Effects of Supplementation of Ferulic Acid (FA) on Growth Performance, Activities of Digestive Enzymes, Antioxidant Capacity and Lipid Metabolism of Large Yellow Croaker (*Larimichthys crocea*) Larvae

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Research Article

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Abstract

A 30-day feeding trial was conducted to investigate effects of supplementation of ferulic acid (FA) on growth performance, activities of digestive enzyme, antioxidant responses and lipid metabolism of the large yellow croaker (*Larimichthys crocea*) larvae. Four isonitrogenous and isolipidic micro-diets were formulated with graded levels of FA (0, 20, 40, and 80 mg/kg). Results showed that larvae fed the diet with supplementation of 40 mg/kg FA had significantly higher survival rate, while the specific growth rate was significantly higher in larvae fed diets with 40 and 80 mg/kg FA than the control group ($P < 0.05$). Activities of trypsin in pancreatic segments (PS) and intestinal segments, lipase in PS and Alkaline phosphatase in brush border membrane were significantly increased by supplementation of FA compared to the control group ($P < 0.05$). Supplementation of FA significantly increased activities of total superoxide dismutase and catalase, and reduced the malondialdehyde content compared to the control group. Meanwhile, activities of lysozyme, total nitric oxide synthase and nitric oxide content were significantly improved by supplementation of FA in diets. Furthermore, results revealed that supplementation of FA reduced the lipid accumulation in visceral mass of larvae fed the diet with 40 mg/kg FA probably through inhibiting gens expression of lipogenesis-related genes (*scd1*, *fas* and *dgat2*) and promoting expression of lipid catabolism-related genes (*aco, cpt1*, and *hl*). In conclusion, appropriate supplementation of 40 mg/kg FA could improve the survival and growth performance of large yellow croaker larvae through increasing digestive, antioxidant capacity and promoting lipid metabolism.

Introduction

Ferulic acid (FA) is a hydroxycinnamic acid extracted from natural products, especially the Chinese herb *Ferula sinkiangensis K. M. Shen* (Silva and Batista 2017; Zhu et al. 2020). As a promising therapeutic phytochemical (Chaudhary et al. 2019), FA had been confirmed of a wide range of functions in regulating enzyme activities (Silva and Batista 2017), promoting abilities of anti-oxidation (Sarker and Oba 2020; Soobrattee et al. 2005), anti-inflammation (Zhou et al. 2020) and anti-cancer (Chaudhary et al. 2018; Middleton et al. 2000). In recent years, researches in the application of FA were carried out in aquatic animals. Previous studies have shown that supplementation of FA can improve the growth performance, antioxidant capacity and immune response of Nile tilapia (*Oreochromis niloticus*), and promote the development of digestive system by improving intestinal morphology and microbiome composition (Dawood et al. 2020; Yu et al. 2020a, b). Moreover, the supplementation of FA in diets enhanced the integument color and inhibited oxidative stress in Red Sea Bream (*Pagrus major*) (Maoka et al. 2008).

Large yellow croaker (*Larimichthys crocea*) is a carnivorous marine fish species which was widely cultured in southeast China due to delicious taste and commercial value (Feng et al. 2017). Compared with juvenile and adult fish, larval phase is critical to fulfill the development of organ systems of the physiological functions (Huang et al. 2020; Liu et al. 2020). However, the production of larvae is still hampered by high mortality during the metamorphosis and weaning (Yao et al. 2020). Nutritional studies on fish larvae had focused on feeding habits, digestive physiology and nutritional requirements (Ai et al. 2008; Feng et al. 2017; Zhao et al. 2013), while few studies on the promotion of larval survival and growth by Chinese herbs were published.

Nevertheless, there was no report even concerned about activities of digestive enzyme and the lipid metabolism of supplementation of FA in marine fish larvae. Thus, the present study intended to evaluate the potential effects of supplementation of FA on growth performance, activities of digestive enzyme, antioxidant capacity and lipid metabolism of large yellow croaker larvae.

Materials And Methods

Feed ingredients and diets formulation

Four isonitrogenous (50.0% crude protein) and isoenergetic (16.7% crude lipid) micro-diets were formulated with graded levels of FA (0, 20, 40, and 80 mg/kg dry diet) (Table 1). The purity of FA was above 99.0%, purchased from Beijing Solarbio Technology Co., Ltd in China.

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

Ingredient % dry diet	Diets (Ferulic Acid %)			
	Diet1 (0)	Diet2 (20)	Diet3 (40)	Diet4 (80)
White fish meal [a]	45	45	45	45
Krill meal [a]	22	22	22	22
Squid viscera meal [a]	3	3	3	3
Yeast hydrolysate [a]	3.5	3.5	3.5	3.5
Soybean Lecithin [b]	5	5	5	5
α-starch	2.9	2.9	2.9	2.9
Calcium bis	2	2	2	2
Vitamin premix [c]	1.5	1.5	1.5	1.5
Mineral premix [d]	1	1	1	1
Ascorbyl polyphosphate	0.2	0.2	0.2	0.2
Sodium alginate	2	2	2	2
Mould inhibitor	0.05	0.05	0.05	0.05
Antioxidant	0.05	0.05	0.05	0.05
Choline choride	0.2	0.2	0.2	0.2
Fish oil	6.5	6.5	6.5	6.5
Strong flour	5	5	5	5
Microcrystalline Cellulose	0.1	0.098	0.096	0.092
Ferulic acid [e]	0	0.002	0.004	0.008
Analyzed nutrients composition (dry matter basis)				
Crude protein (%)	49.92	50.11	50.30	50.37
Crude lipid (%)	16.83	16.84	16.89	16.70

[a] Commercially available from Great Seven Biotechnology Co., Ltd in Shandong, China; elementary composition (dry matter): White fish meal, crude protein, 71.73%, crude lipid, 4.76%; Krill meal, crude protein, 64.86%, crude lipid, 8.0%; Squid viscera meal, crude protein, 81.81%, crude lipid, 5.16%.

[b] Commercially available from Beijing Huaxia Houde Co., Ltd. (Beijing, China).

Ingredient % dry diet	Diets (Ferulic Acid %)			
	Diet1 (0)	Diet2 (20)	Diet3 (40)	Diet4 (80)
[c] Composition of vitamin premix (IU or g kg^{-1}): vitamin A palmitate, 3000000 IU; vitamin D_3, 1200000 IU; DL-α-vitamin E, 40.0 g/kg; menadione, 8.0 g/kg; thiamine-HCl, 5.0 g/kg; riboflavin, 5.0 g/kg; D-calcium pantothenate, 16.0 mg/kg; pyridoxine-HCl, 4.0 mg/kg; inositol, 200.0 mg/kg; biotin, 8.0 mg/kg; folic acid, 1.5 mg/kg; 4-aminobenzoic acid, 5.0 mg/kg; niacin, 20.0 mg/kg; vitamin B_{12}, 0.01 mg/kg; L-ascorgyl-2-monophosphate-Na (3%), 2000.0 mg/kg.				
[d] Composition of mineral premix (g/kg premix): $Ca(H_2PO_4)\cdot H_2O$, 675.0; $C_0SO_4\cdot H_2O$, 0.15; $CuSO_4\cdot H_2O$, 5.0; $FeSO_4\cdot 7H_2O$, 50.0; KCl, 0.1; $MgSO_4\cdot 2H_2O$, 101.7; $MnSO_4\cdot 2H_2O$, 18.0; NaCl, 80.0; $NaSeO_3\cdot H_2O$, 0.05; $ZnSO_4\cdot 7H_2O$, 20.0.				
[e] The ferulic acid was purchased from Beijing Solarbio Technology Co., Ltd in Beijing, China. The purity was ≥ 99.0%.				

Micro-diet was manufactured by extrusion-spheronization method using axial single screw spherical extruder and spheronization. All formulated diets were packed in separate silver bags and stored at -20℃ until use.

Experimental procedures

Disease-free, homogenous size of large yellow croaker larvae were supplied and reared at Marine and Fishery Science and Technology Innovation Base, Zhejiang, China. The average body weight of large yellow croaker larvae was 2.58 ± 0.30 mg. All larvae were fed with rotifers (*Brachionus plicatilis*) (0.5-1.5 x 10^4 individual/L) from 3 to 8 DAH, brine shrimp (*Artemia nauplii*) (1.0-1.5 x 10^3 individual/L) from 6 to 11 DAH, and the mix of live copepodas (*Calanus sinicus*) and the control feed of experiment from 10 to 14 DAH. Then all the larvae were completely fed with experimental diet. The experiment was carried out in 12 white plastic tanks with a water volume of 220 L and density of randomly selected 3000 larvae per tank. Four experimental diets were randomly allocated to triplicate groups of larvae. From 15 to 45 DAH, larvae were manually fed to satiation with the experimental diets seven times (06:30, 09:30, 12:30, 15:30, 18:30, 21:30, and 23:30) daily. The seawater was filtered through the secondary sand filter and was changed 150-200% daily. During the rearing period, water quality was controlled in temperature of 24.5 ± 1.5 ℃, pH of 8.0 ± 0.2 and salinity of 23 ± 2 g/L.

Sampling and dissection

At the beginning of the experiment, initial body length (IBL) and initial body weight (IBW) were measured by fifty larvae of 15 DAH collected randomly from each tank. At the termination, the survival rate (SR) was determined by counting the remaining individuals in each tank. All 45 DAH larvae were fasting for 24 h at the end of the experiment to empty digestive tract, and then were sampled. Fifty individuals were randomly selected from each tank to monitor the final body length (FBL) and final body weight (FBW). Twenty larvae in each tank were dissected on ice to obtain visceral masses containing a crude mixture of pancreas, liver, heart, spleen, and intestine. The visceral masses were quickly put into 2.0 mL RNase-free cryogenic vials, then immediately frozen in liquid nitrogen for gene expression analysis. Fifty larvae were separated under a

dissecting microscope on a glass plate maintained at 0 ℃ to obtain pancreatic segments (PS) and intestinal segments (IS) for the determination of activities of digestive enzyme.

Body composition

After sampling, remaining larvae were taken out from each tank to measure total crude protein, crude lipid, and moisture. The moisture content was determined by placing the sample in a ventilated drying oven at 105 ℃ until the larval weight was constant. The crude protein and the crude lipid of the sample were determined following the method of Association with Official Analytical Chemists (AOAC 2003).

Digestive enzyme activities assay

Samples of 0.2-0.3 g PS and IS were homogenized in 2 mL 0 ℃ phosphate-buffered saline (pH = 7.4) respectively and centrifuged at 3300 g for 10 mins. The supernatant was collected for further determination. Purified brush border membranes (BBM) from homogenate of the intestinal segment were obtained according to a method described by Crane et al. (1979). The activity of trypsin was assayed in accordance with Holm et al. (1988). The activity of leucine-aminopeptidase (LAP) was assayed according to Ji et al. (2013) and Maroux et al. (1973). Several assay kits including total protein quantitative assay kit, α-Amylase assay kit, lipase assay kit, and alkaline phosphatase (AKP) assay kit were purchased from Nanjing Jiancheng Institute of Biological Engineering, China. All experiments were carried out in strict accordance with the instructions.

Antioxidant and innate immune enzyme activities assay

The visceral mass of fish larvae was weighed and homogenized in 0 ℃ phosphate-buffered saline (pH = 7.4). The proportion of tissue (g) and saline (mL) was 1:9. The homogenate of visceral mass was then centrifuged at 3300 g for 10 mins, and the supernatant was used for the assay of activities of antioxidant and innate immune enzyme. Activities of total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC) catalase (CAT), and Malondialdehyde (MDA) content as well as activities of lysozyme (LZ), total nitric oxide synthase (T-NOS), inducible nitric oxide synthase (iNOS) and nitric oxide (NO) content in visceral mass were determined by commercial reagents and kits (Nanjing Jiancheng Bio-Engineering Institute, China).

RNA extraction and real-time quantitative PCR

The sample was pulverized in liquid nitrogen and added with Trizol reagent (Takara, Japan). The total RNA was extracted according to the manufacturer's protocol. The integrity of RNA was evaluated by electrophoresis, and the total RNA concentration was measured using a Nano Drop® 2000 spectrophotometer (Thermo Fisher Scientific, USA). RNase-Free DNase (Takara, Japan) was using to remove the DNA contaminant in RNA. The RNA was reversely transcribed to cDNA by Prime Script-RT reagent Kit (Takara, Japan). The real-time quantitative polymerase chain reaction was carried out in a quantitative thermal cycler (CFX96TM Real-Time System, BIO-RAD, USA). The primers sequence of stearoyl-coenzyme A desaturase 1 (*scd1*), fatty acid synthase (*fas*), diacylgycerol acyltransferase (*dgat2*), sterol-regulatory element binding protein 1 (*srebp1*), acyl-CoA oxidase (*aco*), carnitine palmitoyl transferase 1 (*cpt1*), peroxisome proliferators-activated receptor (*pparα*), hepatic lipase (*hl*), lipoprteinlipase (*lpl*), fatty acid binding protein 3 (*fabp3*), fatty acid binding protein 10 (*fabp10*), fatty acid binding protein 11 (*fabp11*),

microsomal TAG transfer protein (*mtp*), apolipoprotein b100 (*apob100*) and *β-actin*, were synthesized based on the published sequences from Cai et al. (2017) Xu et al. (2015), Yan et al. (2015), and Zuo et al. (2013) (Table 2). Real-time quantitative PCR temperature profile was 95 ℃ for 2 mins, followed by 39 cycles of 95 ℃ for 10 s, 59 ℃ for 10 s, and 72 ℃ for 20 s. The fluorescence data acquired during the extension phase were normalized to *β-actin* via $2^{-\Delta\Delta CT}$ methods as described by Livak and Schmittgen (2001).

Table 2
Primers used for quantitative PCR.

Gene	Forward(5'-3')	Reverse(5'-3')	Accession number
scd1	AAAGGACGCAAGCTGGAACT	CTGGGACGAAGTACGACACC	Xu et al. (2015)
fas	CAGCCACAGTGAGGTCATCC	TGAGGACATTGAGCCAGACAC	Cai et al. (2017)
dgat2	TTCGGTGCTTTCTGCAACTTCG	AAGGATGGGGAAGCGGAAGT	Yan et al. (2015)
srebp1	TCTCCTTGCAGTCTGAGCCAAC	TGAGGACATTGAGCCAGACAC	Cai et al. (2017)
aco	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	Yan et al. (2015)
cpt1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	Yan et al. (2015)
pparα	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC	Zuo et al. (2013)
hl	TCCGTCCATCTATTCATTGACTCTC	GCCACTGTGAACCTTCTTGATATTG	Cai et al. (2017)
lpl	GAGAGGATTCATCTGCTGGGTTAC	ACATCAACAAACTGGGCGTCATC	Yan et al. (2015)
fabp3	CCAAACCCACCACTATCATCTCAG	GCACCATCTTTCCCTCCTCTATTG	Yan et al. (2015)
fabp10	CAATGGAACATGGCAGGTTTACG	TGATTGGCTTGATGTCCTTGGC	Yan et al. (2015)
fabp11	CAGGTGGGCAATCGGACCAA	GGCTCGTTGAGCTTGAACTTGA	Yan et al. (2015)
mtp	ATGTCCAAAATGTTCTCCATGTCTG	ATGTCAATAGCCAACCCTCCTTG	Cai et al. (2017)
apob100	AGAGTGTTGTCCAGGATAAAGATGC	CAGGGCTCAGGGTCTCAGTC	Cai et al. (2017)
β-actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	Yan et al. (2015)

scd1, stearoyl-CoA desaturase 1; *fas*, fatty acid synthase; *dgat2*, diacylgycerol acyltransferase 2; *srebp1*, sterol-regulatory element binding protein 1; *aco*, acyl-CoA oxidase; *cpt-1*, carnitine palmitoyl transferase-1; *pparα*, peroxisome proliferators-activated receptor; *hl*, hepatic lipase; *lpl*, lipoprtein lipase; *fabp3*, fatty acid binding protein 3; *fabp10*, fatty acid binding protein 10; *fabp11*, fatty acid binding protein 11; *mtp*, microsomal TAG transfer protein; *apob100*, apolipoprotein b100.

Calculations and statisticasl analysis

The parameters were calculated as follows:

Survival rate (SR, %) = $N_t \times 100 / N_0$

Specific growth rate (SGR, %/day) = $(\text{Ln } W_t - \text{Ln } W_0) \times 100 / d$

where N_t is the final number of larvae in each tank, and N_0 is the initial number of larvae in each tank; W_t is the final wet body weight (g), and W_0 is the initial wet body weight; d is the experimental period in days.

All data were subjected to perform statistical analysis by using SPSS Statistics 25.0 software (SPSS Inc., USA). The data were firstly analyzed by using one-way analysis of variance (ANOVA), and then determined by Tukey's multiple range test. The level of significance was chosen at $P < 0.05$. Results were expressed as mean ± S.E.M. (Standard error of means).

Results

Survival, growth performance and body composition

With the supplementation of FA increasing from 0 to 40 mg/kg, the SR of large yellow croaker larvae significantly increased from 15.50 to 21.42% ($P < 0.05$) (Table 3). Meanwhile, larvae fed the diet with 40 and 80 mg/kg FA showed significantly higher SGR than the control group ($P < 0.05$) (Table 3). The crude protein of larvae showed an increasing trend, while the total lipid was decreasing from 20.45 to 19.32% with FA supplementation from 0 to 20 mg/kg, but no significant differences were observed among dietary treatments ($P > 0.05$) (Table 4).

Table 3
Effects of supplementation of ferulic acid (FA) on survival and growth performance of large yellow croaker larvae (Means ± S.E.M., n = 3)[1].

Parameters	Diets (Ferulic acid supplementation level mg/kg)			
	Diet1 (0)	Diet2 (20)	Diet3 (40)	Diet4 (80)
Final body length (FBL, mm)	16.47±0.32	18.31±0.32	18.69±0.93	18.65±1.34
Final body weight (FBW, mg)	80.21±6.05^{b}	108.18±7.72^{ab}	124.81±6.14^{a}	121.76±8.93^{a}
Specific growth rate (SGR %/day)	11.44±0.24^{b}	12.57±0.20^{ab}	12.87±0.43^{a}	12.83±0.24^{a}
Survival rate (SR %)	15.50±1.16^{b}	16.44±0.34^{ab}	21.42±1.29^{a}	18.72±1.69^{ab}

[1] Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 4
Effects of supplementation of ferulic acid (FA) on body composition in large yellow croaker (*Larmichthys crocea*) larvae. (Means ± S.E.M., n = 3)[1].

Parameters	Diets (Ferulic acid supplementation level mg/kg)			
	Diet1 (0)	Diet2 (20)	Diet3 (40)	Diet4 (80)
Crude protein (%)	57.91±0.38	58.20±0.92	59.11±0.72	58.74±0.77
Crude lipid (%)	20.45±0.19	19.32±0.43	19.53±0.73	19.91±0.48
Moisture (%)	87.79±0.34	88.39±1.02	88.78±0.81	90.69±0.48

[1] Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Activities of digestive enzyme

No significant difference in the activity of amylase in larval PS and IS were observed among all dietary treatments ($P > 0.05$) (Table 5). The activity of trypsin in PS of larvae fed the diet with 20 mg/kg FA and trypsin in IS in larvae fed diets with 20 and 40 mg/kg FA were significantly higher than the control group ($P < 0.05$) (Table 5). Meanwhile, the activity of lipase in larval PS were significantly higher in larvae fed diets with 20 and 40 mg/kg FA than the control group ($P < 0.05$) (Table 5). The activity of AKP in BBM of larvae fed diets with 20 and 40 mg/kg FA had significantly higher activities compared to the control group ($P < 0.05$), while no difference was observed among dietary treatments in LAP of larvae BBM ($P > 0.05$) (Table 5).

Table 5
Effects of supplementation of ferulic acid (FA) on activities of main digestive enzymes of large yellow croaker larvae (Means ± S.E.M., n = 3)[1].

Parameters		Diets (Ferulic acid supplementation level mg/kg)			
		Diet1 (0)	Diet2 (20)	Diet3 (40)	Diet4 (80)
Amylase (U/mg·protein)	PS	0.30±0.04	0.35±0.02	0.24±0.04	0.29±0.01
	IS	0.27±0.03	0.26±0.02	0.24±0.04	0.28±0.03
Trypsin (U/mg·protein)	PS	0.45±0.12^{b}	0.93±0.10^{a}	0.82±0.12^{ab}	0.44±0.37^{b}
	IS	1.07±0.13^{b}	2.03±0.12^{a}	1.85±0.12^{a}	1.62±0.18^{ab}
Lipase (mU/mg·protein)	PS	0.97±0.10^{b}	1.50±0.10^{a}	1.50±0.08^{a}	1.28±0.11^{ab}
	IS	0.98±0.21	1.32±0.12	1.64±0.23	1.25±0.16
AKP (U/mg·protein)	BBM	1.05±0.06^{b}	1.72±0.15^{a}	1.66±0.14^{a}	1.35±0.22^{ab}
LAP (U/mg·protein)	BBM	12.17±0.55	13.24±0.63	13.43±1.73	14.74±0.86

Abbreviations: AKP, Alkaline-phosphatase; LAP, Leucine-aminopeptidase; PS, Pancreatic segments; IS, Intestinal segments; BBM: brush border membranes.

[1]·Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Antioxidant and innate immunity capacity

The activity of T-SOD was significantly higher in larvae fed the diet with 40 mg/kg FA compared to the control group ($P < 0.05$) (Fig. 1A). However, the activity of T-AOC was not significantly different among dietary treatments ($P > 0.05$) (Fig. 1B). The activity of CAT in larvae fed diets with 20 and 40 mg/kg FA was significantly higher than the control group ($P < 0.05$) (Fig. 1C). Meanwhile, the MDA content in larvae fed diets with 20 and 40 mg/kg FA were significantly lower than the control group ($P < 0.05$) (Fig. 1D). The

activity of LZ in visceral mass was significantly higher in larvae fed diets with 20 and 40 mg/kg FA than the control group ($P < 0.05$) (Fig. 2A),while larvae fed the diet with 40 mg/kg FA had the significantly higher activity of T-NOS compared to the control group ($P < 0.05$) (Fig. 2B). No significant difference was observed in the activity of iNOS among dietary treatments ($P < 0.05$) (Fig. 2C).The NO content in larvae fed diets with 40 and 80 mg/kg FA were significantly higher than the control group ($P < 0.05$) (Fig. 2D).

Triglyceride (TG) content and mRNA expression of lipid metabolism-related genes in visceral mass

TG content of visceral mass in larvae fed the diet with 40 mg/kg FA was significantly lower than the other groups ($P < 0.05$) (Fig. 3A). In terms of the lipogenesis-related mRNA expression, mRNA expression of *scd1* and *fas* were significantly lower in larvae fed the diet with 80 mg/kg FA than the control group ($P < 0.05$), while supplementation of 40 mg/kg FA significantly reduced the mRNA expression of *dgat2* ($P < 0.05$) (Fig. 3B). For the fatty acid catabolism-related genes expression, compared to the control group, larvae fed the diet with 80 mg/kg FA had significantly higher mRNA expression of *aco*, and larvae fed diets with 40 and 80 mg/kg FA had significantly higher mRNA expression of *cpt1* and *hl* ($P < 0.05$) (Fig. 3C). No significant difference was found in the mRNA expression of *ppara* and *lpl* among all treatments ($P > 0.05$) (Fig. 3C). For the lipid transport-related genes, the mRNA expression of *fabp3* was significantly lower in larvae fed diets with 20 and 80 mg/kg FA ($P < 0.05$) and the mRNA expression of *fabp11* was significantly reduced by supplementation of 20 and 40 mg/kg FA ($P < 0.05$) compared to the control group (Fig. 3D). Larvae fed the diet with 40 mg/kg FA had significantly higher *fatp1* expression than the control group ($P < 0.05$) (Fig. 3D). No significant differences was found in the mRNA expression of *mtp*, *fabp10* and *apob100* among all dietary treatments ($P > 0.05$) (Fig. 3D).

Discussion

Results of the present study demonstrated that the survival and growth performance of large yellow croaker larvae were significantly enhanced by supplementation of 40 mg/kg FA, which was consistent with previous findings of farm livestocks in lambs (Macías-Cruz et al. 2014; Valadez-García et al. 2021), heifers (Pena-Torres et al. 2021), weaned piglets (Wang et al. 2020), and fish in Nile tilapia (Dawood et al. 2020; Yu et al. 2020a, b), and zebrafish (*Danio rerio*) (Wen and Ushi 2017). These researches demonstrated that supplementation of dietary FA could promote the survival and growth performance of large yellow croaker larvae, which were probably due to its improvement in the activities of digestive enzyme, anti-oxidation capacity and lipid metabolism.

Improving the activity of digestive enzyme generally led to the advancement efficiency of nutrient digestion and absorption, and then enhance digestive system function of fish larvae (Izquierdo et al. 2000; Khoa et al. 2021). Previous studies have found that enhancing digestive enzyme activities can promote the survival and growth of fish larvae (Imentai et al. 2020), which was similar to the results of the present study. AKP, concentrated in BBM, is an important indicator of digestive and absorptive functions of the intestine (Yuan et al. 2020). In the present study, FA in diets not only improved the activity of trypsin (PS and IS) and lipase

(PS), but also significantly improved activities of AKP (BBM), indicating the improvement of digest ability of large yellow croaker larvae. These results indicated that FA could improve activities of digestive enzymes of large yellow croaker larvae, which were consistent with the results in Nile tilapia (Yu et al. 2020b).

The antioxidant function of fish larvae is not mature, which made the larvae susceptible to the influence of external environment and resulted in slower growth (Birnie-Gauvin et al. 2017). Various kinds of stresses may trigger the production of reactive oxygen species (ROS), then cause oxidative injuries such as lipid peroxidation and DNA damage (Martínez-Álvarez et al. 2005). In order to cope with the stresses, antioxidant defenses system has been developed, containing T-SOD, CAT, and T-AOC (Martínez-Álvarez and Morales 2005). In the present study, activities of SOD and CAT in larvae fed diets with FA were significantly higher than the control group. The result was similar to the study in Nile tilapia (Yu et al. 2016, 2020a), which found that the supplementation of dietary FA could decrease the MDA content and increase activities of SOD and CAT. MDA is the biomarker of oxidative damage (Del et al. 2005). In the present study, MDA content in larvae fed diets with 20 and 40 mg/kg FA were significantly decreased compared to the control group, suggesting that FA reduced the oxidative damage of large yellow croaker larvae. Also, Maoka et al. (2008) reached a similar conclusion that the addition of dietary FA significantly reduced the hepatic MDA content of Red Sea Bream. Therefore, diets supplemented with 20 to 40 mg/kg FA could enhance the antioxidant capacity of large yellow croaker larvae by increasing activities of antioxidant enzymes and reducing the damage of oxygen free radicals.

Antioxidant capacity was positively correlated with reduced inflammation (Li et al. 2019; Mukherjee et al. 2013). Herbal extract have been shown a positive effect on the immunity of fish (Giri et al. 2019; Nootash et al. 2013; Wang et al. 2015; Zemheri-Navruz et al. 2019). The promotion of non-specific immune system not only can help fish to overcome infectious factors but also has an important preventative role (Galina et al. 2009; Punitha et al. 2008; Reverter et al. 2014). The immune function of FA had already been shown in rats (Katayama et al. 2017; Sadar et al. 2016; Zheng et al. 2019) and carp (*Cyprinus carpio*) (Ahmadifar et al. 2019). In the present study, FA in diets significantly increased activities of LZ, T-NOS, and NO content of large yellow croaker larvae. Results were similarly to the Nile tilapia, which significantly enhanced the activity of LZ and NO content (Yu et al. 2020a). The obtained result in this study indicated the potential effect of FA in large yellow croaker larvae on enhancement of immunity and inhibition of stress, similar to the previous study in Nile tilapia (Dawood et al. 2020).

Due to high energy consumption of larvae, promoting lipid catabolism would supply more energy and spare more protein to increase the growth performance (Li et al. 2016). According to previous researches, FA had shown its excellent function on improving lipid metabolism in mammals (Koh et al. 2017; Guo et al. 2019; Wang et al. 2020; Wang et al. 2019) and fish (Yu et al. 2016). In the present study, the supplementation of 40 mg/kg FA had significantly reduced TG content in visceral mass, which was similar to the results in the Nile tilapia (Yu et al. 2016). The results indicated that FA could reduce the lipid accumulation of fish (Cohen et al. 2011). The mRNA expression of *scd1*, *fas* and *dgat2* were significantly down-regulated by supplementation of FA compared to the control group. *Scd1*, *fas* and *dgat2* are crucial in the process of adipocyte synthesis, indicating that FA could decrease the lipogenesis of large yellow croaker larvae (Carobbio et al. 2013; Harris et al. 2011). Compared with the control group, supplementation of FA activated

aco, *cpt1* and *hl* genes thereby activating fatty acid oxidation and accelerating the lipid consumption process. The result was similar in mice with Ma et al. (2019). The mRNA expression of *fabp3* and *fabp11* in larvae fed diets with FA were markedly decreased compared to the control group. Results above showed that FA could reduce the lipogenesis and accumulation of large yellow croaker larvae, which was achieved by reducing the expression of lipogenesis-related genes, up-regulating the expression of lipid catabolism-related genes and down-regulating the relative expression of transport-related genes. Combined with the growth of large yellow croaker larvae in this study, we have reason to suspect that supplementation of FA in diets can promote the SR and SGR of large yellow croaker larvae through improving the lipid metabolism of larvae.

Conclusions

In conclusion, results of the present study demonstrated that appropriate supplementation of FA (40 mg/kg) could promote growth performance of large yellow croaker larvae, which was probably due to its improvement in activities of digestive enzymes, antioxidant capacity and lipid metabolism.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contribution

The authors' contributions were as follows: Q. A. and K. M. formulating the research question; W. X., W. H., C. Y., Y. L. and Z. Y. designed the study and conducted the research; W. X. analysed the data, interpreted the findings and wrote the article. All authors revised the article. We appreciate W. L., X. C., Y. G., and D. X. for their help in revising the article.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

Code availability

Not applicable.

Consent to participate

The authors agree to collaborate and publish this article.

Ethics approval

All experiments in this study were in accordance with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017).

Consent for publication

We will transfer the copyright of the article to editorial office for publishing.

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Figures

Figure 1

Effects of supplementation of ferulic acid (FA) on antioxidant capability in visceral mass of large yellow croaker larvae. A: T-SOD, total superoxide dismutase; B: CAT, catalase; C: T-AOC, total antioxidant capacity; D: MDA, malondialdehyde. Values are means (n = 3), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test).

Figure 2

Effects of supplementation of ferulic acid (FA) on immune capability of large yellow croaker larvae. A: LZ, lysozyme; B: T-NOS, total nitric oxide synthase; C: iNOS, inducible nitric oxide synthase; D: NO, Nitric Oxide. Values are means (n = 3), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test).

Figure 3

Effects of supplementation of ferulic acid (FA) on triglyceride content and relative mRNA expression of genes involved in lipid metabolism in visceral mass of large yellow croaker larvae. A: the triglyceride content; B: the relative mRNA expression of genes involved in lipogenesis; C: the relative mRNA expression of genes involved in lipid consumption; D: the relative mRNA expression of genes involved in lipid transportation. Values are means (n = 3), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test).