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Effect of dietary bile acid (BA) on the growth performance, body composition, antioxidant responses and expression of lipid metabolism-related genes of juvenile large yellow croaker (*Larimichthys crocea*) fed high-lipid diets

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ABSTRACT

The present study was conducted to investigate the effects of dietary bile acid (BA) on the growth performance, body composition, antioxidant responses and expression of lipid metabolism-related genes of juvenile large yellow croaker (*Larimichthys crocea*) (average weight, 12.00 ± 0.20 g). An optimal lipid diet formulated with 4.0% fish oil and 4.0% soybean oil was set as the positive control diet (MO), while a high-lipid diet formulated with 6.5% fish oil and 6.5% soybean oil was used as the negative control diet (HO). The other three diets were supplemented with 150 (HOB150), 300 (HOB300) and 450 mg/kg (HOB450) BA on the basis of the negative control diet. The results showed that the survival rate (SR) was not significantly different among dietary treatments ($P > 0.05$). Compared with the positive control, there were no significant differences in final body weight (FBW) and weight gain rate (WGR) among fish fed diets with high lipid contents. With increasing supplementation of BA in diets, the FBW and WGR gradually increased. Compared with the negative control, fish fed the diet with 300 mg/kg BA had significantly higher FBW and WGR ($P < .05$). The liver lipid content and liver malondialdehyde (MDA) content significantly decreased with increasing dietary BA ($P < .05$). In fish fed diets with 300 mg/kg-450 mg/kg BA, the activities of lipoprotein lipase (LPL) and hepatic lipase (HL) were significantly increased ($P < .05$). The sterol regulatory element binding protein-1 (SREBP-1) mRNA expression levels in the liver significantly decreased with increasing dietary BA ($P < .05$). Furthermore, increasing dietary BA from 150 mg/kg to 450 mg/kg significantly improved the mRNA expression levels of peroxisome proliferator activated receptor α (PPARα), carnitine palmitoyltransferase 1 (CPT1) and acyl-CoA oxidase (ACO) in the liver ($P < .05$). These results suggested that dietary BA supplementation could improve the digestion and absorption capacity of lipids, which might be the reason for the improvement in the growth performance of large yellow croaker.

1. Introduction

Dietary lipids play an important role in fish feed as the main source of energy and essential fatty acids (Sheridan, 1988). Many previous studies have shown that high-lipid diets can increase fish growth (Boujard et al., 2004; Martins et al., 2007). High lipid diets has been increasingly used for cost-effective farming and protein sparing effect in aquaculture in recent years, indicating that may be more helpful for improving protein utilization and maximizing nitrogen retention in fish (Boujard et al., 2004). Long-term feeding with a high-lipid diet may results in disturbance of lipid metabolism, thus affecting growth and physiological, which is very harmful to farmed fish (Jantrarotai et al., 1994; Lee et al., 2002; Tibbetts et al., 2015). While BA can reduce abnormal lipid deposition and promote healthy growth by improving

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lipid metabolism (Goodwin et al., 2000; Pedro et al., 2009; Li and Apte, 2015).

Bile acid (BA) is one of the main components of bile (Einarsson et al., 1991) and is also a natural emulsifier (Alrefai and Gill, 2007). Previous studies in mammals have shown that BA can effectively emulsify lipids to form small chylous particles, which can expand the contact surface between lipids and lipase to accelerate the metabolism of lipids *in vivo* (Kullak-Ublick et al., 2004; Russell, 2009; Li and Apte, 2015). Meanwhile, BA can be combined with glyceride fatty acids to form a water-soluble complex so that lipids can be used efficiently to improve feed efficiency (Einarsson et al., 1991). At the same time, BA was shown to maintain cholesterol homeostasis in the body (Goodwin et al., 2000, Pedro et al., 2009, Wang and Eckel, 2009, Zidong Donna et al., 2014). The mechanism of lipid metabolism in fish is still unclear; however, lipid digestion, absorption, and transport in fish is similar to that in mammals (Tocher, 2003). Given the functional properties of BA and studies in mammals, BA may play an important role in the lipid metabolism of fish. Maybe BA can solve the series of adverse effects caused by fish's high lipid diet.

The purpose of this study was to investigate the effect of BA supplementation in a high-lipid diet on the growth, health and lipid metabolism of juvenile large yellow croaker. Large yellow croaker (*Larimichthys crocea*) is an economically important fish in China. However, growth retardation and an increase in lipid deposition were observed in large yellow croaker fed a high-lipid diet. Previous studies have found that high-lipid feeding of large yellow croaker resulted in a series of problems related to growth and lipid metabolism (Clouet et al., 2010; Wang et al., 2016; Jing et al., 2017).

2. Materials and methods

2.1. Experimental diets

A diet formulated containing approximately 45% crude protein and 18% crude lipid was used as the negative control (HO), and another diet containing approximately 45% crude protein and 13% crude lipid was formulated as the positive control (MO). Practical diets containing graded levels of BA (150 mg/kg, 300 mg/kg and 450 mg/kg) were prepared based on a high-lipid basic diet. All ingredients were separately crushed over 80 um mesh. Then, a small amount of the ingredients was fully mixed, and the remaining amount of ingredients was added to the mixer and fully mixed in oil and a small amount of water. Next, the mixture was rubbed over a 60-mesh sieve and mixed well. Pure water (300 g /kg) was added to produce a stiff dough. After this, the dough was pelleted by being pressed on a sieve in a feed mill with 4-mm holes. Diets were dried in a ventilated oven at 40 °C until attaining a moisture level below 5% and were stored at −20 °C in opaque bags (Table 1).

2.2. Experimental procedure

All large yellow croaker juveniles were obtained from Fu Fa Aquatic Products, Ningde, China. Before the start of the experiment, the same batch of fish was acclimatized to a floating sea cage (4 m*4 m*4 m) for 2 weeks. Sixty fish of similar size (12.00 ± 0.20 g) were randomly distributed among 15 sea cages (1 m*1 m *1.5 m). Each diet was randomly assigned to three cages. Fish were fed to apparent satiation twice daily (05:00 and 17:00) for 10 weeks. At the beginning of the trial, fish were starved for 24 h and then weighed after being anesthetized. Environmental conditions (temperature: 24.3–29.1 °C, salinity 26.2–28.7‰, oxygen level: 6.2–7.4 mg/L) were good for acclimation.

2.3. Sample collection and analysis

At the end of the feeding trial, fish were starved 24 h prior to sampling. After anesthetizing with eugenol (1:10,000), for each cage, total fish were taken to measure the number and body weight, and three fish were collected randomly and stored at −20 °C for future biochemical analyses. The livers were dissected from the fish, weighed individually to calculate the hepatosomatic index (HSI), and the livers from twelve fish per cage were immediately placed in liquid nitrogen and stored at −80 °C before analysis.

The sample was put in the 105 °C ventilation drying oven to dry the sample to constant weight with reference to the Association with Official Analytical Chemists (AOAC, 1995). The crude protein was determined by the Kjeldahl method (N*6.25, FOSS, KjeltecTM 8200). The crude lipid (a general term for substances soluble in petroleum ether) of the sample was determined by Soxhlet extraction (FOSS Soxtec 2050). The ash by combustion in a muffle furnace at 550 °C for 4 h. The fatty acid composition was analyzed using the procedures described by Metcalfe et al. (1966) with some modification (Metcalfe et al. 1966; Ai et al., 2008; Zuo et al., 2012). Fatty acid methyl esters were identified and quantified by an HP6890 gas chromatograph (Agilent Technologies, Santa Clara, California, USA) with a capillary column (007-CW, Hewlett Packard, Palo Alto, CA, Sargent, 1999 USA) and a flame ionization detector.

The content of BA in the feed was determined as follows:

Accurately according to the sample in a 150 mL with triangle in a bottle and then add the extract (50% methanol), ultrasonic extraction 20 min, will plug with triangle on the bottle in the clear liquid in 100 mL centrifuge tube, to 5000 r/min speed centrifuge for 10 min, and then in the centrifuge tube on clear liquid (need) filter such as floats, pour into standby in another centrifuge tube, the solution for the extraction of the sample fluid.

HLB solid phase extraction column (HLB solid phase extraction column of 100 mg/3 cc specification) was successively activated with 3 mL methanol and 3 mL water, 1 mL of the above sample extract was

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

Ingredients %	Diets				
	MO	HO	HOB150	HOB300	HOB450
Fish meal[a]	33.00	33.00	33.00	33.00	33.00
Soybean meal[a]	24.00	24.00	24.00	24.00	24.00
Wheat starch[a]	16.00	16.00	16.00	16.00	16.00
Wheat gluten meal[a]	5.00	5.00	5.00	5.00	5.00
Fish oil[a]	4.00	6.50	6.50	6.50	6.50
Soybean oil[a]	4.00	6.50	6.50	6.50	6.50
Lecithin[a]	2.00	2.00	2.00	2.00	2.00
Vitamin premix[a],[b]	2.00	2.00	2.00	2.00	2.00
Mineral premix[a],[c]	1.00	1.00	1.00	1.00	1.00
Mold inhibitor[a]	0.10	0.10	0.10	0.10	0.10
Ethoxyquin[a]	0.05	0.05	0.05	0.05	0.05
Choline chloride[a]	0.10	0.10	0.10	0.10	0.10
Attractant[a]	1.00	1.00	1.00	1.00	1.00
Bile acid[b]	0.00	0.00	0.015	0.03	0.045
Microcrystalline cellulose[a]	7.75	2.75	2.74	2.72	2.71
Total	100	100	100	100	100
Ingredient					
Crude protein (%DM)	45.11%	44.82%	44.89%	44.83%	44.97%
Crude Lipid (%DM)	12.86%	18.02%	18.06%	18.23%	18.13%

[a] All of these ingredients were supplied by Great Seven Biotechnology Co., Ltd., China.

[b] Vitamin premix (mg kg-1 or g kg-1 diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vit. B12, 0.1 mg; vit. K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; wheat middling, 14.012 g.

[c] Mineral premix (mg kg-1 or g kg-1 diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2{\cdot}6H_2O$ (1%), 50 mg; $CuSO_4{\cdot}5H_2O$, 10 mg; $FeSO_4{\cdot}H2O$, 80 mg; $ZnSO_4{\cdot}H2O$, 50 mg; $MnSO{\cdot}H_2O$, 60 mg; $MgSO_4{\cdot}7H_2O$, 1200 mg; Ca $(H_2PO_4)_2{\cdot}H_2O$, 3000 mg; NaCl, 100 mg; Zoelite, 15.447 g.

removed through the column, and then was successively eluted with 2 mL extract (50% methanol) and 2 mL eluent (60% methanol), and then drained. Elution was performed with 2 mL methanol and filtered with 0.22 m filter membrane for determination by liquid chromatography-tandem mass spectrometer. The mixed standard working fluid with concentration of 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 and 2.00 g/mL was filtered by 0.22 m filter membrane and then tested by liquid chromatography-tandem mass spectrometer. The standard curve was drawn with the characteristic ion mass chromatographic peak area as the ordinate and the concentration of the mixed standard working fluid as the abscissa.

Column: C18 column: column inner diameter 2.1 mm, column length 50 mm, particle size 1.7 m, or equivalent.

Mobile phase: A phase is 0.1% formic acid solution, B phase is 0.1% formic acid acetonitrile solution, gradient elution.

Flow rate: 0.3 mL/min, column temperature: 40 °C, sample volume: 5 mL.

The quantitative determination of The mixed standard working fluid and sample solution were analyzed by computer, and the peak area response value of chromatography was obtained. The response value of the sample solution should be within the linear range of instrument measurement. If the concentration of sample solution exceeds the linear range, it will be re-measured after dilution.

2.4. Antioxidant and lipid metabolism index

Catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (T-AOC), superoxide dismutase (SOD), hepatic lipase (HL) and lipoprotein lipase (LPL) were measured using a test kit (Nanjing Jiancheng Bioengineering Institute China). The reagent preparation, sample pretreatment and method of operation are in accordance with the operating instructions.

2.5. RNA extraction and real-time quantitative PCR

According to the kit instructions, the total sample was ground and milled at −80 °C in liquid nitrogen. Approximately 100-mg samples were added to 1 ml of RNAiso TM Plus from Takara (Japan) for the full reaction, and total RNA was extracted from the samples. The RNA concentration and purity were determined by ultraviolet colorimetry (Nanodrop Thermo Fisher Scientific, USA), and the sample absorbance ratio (260/280 nm) was between 1.8 and 2.0. RNA quality was determined by 1.5% agarose gel electrophoresis. The total RNA concentration was diluted to 500 ng/μl, and reverse transcription was performed immediately with the kit from Takara (Japan).

The real-time quantitative PCR procedure was performed in a total volume of 20 μL. The real-time quantitative PCR was programmed as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 15 s, and 72 °C for 10 s, and melting curve analysis was performed to confirm the specificity of the products. The primer sequences for β-actin, sterol-regulatory element binding protein-1 (SREBP-1), stearoyl-coenzyme A desaturase (SCD-1), fatty acid synthase (FAS), peroxisome proliferator-activated receptor α (PPAR-α), carnitine palmitoyltransferase 1 (CPT-1) and acyl-CoA oxidase (ACO) were calculated and normalized *via* the delta-delta method (Cui-Luan et al., 2009) (Table 2).

2.6. Calculations and statistical analysis

Growth and somatic indices were calculated according to the following:

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

$$\text{Specific growth rate (SGR\%, day}^{-1}\text{)} = \{(\text{Ln}(w_t) - \text{Ln}(w_o))/\text{duration (70 days)}\} \times 100.$$

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

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

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

$$\text{Protein efficiency ratio (PER)} = (w_t - w_o)/P.$$

Where FN is the final number and IN is the initial number of fish in each cage, w_t is the final weight, w_o is the initial weight of fish, F is the feed intake (g), and P is the amount of protein in the diet (%).

SPSS 25.0 one-way ANOVA was used for data statistics, and Tukey's test was used for significance comparison between groups. $P < .05$ indicates a significant difference. All results were expressed as the mean SEM. Different letters in the same line indicate significant differences ($P < .05$).

3. Results

3.1. Growth performance and feed utilization

The survival rate (SR) was independent of the dietary treatments ($P > .05$). With increasing dietary BA, the specific growth rate (SGR) gradually increased thereafter. When fish were fed the diet with 300 mg/kg BA, the SGR was significantly higher than that of the negative control group ($P < .05$). The final body weight (FBW) and the weight gain rate (WGR) had the same trends as the SGR. The feed conversion ratio (FCR) was decreased with increasing dietary BA and was significantly higher than that of the negative control group in fish fed diets with 150 mg/kg-450 mg/kg BA ($P < .05$). Especially in fish fed the diet with 300 mg/kg BA, the FCR was the lowest among dietary treatments. The protein efficiency ratio (PER) was significantly enhanced by BA supplementation. Compared with the negative control group, the PER in fish fed diets with 150 mg/kg-450 mg/kg BA was significantly higher ($P < .05$). When fish were fed the diet with 300 mg/kg BA, the PER reached a plateau and was significantly higher than that of the positive control group ($P < .05$). The HSI in fish fed diets with 150 mg/kg-450 mg/kg BA was significantly lower than that of the negative control group ($P < .05$). However, in the positive control group, the HSI was still significantly lower than that in the other groups ($P < .05$) (Table 3).

3.2. Body composition

With increasing BA in diets, the crude protein gradually increased thereafter, but no significant differences were observed ($P > .05$). However, the crude protein in the positive control group was significantly higher than that in the other groups ($P < .05$). The crude lipids showed no significant differences with increasing dietary BA but were significantly lower in the positive control group than those in the other groups ($P < .05$). As dietary BA increased, the liver lipid content was significantly lower than that of the negative control group ($P < .05$). Especially when fish were fed the diet with 450 mg/kg BA, the liver lipid content was the lowest among fish fed high-lipid diets. However, the liver lipid content in the positive control group was the lowest among the dietary treatments ($P < .05$). In contrast, the muscle lipid content gradually increased with increasing dietary BA and was significantly higher in fish fed high-lipid diets compared to that in the positive control group ($P < .05$). With 300 mg/kg and 450 mg/kg BA supplementation in diets, the muscle lipid content was significantly higher than that in the other groups ($P < .05$). The whole body moisture and ash were higher in the positive control group than those in the other groups, but no significant differences were observed among the dietary treatments ($P > .05$) (Table 4).

Table 2
Primer pair sequences for real-time PCR.

Target gene	Forward (5′-3′)	Reverse (5′-3′)	References
SREBP-1	TCTCCTTGCAGTCTGAGCCAAC	TCAGCCCTTGGATATGAGCCT	Cai et al. (2016)
SCD-1	AAAGGACGCAAGCTGGAACT	CTGGGACGAAGTACGACACC	Cai et al. (2016)
FAS	CAGCCACAGTGAGGTCATCC	TGAGGACATTGAGCCAGACAC	Yan et al. (2015)
PPARα	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC	Yan et al. (2015)
CPT-1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	Yan et al. (2015)
ACO	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	Yan et al. (2015)
β-Actin	CTACGAGGGTTATGCCCTGCC	TGAAGGAGTAACCGCGCTCTGT	Yan et al. (2015)

SREBP-1, sterol-regulatory element binding protein-1; FAS, fatty acid synthase; SCD, stearoyl-coenzyme A desaturase; PPARα, peroxisome proliferator-activated receptor α; CPT1, carnitine palmitoyl transferase 1; ACO, acyl-CoA oxidase.

3.3. Muscle fatty acid composition

As dietary BA increased, C18 was decreased and was significantly lower with 150 mg/kg-450 mg/kg BA supplementation in diets than that in the negative control group ($P < .05$). C18 1n-9 was not significantly influenced by increasing dietary BA but was significantly lower in the positive control group than the level in the other groups ($P < .05$). C18 2n-6 and C18 3n-3 gradually increased thereafter with increasing dietary BA and were significantly higher than the levels in the positive control group when fish were fed the diet with 300 mg/kg BA ($P < .05$). In addition, there were no significant differences in other fatty acids in the muscle among dietary treatments ($P > .05$) (Table 5).

3.4. Liver lipid metabolism and antioxidant capacity

With increasing BA in the diet, the activity of CAT was gradually increased and thereafter decreased and was significantly higher in fish fed the diet with 300 mg/kg BA than activity in the other groups ($P < .05$). Meanwhile, the activity of CAT in the positive control group was the lowest among the dietary treatments ($P < .05$). The liver content of MDA was significantly decreased with increasing dietary BA ($P < .05$), and the highest content of MDA was observed in the negative control group ($P < .05$). SOD gradually increased with increasing dietary BA, but no significant difference was observed among dietary treatments ($P > .05$). With increasing dietary BA, the T-AOC was significantly increased ($P < .05$). The T-AOC was higher in the negative control group than that in the positive control group, but no significant difference was observed ($P > .05$). With increasing BA in the diet, the activity of LPL was significantly increased ($P < .05$). Although the activity of LPL was higher in the negative control group than the activity in the positive control group, no significant difference was observed ($P > .05$). The activity of HL was significantly higher in the negative control group than the activity in the positive control group ($P < .05$). Moreover, with dietary BA increasing from 300 mg/kg to 450 mg/kg, the activity of HL was significantly higher than that in the negative control group ($P < .05$) (Table 6).

3.5. Expression of genes related to lipid metabolism

With increasing supplementation of BA in the diet, the mRNA expression levels of SREBP-1 and SCD were significantly decreased ($P < .05$). Moreover, the mRNA expression levels of FAS in fish fed diets with 300 mg/kg and 450 mg/kg BA were significantly lower than level in the other groups ($P < .05$) (Fig. 1). With increasing supplementation of BA in the diet, the mRNA expression levels of PPAR-α, ACO and CPT-1 were significantly increased ($P < .05$). The mRNA expression levels of PPAR-α, ACO and CPT-1 in the negative control group were significantly lower than expression levels in the other groups ($P < .05$) (Fig. 2).

4. Discussion

The results of the present study showed that no significant differences were observed in the growth performance of large yellow croaker, suggesting that the fish may be able to tolerate high lipid diets within this lipid range (13%–18% lipid). Unlike some previous studies (Boujard et al., 2004; Martins et al., 2007), feeding high-lipid diet did not significantly improve the growth of large yellow croaker. This indicates that simply increasing the lipid level of feed cannot achieve the purpose of promoting growth. Some previous studies have shown no significant change or even decrease in growth after fish fed high lipid diets. It's quite possible because excessive energy in diets can lead to decrease feed consumption (reducing total protein intake) and reduce the utilization of other nutrients (Chou and Shiau, 1996; Lovell, 1998; Wang et al., 2005). However, growth was promoted with supplementation of BA increasing from 300 mg/kg to 450 mg/kg. Although no information about the effects of dietary BA on aquatic animals is available, previous studies showed that ursodeoxycholic acid (UDCA) could improve the growth of juvenile eel and yellowtail (Deshimaru, 1982; Maita et al., 1996). Meanwhile, the addition of chenodeoxycholic acid (CDCA) could significantly improve the growth of large yellow

Table 3
Effects of dietary bile acid on the growth response and survival of large yellow croaker.

Index	Diets				
	MO	HO	HOB150	HOB300	HOB450
Initial body weight (g)	11.99 ± 0.15	11.95 ± 0.14	12.02 ± 0.17	12.09 ± 0.09	11.93 ± 0.24
Final body weight (g)	57.59 ± 0.46^{a}	58.68 ± 0.89^{a}	58.4 ± 1.13^{a}	63.71 ± 0.68^{b}	61.63 ± 1.61^{ab}
Weight gain rate (%)	380.52 ± 10.34^{a}	390.95 ± 1.03^{a}	385.86 ± 12.5^{a}	427.15 ± 3.78^{b}	416.64 ± 11.63^{ab}
Specific growth rate (% day^{-1})	2.24 ± 0.03^{a}	2.27 ± 0.01^{a}	2.26 ± 0.04^{a}	2.37 ± 0.01^{b}	2.35 ± 0.03^{ab}
Survival rate (%)	85.00 ± 5.30	82.22 ± 4.94	86.67 ± 3.47	83.89 ± 2.00	88.89 ± 2.94
Feed conversion ratio	1.37 ± 0.02^{a}	1.46 ± 0.02^{b}	1.36 ± 0.01^{a}	1.30 ± 0.02^{a}	1.34 ± 0.02^{a}
Protein efficiency ratio	1.61 ± 0.03^{ab}	1.54 ± 0.03^{a}	1.66 ± 0.01^{bc}	1.74 ± 0.02^{c}	1.68 ± 0.02^{bc}
Hepato-somatic index (%)	1.28 ± 0.02^{a}	1.67 ± 0.06^{c}	1.40 ± 0.04^{b}	1.47 ± 0.03^{b}	1.44 ± 0.04^{b}

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

Table 4
Proximate composition (dry weight %) in whole body and the lipid content of the liver and muscle in large yellow croaker.

Index	Diets				
	MO	HO	HOB150	HOB300	HOB450
Moisture (%)	74.03 ± 0.41	72.85 ± 0.55	72.78 ± 0.20	72.44 ± 0.52	71.70 ± 0.89
Ash (%)	12.94 ± 0.15	12.04 ± 0.15	11.87 ± 0.22	12.01 ± 0.26	11.83 ± 0.70
Crude protein (% d.w.)	59.65 ± 2.52^{b}	54.58 ± 1.6^{a}	54.89 ± 0.97^{a}	55.54 ± 1.71^{a}	53.69 ± 1.23^{a}
Crude lipid (% d.w.)	28.67 ± 1.58^{a}	33.20 ± 0.99^{b}	33.97 ± 0.65^{b}	33.58 ± 0.39^{b}	34.24 ± 1.01^{b}
Liver lipid content (% d.w.)	64.32 ± 4.03^{b}	68.28 ± 1.45^{c}	62.61 ± 0.61^{ab}	62.23 ± 2.13^{ab}	59.99 ± 3.09^{a}
Muscle lipid content (% d.w.)	24.07 ± 1.28^{a}	26.76 ± 1.64^{b}	27.9 ± 2.51^{bc}	29.67 ± 2.2^{c}	29.59 ± 1.91^{c}

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

croaker (Du et al., 2017). Moreover, in this study, the FCR was significantly decreased with 150 mg/kg-450 mg/kg BA, and the PER was significantly increased, which agreed with previous research findings in juvenile eel (Deshimaru, 1982) and yellowtail (Maita et al., 1996), suggesting that the results may be closely related to improving the utilization of dietary lipids. Furthermore, studies in mammals and poultry showed that dietary BA could promote the growth performance, prevent diseases and improve body composition, which might result from the emulsification of lipids by bile acids (Kevresan et al., 2006; Parsaie et al., 2007; Guariento et al., 2008; Russell, 2009).

In the present study, along with the increase in dietary BA content, the trend of the crude lipid was increased compared with the control group, but the differences were not significant. The liver lipid content and the HSI of the experimental fish were increased after feeding with high-lipid diets. The results were consistent with the results of clarias catfish (Jantrarotai et al., 1994), hybrid tilapia (Chou and Shiau, 1996), rockfish (Lee et al., 2002), starry flounder (Ding et al., 2010) and haddock (Tibbetts et al., 2015). BA plays an important role in reducing liver triglyceride deposition, protecting liver health and resisting liver diseases (Cipriani et al., 2009; Jain et al., 2012; Watanabe and Fujita, 2014). Correspondingly, in this study, the increases in liver lipid content and HSI in the high-lipid groups were markedly reduced by dietary BA treatments. Moreover, the muscle lipid content was significantly increased with 300 mg/kg to 450 mg/kg BA. The content ratio of linolenic acid (C18 3n-3) in the muscle was significantly increased with 300 mg/kg BA. C18 3n-3 is a precursor for the synthesis of DHA and EPA (Leonard et al., 2004; Sargent, 1999). Although the muscle DHA and EPA content ratio was not significantly influenced by the BA in this study, the DHA and EPA content ratio was the highest with 300 mg/kg BA supplementation among dietary treatments. However, there is a general lack of knowledge about the effects of BA on fatty acid synthesis in aquatic animals. Studies on mammals have suggested that BA may have an impact on the pathway of arachidonic acid synthesis (Zhang et al., 2017), but the synthesis mechanism in fish needs to be further explored.

Furthermore, antioxidant capacity is one of the most important indicators that reflects growth performance (Mate, 2000; Winzer et al., 2000). Fish fed diets supplemented with BA had significantly higher activities of SOD, CAT, and T-AOC and lower contents of MDA in the liver. The results demonstrated a beneficial effect on the antioxidant capacity of large yellow croaker. Similar results have been reported in mammals in which dietary BA could promote the antioxidant capacity (Kullak-Ublick et al., 2004; Li and Apte, 2015). Thus, the increased ability of antioxidation may be an explanation for the healthy growth performance. This study also found that with the supplementation of BA in the diet, the activities of LPL and HL in the liver were significantly increased. Previous studies in mammals demonstrated that LPL and HL are two important markers for measuring the lipid metabolism ability (Macfarlane et al., 1990; Chamberlain et al., 1991; Lewis et al., 1994, Wang and Eckel, 2009) and could also prevent the accumulation of excessive cholesterol in extrahepatic tissues. To further explore the effect of dietary BA on lipid metabolism, related gene expression was analyzed.

SREBP-1, a key transcription factor for regulating the expression of lipid metabolism-related genes, and SCD, an important catalytic enzyme for the formation of monounsaturated fatty acids during the

Table 5
Effects of dietary bile acid on the fatty acid composition in muscle of large yellow croaker.

Fatty acid (%)	Diets				
	MO	HO	HOB150	HOB300	HOB450
C14	2.34 ± 0.12	2.24 ± 0.09	2.25 ± 0.06	2.36 ± 0.02	2.38 ± 0.03
C16	16.95 ± 0.52	16.88 ± 0.56	16.91 ± 0.21	16.52 ± 0.36	16.54 ± 0.15
C18	4.18 ± 0.09^{a}	4.72 ± 0.16^{b}	4.35 ± 0.05^{a}	4.36 ± 0.07^{a}	4.31 ± 0.24^{a}
C20	1.79 ± 0.08	1.81 ± 0.11	1.63 ± 0.05	1.54 ± 0.03	1.58 ± 0.08
ΣSFA	25.26 ± 0.57	24.24 ± 0.78	23.87 ± 0.15	23.60 ± 0.39	23.61 ± 0.06
C16 1n-7	2.98 ± 0.10	3.04 ± 0.16	3.09 ± 0.09	3.07 ± 0.05	3.08 ± 0.03
C18 1n-9	16.12 ± 0.14^{a}	18.64 ± 0.38^{b}	18.09 ± 0.17^{b}	17.65 ± 0.08^{b}	17.71 ± 0.28^{b}
ΣMUFA	19.10 ± 0.18^{a}	21.67 ± 0.48^{b}	21.18 ± 0.24^{b}	20.72 ± 0.13^{b}	20.80 ± 0.27^{b}
C18 2n-6	24.12 ± 0.18^{a}	25.24 ± 0.63^{ab}	25.58 ± 0.21^{ab}	26.43 ± 0.23^{b}	25.82 ± 0.17^{ab}
C20 4n-6	0.48 ± 0.01	0.46 ± 0.01	0.47 ± 0.03	0.48 ± 0.01	0.54 ± 0.03
Σn-6 PUFA	24.60 ± 0.74^{a}	25.7 ± 0.64^{ab}	26.06 ± 0.24^{ab}	26.91 ± 0.23^{b}	26.35 ± 0.14^{ab}
C18 3n-3	2.83 ± 0.05^{a}	2.94 ± 0.09^{ab}	3.07 ± 0.04^{ab}	3.28 ± 0.03^{b}	3.02 ± 0.04^{ab}
C20 5n-3	2.94 ± 0.17	2.92 ± 0.15	3.14 ± 0.06	3.28 ± 0.07	3.09 ± 0.10
C22 6n-3	5.55 ± 0.13	5.50 ± 0.06	5.78 ± 0.22	5.80 ± 0.18	5.66 ± 0.07
Σn-3 PUFA	11.31 ± 0.24^{a}	11.35 ± 0.17^{a}	11.99 ± 0.19^{ab}	12.22 ± 0.19^{b}	12.01 ± 0.06^{ab}
n-3/n-6 PUFA	0.46 ± 0.02	0.44 ± 0.02	0.46 ± 0.02	0.45 ± 0.03	0.46 ± 0.04

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

Table 6
Effects of dietary bile acid on liver lipid metabolism and the antioxidant capacity of large yellow croaker.

Index	Diets				
	MO	HO	HOB150	HOB300	HOB450
CAT (U/mg prot)	15.34 ± 1.53^{a}	18.39 ± 0.58^{b}	20.69 ± 1.44^{b}	27.45 ± 2.24^{c}	21.74 ± 1.14^{b}
MDA (nmol/mg prot)	12.17 ± 0.86^{a}	16.53 ± 1.52^{b}	13.72 ± 1.13^{a}	12.94 ± 1.13^{a}	13.58 ± 0.44^{a}
SOD (U/mg prot)	141.45 ± 1.12	144.36 ± 4.96	149.35 ± 5.98	152.85 ± 6.95	151.44 ± 6.99
T-AOC (U/mg prot)	1.52 ± 0.09^{a}	1.89 ± 0.19^{ab}	2.07 ± 0.13^{b}	2.16 ± 0.09^{b}	2.13 ± 0.16^{b}
LPL (U/mg prot)	0.86 ± 0.12^{a}	0.98 ± 0.12^{a}	1.42 ± 0.11^{b}	1.61 ± 0.13^{b}	1.50 ± 0.07^{b}
HL (U/mg prot)	0.64 ± 0.06^{a}	1.02 ± 0.16^{b}	1.07 ± 0.14^{b}	1.28 ± 0.09^{c}	1.30 ± 0.18^{c}

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

synthesis of fatty acids, have a close correlation in the process of lipid metabolism (Stefania et al., 2013). In the present study, the mRNA expression levels of SREBP-1 and SCD were significantly downregulated in BA treatments, which may be one of the main reasons that explains the decreased liver lipid content and C18 1n-9 in muscle. With dietary BA increasing from 300 mg/kg to 450 mg/kg, FAS, another key enzyme regulated by SREBP-1 during fatty acid synthesis controlling the synthesis of long-chain fatty acids, was significantly downregulated (Smith et al., 2003). The decreased mRNA expression level of FAS may reduce the level of fatty acids synthesized by the liver, thus slowing down the accumulation of lipid in the liver. On the other hand, β-oxidation is an important method of lipid catabolism in fish. The basic mechanism and regulation of this pathway appear to be similar in mammals and fish (Nanton et al., 2003). PPAR-α, a key mediator of energy metabolism, is mainly expressed in the liver and is involved in regulating the expression of several key enzymes of fatty acid oxidation, such as ACO and CPT-1 (Jiansheng et al., 2011; Kersten, 2014; Souza-Mello, 2015). In this study, the mRNA expression levels of PPAR-α, ACO and CPT-1 in fish fed a high-lipid diet were decreased, but they were significantly upregulated in the BA treatment diets. Moreover, Ning et al. (2016) demonstrated that high expression levels of PPAR-α in Nile tilapia could increase lipid degradation and decrease adipogenesis, which was similar to mammals. Therefore, it was concluded that one of the main reasons for the decrease in lipid content in the liver may be attributed to the activation of PPAR-α regulating the activation of liver fatty acid oxidation by the downstream target genes ACO and CPT-1, which accelerates the lipid consumption process in the liver.

In summary, the results of this study showed that dietary BA could promote growth performance and reduce the liver lipid deposition of large yellow croaker. These beneficial effects could be attributed to the increased activities of lipid metabolism and antioxidant enzymes, as well as the increase in gene expression of lipid oxidation genes and the

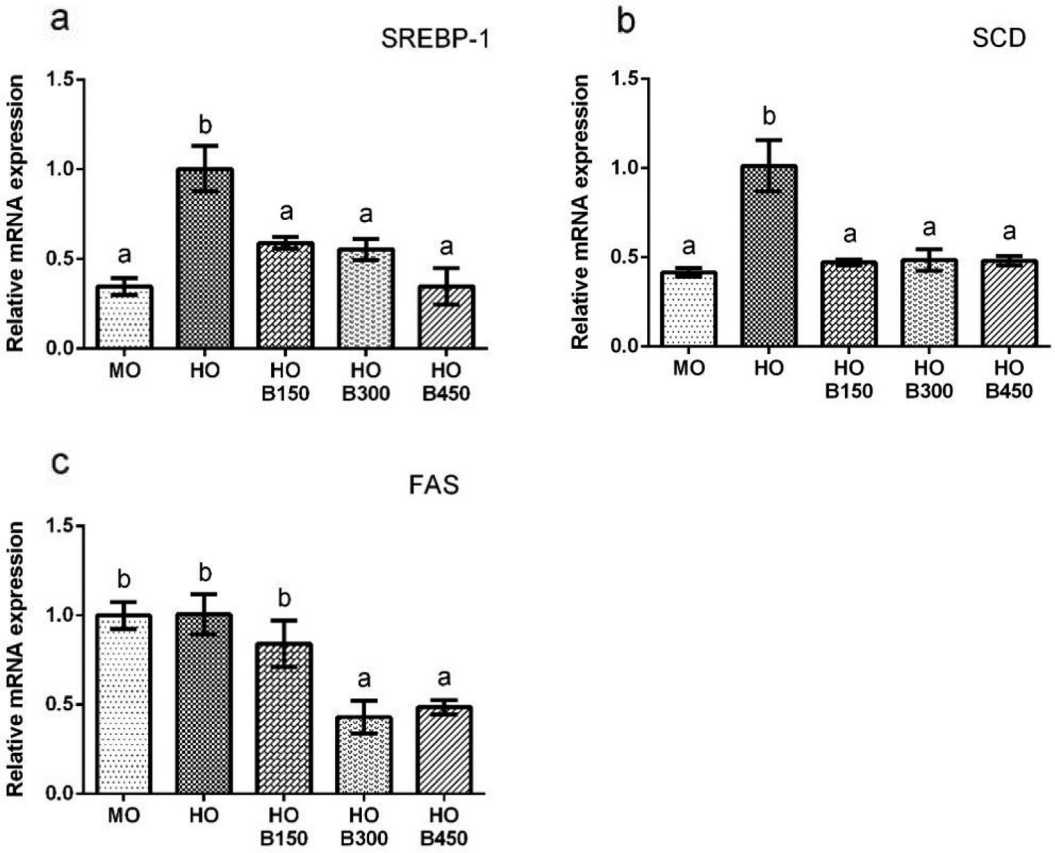

Fig. 1. Effects of high dietary lipid levels and BA on the relative expression of (a) sterol regulatory element binding protein-1 (SREBP-1), (b) stearoyl coenzyme A desaturase (SCD), and (c) fatty acid synthase (FAS) in larval large yellow croaker. Data are presented as the means ± S.E.M. ($n = 3$). Bars bearing the same letters are not significantly different ($P > .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

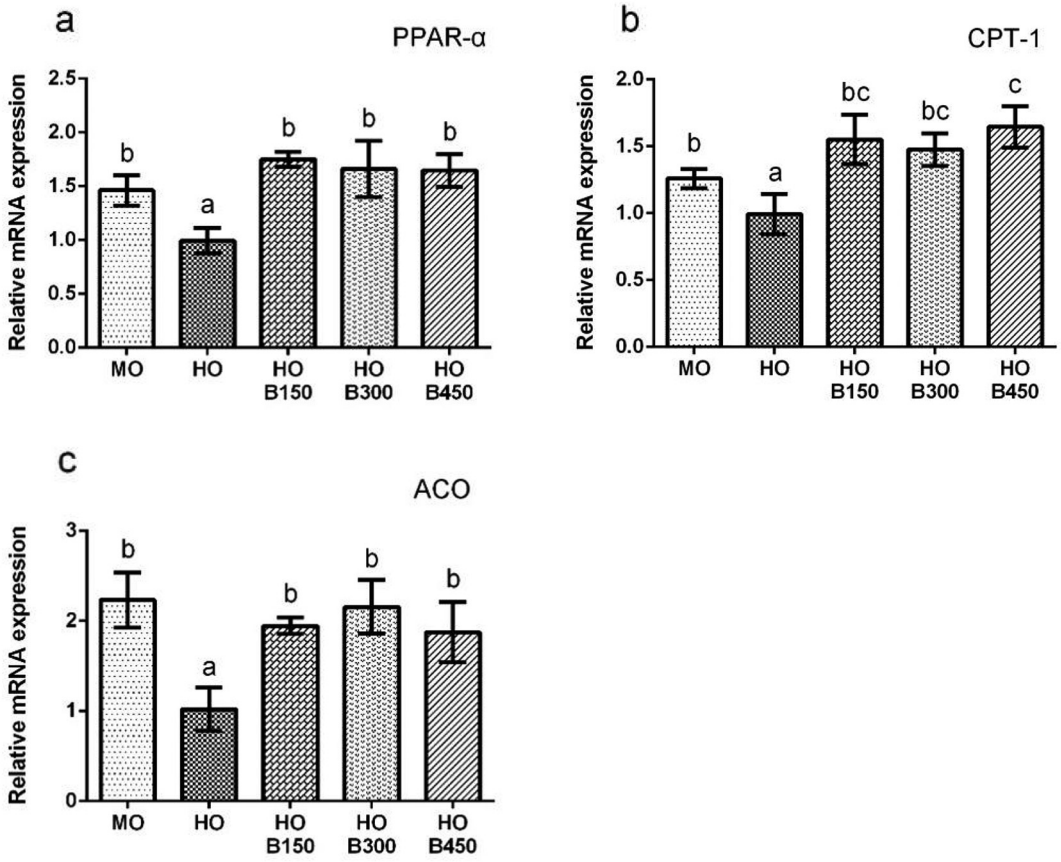

Fig. 2. Effects of high dietary lipid levels and BA on the relative expression of (a) peroxisome proliferator-activated receptor α (PPAR-α), (b) carnitine palmitoyl transferase 1 (CPT-1), and (c) acyl-CoA oxidase (ACO) in juvenile large yellow croaker. Data are presented as the means ± S.E.M. (n = 3). Bars bearing the same letters are not significantly different ($P > .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

decrease in gene expression of lipid synthesis genes in the liver.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

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. Aquac. Nutr. 14, 515–522.

Alrefai, W.A., Gill, R.K., 2007. Bile acid transporters: structure, function, regulation and pathophysiological implications. Pharm. Res. 24, 1803.

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.

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.

Chamberlain, J.C., Thorn, J.A., Galton, D.J., 1991. The hepatic lipase (HL) gene: associations with hypertriglyceridaemia. Atherosclerosis. 90, 224.

Chou, B.S., Shiau, S.Y., 1996. Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus* × *Oreochromis* aureus. Aquaculture. 143, 185–195.

Cipriani, S., Mencarelli, A., Palladino, G., Fiorucci, S., 2009. FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (fa/fa) obese rats. J. Lipid Res. 51 jlr. M001602-001784.

Clouet, P., Huang, L.M., Degrace, P., Zheng, W.H., He, J.G., Tian, L.X., Liu, Y.J., 2010. Utilization of different dietary lipid sources at high level in herbivorous grass carp (Ctenopharyngodon idella): mechanism related to hepatic fatty acid oxidation. Aquac. Nutr. 14, 77–92.

Cui-Luan, Y., Peng, K., Zhi-Yong, W., Pei-Feng, J., Xian-De, L., Ming-Yi, C., Xue-Zhe, H., 2009. Molecular cloning and expression of MyD88 in large yellow croaker, *Pseudosciaena crocea*. Fish Shellfish Immunol. 26, 249–255.

Deshimaru, O.J.N.S., 1982. Suitable levels of lipids and ursodesoxycholic acid in diet for yellowtail. Nippon Suisan Gakkaishi 48, 1265–1270.

Ding, L.Y., Zhang, L.M., Wang, J.Y., Ma, J.J., Meng, X.J., Duan, P.C., Sun, L.H., Sun, Y.Z., 2010. Effect of dietary lipid level on the growth performance, feed utilization, body composition and blood chemistry of juvenile starry flounder (Platichthys stellatus). Aquac. Res. 41, 1470–1478.

Du, J., Xu, H., Li, S., Cai, Z., Mai, K., Ai, Q., 2017. Effects of dietary chenodeoxycholic acid on growth performance, body composition and related gene expression in large yellow croaker (Larimichthys crocea) fed diets with high replacement of fish oil with soybean oil. Aquaculture. 479, 584–590.

Einarsson, K., Ericsson, S., Ewerth, S., Reihner, E., Rudling, M., Stahlberg, D., Angelin, B., 1991. Bile acid sequestrants: mechanisms of action on bile acid and cholesterol metabolism. Eur. J. Clin. Pharmacol. 40 (Suppl. 1), S53–S58.

Goodwin, B., Jones, S.A., Price, R.R., Watson, M.A., Mckee, D.D., Moore, L.B., Galardi, C., Wilson, J.G., Lewis, M.C., Roth, M.E., 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol. Cell 6, 517–526.

Guariento, M., Raimondo, D., Assfalg, M., Zanzoni, S., Pesente, P., Ragona, L., Tramontano, A., Molinari, H., 2008. Identification and Functional Characterization of the Bile Acid Transport Proteins in Non-mammalian Ileum and Mammalian Liver.

Jain, A.K., Stoll, B., Burrin, D.G., Holst, J.J., Moore, D.D., 2012. Enteral bile acid treatment improves parenteral nutrition-related liver disease and intestinal mucosal atrophy in neonatal pigs. Am. J. Physiol Gastrointest. Liver Physiol. 302 (2), G218–G224.

Jantrarotai, W., Sitasit, P., Rajchapakdee, S., 1994. The optimum carbohydrate to lipid ratio in hybrid Clarias catfish (*Clarias macrocephalus*×*C. gariepinus*) diets containing raw broken rice. Aquaculture. 127, 61–68.

Jiansheng, H., Navin, V., Songtao, Y., Yuzhi, J., Liang, B., Aurore, V., Mustapha, C.M., Mushfiquddin, K., Inderjit, S., Gongshe, Y., 2011. Progressive endoplasmic reticulum stress contributes to hepatocarcinogenesis in fatty acyl -CoA oxidase 1-deficient mice.

Am. J. Pathol. 179, 703–713.
Jing, Y., Kai, L., Mai, K., Ai, Q., 2017. Dietary lipid levels affect lipoprotein clearance, fatty acid transport, lipogenesis and lipolysis at the transcriptional level in muscle and adipose tissue of large yellow croaker (*Larimichthys crocea*). Aquac. Res. 48.
Kersten, S., 2014. Integrated physiology and systems biology of PPAR-α. Mol. Metab. 3, 354–371.
Kevresan, S., Kuhajda, K., Kandrac, J., Fawcett, J.P., Mikov, M., 2006. Biosynthesis of bile acids in mammalian liver. Eur. J. Drug Metab. Pharmacokinet. 31, 145.
Kullak-Ublick, G.A., Bruno, S., Meier, P.J., 2004. Enterohepatic bile salt transporters in normal physiology and liver disease. Gastroenterology. 126, 322–342.
Lee, S.M., Jeon, I.G., Lee, J.Y., 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture. 211, 227–239.
Leonard, A.E., Pereira, S.L., Sprecher, H., Huang, Y.S.J.P.I.L.R., 2004. Elongation of long-chain fatty acids. Prog. Lipid. Res. 43, 36–54.
Lewis, M.E.S., Forsythe, I.J., Wilson, B., 1994. Retroviral-mediated gene transfer and expression of human lipoprotein lipase (LPL) in somatic cells. Atherosclerosis 109 (1-2), 65–66.
Li, T., Apte, U., 2015. Bile acid metabolism and signaling in cholestasis, inflammation, and cancer. Adv. Pharmacol. 74, 263–302.
Lovell, T., 1998. Nutrition and Feeding of Fish[M].
Macfarlane, R.B., Harvey, H.R., Bowers, M.J., Patton, J.S., 1990. Serum Lipoproteins in Striped Bass (*Morone saxatilis*): Effects of Star. Can. J. Fish. Aquatic Sci. 47, 739–745.
Maita, M., Tachiki, H., Kaibara, A., Itawaki, R., Ikeda, Y.J.N.S., 1996. Pharmacological effect of Ursodeoxycholic acid in juvenile eel (short paper). Nihon Suisan Gakkaishi. 62, 129–130.
Martins, D.A., Afonso, L.O.B., Hosoya, S., Lewis-Mccrea, L.M., Valente, L.M.P., Lall, S.P., 2007. Effects of dietary lipid level on growth and lipid utilization by juvenile Atlantic halibut (*Hippoglossus hippoglossus, L.*). Aquaculture. 263, 150–158.
Mate, J.M., 2000. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 153, 83–104.
Metcalfe, L.D., Schmitz, A.A., Pelka, J.R., 1966. Rapid Preparation of Fatty Acid Esters from Lipids for Gas Chromatographic Analysis. Analytical Chemistry 38 (3), 514–515.
Nanton, D.A., Lall, S.P., Ross, N.W., McNiven, M.A., 2003. Effect of dietary lipid level on fatty acid β-oxidation and lipid composition in various tissues of haddock, Melanogrammus aeglefinus L. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 135, 95–108.
Ning, L.-J., He, A.-Y., Li, J.-M., Lu, D.-L., Jiao, J.-G., Li, L.-Y., Li, D.-L., Zhang, M.-L., Chen, L.-Q., Du, Z.-Y., 2016. Mechanisms and metabolic regulation of PPARα activation in Nile tilapia (Oreochromis niloticus). BBA - Molecular and Cell Biology of Lipids. 1861, 1036–1048.
Parsaie, S., Shariatmadari, F., Zamiri, M.J., Khajeh, K., 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers compared with a maize-based diet. Br. Poult. Sci. 48 (5), 594–600.
Pedro, B., Beatriz, O., Susana, C., Jorge, D., Luis, C.O., Valente, L.M.P., 2009. Dietary lipid level affects growth performance and nutrient utilisation of Senegalese sole (*Solea senegalensis*) juveniles. Br. J. Nutr. 102, 1007–1014.
Russell, D.W., 2009. Fifty years of advances in bile acid synthesis and metabolism. J. Lipid Res. 50, S120.
Sargent, J., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177.
Sheridan, M.A., 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp. Biochem. Physiol. 90B, 679–690.
Smith, S., Witkowski, A., Joshi, A.K., 2003. Structural and functional organization of the animal fatty acid synthase. Prog. Lipid Res. 42, 289–317.
Souza-Mello, V., 2015. Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver disease. World J. Hepatol. 7, 1012.
Stefania, C., Hagen, R.M., Lelliott, C.J., Marc, S., Gema, M.G., Chong-Yew, T., Audrey, S., Atherton, H.J., Nuria, B., Mikael, B., 2013. Adaptive changes of the Insig1/SREBP1/SCD1 set point help adipose tissue to cope with increased storage demands of obesity. Diabetes. 62, 3697–3708.
Tibbetts, S.M., Lall, S.P., Milley, J.E., 2015. Effect of dietary protein and lipid levels and DP DE-1 ratio on growth, feed utilization and hepatosomatic index of juvenile haddock, Melanogrammus aeglefinus L. Aquac. Nutr. 11, 67–75.
Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.
Wang, H., Eckel, R.H., 2009. Lipoprotein lipase: from gene to obesity. Am. J. Physiol. Endocrinol. Metab. 297, E271.
Wang, J.T., Liu, Y.J., Tian, L.X., Mai, K.S., Du, Z.Y., Wang, Y., Yang, H.J., 2005. Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*). Aquaculture. 249, 439–447.
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. Sci. Rep. 6, 19921.
Watanabe, S., Fujita, K.J.L., 2014. Dietary hyodeoxycholic acid exerts hypolipidemic effects by reducing farnesoid X receptor antagonist bile acids in mouse enterohepatic tissues. 49, 963–973.
Winzer, K., Becker, W., Van Noorden, C.J.F., Köhler, A., 2000. Shorttime induction of oxidative stress in hepatocytes of the European flounder (Platichthys flesus). Mar. Environ. Res. 50, 495–501.
Zhang, J.M., Wang, X.H., Hao, L.H., Wang, H., Zhang, X.Y., Muhammad, I., Qi, Y., Li, G.L., Sun, X.Q.J.E.T.P., 2017. Nrf2 is crucial for the down-regulation of Cyp7a1 induced by arachidonic acid in Hepg2 cells. Environ. Toxicol. Pharmacol. 52, 21–26.
Zidong Donna, F., Julia Yue, C., Klaassen, C.D., 2014. Atorvastatin induces bile acid-synthetic enzyme Cyp7a1 by suppressing FXR signaling in both liver and intestine in mice. J. Lipid Res. 55, 2576–2586.
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.