2*) and *acetyl-CoA carboxylase 1* (*acc1*) was dramatically increased in fish fed the diet with 75 % TO compared with fish fed the diet with 75 % TOC and the control group ($P < 0·05$) (Fig. 3(C)). Expression of genes related to fatty acid synthesis (*elongation of very long-chain fatty acid protein 4* (*elovl4*), *elongation of very long-chain fatty acid protein 5* (*elovl5*) and *fatty*

Table 6. Fatty acid profile (% total fatty acids) in the liver of large yellow croakers (Mean values with their standard errors; *n* 4)*

	FO		75 % TO		75 % TOC	
	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	4·31[a]	0·26	1·77[b]	0·04	1·85[b]	0·02
16 : 0	22·88[a]	0·57	17·69[b]	0·44	18·13[b]	0·14
18 : 0	6·41	0·49	6·50	0·48	6·64	0·51
20 : 0	1·30	0·11	1·44	0·05	1·50	0·13
∑SFA	34·90[a]	0·66	27·40[b]	0·67	28·12[b]	0·56
16 : 1*n*-7	12·93[a]	0·36	6·84[b]	0·16	7·12[b]	0·09
18 : 1*n*-9	22·16[b]	1·16	27·85[a]	0·64	29·46[a]	0·54
∑MUFA	35·09	0·94	34·69	0·57	36·58	0·47
18 : 2*n*-6	7·90[b]	0·54	18·21[a]	0·67	15·46[a]	1·03
20 : 4*n*-6	0·50[a]	0·03	0·25[b]	0·00	0·22[b]	0·01
∑*n*-6 PUFA	8·41[b]	0·53	18·46[a]	0·67	15·69[a]	1·03
18 : 3*n*-3	1·48[c]	0·23	7·46[a]	0·35	6·09[b]	0·08
20 : 5*n*-3 (EPA)	3·34[a]	0·15	0·78[b]	0·02	0·48[b]	0·04
22 : 6*n*-3 (DHA)	2·08[a]	0·10	0·83[b]	0·01	0·54[c]	0·04
∑*n*-3 PUFA	6·90[b]	0·26	9·07[a]	0·32	7·11[a]	0·07
n-3:*n*-6 PUFA	0·83[a]	0·07	0·49[b]	0·02	0·46[b]	0·03
∑*n*-3 LC-PUFA	5·42[a]	0·23	1·61[b]	0·04	1·02[c]	0·08

FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine; LC-PUFA, long-chain PUFA.
[a,b,c] Mean values in a row sharing the same superscript letter or absence of letters are not significantly different determined by Tukey's test ($P > 0·05$).
* Some fatty acids, of which the contents are minor, trace amount or not detected, such as C20 : 0, C22 : 0, C24 : 0, C14 : 1, C20 : 1*n*-9, C22 : 1*n*-11, C20 : 2*n*-6, C18 : 3*n*-6, C20 : 3*n*-6, C18 : 4*n*-3, C22 : 5*n*-3, are not listed in the table.

Table 7. Serum biochemical indexes and enzyme activities of large yellow croakers (Mean values with their standard errors; *n* 4)

	FO		75 % TO		75 % TOC	
	Mean	SEM	Mean	SEM	Mean	SEM
TC (mmol/l)	2·79	0·29	3·45	0·29	2·89	0·30
TAG (mmol/l)	3·71[b]	0·44	5·09[a]	0·40	3·80[a,b]	0·32
HDL-cholesterol (mmol/l)	0·79[a]	0·03	0·56[b]	0·01	0·77[a]	0·06
LDL-cholesterol (mmol/l)	0·75[a,b]	0·07	0·93[a]	0·11	0·63[b]	0·03
ALT (U/l)	55·76	2·21	60·40	2·63	57·86	3·09
AST (U/l)	5·33	0·47	6·12	0·60	5·65	0·50

FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine; TC, total cholesterol; ALT, alanine transaminase; AST, aspartate aminotransferase.
[a,b] Mean values in a row sharing the same superscript letter or absence of letters are not significantly different determined by Tukey's test ($P > 0·05$).

acyl desaturase 6 (*fad6*)) was dramatically increased in fish fed diets with 75 % TO and 75 % TOC compared with the control group ($P < 0·05$) (Fig. 3(D)). The mRNA expression of *cpt-I* and *cluster of differentiation 36* (*cd36*) was significantly increased in fish fed the diet with 75 % TOC compared with fish fed the diet with 75 % TO and the control group ($P < 0·05$) (Fig. 3(A) and (B)). The mRNA expression of *stearoyl-CoA desaturase 1* (*scd1*) and *hepatic lipase* (*hl*) was significantly increased in fish fed the diet with 75 % TOC compared with the control group ($P < 0·05$) (Fig. 3(A) and (D)). There was no significant difference in the mRNA expression of *sterol-regulatory element binding protein 1* (*srebp1*), *fatty acid synthase* (*fas*), *aco, fatty acid transport protein 1* (*fatp1*), *microsomal TAG transfer protein* (*mtp*) or *apob100* among dietary treatments ($P > 0·05$) (Fig. 3(A)–(C)).

Expression of genes related to inflammation

The mRNA expression of pro-inflammatory genes (*tnfα* and *interferon γ* (*ifnγ*)) was significantly increased in fish fed the diet with 75 % TO compared with the control group ($P < 0·05$), while they both were repressed in fish fed the diet with 75 % TOC compared with 75 % TO ($P < 0·05$) (Fig. 4(B)). The mRNA expression of anti-inflammatory genes (*il-10* and *arginase-1* (*arg1*)) was significantly increased in fish fed the diet with 75 % TOC compared with fish fed the diet with 75 % TO and the control group ($P < 0·05$) (Fig. 4(A) and (B)). There was no significant difference in the mRNA expression of *il-1β*, *il-6* or *cyclo-oxygenase-2* (*cox-2*) of fish among dietary treatments ($P > 0·05$) (Fig. 4(A) and (B)).

Discussion

In the present study, specific growth rate significantly increased in fish fed diets with 75 % TO and 75 % TOC compared with the control group. The growth-promoting effect was possibly due to the balance of fatty acids with the supplementation of various TO. In the present study, the content of *n*-3 LC-PUFA in 75 % TO and 75 % TOC diets was approximately 0·83 %, which was supplied in an appropriate level for large yellow croaker. A previous study has reported that large yellow croaker fed

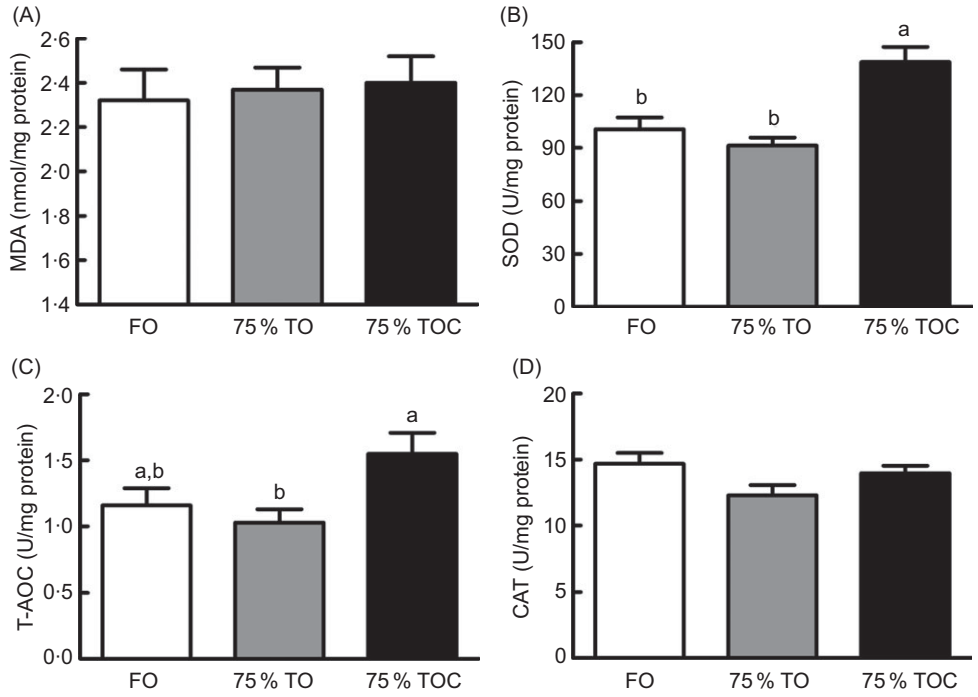

Fig. 1. Concentration of (A) malondialdehyde (MDA) and activities of (B) superoxide dismutase (SOD), (C) total antioxidant capacity (T-AOC) and (D) catalase (CAT) in the liver of large yellow croakers. FO, fish oil; 75 % TO, 75 % fish oil substituted by terrestrial oils; 75 % TOC, 75 % TO supplemented with 800 mg/kg L-carnitine. Values are means (n 4), with their standard errors represented by vertical bars. [a,b] Columns sharing the same letter or absence of letters are not significantly different determined by Tukey's test ($P > 0{\cdot}05$).

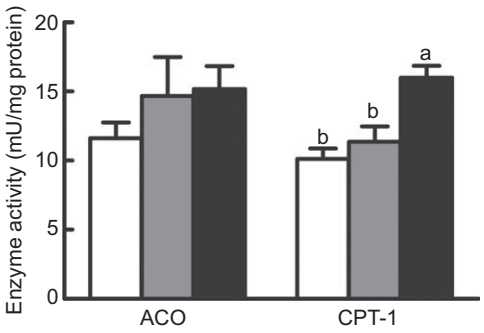

Fig. 2. Enzyme activities of carnitine palmitoyltransferase 1 (CPT-I) and acyl-CoA oxidase (ACO) in the liver of large yellow croakers. (▭), Fish oil (FO); (▬), 75 % FO substituted by terrestrial oils (75 % TO); (■), 75 % TO supplemented with 800 mg/kg L-carnitine. Values are means (n 4), with their standard errors represented by vertical bars. [a,b] Columns sharing the same letter or absence of letters are not significantly different determined by Tukey's test ($P > 0{\cdot}05$).

0·60–0·98 % dietary n-3 LC-PUFA have better growth performance, nonspecific immune responses and disease resistance than the control group (0·15 % n-3 LC-PUFA)[33]. Also, the ratio of ALA:LNA in 75 % TO and 75 % TOC diets was kept in an appropriate value (0·47) for large yellow croaker, while the corresponding ratio in the FO diet (0·13) was lower than the aforementioned ratio in TO diet. A previous study has shown that the ratio of dietary ALA:LNA in 0·5 is beneficial for the growth of large yellow croaker[44].

Previously, Turchini *et al.*[2] found that 60–75 % of dietary FO could be replaced by alternative TO in almost all finfish species when the requirement of essential fatty acid was met. However, a high level of TO might increase abnormal lipid deposition of fish[45]. In this experiment, significantly higher contents of LDL-cholesterol and TAG as well as hepatic lipid content were found in fish fed the diet with 75 % TO than the control group. Similar results were also observed in Malaysian mahseer (*Tor tambroides*)[46], rainbow trout (*Oncorhynchus mykiss*)[47] and turbot[6]. Previous studies have demonstrated that oleic acid is more likely to increase steatosis and plasma lipid concentrations than palmitic acid[48,49] and PUFA[50,51]. Accordingly, the increased lipid deposition of experimental fish could be due to the increase of dietary oleic acid in the 75 % TO diet compared with the FO diet. However, hepatic lipid deposition and the content of LDL-cholesterol were decreased in fish fed the diet with 75 % TOC compared with 75 % TO. Sang *et al.*[27] reported that the supplementation of 800 mg/kg L-carnitine significantly improved growth performance and reduced serum TAG and total cholesterol levels in large yellow croaker compared with the control group ($P < 0{\cdot}05$). The hypolipidemic effect was probably due to the role of L-carnitine in facilitating the transport of long-chain fatty acids into mitochondria for β-oxidation[52,53], as contents of C18 : 3n-3, DHA and total n-3 LC-PUFA were dramatically decreased, and the content of n-6 PUFA was also numerically lower in the liver of fish fed the diet with 75 % TOC compared with 75 % TO.

The decreased content of PUFA could be attributed to changes of fatty acid oxidation and synthesis in fish fed the diet with 75 % TOC compared with 75 % TO. As for fatty acid utilisation and oxidation, CPT-I is a rate-limiting enzyme that controls the mitochondrial uptake of long-chain acyl-CoA and facilitates

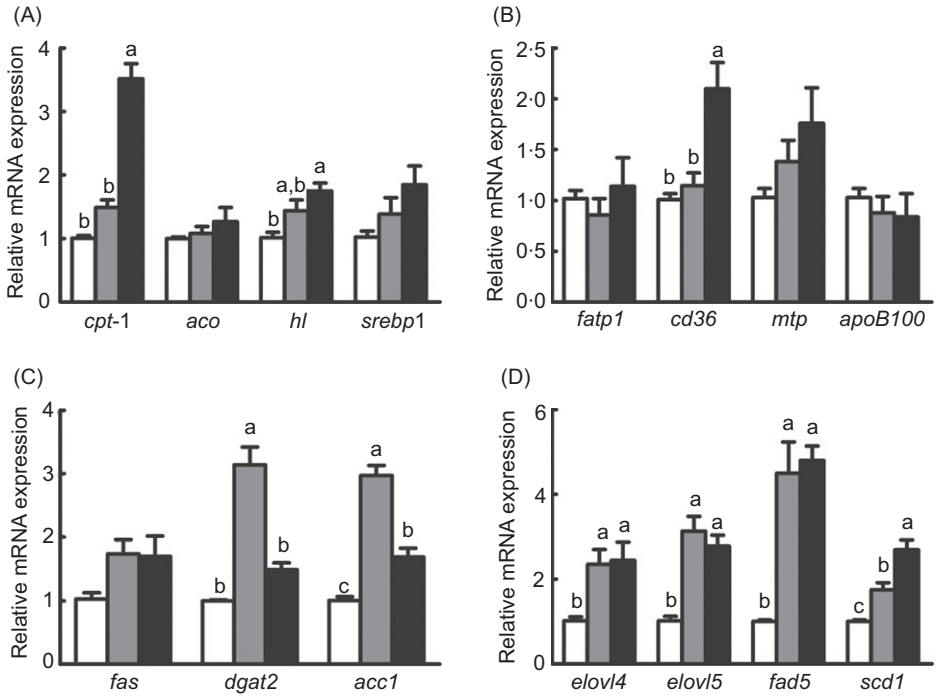

Fig. 3. Expression of genes related to lipid metabolism in the liver of large yellow croakers. *cpt-I*, Carnitine palmitoyltransferase I; *aco*, acyl-CoA oxidase; *hl*, hepatic lipase; *srebp1*, sterol-regulatory element binding protein 1; *fas*, fatty acid synthase; *fatp1*, fatty acid transport protein 1; *cd36*, cluster of differentiation 36; *mtp*, microsomal TAG transfer protein; *dgat2*, diacylglycerol acyltransferase 2; *acc1*, acetyl-CoA carboxylase 1; *elovl4*, long-chain fatty acid protein 4; *elovl5*, elongation of very long-chain fatty acid protein 5; *fad6*, fatty acyl desaturase 6; *scd1*, stearoyl-CoA desaturase 1. (▭), Fish oil (FO); (▬), 75 % FO substituted by terrestrial oils (75 % TO); (■), 75 % TO supplemented with 800 mg/kg L-carnitine. Values are means (n 4), with their standard errors represented by vertical bars. [a,b,c] Columns sharing the same letter or absence of letters are not significantly different determined by Tukey's test ($P > 0{\cdot}05$).

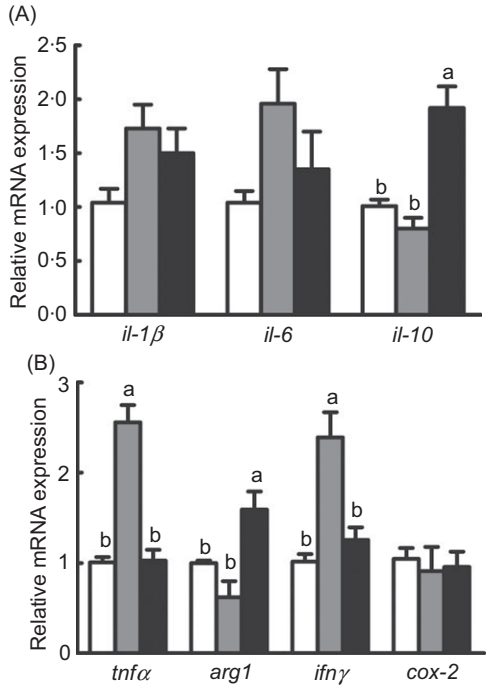

Fig. 4. Expression of genes related to inflammation in the liver of large yellow croakers. *arg-1*, Arginase-1; *ifnγ*, interferon γ; *cox-2*, cyclo-oxygenase-2. (▭), Fish oil (FO); (▬), 75 % FO substituted by terrestrial oils (75 % TO); (■), 75 % TO supplemented with 800 mg/kg L-carnitine. Values are means (n 4), with their standard errors represented by vertical bars. [a,b] Columns sharing the same letter or absence of letters are not significantly different determined by Tukey's test ($P > 0{\cdot}05$).

long-chain fatty acids for β-oxidation[54]. CD36 is a key NEFA uptake transporter that involves transferring fatty acids into cells[55] or mitochondria[56,57] for utilisation. HL is a pivotal TAG lipase that contributes to vascular lipoprotein degradation, TAG hydrolysis and the uptake of lipoprotein into the liver[58]. In the present study, the mRNA expression of *cpt-I*, *cd36* and *hl* as well as the activity of CPT-I was significantly increased in fish fed the diet with 75 % TOC compared with 75 % TO. These results showed that L-carnitine facilitated the utilisation of lipid in the diet and promoted fatty acid oxidation. As for fatty acid synthesis, *elovl4, elovl5* and *fad6* were key genes for the biosynthesis of arachidonic acid, EPA and DHA from LNA and ALA in fish[12]. No significant difference was observed in the mRNA expression of *elovl4*, *elovl5* or *fad6* in fish fed diets with 75 % TOC and 75 % TO, which meant that the supplementation of L-carnitine had no significant effect on the capacity of LC-PUFA biosynthesis. Further, vertebrates cannot synthesise C18 : 2*n*-6 and C18 : 3*n*-3 from C16 : 1*n*-7 and C18 : 1 *n*-9[12], and the capacity of biosynthesis of C20 and C22 LC-PUFA from C18 PUFA is very limited in most marine fish species[59]. Above all, the unchanged PUFA synthesis and increased PUFA oxidation could be attributed to the decreased content of PUFA in fish fed the diet with 75 % TOC compared with 75 % TO. Similarly, dietary L-carnitine promoted the utilisation of fatty acid and decreased the content of PUFA in red porgy (*Pagrus pagrus*, L.)[60], cobia (*Rachycentron canadum*)[61] and beluga sturgeon (*Huso huso*)[62].

Antioxidant capacity is one of the most commonly used indexes to evaluate the physical condition of fish[63]. In this study, the activity of T-AOC showed a decreasing trend (from

1·16 to 1·03) in fish fed the diet with 75 % TO compared with the control group. When the 75 % TO diet was supplemented with 800 mg/kg L-carnitine, the activity of superoxide dismutase and T-AOC was dramatically increased in fish compared with fish fed the diet with 75 % TO. A previous study has reported that L-carnitine induces Nrf2/Keap1 pathway activation *in vivo* and *in vitro* and increases the antioxidant enzyme activities (catalase, T-superoxide dismutase, GSH-PX) in *Rhynchocypris lagowski*[64]. Further, L-carnitine played some crucial physiological roles in the inhibition of superoxide radical formation[65]. The increased antioxidant capacity could be attributed to the modulatory effects of L-carnitine on critical antioxidant enzymes in fish fed the diet with 75 % TOC compared with 75 % TO.

In addition to antioxidant capacity, the inflammation in fish was also an important indicator to evaluate the health status. In the present study, the mRNA expression of pro-inflammatory genes (*tnfα* and *ifnγ*) was significantly higher in fish fed the diet with 75 % TO than the control group. However, the mRNA expression of the pro-inflammatory genes was decreased in fish fed the diet with the 75 % TOC compared with 75 % TO. The mRNA expression of anti-inflammatory genes (*il-10* and *arg-1*) was increased in fish fed the diet with the 75 % TOC compared with 75 % TO. Previous studies have demonstrated that dietary L-carnitine can improve the immunity and disease resistance of juvenile narrow clawed crayfish (*Astacus leptodactylus leptodactylus*)[66], black carp (*Mylopharyngodon piceus*)[67] and common carp (*Cyprinus carpio* L.)[68]. L-Carnitine exerted protective effects by inhibiting the generation of ammonia and xenobiotics in rat hepatocytes[69]. Future research is required to explore the specific mechanism how L-carnitine decreased the inflammation of fish fed the diet with 75 % TOC compared with 75 % TO.

In conclusion, dietary TO mixture (75 % TO and 75 % TOC diets) increased the growth of large yellow croaker compared with the control group, which was probably due to the appropriate level of *n*-3 LC-PUFA and the appropriate ratio of ALA:LNA. Although the growth was not significantly different, the supplementation of 800 mg/kg L-carnitine in the 75 % TO diet (75 % TOC) could increase antioxidant capacity, fatty acid oxidation and decrease lipid abnormal deposition and the expression of inflammatory genes compared with 75 % TO.

Acknowledgements

This work was supported by the Scientific and Technological Innovation of Blue Granary (grant number: 2018YFD0900402), National Science Fund for Distinguished Young Scholars of China (grant number: 31525024), the China Agriculture Research System (grant number: CARS47-11), Key Program of National Natural Science Foundation of China (grant number: 31830103), Leading Talent of Technological Innovation of Ten-Thousands Talents Program (grant number: CS31117200001) and Aoshan Talents Program Supported by Qingdao National Laboratory for Marine Science and Technology (grant number: 2015ASTP).

The authors' contributions were as follows: Q. A. contributed to funding acquisition, project administration and supervision; Q. A. and K. M. designed the research; X. L., Q. C. and Q. C. conducted the research; X. L. analysed the data and wrote the paper. We appreciate Yunqiang Zhang, Jiamin Li, Weiwei Dai, Xiufei Cao, Ye Gong, Tao Ding and Md Golam Sajed Riar for their help in revising the article.

The authors declare that there are no conflicts of interests associated with the manuscript.

References

1. Pickova J & Mørkøre T (2007) Alternate oils in fish feeds. *Eur J Lipid Sci Tech* **109**, 256–263.
2. Turchini GM, Torstensen BE & Ng WK (2009) Fish oil replacement in finfish nutrition. *Rev Aquacult* **1**, 10–57.
3. Mourente G, Good JE, Thompson K, *et al.* (2007) Effects of partial substitution of dietary fish oil with blends of vegetable oils, on blood leucocyte fatty acid compositions, immune function and histology in European sea bass (*Dicentrarchus labrax* L.). *Br J Nutr* **98**, 770–779.
4. Montero D, Mathlouthi F, Tort L, *et al.* (2010) Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream *Sparus aurata*. *Fish Shellfish Immunol* **29**, 1073–1081.
5. Bell JG, McEvoy J, Tocher DR, *et al.* (2001) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J Nutr* **131**, 1535–1543.
6. Peng M, Xu W, Mai KS, *et al.* (2014) Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil. *Aquaculture* **433**, 442–449.
7. Mozanzadeh MT, Agh N, Yavari V, *et al.* (2016) Partial or total replacement of dietary fish oil with alternative lipid sources in silvery-black porgy (*Sparidentex hasta*). *Aquaculture* **451**, 232–240.
8. Emre Y, Kurtoğlu A, Emre N, *et al.* (2016) Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and haematological parameters of juvenile meagre, *Argyrosomus regius*. *Aquac Res* **47**, 2256–2265.
9. Torrecillas S, Mompel D, Caballero MJ, *et al.* (2017) Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). *Aquaculture* **468**, 386–398.
10. Sargent JR, McEvoy LA & Bell JG (1997) Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* **155**, 117–127.
11. Sargent J, Bell G, McEvoy L, *et al.* (1999) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* **177**, 191–199.
12. Tocher DR (2010) Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac Res* **41**, 717–732.
13. Wu FC & Chen HY (2012) Effects of dietary linolenic acid to linoleic acid ratio on growth, tissue fatty acid profile and immune response of the juvenile grouper *Epinephelus malabaricus*. *Aquaculture* **324**, 111–117.
14. Menoyo D, Lopez-Bote CJ, Diez A, *et al.* (2007) Impact of *n*-3 fatty acid chain length and *n*-3/*n*-6 ratio in Atlantic salmon (*Salmo salar*) diets. *Aquaculture* **267**, 248–259.
15. Berge GM, Witten PE, Baeverfjord G, *et al.* (2009) Diets with different *n*-6/*n*-3 fatty acid ratio in diets for juvenile Atlantic salmon, effects on growth, body composition, bone development and eicosanoid production. *Aquaculture* **296**, 299–308.

16. Tan XY, Luo Z, Xie P, *et al.* (2009) Effect of dietary linolenic acid/linoleic acid ratio on growth performance, hepatic fatty acid profiles and intermediary metabolism of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture* **296**, 96–101.
17. Henderson RJ & Sargent JR (1985) Chain-length specificities of mitochondrial and peroxisomal beta-oxidation of fatty acids in livers of rainbow trout (*Salmo gairdneri*). *Comp Biochem Phys B* **82**, 79–85.
18. Monteiro M, Matos E, Ramos R, *et al.* (2018) A blend of land animal fats can replace up to 75 % fish oil without affecting growth and nutrient utilization of European seabass. *Aquaculture* **487**, 22–31.
19. Yan J, Liao K, Wang TJ, *et al.* (2015) Dietary lipid levels influence lipid deposition in the liver of large yellow croaker (*Larimichthys crocea*) by regulating lipoprotein receptors, fatty acid uptake and triacylglycerol synthesis and catabolism at the transcriptional level. *PLOS ONE* **10**, e0129937.
20. Ai QH, Zhao JZ, Mai KS, *et al.* (2008) Optimal dietary lipid level for large yellow croaker (*Pseudosciaena crocea*) larvae. *Aquacult Nutr* **14**, 515–522.
21. Harpaz S (2005) L-Carnitine and its attributed functions in fish culture and nutrition-a review. *Aquaculture* **249**, 3–21.
22. Li JM, Li LY, Zhang YX, *et al.* (2019) Functional differences between L-and D-carnitine in metabolic regulation evaluated using a low-carnitine Nile tilapia model. *Br J Nutr* **122**, 625–638.
23. Chen YF, Sun ZZ, Liang ZM, *et al.* (2020) Addition of L-carnitine to formulated feed improved growth performance, antioxidant status and lipid metabolism of juvenile largemouth bass, *Micropterus salmoides*. *Aquaculture* **518**, 734434.
24. Schreiber S, Becker K, Bresler V, *et al.* (1997) Dietary L-carnitine protects the gills and skin of guppies (*Poecilia reticulata*) against anionic xenobiotics. *Comp Biochem Phys C* **117**, 99–102.
25. Li LY, Limbu SM, Ma Q, *et al.* (2018) The metabolic regulation of dietary L-carnitine in aquaculture nutrition: present status and future research strategies. *Rev Aquacult* **11**, 1228–1257.
26. Schlechtriem C, Bresler V, Fishelson L, *et al.* (2004) Protective effects of dietary L-carnitine on tilapia hybrids (*Oreochromis niloticus* × *Oreochromis aureus*) reared under intensive pond-culture conditions. *Aquacult Nutr* **10**, 55–63.
27. Sang WG, Deng SQ, Shentu JK, *et al.* (2012) Effects of dietary L-carnitine and betaine on serum biochemical indices of large yellow croaker (*Pseudosciaena crocea*) cultured in floating net cages. *Adv J Food Sci Technol* **4**, 304–308.
28. Ai QH, Mai KS, Tan BP, *et al.* (2006) Effects of dietary vitamin C on survival, growth, and immunity of large yellow croaker, *Pseudosciaena crocea*. *Aquaculture* **261**, 327–336.
29. Li YN, Pang YN, Xiang XJ, *et al.* (2019) Molecular cloning, characterization, and nutritional regulation of Elovl6 in large yellow croaker (*Larimichthys crocea*). *Int J Mol Sci* **20**, 1801.
30. AOAC (1995) *Official Methods of Analysis of the Association of Analytical Chemistry*, 15th ed. Arlington: AOAC.
31. Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
32. Metcalfe LD, Schmitz AA & Pelka JR (1966) Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* **38**, 514–515.
33. Zuo RT, Ai QH, Mai KS, *et al.* (2012) Effects of dietary *n*-3 highly unsaturated fatty acids on growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow croaker (*Larimichthys crocea*) following natural infestation of parasites (*Cryptocaryon irritans*). *Fish Shellfish Immunol* **32**, 249–258.
34. Suarez RK & Hochachka PW (1981) Preparation and properties of rainbow trout liver mitochondria. *J Comp Physiol* **143**, 269–273.
35. Aprille JR & Asimakis GK (1980) Postnatal development of rat liver mitochondria: state 3 respiration, adenine nucleotide translocase activity, and the net accumulation of adenine nucleotides. *Arch Biochem Biophys* **201**, 564–575.
36. Bieber LL & Fiol C (1986) Purification and assay of carnitine acyltransferases. *Method Enzymol* **123**, 276–284.
37. Chen GH, Luo Z, Chen F *et al.* (2017) PPARα, PPARγ, SREBP-1 pathways mediated waterborne iron (Fe)-induced reduction in hepatic lipid deposition of javelin goby *Synechogobius hasta*. *Comp Biochem Phys C* **197**, 8–18.
38. Zheng JL, Luo Z, Liu CX, *et al.* (2013) Differential effects of the chronic and acute zinc exposure on carnitine composition, kinetics of carnitine palmitoyltransferases I (CPT I) and mRNA levels of CPT I isoforms in yellow catfish *Pelteobagrus fulvidraco*. *Chemosphere* **92**, 616–625.
39. Small GM, Burdett K & Connock MJ (1985) A sensitive spectrophotometric assay for peroxisomal acyl-CoA oxidase. *Biochem J* **227**, 205–210.
40. Zheng JL, Zeng L, Shen B, *et al.* (2016) Antioxidant defenses at transcriptional and enzymatic levels and gene expression of Nrf2-Keap1 signaling molecules in response to acute zinc exposure in the spleen of the large yellow croaker *Pseudosciaena crocea*. *Fish Shellfish Immunol* **52**, 1–8.
41. Li XS, Ji RL, Cui K, *et al.* (2019) High percentage of dietary palm oil suppressed growth and antioxidant capacity and induced the inflammation by activation of TLR-NF-κB signaling pathway in large yellow croaker (*Larimichthys crocea*). *Fish Shellfish Immunol* **87**, 600–608.
42. Nolan T, Hands RE & Bustin SA (2006) Quantification of mRNA using real-time RT-PCR. *Nat Protoc* **1**, 1559.
43. Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.
44. Zuo RT, Mai KS, Xu W, *et al.* (2015) Dietary ALA, but not LNA, increase growth, reduce inflammatory processes, and increase anti-oxidant capacity in the marine finfish *Larimichthys crocea*. *Lipids* **50**, 149–163.
45. Menoyo D, Izquierdo MS, Robaina L, *et al.* (2004) Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. *Br J Nutr* **92**, 41–52.
46. Kamarudin MS, Ramezani-Fard E, Saad CR *et al.* (2012) Effects of dietary fish oil replacement by various vegetable oils on growth performance, body composition, fatty acid profile of juvenile Malaysian mahseer, *Tor tambroides*. *Aquacult Nutr* **18**, 532–543.
47. Yıldız M, Eroldoğan TO, Ofori-Mensah S, *et al.* (2018) The effects of fish oil replacement by vegetable oils on growth performance and fatty acid profile of rainbow trout: re-feeding with fish oil finishing diet improved the fatty acid composition. *Aquaculture* **488**, 123–133.
48. Moravcová A, Ervinková Z, Kuera O, *et al.* (2015) The effect of oleic and palmitic acid on induction of steatosis and cytotoxicity on rat hepatocytes in primary culture. *Physiol Res* **64**, S627.
49. Ricchi M, Odoardi MR, Carulli L, *et al.* (2009) Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. *J Gastroenterol Hepatol* **24**, 830–840.
50. Juárez-Hernández E, Chávez-Tapia NC, Uribe M, *et al.* (2016) Role of bioactive fatty acids in nonalcoholic fatty liver disease. *Nutr J* **15**, 72–72.
51. Fernandez ML & West KL (2005) Mechanisms by which dietary fatty acids modulate plasma lipids. *J Nutr* **135**, 2075–2078.
52. Li JM, Li LY, Qin X *et al.* (2017) Systemic regulation of L-carnitine in nutritional metabolism in zebrafish, *Danio rerio*. *Sci Rep* **7**, 40815.

British Journal of Nutrition

53. Ozorio ROA, Van Ginneken V, Bessa RJB, *et al.* (2009) Effects of exercise on L-carnitine and lipid metabolism in African catfish (*Clarias gariepinus*) fed different dietary L-carnitine and lipid levels. *Br J Nutr* **103**, 1139–1150.
54. Bonnefont JP, Djouadi F, Prip-Buus C, *et al.* (2004) Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. *Mol Aspects Med* **25**, 1–520.
55. Zhou J, Febbraio M, Wada T, *et al.* (2008) Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARγ in promoting steatosis. *Gastroenterology* **134**, 556–567.e551.
56. Bonen A, Campbell SE, Benton CR, *et al.* (2004) Regulation of fatty acid transport by fatty acid translocase/CD36. *Proc Nutr Soc* **63**, 245–249.
57. Campbell SE (2004) A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. *J Biol Chem* **279**, 36235–36241.
58. Holmes RS, VandeBerg JL & Cox LA (2011) Vertebrate hepatic lipase genes and proteins: a review supported by bioinformatic studies. *Open Access Bioinformatics* **2011**, 85–95.
59. Castro LFC, Tocher DR & Monroig O (2016) Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire. *Prog Lipid Res* **62**, 25–40.
60. Nogueira N, Cordeiro N, Canada P, *et al.* (2010) Separate and combined effects of cyclic fasting and L-carnitine supplementation in red porgy (*Pagrus pagrus*, L. 1758). *Aquac Res* **41**, 795–806.
61. Wang JT, Han T, Tian LX, *et al.* (2007) Effect of dietary L-Carnitine supplements on growth and body composition of juvenile cobia (*Rachycentron canadum*). *J Zhejiang Ocean Univ* **26**, 125–131.
62. Mohseni M, Ozorio R, Pourkazemi M, *et al.* (2008) Effects of dietary L-carnitine supplements on growth and body composition in beluga sturgeon (*Huso huso*) juveniles. *J Appl Ichthyol* **24**, 646–649.
63. Tan XH, Sun ZZ, Liu QY, *et al.* (2018) Effects of dietary ginkgo biloba leaf extract on growth performance, plasma biochemical parameters, fish composition, immune responses, liver histology, and immune and apoptosis-related genes expression of hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus*♀) fed high lipid diets. *Fish Shellfish Immunol* **72**, 399–409.
64. Zhang DM, Guo ZX, Zhao YL, *et al.* (2019) L-Carnitine regulated Nrf2/Keap1 activation *in vitro* and *in vivo* and protected oxidized fish oil-induced inflammation response by inhibiting the NF-κB signaling pathway in *Rhynchocypris lagowski* Dybowski. *Fish Shellfish Immunol* **93**, 1100–1110.
65. Gülçin İ (2006) Antioxidant and antiradical activities of L-carnitine. *Life Sci* **78**, 803–811.
66. Safari O, Atash MMS & Paolucci M (2015) Effects of dietary L-carnitine level on growth performance, immune responses and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823. *Aquaculture* **439**, 20–28.
67. Ming JH, Ye JY, Zhang YX *et al.* (2013) The influence of maggot meal, L-carnitine on growth, immunity, antioxidant indices, disease resistance of black carp (*Mylopharyngodon piceus*). *J Chinese Cereals Oils Assoc* **28**, 80–86.
68. Chen XM, Guo GL, Sun L, *et al.* (2017) Modulatory role of L-carnitine against microcystin-LR-induced immunotoxicity and oxidative stress in common carp. *Fish Physiol Biochem* **43**, 1081–1093.
69. Nakamura Y, Iida H, Nakatake R, *et al.* (2018) L-Carnitine has a liver-protective effect through inhibition of inducible nitric oxide synthase induction in primary cultured rat hepatocytes. *Funct Foods Health Dis* **8**, 212–227.