DOI: 10.1111/are.13574

ORIGINAL ARTICLE

WILEY Aquaculture Research

Effects of dietary tea polyphenols on growth, biochemical and antioxidant responses, fatty acid composition and expression of lipid metabolism related genes of large yellow croaker (*Larimichthys crocea*)

Renlei Ji[1] | Yicong Li[1] | Xueshan Li[1] | Xiaojun Xiang[1] | Yongnan Li[1] | Si Zhu[1] | Bo Yang[1] | Yanjiao Zhang[1] | Kangsen Mai[1,2] | Qinghui Ai[1,2]

[1]Key Laboratory of Aquaculture Nutrition and Feed (Ministry of Agriculture), Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao, Shandong, China

[2]Laboratory for Marine Fisheries and Aquaculture, Qingdao National Laboratory for Marine Science and Technology, Qingdao, Shandong, China

Correspondence
Qinghui Ai, Fisheries College, Ocean University of China, Qingdao, Shandong, China
Email: qhai@ouc.edu.cn

Funding information
The National Natural Science Foundation of China, Grant/Award Number: 31372541; The China Agriculture Research System, Grant/Award Number: CARS47-11; AoShan Talents Program Supported by Qingdao National Laboratory for Marine Science and Technology, Grant/Award Number: 2015ASTP-0S05; The National Science Fund for Distinguished Young Scholars of China, Grant/Award Number: 31525024

Abstract

The study was conducted to investigate the effects of dietary tea polyphenols (TP) on growth performance, biochemical and antioxidants responses, fatty acid composition, and lipid metabolism-related gene expressions of large yellow croaker (*Larimichthys crocea*). Four diets were formulated with different levels of TP (0.00%, 0.01%, 0.02% and 0.05%). Results showed that growth performance of *L. crocea* were not different among dietary treatments. Compared with the control group, fish in 0.02% TP group had lower body and hepatic lipid content and lower total cholesterol content. The minimum content of triglycerides and low-density lipoprotein-cholesterol were found in 0.05% TP group. Hepatic n-6 PUFA and n-3 PUFA were significantly higher in TP supplementation groups. Malondialdehyde content was lower in TP supplementation groups, and superoxide dismutase activity was higher in 0.01% TP group than the control group. The mRNA expressions of carnitine palmitoyltransferase1, acyl-CoA oxidase and peroxisome proliferators-activated receptor α were up-regulated in 0.01% and 0.02% TP groups, while lipoprotein lipase expression was down-regulated in TP supplementation groups than the control group. Results suggested that 0.01%–0.02% TP supplementation could reduce the deposition of liver lipid of *L. crocea* caused by high-lipid diet, which might be due to the increase in lipid oxidation related gene expressions.

KEYWORDS
antioxidant capacity, fatty acid composition, gene expression, large yellow croaker, tea polyphenols

1 | INTRODUCTION

High lipid diets (HLD) has been increasingly used for cost-effective farming and protein sparing effect in aquaculture in recent years, indicating that HLD may be more helpful for improving protein utilization and maximizing nitrogen retention in fish (Boujard et al., 2004). However, HLD often lead to abnormal lipid accumulation in the liver with the low disease resistance and hepatocyte injury of farmed fish (Ai et al., 2004; Du et al., 2008), which is harmful to the health and production of fish (Cabello, 2006). More researches are needed to explore a way to reverse the negative effects of HLD.

Tea polyphenols (TP), the main constituent of tea, have been reported to have various pharmacological and biological functions,

such as antioxidant (Benzie & Szeto, 1999), antibacterial and antiviral (Hattori, Kusumoto, Namba, Ishigami & Hara, 1990; Nakayama et al., 1993), anti-obesity (Murase, Nagasawa, Suzuki, Hase & Tokimitsu, 2002), and hypocholesterolemic activities (Ikeda et al., 2003). Besides, studies of lipid metabolism in animals and cells have found that TP reduced the contents of triacylglycerol, total cholesterol and low-density lipoprotein-cholesterol (Chan et al., 1999; Nanjo, Hara & Kikuchi, 1994), inhibited body and hepatic fat accumulation (Chaudhari & Hatwalne, 1977; Ishigaki, Tonooka, Matsumoto & Hara, 1991). Numerous studies have been carried out to investigate the medicinal properties and health benefits of TP on mammals. However, few reports have focused on the effects TP supplementation on the growth and lipid metabolism in fish.

Large yellow croaker (*Larimichthys crocea*), with its high level of production and delicious taste, has been widely cultured in southeast China. Furthermore, previous studies found that the lipid metabolism of the large yellow croaker is similar to other fish species and mammals, and could be a suitable research model for lipid metabolism in marine fish (Cai, Feng, Xiang, Mai & Ai, 2016; Yan et al., 2015; Zuo et al., 2012). Thus, this study was carried out to determine the effects of the supplementation of TP on growth, antioxidant capacity and lipid deposition in the liver of large yellow croaker fed HLD.

2 | MATERIALS AND METHODS

2.1 | Feed ingredients and diet formulation

Four isoproteic (43% crude protein) and isolipidic (18% crude lipid) diets were formulated to contain graded levels of TP (0.00%, 0.01%, 0.02% and 0.05%) (Table 1). Previous studies found that 12% dietary lipid content was an appropriate lipid content for large yellow croaker (Yi et al., 2014) and 18% dietary lipid content was a high lipid content for large yellow croaker (Wang, Yan, Xu, Ai & Mai, 2016; Yan et al., 2015). White fish meal, wheat gluten meal, casein, wheat meal and soybean meal were used as protein sources, whereas fish oil and soybean oil were used as the main lipid sources. Ingredients and nutrient composition of the four experimental diets are given in details (Table 1).

Dietary ingredients were ground into the fine powder through 320 μm mesh and weighed. All the ingredients were thoroughly mixed until they were homogenous in the mixer. After that, oil mixture and water were thoroughly mixed with all ingredients respectively. Pellets (4 × 5 mm and 5 × 5 mm) were made by automatic fish granulator (F-26, South China University of Technology), placed into an oven at 55°C for drying 12 hr, sealed in bags, and stored frozen (−20°C) in the feeding trial.

2.2 | Experimental procedure

Large yellow croaker was bought from the Fu Fa Aquatic Products, Ningde, China. Before the start of the experiment, the same batch of fish was reared in floating sea cages (2 × 4 × 2 m) and the control diet was fed for 2 weeks to adapt to the experimental conditions and diets.

At the beginning of the experiment, the fish were fasted for 24 hr and weighed. Sixty fish of similar sizes (15.88 ± 0.12 g) were distributed into 12 sea cages (1 × 1 × 2 m), and each diet was randomly allocated to triplicate groups of fish. Fish were fed twice daily at 05:00 and 17:00 for 70 days. During the experimental period, water temperature ranged from 26.5 to 31.0°C, salinity was from 32‰ to 36‰ and dissolved oxygen was approximately 7 mg/L. At the end of the experiment, the fish fasted for 24 hr before harvest. Total number and body weight of fish in each cage were measured for analysis of weight gain (WG), survival rate (SR) and specific growth rate (SGR). The body weight and liver weight were measured for analysis of hepato-somatic index (HSI) and viscera-somatic index (VSI).

TABLE 1 Formulation and proximate analysis of the experimental diets (% dry weight)

	Tea polyphenols level (% dry weight)			
Ingredient	**0**	**0.01**	**0.02**	**0.05**
White fish meal	29.00	29.00	29.00	29.00
Wheat gluten meal	8.00	8.00	8.00	8.00
Casein	8.00	8.00	8.00	8.00
Wheat meal	15.50	15.49	15.48	15.45
Soybean meal	18.00	18.00	18.00	18.00
Fish oil	8.00	8.00	8.00	8.00
Soybean oil	6.00	6.00	6.00	6.00
Phospholipid	2.00	2.00	2.00	2.00
Mineral premix	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00
Attractant	0.30	0.30	0.30	0.30
Sodium alginate	1.00	1.00	1.00	1.00
Ethoxyquin	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.10
Tea polyphenols	0.00	0.01	0.02	0.05
(Total)	100.00	100.00	100.00	100.00
Proximate analysis (n = 3)				
Crude protein %	42.57	42.78	43.01	42.89
Crude Lipid %	17.78	17.58	17.32	17.76

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

Mineral premix (mg or g kg-1diet): $CuSO_4.5H_2O$, 10 mg; Na_2SeO_3 (1%), 25 mg; $ZnSO_4.H_2O$, 50 mg; $CoCl_2.6H_2O$ (1%), 50 mg; $MnSO_4.H_2O$, 60 mg; $FeSO_4.H_2O$,80 mg; $Ca(IO_3)_2$, 180 mg; $MgSO_4.7H_2O$, 1,200 mg; zeolite, 18.35 g.

Vitamin premix (mg or g kg-1diet): vitamin D, 5 mg; vitamin K, 10 mg; vitaminB12, 10 mg; vitamin B6, 20 mg; folic acid, 20 mg; vitamin B1, 25 mg; vitamin A,32 mg; vitamin B2, 45 mg; pantothenic acid, 60 mg; biotin, 60 mg; niacin acid,200 mg; a-tocopherol, 240 mg; inositol, 800 mg; ascorbic acid, 2,000 mg; microcrystalline cellulose, 16.47 g.

Tea polyphenols: supplied by solar China; Concentration ≥ 95%,

Attractant: glycine and betaine.

2.3 | Chemical analysis of diets and fish

Crude protein, crude lipid, moisture and ash content in the diet and whole body were determined following the procedures of the Association of Official Analytical Chemists (AOAC, 1995). Briefly, the moisture content was determined by drying to constant weight at 105°C, and crude protein content (N × 6.25) was determined by the Kjeldahl method. Crude lipid content was determined by ether extraction using Soxhlet method. The fatty acid composition were analysed using the procedures described by Metcalfe, Schmitz and Pelka (1966) with some modification (Ai et al., 2008; Zuo et al., 2012). Fatty acid methyl esters were identified and quantified by HP6890 gas chromatograph (Agilents Technologies, Santa Clara, California, USA) with the capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector.

2.4 | Blood collection

Following the feeding trial, fish were fasted for 24 hr, then anaesthetized with MS222 (1:10,000) (Shanghai Reagent, China). Blood samples were obtained from the caudal vein with 1 ml syringes of 10 fish from each cage and allowed to clot in the cold for 6-8 hr. The clot was removed and residual blood cells were separated from the serum by centrifugation (3,500 r/min for 10 min). The serum stored at −80°C for later analysis.

2.5 | Plasma biochemical parameters

Aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c) are tested by the Mindry BS-180 automatic biochemical analyzer (Mindry China).

2.6 | Antioxidant index

Malondialdehyde (MDA), total antioxidant capacity (T-AOC) and Superoxide dismutase (SOD) were measured using a test kit (Nanjing Jiancheng Bioengineering Institute China). The reagent preparation, sample pretreatment and method of operation are in accordance with operating instructions.

2.7 | RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from liver using Trizol Reagent (Invitrogen, USA) and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. Then, RNA reverse transcribed to cDNA by Prime Script-RT reagent Kit (Takara, Japan). RT-PCR was performed in a quantitative thermal cycler (CFX96™ Real-Time System, BIO-RAD, USA). The amplification was performed in a total volume of 25 μl, containing 1 μl of each primer, 1 μl cDNA product, 12.5 μl SYBR-Premix ExTaqII (Takara, Japan) and 9.5 μl RNA-free water. The real-time PCR program was as follows Zuo et al. (2012).The primer sequences of palmitoyltransferase1(CPT1), acyl-CoA oxidase (ACO), lipoprotein lipase (LPL) and peroxisome proliferators-activated receptorα (PPARα) were from Cai et al. (2016) and Yan et al. (2015) and listed in Table 2. To calculate the expression of CPT 1, ACO, LPL and PPAR α, the comparative ct method ($2^{-\Delta\Delta t}$method) was used as described by Yao et al. (2009).

3 | CALCULATIONS AND STATISTICAL ANALYSIS

$$\text{Weight gain}\,(\text{WG}\%) = 100 \times (\text{Wt} - \text{Wo})/\text{Wo}$$

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

$$\text{Specific growth rate}\,(\text{SGR}\%) = 100 \times (\text{Ln Wt} - \text{Ln Wo})/\text{t}$$

$$\text{Hepato-somatic index}\,(\text{HSI}\%) = 100 \times \text{liver weight}/\text{body weight}$$

$$\text{Viscera-somatic index}\,(\text{VSI}\%) = 100 \times \text{visceral weight}/\text{body weight}$$

where FN is the final number and IN is the initial number of fish in each cage. Wt is the final weight, Wo is the initial weight of fish and t is the experiment period.

The results were presented as means ± *SEM* (standard error of the mean). One-way ANOVA was used to analyse all the data. When there were significant differences ($p < .05$), the group means were further compared using Tukey's multiple range test. All statistical analyses were performed using SPSS 19.0 (SPSS, IL, USA).

4 | RESULTS

4.1 | Survival rate and growth

The survival rate was independent of the dietary treatments ($p > .05$). Although no significant differences were observed in

TABLE 2 Sequences of the PCR primers used in this study

Target gene	Forward (5′–3′)	Reverse (5′–3′)	Reference
CPT1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	Yan et al. (2015)
ACO	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	Yan et al. (2015)
LPL	GAATTCAACGCGGAAACACAG	ACGCTCATAGAGGGCAGACAC	Yan et al. (2015)
PPARα	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC	Cai et al. (2016)
β-actin	CTACGAGGGTTATGCCCTGCC	TGAAGGAGTAACCGCGCTCTGT	Yan et al. (2015)

CPT1, carnitine palmitoyltransferase1; ACO, acyl-CoA oxidase; LPL, lipoprotein lipase; PPARα, peroxisome proliferators-activated receptor α.

TABLE 3 Growth response and survival of large yellow croaker fed diets with graded levels of tea polyphenols level (Means ± *SEM*)

	Tea polyphenols level (% dry weight)			
	0	0.01	0.02	0.05
Initial body weight g	15.89 ± 0.02	15.89 ± 0.23	15.88 ± 0.09	15.87 ± 0.13
Final body weight g	36.71 ± 3.05	37.96 ± 2.45	37.53 ± 0.37	36.80 ± 0.50
Weight gain %	131.02 ± 18.88	138.98 ± 17.39	136.52 ± 14.24	131.77 ± 2.37
Specific growth rate %	1.21 ± 0.07	1.26 ± 0.05	1.24 ± 0.04	1.20 ± 0.02
Survival rate %	86.67 ± 1.67	87.78 ± 2.55	85.00 ± 1.67	85.44 ± 0.96
Hepato-somatic index %	2.96 ± 0.31	2.92 ± 0.39	3.03 ± 0.12	2.93 ± 0.26
Viscera-somatic index %	8.53 ± 0.32	8.04 ± 0.20	7.98 ± 0.16	8.15 ± 0.28

Data are expressed as means ± *SEM*. Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$). *SEM*, standard error of means. ($n = 3$).

growth among dietary treatments ($p > .05$), growth had increased with dietary TP increasing from 0.01% to 0.02% and thereafter decreased (Table 3). There were no significant difference in HSI and VSI among dietary treatments ($p > .05$).

4.2 | Whole body composition

The lipid content in whole body and liver were decreased with dietary TP increasing from 0.01% to 0.02% and thereafter increased (Table 4). In whole body and liver, fish fed the diet with 0.02% TP had the lowest lipid ($p < .05$), while muscle lipid content was not significantly different among dietary treatments ($p > .05$). No significant differences were observed in crude protein, the moisture of whole body among dietary treatments ($p > .05$).

4.3 | Serum biochemical indexes

The content of TG, TC and LDL-c decreased whereas the HDL-c content increased with TP increasing from 0.01% to 0.05% (Figure 1). Fish fed the diet with 0.02% TP had significantly lower TC content compared with control group ($p < .05$), and also had lower content of TG and LDL-c compared with control group without significant differences ($p > .05$). The content of TG and LDL-c was significantly lower in fish fed the diet with 0.05% TP compared with control group ($p < .05$). On the contrary, the HDL-c content was significantly higher in 0.02% TP supplementation group compared with control group ($p < .05$). Compared with the control group, the activity of AST and ALT were significantly lower in 0.02%TP group and 0.01% TP group respectively ($p < .05$).

4.4 | Fatty acid composition

The hepatic saturated fatty acids (SFA) gradually decreased whereas the n-6 PUFA and n-3 PUFA of the liver increased by dose-dependent with the dietary TP increasing from 0.01% to 0.05% (Table 5), but there were no significant differences in SFA, MUFA, n-3 PUFA, n-6 PUFA and other fatty acid in the muscle among treatment groups ($p > .05$) (Table 6). In the liver, the SFA and stearic acid were significantly lower in fish fed the diet with 0.01% TP compared with the control group ($p < .05$) and the lowest SFA appeared in 0.05% TP group ($p < .05$). The lowest C20:0 was observed in 0.05% TP group ($p < .05$). The C18:1n-9 was significantly lower in 0.02% TP group compared with control group ($p < .05$). The n-6 PUFA and C18:2n-6 were significantly higher in fish fed the diet with 0.02% and 0.05% TP compared with the control group ($p < .05$). The n-3 PUFA, C18:3n-3 and C20:3n-3 were significantly higher in 0.02% TP supplementation group compared with control group ($p < .05$). Although there was an upward trend of n-3 LC-PUFA, no significant difference was found among treatment groups ($p > .05$). No significant differences were observed in n-3 PUFA/n-6 PUFA, MUFA and other fatty acid among treatment groups ($p > .05$).

TABLE 4 Effects of tea polyphenols on body composition of large yellow croaker (Means ± *SEM*)

	Tea polyphenols level (% dry weight)			
Index	0	0.01	0.02	0.05
Moisture (%)	72.22 ± 1.62	72.11 ± 0.79	71.46 ± 0.31	71.84 ± 0.64
Crude protein (%w.w.)	15.14 ± 0.46	15.23 ± 0.54	15.81 ± 0.25	15.33 ± 0.14
Crude lipid (% w.w.)	9.36 ± 0.17^{b}	8.89 ± 0.17^{ab}	8.55 ± 0.32^{a}	8.80 ± 0.35^{ab}
Liver lipid content (% w.w.)	39.91 ± 0.78^{c}	38.42 ± 1.32^{c}	33.90 ± 0.30^{a}	35.87 ± 0.33^{b}
Muscle lipid content (% w.w)	9.58 ± 0.75	9.12 ± 0.97	9.35 ± 1.46	9.16 ± 0.50

Data are expressed as means ± *SEM*. Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$). *SEM*, standard error of means. ($n = 3$). w.w. means wet weight.

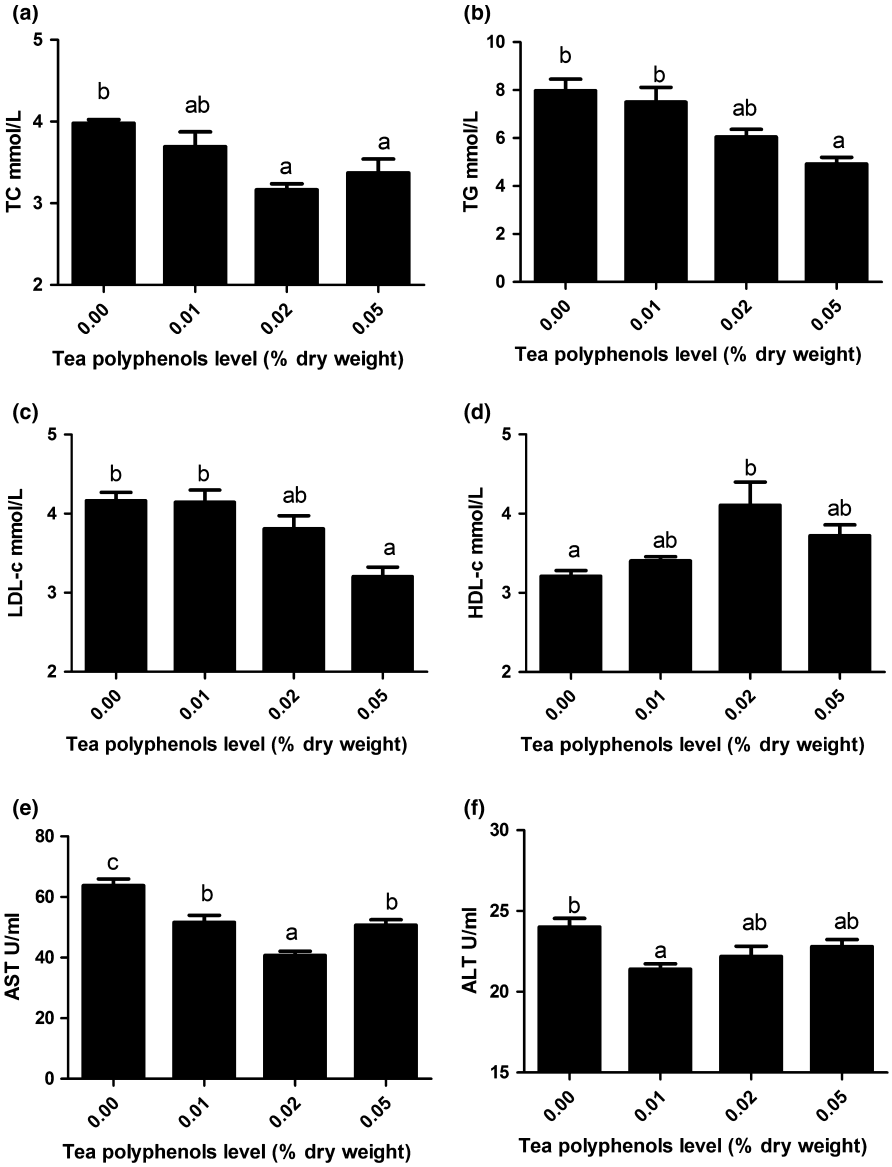

FIGURE 1 The serum biochemical indexes (a) TC. (b) TG. (c) LDL-c. (d) HDL-c. (e) AST. (f) ALT of large yellow croaker fed the experimental diets. Results are expressed as means + *SEM* ($n = 3$). Bars bearing with different letters are significantly different by Tukey's test ($p < .05$)

4.5 | Antioxidant indexes

Malondialdehyde content was significantly lower in TP supplementation groups compared with the control group ($p < .05$) (Figure 2a), and fish fed the diet with 0.02% TP had the lowest MDA ($p < .05$). The activity of SOD was higher in TP supplementation groups compared with the control group. Especially, fish fed the diet with 0.01% TP was significantly higher than the control group ($p < .05$) (Figure 2c). Also, fish fed the diet with 0.02% TP had significantly higher T-AOC compared with the control group ($p < .05$) (Figure 2b).

4.6 | Lipid metabolism related gene expression in the liver

The mRNA expression levels of CPT1, PPARα and ACO significantly increased in fish fed diets with 0.01% and 0.02% TP ($p < .05$) and thereafter significantly dropped when dietary TP increased to 0.05% compared with fish fed the diet with 0.02% TP ($p < .05$) (Figure 3a, b,c). The mRNA expressions of LPL in TP treatment groups were significantly lower than the control group ($p < .05$) (Figure 3d).

5 | DISCUSSION

In this study, although no significant differences were observed in growth performance of large yellow croaker among dietary treatments, growth had been increased with supplementation of TP increasing from 0.01% to 0.02%. Previous studies in mammals have found that TP or tea extracts can significantly reduce body weight (Bose et al., 2008; Tian et al., 2013; Wolfram, Wang & Thielecke, 2006). The differences were probably due to the different animal species used.

Results of this study found that 0.02% dietary TP significantly decreased lipid content of whole body and liver which is consistent

TABLE 5 Effects of tea polyphenols on fatty acid composition in the liver of large yellow croaker (Means ± *SEM*) (% of total fatty acids)

	Tea polyphenols level (% dry weight)			
Fatty acid (%)	**0**	**0.01**	**0.02**	**0.05**
C14:0	2.97 ± 0.24	3.02 ± 0.10	3.12 ± 0.19	3.32 ± 0.15
C16:0	17.00 ± 0.94	16.45 ± 0.47	15.64 ± 0.13	15.90 ± 0.93
C18:0	6.22 ± 0.60^{c}	5.30 ± 0.17^{b}	4.87 ± 0.30^{b}	3.82 ± 0.39^{a}
C20:0	1.21 ± 0.06^{b}	1.23 ± 0.05^{b}	1.18 ± 0.05^{b}	1.09 ± 0.02^{a}
∑SFA	27.40 ± 0.58^{c}	26.01 ± 0.35^{b}	24.82 ± 0.42^{a}	24.14 ± 0.69^{a}
C16:1n-7	7.82 ± 0.78	7.90 ± 0.39	7.60 ± 0.21	8.60 ± 0.59
C18:1n-9	20.32 ± 0.83^{b}	19.55 ± 0.65^{ab}	18.97 ± 0.47^{a}	19.35 ± 0.47^{ab}
∑MUFA	28.14 ± 1.34	27.45 ± 0.68	26.57 ± 0.44	27.95 ± 1.04
C18:2n-6	23.64 ± 1.36^{a}	24.14 ± 0.27^{ab}	25.69 ± 0.10^{b}	25.78 ± 0.95^{b}
C20:4n-6	0.53 ± 0.03	0.54 ± 0.04	0.57 ± 0.02	0.54 ± 0.03
∑n-6 PUFA	24.17 ± 1.33^{a}	24.67 ± 0.31^{ab}	26.26 ± 0.08^{b}	26.32 ± 0.99^{b}
C18:3n-3	2.27 ± 0.09^{a}	2.35 ± 0.08^{a}	2.48 ± 0.02^{b}	2.56 ± 0.07^{b}
C20:5n-3	2.89 ± 0.12^{a}	3.03 ± 0.22^{ab}	3.32 ± 0.15^{c}	3.22 ± 0.08^{bc}
C22:6n-3	2.34 ± 0.03	2.31 ± 0.20	2.45 ± 0.16	2.24 ± 0.28
∑n-3 PUFA	7.50 ± 0.20^{a}	7.68 ± 0.48^{ab}	8.26 ± 0.31^{b}	8.01 ± 0.39^{ab}
n-3/n-6 PUFA	0.31 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	0.31 ± 0.01
∑n-3 LC-PUFA	5.23 ± 0.13	5.34 ± 0.41	5.78 ± 0.29	5.45 ± 0.35

Data are expressed as means ± *SEM*. Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$). *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; LC-PUFA, long chain-polyunsaturated fatty acids.

TABLE 6 Effects of tea polyphenols on fatty acid composition in the muscle of large yellow croaker (Means ± *SEM*) (% of total fatty acids)

	Tea polyphenols level (% dry weight)			
Fatty acid (%)	**0**	**0.01**	**0.02**	**0.05**
C14:0	3.88 ± 0.42	3.81 ± 0.23	3.81 ± 0.50	3.59 ± 0.26
C16:0	18.59 ± 0.79	18.08 ± 0.50	18.62 ± 1.75	17.90 ± 0.27
C18:0	4.64 ± 0.51	4.81 ± 0.02	5.06 ± 0.14	4.81 ± 0.33
C20:0	1.17 ± 0.14	1.29 ± 0.19	1.15 ± 0.20	1.19 ± 0.04
∑SFA	28.28 ± 1.03	27.99 ± 0.70	28.63 ± 2.27	27.49 ± 0.19
C16:1n-7	6.62 ± 0.49	6.51 ± 0.24	6.68 ± 0.69	6.17 ± 0.28
C18:1n-9	16.93 ± 0.26	17.17 ± 0.33	17.45 ± 0.23	17.04 ± 0.40
∑MUFA	23.55 ± 0.74	23.69 ± 0.52	24.13 ± 0.84	23.21 ± 0.22
C18:2n-6	22.68 ± 1.59	22.56 ± 1.29	22.82 ± 1.23	23.00 ± 0.26
C20:4n-6	0.70 ± 0.13	0.77 ± 0.88	0.77 ± 0.03	0.73 ± 0.03
∑n-6 PUFA	23.38 ± 1.46	23.33 ± 1.33	23.59 ± 1.22	23.73 ± 0.28
C18:3n-3	2.30 ± 0.23	2.27 ± 0.08	2.23 ± 0.11	2.33 ± 0.14
C20:5n-3	4.63 ± 0.32	4.41 ± 0.09	4.25 ± 0.21	4.46 ± 0.16
C22:6n-3	4.62 ± 0.42	4.24 ± 0.36	4.28 ± 0.07	4.17 ± 0.12
∑n-3 PUFA	11.55 ± 0.72	10.92 ± 0.37	10.75 ± 0.33	10.96 ± 0.18
n-3/n-6 PUFA	0.50 ± 0.02	0.47 ± 0.03	0.46 ± 0.03	0.46 ± 0.01

Data are expressed as means ± *SEM*. Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$). *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.

with studies in mammals that TP has the function of lipid-lowering and anti-obesity effects (Bursill & Roach, 2007; Tian et al., 2013; Wolfram et al., 2006). Correspondingly, the content of plasma TC, TG and LDL-c were decreased in TP supplementation groups, and 0.02%–0.05% TP supplementation can significantly increase plasma HDL-c content. Furthermore, in this study, TP supplementation

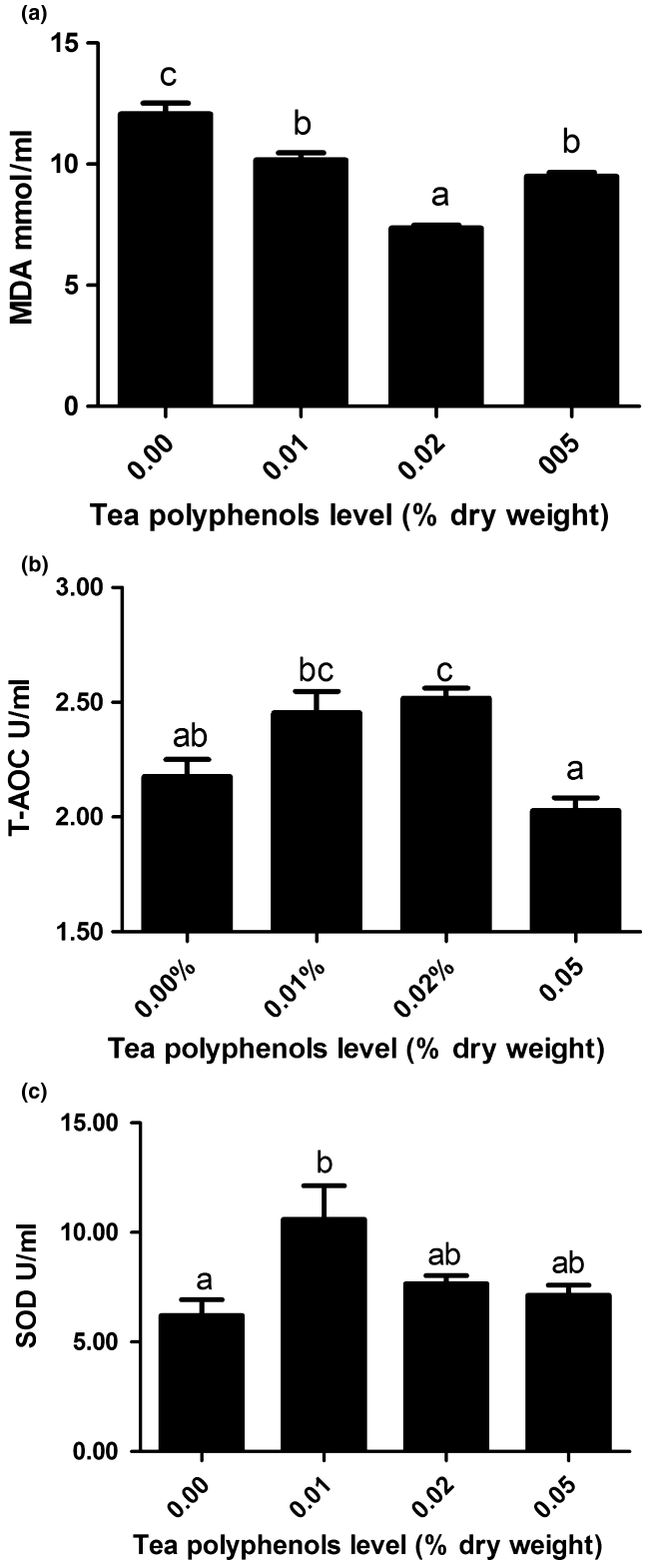

FIGURE 2 Antioxidant indexes (a) MDA. (b) T-AOC. (c) SOD in the serum of large yellow croaker fed the experimental diets. Results are expressed as means + *SEM* ($n = 3$). Bars bearing with different letters are significantly different by Tukey's test ($p < .05$)

decreased activity of plasma ALT and AST compared with the control group, indicating that TP supplementation could improve liver damage to some extent.

In the liver, with the decrease in hepatic lipid content, SFA significantly decreased, while the n-6 PUFA and n-3 PUFA significantly increased. Studies suggested that SFA and MUFA were more prone to lipolysis in vivo (NRC 2011), and some studies in mammals found that TP can promote the lipid oxidation (Lin & Lin-Shiau, 2006; Murase et al., 2002). Besides, previous studies in mammals have demonstrated that the synthesis of SFA and MUFA were inhibited by TP or tea extract (Yeh, Chen, Chiang, Lin-Shiau & Lin, 2003). The relative content of n-3 PUFA and n-6 PUFA increased with TP increasing from 0.01% to 0.05%, which may be due to the decrease in SFA and MUFA or the absorption of LC-PUFA interfered by TP.

Furthermore, antioxidant capacity and health are related to growth performance. In this study, MDA content was significantly decreased in TP treatment groups compared with the control group. Similar results had been found in humans that TP could decease MDA content by inhibiting the absorption and accumulation of plasma MDA from the diet (Gorelik, Ligumsky, Kohen & Kanner, 2008). What's more, in this study, T-AOC and the activity of SOD were increased in fish fed diets with 0.01% and 0.02% TP. Therefore, in this study, TP treatment could partly improve hepatocyte injury, the antioxidant activity and health of fish, which may be an explanation for the good growth performance and lower liver lipid content in large yellow croaker.

Previous studies in mammals have shown that tea catechins and EGCG (catechins, epicatechin, epigallocatechin gallate), two of the main components of TP, could restraint the development of fatty liver which induced by HLD and increase the oxidation and lipolysis of lipid (Bose et al., 2008; Murase et al., 2002). However, the lipid metabolism in fish remains to be elucidated, and it is always an interaction of fatty acid transport, synthesis and oxidation which is similar to mammals (Tocher, 2003). LPL involved in hydrolyzing triglyceride and the process of transferring free fatty acids into the cell (Mead, Irvine & Ramji, 2002; Oku, Koizumi, Okumura, Kobayashi & Umino, 2006). In this study, the mRNA expressions of LPL were significantly down-regulated in TP treatment groups compared with the control group, indicating that lipid absorption was reduced. PPARα has been confirmed to play an important role in increasing oxidation and lipolysis of lipid to reduce lipid accumulation (Goto et al., 2011; Kersten et al., 1999). The activation of PPARα upregulated gene expressions of CPT1 and ACO, which related to oxidation of fatty acids (Kersten, 2014; Mandard, Müller & Kersten, 2004). Thus, higher CPT1, ACO expression in 0.01% and 0.02 groups may be due to the up-regulation of PPARα. However, Gene expressions of ACO, CPT1 and PPARα decreased in fish fed the diet with 0.05% TP treatment group compared with 0.01% and 0.02% TP groups. One possible reason may be that excessive addition of TP may produce a resistance to nutrition, and high doses of caffeine that TP contain inhibited the gene expressions of ACO, CPT1 and PPARα. Therefore, it could be speculated that dietary TP may improve the growth performance and decreased lipid content in whole body and the liver of large yellow croaker by improving oxidation and lipolysis of lipid.

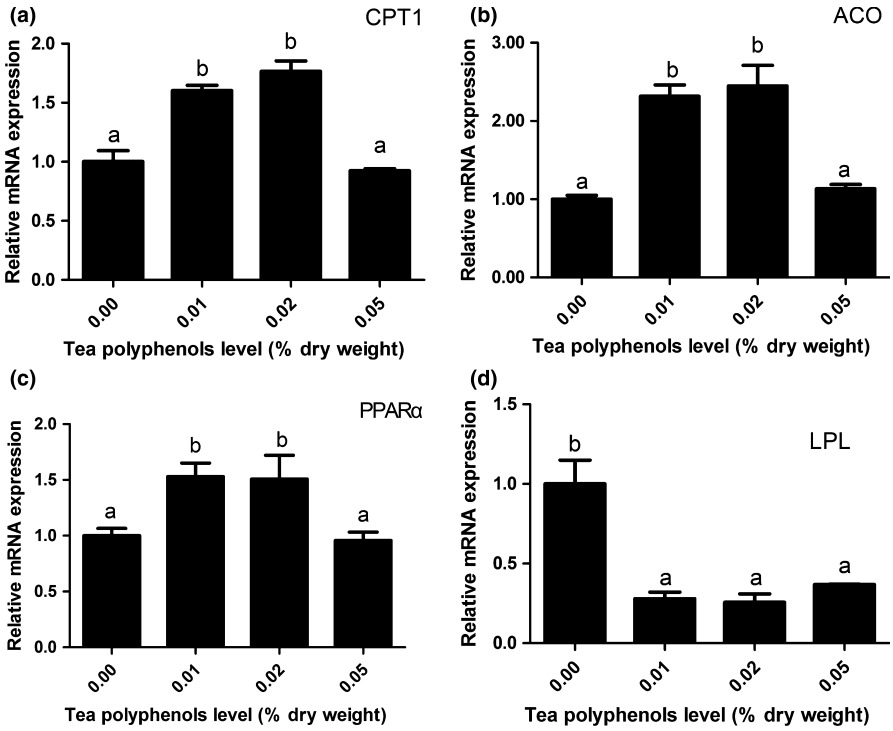

FIGURE 3 Expression of genes related to (a) CPT 1. (b) ACO. (c) PPARα. (d) LPL in the liver of large yellow croaker fed the experimental diets. Results are expressed as means + *SEM* ($n = 3$). Bars bearing with different letters are significantly different by Tukey's test ($p < .05$)

In conclusion, results of this study show that 0.01%–0.02% TP supplementation could reduce the deposition of liver lipid of *L. crocea* caused by high-lipid diet, which could be due to the increase in gene expression of lipid oxidation related gene.

ACKNOWLEDGMENT

This research was supported by the National Natural Science Foundation of China (grant no. 31372541), the China Agriculture Research System (CARS47-11), The AoShan Talents Program Supported by Qingdao National Laboratory for Marine Science and Technology (Grant no. 2015ASTP-0S05) and the National Science Fund for Distinguished Young Scholars of China (Grant no. 31525024) We thank Junzhi Zhang, Qiuchi Chen, Kun Cui, Qiang Chen, Wei Fang for their help during the experiment.

CONFLICT OF INTEREST

There are no conflicts of interest to report.

ORCID

Yanjiao Zhang http://orcid.org/0000-0002-7471-9021
Qinghui Ai http://orcid.org/0000-0002-3958-474X

REFERENCES

Ai, Q., Mai, K., Zhang, C., Xu, W., Duan, Q., Tan, B., & Liufu, Z. (2004). Effects of dietary vitamin C on growth and immune response of Japanese seabass, Lateolabrax japonicus. *Aquaculture*, *242*, 489–500. https://doi.org/10.1016/j.aquaculture.2004.08.016

Ai, Q., Zhao, J., Mai, K., Xu, W., Tan, B., Ma, H., & Liufu, Z. (2008). Optimal dietary lipid level for large yellow croaker (Pseudosciaena crocea) larvae. *Aquaculture Nutrition*, *14*, 515–522. https://doi.org/10.1111/j.1365-2095.2007.00557.x

Association of Official Analytical Chemists (AOAC) (1995). *Official methods of analysis of official analytical chemists international*(16th edn). Association of Official Analytical Chemists, Arlington, VA.

Benzie, I. F., & Szeto, Y. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *47*, 633–636. https://doi.org/10.1021/jf9807768

Bose, M., Lambert, J. D., Ju, J., Reuhl, K. R., Shapses, S. A., & Yang, C. S. (2008). The major green tea polyphenol,(-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat–fed mice. *The Journal of Nutrition*, *138*, 1677–1683.

Boujard, T., Gélineau, A., Covès, D., Corraze, G., Dutto, G., Gasset, E., & Kaushik, S. (2004). Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (Dicentrarchus labrax) fed high fat diets. *Aquaculture*, *231*, 529–545. https://doi.org/10.1016/j.aquaculture.2003.11.010

Bursill, C. A., & Roach, P. D. (2007). A green tea catechin extract upregulates the hepatic low-density lipoprotein receptor in rats. *Lipids*, *42*, 621–627. https://doi.org/10.1007/s11745-007-3077-x

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental microbiology*, *8*, 1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Cai, Z., Feng, S., Xiang, X., Mai, K., & Ai, Q. (2016). Effects of dietary phospholipid on lipase activity, antioxidant capacity and lipid metabolism-related gene expression in large yellow croaker larvae (Larimichthys crocea). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *201*, 46–52. https://doi.org/10.1016/j.cbpb.2016.06.007

Chan, P. T., Fong, W. P., Cheung, Y. L., Huang, Y., Ho, W. K. K., & Chen, Z.-Y. (1999). Jasmine green tea epicatechins are hypolipidemic in hamsters (Mesocricetus auratus) fed a high fat diet. *The Journal of nutrition*, *129*, 1094–1101.

Chaudhari, P., & Hatwalne, V. (1977). Effect of epicatechin on liver lipids of rats fed with choline deficient diet. *Indian Journal of Nutrition and Dietetics*, *14*, 136–139.

Du, Z., Clouet, P., Huang, L., Degrace, P., Zheng, W., He, J., … Liu, Y. (2008). Utilization of different dietary lipid sources at high level in herbivorous grass carp (Ctenopharyngodon idella): Mechanism related to hepatic fatty acid oxidation. *Aquaculture Nutrition*, *14*, 77–92. https://doi.org/10.1111/j.1365-2095.2007.00507.x

Gorelik, S., Ligumsky, M., Kohen, R., & Kanner, J. (2008). A novel function of red wine polyphenols in humans: Prevention of absorption of cytotoxic lipid peroxidation products. *The FASEB Journal*, *22*, 41–46. https://doi.org/10.1096/fj.07-904

Goto, T., Lee, J.-Y., Teraminami, A., Kim, Y.-I., Hirai, S., Uemura, T., … Kawada, T. (2011). Activation of peroxisome proliferator-activated receptor-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. *Journal of lipid research*, *52*, 873–884. https://doi.org/10.1194/jlr.M011320

Hattori, M., Kusumoto, I. T., Namba, T., Ishigami, T., & Hara, Y. (1990). Effect of tea polyphenols on glucan synthesis by glucosyltransferase from Streptococcus mutans. *Chemical and Pharmaceutical Bulletin*, *38*, 717–720. https://doi.org/10.1248/cpb.38.717

Ikeda, I., Kobayashi, M., Hamada, T., Tsuda, K., Goto, H., Imaizumi, K., … Kakuda, T. (2003). Heat-epimerized tea catechins rich in gallocatechin gallate and catechin gallate are more effective to inhibit cholesterol absorption than tea catechins rich in epigallocatechin gallate and epicatechin gallate. *Journal of Agricultural and Food Chemistry*, *51*, 7303–7307. https://doi.org/10.1021/jf034728l

Ishigaki, A., Tonooka, F., Matsumoto, N., & Hara, Y. (1991). *Suppression of the accumulation of body and liver fat by tea catechin*. In: Shizuoka, Japan: Organizing Committee of International Symposium on Tea Science, pp. 309–313.

Kersten, S. (2014). Integrated physiology and systems biology of PPARα. *Molecular Metabolism*, *3*, 354–371. https://doi.org/10.1016/j.molmet.2014.02.002

Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B., & Wahli, W. (1999). Peroxisome proliferator–activated receptor α mediates the adaptive response to fasting. *Journal of Clinical Investigation*, *103*, 1489. https://doi.org/10.1172/JCI6223

Lin, J. K., & Lin-Shiau, S. Y. (2006). Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. *Molecular nutrition & food research*, *50*, 211–217. https://doi.org/10.1002/mnfr.200500138

Mandard, S., Müller, M., & Kersten, S. (2004). Peroxisome proliferator-activated receptor α target genes. *Cellular and Molecular Life Sciences CMLS*, *61*, 393–416. https://doi.org/10.1007/s00018-003-3216-3

Mead, J. R., Irvine, S. A., & Ramji, D. P. (2002). Lipoprotein lipase: Structure, function, regulation, and role in disease. *Journal of molecular medicine*, *80*, 753–769. https://doi.org/10.1007/s00109-002-0384-9

Metcalfe, L., Schmitz, A. A., & Pelka, J. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical chemistry*, *38*, 514–515. https://doi.org/10.1021/ac60235a044

Murase, T., Nagasawa, A., Suzuki, J., Hase, T., & Tokimitsu, I. (2002). Beneficial effects of tea catechins on diet-induced obesity: Stimulation of lipid catabolism in the liver. *International Journal of Obesity*, *26*, 1459. https://doi.org/10.1038/sj.ijo.0802141

Nakayama, M., Suzuki, K., Toda, M., Okubo, S., Hara, Y., & Shimamura, T. (1993). Inhibition of the infectivity of influenza virus by tea polyphenols. *Antiviral research*, *21*, 289–299. https://doi.org/10.1016/0166-3542(93)90008-7

Nanjo, F., Hara, Y., & Kikuchi, Y. (1994). *Effects of tea polyphenols on blood rheology in rats fed a high-fat diet*. ACS Publications.

NRC (2011). *NRC, 2011: Nutrient requirements of fish and shrimp*. Washington, DC: National Academies Press.

Oku, H., Koizumi, N., Okumura, T., Kobayashi, T., & Umino, T. (2006). Molecular characterization of lipoprotein lipase, hepatic lipase and pancreatic lipase genes: Effects of fasting and refeeding on their gene expression in red sea bream Pagrus major. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *145*, 168–178. https://doi.org/10.1016/j.cbpb.2006.06.008

Tian, C., Ye, X., Zhang, R., Long, J., Ren, W., Ding, S., … Xu, S. (2013). Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPARγ-adiponectin pathway. *PLoS ONE*, *8*, e53796. https://doi.org/10.1371/journal.pone.0053796

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in fisheries science*, *11*, 107–184. https://doi.org/10.1080/713610925

Wang, T., Yan, J., Xu, W., Ai, Q., & Mai, K. (2016). Characterization of Cyclooxygenase-2 and its induction pathways in response to high lipid diet-induced inflammation in Larmichthys crocea. *Scientific Reports*, Nat. Publ. Group 1–13. https://doi.org/10.1038/srep19921

Wolfram, S., Wang, Y., & Thielecke, F. (2006). Anti-obesity effects of green tea: From bedside to bench. *Molecular nutrition & food research*, *50*, 176–187. https://doi.org/10.1002/mnfr.200500102

Yan, J., Liao, K., Wang, T., Mai, K., Xu, W., & Ai, Q. (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. https://doi.org/10.1371/journal.pone.0129937

Yao, C.-L., Kong, P., Wang, Z.-Y., Ji, P.-F., Liu, X.-D., Cai, M.-Y., & Han, X.-Z. (2009). Molecular cloning and expression of MyD88 in large yellow croaker, Pseudosciaena crocea. *Fish & shellfish immunology*, *26*, 249–255. https://doi.org/10.1016/j.fsi.2008.10.014

Yeh, C., Chen, W., Chiang, C., Lin-Shiau, S., & Lin, J. (2003). Suppression of fatty acid synthase in MCF-7 breast cancer cells by tea and tea polyphenols: A possible mechanism for their hypolipidemic effects. *The pharmacogenomics journal*, *3*, 267. https://doi.org/10.1038/sj.tpj.6500192

Yi, X., Zhang, F., Xu, W., Li, J., Zhang, W., & Mai, K. (2014). Effects of dietary lipid content on growth, body composition and pigmentation of large yellow croaker Larimichthys croceus. *Aquaculture*, *434*, 355–361. https://doi.org/10.1016/j.aquaculture.2014.08.035

Zuo, R., Ai, Q., Mai, K., Xu, W., Wang, J., Xu, H., … Zhang, Y. (2012). Effects of dietary docosahexaenoic to eicosapentaenoic acid ratio (DHA/EPA) on growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow croaker (Larmichthys crocea) following natural infestation of parasites (Cryptocaryon irritans). *Aquaculture*, *334*, 101–109. https://doi.org/10.1016/j.aquaculture.2011.12.045

How to cite this article: Ji R, Li Y, Li X, et al. Effects of dietary tea polyphenols on growth, biochemical and antioxidant responses, fatty acid composition and expression of lipid metabolism related genes of large yellow croaker (*Larimichthys crocea*). *Aquac Res*. 2018;49:1210–1218. https://doi.org/10.1111/are.13574