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Original Research Article

Dietary lysophospholipids improves growth performance and hepatic lipid metabolism of largemouth bass (*Micropterus salmoides*)

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ABSTRACT

This study was conducted to evaluate the influence of dietary lysophospholipids combined with 1% dietary fish oil reduction on the growth performance and hepatic lipid metabolism of largemouth bass (*Micropterus salmoides*). Five isonitrogenous feeds were prepared with lysophospholipids at 0% (fish oil group, FO), 0.05% (L-0.05), 0.1% (L-0.1), 0.15% (L-0.15) and 0.2% (L-0.2), respectively. The dietary lipid was 11% in the FO diet and 10% in the other diets. Largemouth bass were fed for 68 d (initial body weight = 6.04 ± 0.01 g) with 4 replicates per group and 30 fish per replicate. The results showed that the fish fed diet containing 0.1% lysophospholipids had higher digestive enzyme activity and obtained better growth performance compared to the fish fed FO diet ($P < 0.05$). The feed conversion rate in the L-0.1 group was significantly lower than that in the other groups. Serum total protein and triglyceride contents in L-0.1 group were significantly higher than those in other groups ($P < 0.05$) and the contents of total cholesterol and low-density lipoprotein cholesterol in L-0.1 group were significantly lower than those in FO group ($P < 0.05$). The activity and genes expression of hepatic glucolipid metabolizing enzymes in L-0.15 group were significantly increased compared to those in FO group ($P < 0.05$). Reducing 1% fish oil along with 0.1% lysophospholipids added to the feed could improve the digestion and absorption of nutrients, enhance the activity of liver glycolipid metabolizing enzymes, and thus effectively promote the growth of largemouth bass.

1. Introduction

Largemouth bass (*Micropterus salmoides*), also known as “freshwater grouper”, is an important freshwater economic fish in China, with fast growth rate, strong disease resistance and delicious meat quality (Chen et al., 2021; Wu et al., 2021). As a carnivorous fish, largemouth bass has a limited ability to utilize carbohydrates and prefers to use lipids and proteins for energy. Lipids are not only one of the main sources of energy, but also improve the utilization of protein by reducing its consumption as energy (Li et al., 2020). However, higher dietary lipid or fish oil would present liver lesions, lower growth performance and immunity on fish such as grouper (*Epinephelus malabaricus*), largemouth bass, grass carp (*Ctenopharyngodon idella*), and juvenile turbot (*Psetta maxima*) (Lin and Shiau, 2003; Du et al., 2008; Sevgili et al., 2014).

Fish oil is the best lipid raw material containing premium essential fatty acids for the growth and development of aquatic animals, since the availability of energy is accounting for 70% to 80% (Turchini et al., 2009). However, the yield of fish oil has been down due to the limited natural fishery resources and the increasing of consumption of aquatic products (Tacon and Metian, 2008), and then the price of fish oil was up (Jiang et al., 2015). Therefore, it is especially important to reduce the amount of lipid in the feed and to improve lipid utilization. Emulsifiers such as phospholipids and bile acids were usually added to help emulsify dietary lipid into chylomicron that are suspended in digestive juice for better absorption (Romański, 2007; Jiang et al., 2018; Román Padilla et al., 2017).

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Lysophospholipid, as derivatives of lecithin, is the product obtained by removing of one molecule of hydrophobic fatty acids through the enzymatic digestion of phospholipase or lipase (Zhang, 2007). By reducing hydrophobic groups, the hydrophilicity and emulsification properties of lysophospholipids are higher than those of the lecithin, for example, the critical micelle concentration of lysophospholipids is lower (0.02 to 0.2 mmol/L) than that of lecithin (0.3 to 2 mmol/L) (Khan et al., 2018), and the emulsification capacity is 5 times higher than that of phospholipids (Zhang, 2007). When terrestrial animals ingested the diets with lysophospholipids, better growth performance could be found such as improving digestibility, promoting lipid metabolism and energy utilization (Wealleans et al., 2020; Zhang et al., 2011; Xing et al., 2004). In diets of channel catfish (*Ictalurus punctatus*) and turbot (*Scophthalmus maximus* L), lysophospholipids also exerted the function to improve feed utilization, protein efficiency and antioxidant properties (Liu et al., 2019; Li et al., 2019), and even reduce the amount of dietary lipid of tiger shrimp (*Penaeus monodon*) feed (Khan et al., 2018). The permeability of the bilayer membrane structure located intestinal mucosal cells was been altered by lysophospholipids so that the size of the membrane pores was enlarged and then the conversion of fatty acids was promoted into micelles, which improved the utilization of nutrients and energy, especially utilization of the dietary lipid (Kim et al., 2018). Another thing, lysophospholipids could take part in the lipid transport and metabolism through affecting *FAS*, *CPT-1* and *APOA* genes expression in the liver (Xu et al., 2022).

The purpose of this study was to explore the effects of dietary lysophospholipids combined with 1% dietary fish oil reduction on growth performance, histology of intestinal and liver, hepatic lipid metabolism of largemouth bass.

2. Materials and methods

2.1. Animal ethics statement

Fish handling and experimental protocols in this study were approved by the ethics committee at Guangdong Ocean University (GDOU-01/2019).

2.2. Diets and experimental design

Five isonitrogenous diets were designed for this experiment. The control group (FO) contained 11% of crude lipid with fish oil (5%) as the fat source, and the experimental group contained 10% of crude lipid with fish oil (4%) and 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively (purity 25%, Kemin AquaScience, Zhuhai, China).

The raw materials were crushed and passed through a 60-mesh sieve, accurately weighed according to the diet formula (Table 1). The raw materials were mixed evenly using the step-by-step expansion method, mixed in a V-mixer (B30, Guangzhou Panyu Lifeng Food Machinery Factory, Guangzhou, China) for 15 min, then fish oil and water were added. The sinkable puffed pellet feed was made by a dry puffing machine with a 3-mm diameter die. The diets were dried at constant temperature drying oven at 90 °C for 30 min and then at room temperature 25 °C for 48 h, until the moisture content was about 10%. The diets were turned twice a day to prevent mildew during dry period and stored at −20 °C.

2.3. Fish and feeding trial

The experiment was conducted at the Marine Biology Research Base of Guangdong Ocean University (Zhanjiang, China). Healthy juvenile largemouth bass purchased from Zhenghe Company (Zhuhai, China) were temporarily reared in 1 m^3 buckets and fed commercial diets (48% crude protein, 7% crude lipid) during the 2-week domestication phase. Fasting was carried out for 24 h before the start of the feeding trial. A total of 600 healthy largemouth bass with the approximate size (6.04 ± 0.01 g) were randomly divided into 5 groups with 4 replicates. Fish were cultured in 20 buckets (0.3 m^3) assembled in a hydrostatic aquaculture system and fed twice daily (08:00 and 16:00) until apparent satiation. At the beginning of the experiment, the culture water was filtered, precipitated, chlorine dioxide disinfected. The water for breeding was aeration treated and then replaced daily with a replacement rate of 70%. Water quality were dissolved oxygen concentration ≥5.0 mg/L, pH value 7.5 to 8.5, temperature 29.0 to 32.0 °C, and ammonia nitrogen ≤0.05 mg/L. The feeding trial lasted for 68 d.

Table 1
Ingredients and nutritional composition (% as DM basis).

Item	Diets[1]				
	FO	L-0.05	L-0.1	L-0.15	L-0.2
Ingredients					
Domestic defatted fish meal[2]	20.00	20.00	20.00	20.00	20.00
Imported steam fish meal[3]	30.00	30.00	30.00	30.00	30.00
Soybean meal[4]	10.35	10.35	10.35	10.35	10.35
Fermented soybean meal[5]	5.00	5.00	5.00	5.00	5.00
Imported chicken powder[6]	10.00	10.00	10.00	10.00	10.00
Plasma protein powder[7]	2.00	2.00	2.00	2.00	2.00
Wheat gluten flour[8]	1.50	1.50	1.50	1.50	1.50
Cassava starch[9]	4.00	4.00	4.00	4.00	4.00
Wheat flour[10]	6.15	6.15	6.15	6.15	6.15
Seaweed powder[11]	2.00	2.95	2.90	2.85	2.80
Fish oil[2]	5.00	4.00	4.00	4.00	4.00
Lysophospholipid[12]	0.00	0.05	0.10	0.15	0.20
Vitamin premix[5]	1.00	1.00	1.00	1.00	1.00
Mineral premix[5]	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00
Nutritional composition					
Moisture	7.91	8.10	8.52	8.67	8.22
Crude protein	50.51	50.81	50.75	50.95	50.16
Crude lipid	11.32	10.38	10.33	10.29	10.41
Ash	13.55	12.90	12.98	13.81	13.51

[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.
[2] Produced by Minato Kenko Fishmeal Plant (Rongcheng, China).
[3] Produced by CFG Investment S.A.C. Plant (Chimbote, Peru).
[4] Produced by China National Cereals, Oils and Foodstuffs Corporation (Dongduan, China).
[5] Produced by Guangdong Yuehai Feed Group (Zhanjaing, China).
[6] Produced by Pilgrim's Pride Corporation, Plant Moorefield (America).
[7] Produced by Geron Biological Products Co. (Nanning, China).
[8] Produced by Huafeng Powder Co. (Xinxiang, China).
[9] Produced by Danming Biological Engineering Co. (Handan, China).
[10] Produced by Yihai Kerry Cereals, Oils & Food Industry Co. (Dongguan, China).
[11] Produced by Haixing Yuan Biotechnology Co. (Qingdao, China).
[12] Produced by Kemin AquaScience (Zhuhai, China).

2.4. Sample collection and analysis

2.4.1. Growth performance

The experimental fish were fasted for 24 h before the end of the feeding trial. After anesthetized with eugenol (100 mg/L, Sinopharm Chemical Reagent Co., Ltd, Beijing, China), fish in each bucket were counted and weighed to determine survival rate (SR), weight gain rate (WGR), and specific growth rate (SGR), respectively. The amount of feed intake was recorded during culture and used to calculate feeding rate (FR), feed conversion ratio (FCR).

Five fish were randomly collected from each tank to measure body length and weight, and then dissected to strip and weigh the viscera and liver to calculate the condition factor, hepatosomatic index (HSI), and visceral somatic index (VSI).

2.4.2. Body composition determination

Three fish were randomly collected from each bucket and stored at −20 °C to test whole fish composition. Another three fish were taken from each bucket, and the liver and dorsal muscle were dissected and stored at −20 °C to test the liver and muscle nutrient composition. Approximate nutrient contents in whole fish, liver, muscle, and diets were analyzed by using standard methods of the Association of Official Analytical Chemists International (AOAC, 2000). Moisture was determined by drying in an oven at 105 °C until constant weight. The crude protein (CP) was determined by the Kjeldahl method (N × 6.25). The crude lipid (CL) was extracted by Soxhlet extraction with petroleum ether. The ash was determined by combustion in a muffle furnace at 550 °C until constant weight.

2.4.3. Measurement of serum biochemical indicators

Blood collected from the tail vein of 6 randomly selected fish from each bucket were stored at 4 °C for 12 h and then centrifuged at 4 °C at 1,062 × *g* for 15 min to separate serum.

The kits (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China) and microplate reader (Thermo Multiskan GO 1510, USA) were used to measure serum total protein (TP, No. A045-4-2), triglyceride (TG, No. A110-1-1), total cholesterol (TC, No. A111-1-1), high-density lipoprotein cholesterol (HDL-C, No. A112-1-1), low-density lipoprotein cholesterol (LDL-C, No. A113-1-1), aspartate transaminase (AST, No. C010-2-1) and alanine aminotransferase (ALT, No. C009-2-1).

2.4.4. Liver and intestinal slices

Two fish were randomly selected from each barrel, dissected to sample the liver and intestine. The samples were preserved in 4% formaldehyde solution and sent to make oil red O stained slice for liver and H&E stained for liver and intestine (Wuhan servicebio Biotechnology Co., Ltd., China).

2.4.5. Analysis of activities of digestion enzymes and glycolipid metabolizing enzyme

The liver and intestine were sampled from 3 fish randomly selected from each barrel, and quickly placed in liquid nitrogen and subsequently stored at −80 °C for enzyme activity assay. Refer to the kits (Shanghai EnzymeLink Ltd., Shanghai, China) instructions to extract the tissue supernatant, which could be used to analysis for enzyme activities, including total protease (Protease, No. ml076612), phosphoenolpyruvate carboxykinase (PEPCK, No. ml036430), glucokinase (GK, No. ml024808), glucose-6-phosphatedehydrogenase (G-6-PDH, No. ml546427), fatty acid synthase (FAS, No. ml036370), acetyl coenzyme A carboxylase (ACC, No. ml022714), lipoprotein lipase (LPL, No. ml036373), hormone-sensitive lipase (HSL, No. ml026143), and carnitine ester acyl-transferase-1 (CPT-1, No. ml036411) in liver. The activity of α-amylase (No. C016-1-1) and lipase (No. A054-2-1) in liver and intestine were measured using the kits (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China).

2.4.6. Determination of expression of genes related to hepatic lipid metabolism

Three fish were randomly collected from each bucket, and the livers were dissected and quickly placed in enzyme-free EP tubes containing RNA-later (Ambion, USA) and stored at −80 °C for gene mRNA determination. Total RNA was extracted from each fish livers using Trizol reagent (Beijing GMO Technology Co., Ltd., China) and treated with DNase reagent (Takara, Japan) to remove potentially contaminating DNA. The integrity and quality of total RNA was verified by 1% agarose gel electrophoresis and assessed using a spectrophotometer (ND-1000, Nano-Drop Technologies, Wilmington, USA). Reverse transcription assays were performed to obtain cDNA using the Prime ScriptTM RT kit (Takara, Japan). The qRT-PCR reactions were performed according to the instructions of the SYBR Premix Ex TaqTM II kit. The qRT-PCR reactions were performed in a fluorescence quantitative thermal cycler (Bio-Rad CFX96, USA) at the following conditions: 95 °C for 30 s; 95 °C for 5 s, 60 °C for 20 s, 40 cycles; 65 °C for 15 s. The internal reference gene was *ef1α*. The expression levels of the target genes were calculated followed the $2^{-\Delta\Delta Ct}$ method. The primer sequences of the genes are shown in Table 2.

2.5. Calculations and statistical methods

The data were analyzed by one-way analysis of variance (ANOVA) with SPSS 21.0. When there were significant differences between groups, Tukey's multiple comparison test was performed with differences significant at $P < 0.05$.

3. Results

3.1. Growth and feed utilization

The effects of dietary lysophospholipids levels on growth and feed utilization of largemouth bass were shown in Table 3. FBW, WGR and SGR were significantly higher in the L-0.1 and L-0.15 groups than those in the rest of the groups ($P < 0.05$). Feeding rate in the groups both L-0.05 and L-0.1 was significantly lower than that in the FO and L-0.2 groups ($P < 0.05$). FCR in the L-0.1 group was significantly lower than that in the FO group, L-0.05 and L-0.2 groups ($P < 0.05$).

The condition factor of fish in group L-0.1 was significantly higher than that in group L-0.05 ($P < 0.05$), while there was no significant difference compared with other groups ($P > 0.05$). There was no significant difference in HSI and VSI among all groups ($P > 0.05$).

3.2. Nutritional analysis of whole fish, muscle and liver

The composition of whole fish, liver and muscle of largemouth bass are shown in Table 4. There was no significant difference in

Table 2
Primer information.

Gene	Primer sequence (5′–3′)	GenBank no.
lpl	F:AACCGCAATCCCTCGCC R:AAGGTCTGTGTTTCTGAGTTGA	XM_038715978.1
hsl	F:CACTAACACCCCCACACCAA R:CAGAGTCATCCAGCAAGGCA	XM_038725628.1
cpt-1	F:AACGGATGGAGGCTTTGACC R:CTACACCTGGGACACGACTG	XM_038695351.1
atgl	F:CTTTTGGCACTCAGGGTCGT R:TACGGTGGGGTCATCAAGGT	XM_038705351.1
apoa	F:GACACTGGGGATGGAAAGCA R:CACATACGAGCAGAGCGAGT	XM_038692601.1
fas	F:TTACACTGCCACAGCAACCA R:TGCCCCTCCTACTACACCTC	XM_038735140.1
acc	F:TAGTCCAGTGCCCATCCTCA R:CCAGAAAAGCCCCTCCAGTT	XM_038709737.1
dgat	F:GCAACATCAAGCCGTCCGACTC R:AGCACAGCGAGCCAGAGGTAAT	XM_038705876.1
apob	F:GTGTTTGCTGTGCTGCTCCT R:GCTCCGTATCGTCTTTGGG	XM_038727548.1
ef1α	F:TGCTGCTGGTGTTGGTGAGTT R:TTCTGGCTGTAAGGGGGCTC	XM_038724777.1

lpl = lipoprotein lipase; *hsl* = hormone-sensitive triglyceride lipase; *cpt-1* = carnitine palmitoyl transferase 1; *atgl* = adipose triglyceride lipase; *apoa* = apolipoprotein A; *fas* = fatty acid synthetase; *acc* = acetyl-CoA carboxylase; *dgat* = diacylglycerol acyltransferase; *apob* = apolipoprotein B; *ef1α* = elongation factors 1α.

Table 3
Effects of lysophospholipid on growth performance and feed utilization of largemouth bass ($n = 3$)[1].

Index[2]	FO	L-0.05	L-0.1	L-0.15	L-0.2
IBW, g	6.04 ± 0.00	6.04 ± 0.01	6.04 ± 0.01	6.04 ± 0.01	6.03 ± 0.01
FBW, g	41.58 ± 1.43^{b}	37.29 ± 2.72^{a}	47.52 ± 1.91^{c}	45.18 ± 2.18^{c}	40.99 ± 0.83^{b}
SGR, %/d	2.92 ± 0.05^{b}	2.75 ± 0.11^{a}	3.12 ± 0.06^{c}	3.05 ± 0.08^{c}	2.90 ± 0.03^{b}
WGR, %	588.63 ± 23.81^{b}	517.99 ± 45.54^{a}	684.11 ± 31.55^{c}	647.87 ± 35.98^{c}	578.98 ± 13.83^{b}
FR, %/d	1.89 ± 0.45^{b}	1.78 ± 0.12^{a}	1.77 ± 0.43^{a}	1.84 ± 0.15^{ab}	1.90 ± 0.37^{b}
FCR	0.79 ± 0.03^{bc}	0.83 ± 0.01^{c}	0.75 ± 0.02^{a}	0.78 ± 0.02^{ab}	0.81 ± 0.03^{bc}
SR, %	96.67 ± 3.35	93.33 ± 3.35	93.33 ± 3.35	96.67 ± 3.35	94.43 ± 1.96
CF, g/cm^3	1.82 ± 0.08^{ab}	1.79 ± 0.09^{a}	1.99 ± 0.11^{b}	1.87 ± 0.04^{ab}	1.83 ± 0.04^{ab}
HSI, %	2.98 ± 0.19	2.89 ± 0.22	3.07 ± 0.25	3.03 ± 0.11	2.83 ± 0.25
VSI, %	7.26 ± 0.17	7.48 ± 0.48	6.72 ± 0.21	7.32 ± 0.17	7.39 ± 0.16

IBW = initial body weight; FBW = finally body weight; SGR = special growth rate; WGR = weight gain rate; FR = feeding rate; FCR = feed conversion rate; SR = survival rate; CF = condition factor; HSI = hepatosomatic index; VSI = visceral somatic index.
[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.
[2] The indicators of fish growth performance were calculated using the following formula: SR (%) = final fish number/initial fish number × 100, WGR (%) = [(final weight, g) − (initial weight, g)]/(initial weight, g) × 100, SGR (%/d) = [ln (final weight, g) − ln (initial weight, g)]/days of feeding trial × 100, CF (g/cm^3) = [(body weight, g)/(body length^3, cm^3)] × 100, FCR = (dry diet fed, g)/[(final body weight, g) − (initial body weight, g)], FR (%/d) = 100 × (feed consumed, g)/{[(initial body weight, g) + (final body weight, g)]/2}/days, VSI (%) = 100 × [(viscera weight, g)/body weight, g)], HSI (%) = 100 × [(liver weight, g)/(body weight, g)].

moisture and crude protein contents in whole fish, liver and muscle among the groups ($P > 0.05$). There was no significant difference in CL content of whole fish and muscle between the L-0.05 and L-0.1 groups ($P > 0.05$), but the CL content of these 2 groups was significantly lower than that of the FO group ($P < 0.05$). The lowest liver glycogen and myoglycogen contents of the fish were found in FO group ($P < 0.05$). The highest muscle glycogen contents were observed in L-0.15 group, which significantly higher than FO, L-0.05 and L-0.2 groups ($P < 0.05$). The liver glycogen content increased with the increase of dietary lysophospholipids, and the highest value was found in L-0.2 group ($P < 0.05$).

3.3. Serum biochemical indices

The effect of lysophospholipids on the serum biochemical indexes of largemouth bass is shown in Table 5. Serum TP and TG contents in group L-0.1 were significantly higher than those in other groups ($P < 0.05$). The contents of TC and LDL-C in L-0.1 group were not significantly different compared to the L-0.15 group, but were significantly lower than those in FO group ($P < 0.05$). The HDL-C contents in L-0.1 group were significantly higher than that in FO group ($P < 0.05$).

3.4. Activities of digestive enzymes in liver and intestine

The activities of digestive enzymes in liver and intestine are shown in Table 6. Lipase activities in liver and intestine showed an upward trend with the increase of dietary lysophospholipids. Hepatic lipase in L-0.05 to L-0.2 groups showed a significantly higher activities than that in the FO group ($P < 0.05$) and intestinal lipase in L-0.1 to L-0.2 groups showed significantly higher than that in FO and L-0.05 groups ($P < 0.05$). Higher amylase activities in liver and intestine were observed when dietary lysophospholipids was up to 0.1% ($P < 0.05$). There was no significant difference in the activities of fish liver protease in the L-0.05 group compared to the FO group ($P > 0.05$), while there was a significant higher activity of hepatic protease in the fish raised in the L-0.1 to L-0.2 groups ($P < 0.05$). The highest activities of intestinal protease were observed in L-0.2 group ($P < 0.05$).

3.5. Histological sections of the liver and intestine

The liver sections are demonstrated in Fig. 1. Compared to the FO group, the area of lipid droplets in the liver was significantly reduced when fish consumed the diet containing the lysophospholipids

Table 4
Effects of lysophospholipid on whole fish, muscle and liver composition of largemouth bass (%, fresh weight basis)[1].

Item	FO	L-0.05	L-0.1	L-0.15	L-0.2
Whole fish					
Moisture	71.96 ± 0.34	73.24 ± 0.21	72.09 ± 0.85	72.86 ± 0.53	69.96 ± 2.25
CP	16.58 ± 0.30	16.40 ± 0.32	17.06 ± 0.41	16.63 ± 0.61	16.35 ± 0.29
CL	6.04 ± 0.08^{c}	5.16 ± 0.11^{a}	5.40 ± 0.15^{ab}	5.52 ± 0.19^{ab}	6.11 ± 0.05^{c}
Ash	4.18 ± 0.09	4.04 ± 0.12	4.07 ± 0.11	4.15 ± 0.19	4.57 ± 0.90
Muscle					
Moisture	78.13 ± 1.99	78.39 ± 0.34	77.97 ± 0.11	77.99 ± 0.26	78.33 ± 0.29
CP	18.53 ± 1.64	18.38 ± 0.60	18.88 ± 1.00	18.79 ± 2.22	18.52 ± 0.64
CL	0.69 ± 0.04^{b}	0.54 ± 0.02^{a}	0.57 ± 0.05^{a}	0.59 ± 0.02^{a}	0.65 ± 0.03^{ab}
Glycogen, mg/g	0.28 ± 0.26^{a}	0.34 ± 0.66^{ab}	0.40 ± 0.09^{bc}	0.46 ± 0.12^{c}	0.38 ± 0.24^{b}
Liver					
Moisture	73.07 ± 0.54	71.55 ± 0.33	72.07 ± 1.71	73.3 ± 1.33	74.55 ± 0.41
CP	7.65 ± 0.46	7.85 ± 0.45	7.97 ± 0.31	7.83 ± 0.26	7.35 ± 0.29
CL	2.23 ± 0.13^{b}	2.00 ± 0.11^{b}	2.09 ± 0.06^{b}	1.92 ± 0.09^{b}	1.68 ± 0.06^{a}
Glycogen, mg/g	31.44 ± 56.28^{a}	40.48 ± 30.41^{b}	44.64 ± 55.73^{b}	45.37 ± 48.48^{b}	55.27 ± 52.58^{c}

CP = crude protein; CL = crude lipid.
[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

Table 5
Effect of lysophospholipid on serum biochemical indexes of largemouth bass.[1]

Index	FO	L-0.05	L-0.1	L-0.15	L-0.2
TP, mg/mL	8.05 ± 0.33[a]	8.38 ± 0.33[a]	10.84 ± 1.08[b]	7.78 ± 1.51[a]	7.60 ± 2.03[a]
TG, mmol/L	1.74 ± 0.12[a]	2.31 ± 0.08[b]	2.92 ± 0.11[c]	2.48 ± 0.09[b]	2.46 ± 0.14[b]
TC, mmol/L	6.49 ± 0.75[c]	5.48 ± 0.76[b]	4.50 ± 0.47[a]	4.33 ± 0.27[a]	5.98 ± 0.28[bc]
HDL-C, mmol/L	3.85 ± 0.74[a]	5.77 ± 0.64[ab]	7.69 ± 1.0[b]	7.05 ± 1.23[b]	5.13 ± 1.05[ab]
LDL-C, mmol/L	4.39 ± 0.63[b]	4.06 ± 0.83[b]	3.16 ± 0.19[a]	3.63 ± 0.21[ab]	4.41 ± 0.22[b]
Glu, mmol/L	4.18 ± 0.42[a]	5.34 ± 0.78[b]	5.54 ± 0.82[b]	6.06 ± 1.21[b]	6.30 ± 0.16[b]
AST, U/L	8.27 ± 1.71	10.87 ± 1.93	9.14 ± 0.42	9.97 ± 2.07	9.86 ± 1.35
ALT, U/L	11.11 ± 0.78	9.19 ± 1.27	8.26 ± 1.51	7.93 ± 1.10	8.76 ± 0.49

TP = total protein; TG = triglyceride; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Glu = glucose; AST = aspartate transaminase; ALT = alanine aminotransferase.
[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

Table 6
Effects of lysophospholipid on the activities of digestive enzymes in liver and intestine of largemouth bass.[1]

Index	FO	L-0.05	L-0.1	L-0.15	L-0.2
Liver					
Lipase, U/g	3.91 ± 0.32[a]	5.03 ± 0.41[b]	5.08 ± 0.73[b]	5.94 ± 0.77[bc]	6.29 ± 0.35[c]
Amylase, IU/g	2.32 ± 0.22[b]	2.09 ± 0.08[a]	3.09 ± 0.08[c]	2.92 ± 0.13[c]	3.01 ± 0.08[c]
Protease, IU/g	2.02 ± 0.15[a]	1.99 ± 0.08[a]	2.44 ± 0.27[b]	2.90 ± 0.07[c]	3.12 ± 0.04[c]
Intestinal tract					
Lipase, U/g	7.54 ± 0.33[a]	8.32 ± 0.41[a]	9.34 ± 0.39[b]	12.01 ± 0.66[c]	12.14 ± 0.27[c]
Amylase, IU/g	5.04 ± 0.26[a]	5.27 ± 0.35[a]	6.11 ± 0.10[b]	5.97 ± 0.31[b]	6.42 ± 0.52[b]
Protease, IU/mg	25.44 ± 0.49[a]	26.32 ± 2.74[a]	27.87 ± 0.36[a]	27.94 ± 0.87[a]	31.92 ± 1.38[b]

[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

accompanied by 1% reduction in fish oil (Figs. 1A and 2). The liver section stained by H&E showed that enlarged hepatocytes and loss of nuclei and cytoplasmic vacuolization in many cells were found in the L-0.05 and L-0.2 groups (Fig. 1B).

The morphological data and H&E stained section of the intestine are shown in Table 7 and Fig. 3. The thickness of the muscle layer in the L-0.1 group was significantly higher than that in the L-0.05, L-0.15 and L-0.2 groups ($P < 0.05$), and was no significant difference with the FO group ($P > 0.05$). The height of the folds in the L-0.1 group was significantly higher than that in the L-0.05 group ($P < 0.05$), and was no significant difference with the other groups ($P > 0.05$). The fold width of fish raised in the FO group was significantly lower than that fish consumed diet containing lysophospholipids ($P < 0.05$).

3.6. Hepatic metabolic enzyme activity

3.6.1. Activity of enzymes related to hepatic glucose metabolism

The activities of hepatic glucose metabolizing enzymes are shown in Table 8. With the increase of dietary lysophospholipids supplementation, PEPCK, GK and G-6-PDH activities in the fish liver were significantly influence. The PEPCK and GK activities in the livers of group L-0.05 and L-0.1 were not obviously different from each other ($P > 0.05$), but much higher than that of the fish oil group, and much lower than both group L-0.15 and L-0.2 ($P < 0.05$). G-6-PDH activities in the L-0.15 and L-0.2 groups were significantly higher than those in the FO group ($P < 0.05$).

3.6.2. Activity of enzymes related to hepatic lipid metabolism

The hepatic lipid metabolizing enzyme activities are shown in Table 9. The FAS, ACC, LPL and HSL activities in the fish liver were enhanced with the increase of dietary lysophospholipids. The FAS, ACC, LPL and HSL activities were the highest in the L-0.2 group, and which were significantly higher than in FO group ($P < 0.05$). CPT-1 activities in L-0.15 group were significantly higher than the FO, L-0.05 and L-0.2 groups ($P < 0.05$), and without significantly difference with L-0.1 group ($P > 0.05$).

3.7. Hepatic lipid metabolism genes expression

The mRNA expressions of hepatic lipid metabolism genes are shown in Fig. 4. Compared with the FO group, the mRNA expression levels of genes *lpl*, *hsl*, *cpt-1*, *atgl*, *fas*, *acc* and *dgat* were significantly upregulated in L-0.15 and L-0.2 groups ($P < 0.05$). And compare to the FO group, the mRNA expression of *apob* was significantly downregulated in the L-0.1 group ($P < 0.05$). There was no significant change between the last two groups (L-0.15 and L-0.2 groups) ($P > 0.05$).

4. Discussion

Lysophospholipid was a highly emulsifying emulsifier that facilitate the digestion and absorption of lipids in the intestinal tract and increased the weight gain of broilers (*Gallus gallus domesticus*) (Zhang et al., 2011; Wealleans et al., 2020) and weaned piglets (*Susscrofa domestica*) (Xing et al., 2004). In this experiment, the 1% reduction of dietary crude lipid with supplemental lysophospholipids improved fish growth compared to the basal diet with 11% dietary crude lipid. Growth performance and feed utilization of largemouth bass fed diet with 0.1% of lysophospholipids were enhanced significantly compare to the fish fed control diet (FO), which indicated that supplementation with 0.1% lysophospholipids helped to enhance the dietary lipid utilization depending on its emulsification effect, allowing fish to obtain enough energy despite the 1% reduction in fish oil. Although the addition of 0.0125% to 0.05% lysophospholipids in the channel catfish diet has

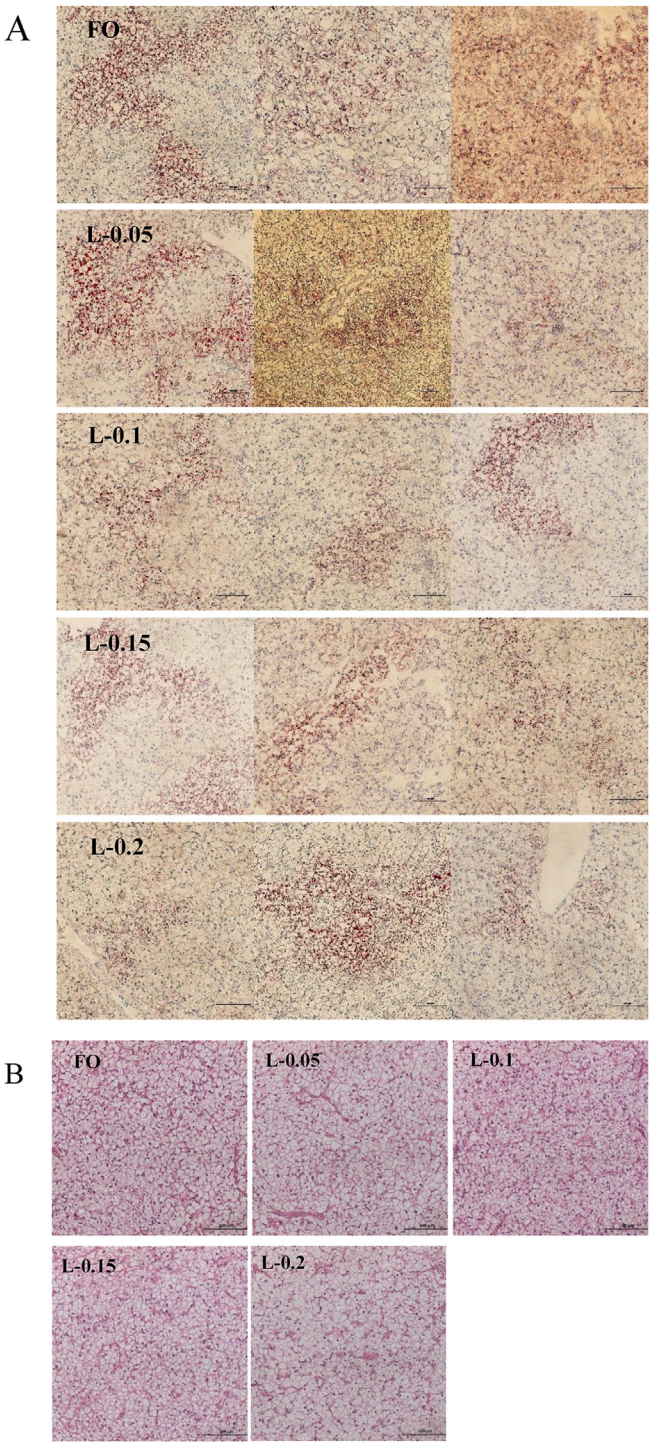

Fig. 1. Liver sections of largemouth bass. (A) Oil red O stained (magnification 200×), (B) H&E stained (magnification 200×). FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

no significantly effect on the growth (Liu et al., 2019), optimal level of lysophospholipids could also promote the growth of turbot (Li et al., 2019) and hybrid tilapia (*Oreochromis aureus* ♂ × *Oreochromis niloticus* ♀) (Tao et al., 2010). It should be the reason for the differences in the lysophospholipids dose, composition of the formulation and the fish species.

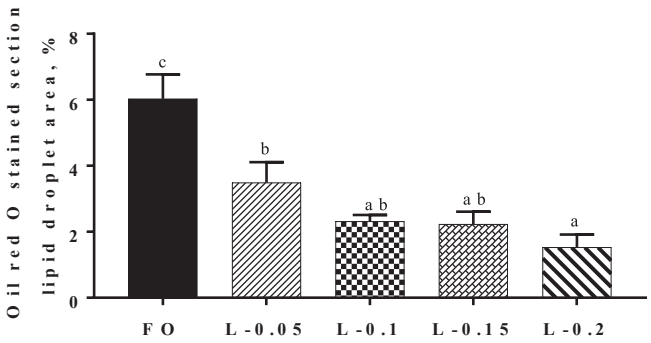

Fig. 2. Lipid droplet area of section stained by Oil red O. Bars with different letters differ significantly. FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

Feeding rate in group L-0.1 was significantly lower than that in FO group, which indicated the feed intake was already sufficient to meet the energy requirements of the fish. The addition of lysophospholipids helped to reduce FR, which has also been shown in channel catfish (Liu et al., 2019), crucian carp (*Carassais auratus gibelio*) (Li et al., 2010) and laying hens (Han et al., 2010). When the dietary lysophospholipids was up to 0.2%, the growth of largemouth bass was significantly reduced. It was probably that the excessive lysophospholipids were cytotoxic, which would damage the phospholipid layer of the cell membrane and induce oxidative stress due to the formation of endogenous reactive oxygen species, thus cause pathological reactions such as abnormal liver function (Goetzl and Tigyi, 2004; Poli et al., 2004).

The higher emulsification capacity of lysophospholipids facilitates the formation of a stable microemulsion system, reduces the volume of celiac particles, and increases the contact area between celiac and intestinal villi (Li et al., 2018). The chyme could have more chance to meet the digestive enzymes, on the other hand, the activities of digested enzyme were stimulated by more substrates, which promoted the digestion and absorption of the feed (Krogdahl et al., 2015). In this trial, the activities of the lipase, amylase and protease were higher when dietary lysophospholipids were over 0.1% than in FO group. In addition, lysophospholipids could alter the permeability of the bilayer membrane structure located intestinal mucosal cells, increase the size of the membrane pores, and promote the conversion of fatty acids into micelles, thereby improving the utilization of nutrients and energy by animals (Kim et al., 2018; François et al., 2000), especially utilization of the dietary lipid.

And lysophospholipids likewise affected the intestinal histomorphology of animals to varying degrees (Liu et al., 2019; Boontiam et al., 2017). Lysophospholipids could promote mitosis of activated cells at the tip of the intestinal villi, thereby increasing the surface area of the intestinal villi (Khonyoung et al., 2015; Boontiam et al., 2017). In this experiment, the fold width in fish fed diet with 0.1% lysophospholipids was significantly higher than that in FO group, which indicated an expansion in intestinal surface area. This phenomenon can help the absorption of nutrients and thus improve the growth of largemouth bass, which is consistent with the growth performance of the fish.

Serum TG and TC are important indicators to reflect the lipid metabolic status (Kjær et al., 2009). Compared to the FO group, the significantly higher serum TG and HDL-C were observed and lower TC and LDL-C were found when fish consumed diets with lysophospholipids above 0.05%. The same results were observed from juvenile turbot (Li et al., 2019) and channel catfish (Liu et al., 2019). With the decrease of serum TC concentration, the content of serum

Table 7
Effect of lysophospholipid on intestinal development of largemouth bass.[1]

Index	FO	L-0.05	L-0.1	L-0.15	L-0.2
Muscular layer thickness, μm	115.47 ± 14.52^{bc}	91.00 ± 4.39^{a}	131.36 ± 12.89^{c}	111.60 ± 12.59^{b}	97.31 ± 11.57^{ab}
Fold height, μm	546.84 ± 51.26^{b}	468.79 ± 41.97^{a}	535.80 ± 21.87^{b}	534.09 ± 48.14^{b}	535.80 ± 21.87^{b}
Fold width, μm	59.20 ± 10.40^{a}	77.05 ± 13.37^{b}	79.71 ± 9.59^{b}	79.93 ± 11.97^{b}	74.82 ± 9.71^{b}

$^{a, b, c}$ Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

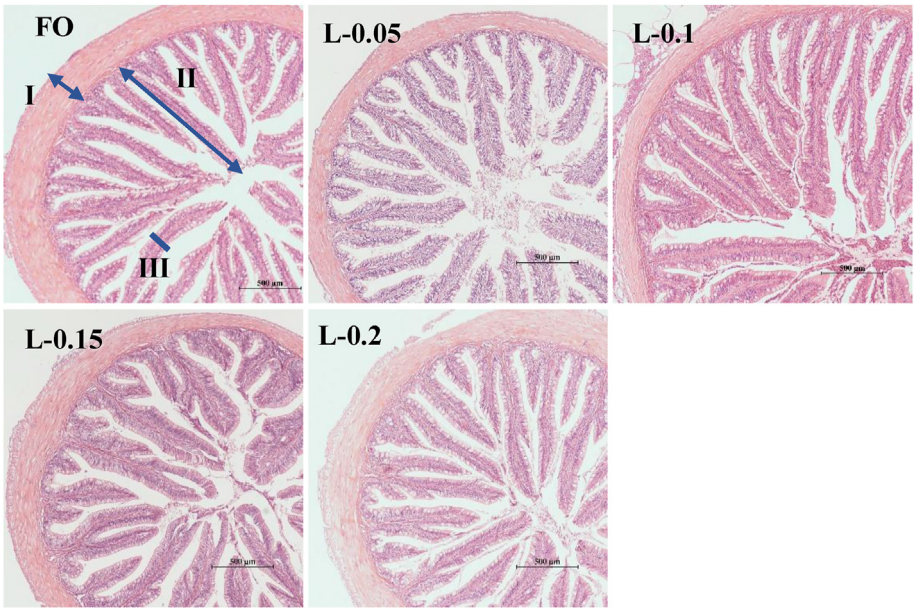

Fig. 3. Intestinal H&E stained sections (magnification 100×; I: muscular layer thickness, II: fold height, III: fold width). FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

Table 8
Effects of lysophospholipid on the activities of enzymes related to glucose metabolism in liver of largemouth bass.[1]

Index	FO	L-0.05	L-0.1	L-0.15	L-0.2
PEPCK, IU/g	0.29 ± 0.05^{a}	0.46 ± 0.09^{b}	0.46 ± 0.05^{b}	0.61 ± 0.09^{c}	0.64 ± 0.08^{c}
GK, mU/g	10.42 ± 1.02^{a}	12.37 ± 1.32^{b}	11.79 ± 0.53^{b}	13.73 ± 0.51^{c}	16.97 ± 0.44^{d}
G-6-PDH, U/g	0.12 ± 0.01^{a}	0.12 ± 0.02^{ab}	0.14 ± 0.02^{abc}	0.15 ± 0.01^{bc}	0.16 ± 0.01^{c}

PEPCK = phosphoenolpyruvate carboxykinase; GK = glucokinase; G-6-PDH = glucose-6-phosphate dehydrogenase.
$^{a, b, c}$ Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

Table 9
Effects of lysophospholipid on activities of enzymes related to lipid metabolism in liver of largemouth bass.[1]

Index	FO	L-0.05	L-0.1	L-0.15	L-0.2
FAS, U/g	23.19 ± 1.48^{a}	25.68 ± 1.85^{ab}	27.37 ± 0.53^{ab}	30.68 ± 2.36^{b}	32.56 ± 3.01^{b}
ACC, U/g	0.24 ± 0.02^{ab}	0.20 ± 0.04^{a}	0.28 ± 0.05^{bc}	0.23 ± 0.03^{ab}	0.31 ± 0.02^{c}
LPL, U/g	5.74 ± 0.17^{a}	5.86 ± 0.15^{a}	6.47 ± 0.30^{ab}	7.92 ± 1.00^{b}	8.97 ± 0.53^{c}
HSL, U/g	6.03 ± 0.59^{a}	7.90 ± 0.84^{b}	8.30 ± 0.38^{bc}	9.24 ± 0.45^{cd}	9.77 ± 0.92^{d}
CPT-1, U/g	2.71 ± 0.54^{a}	3.63 ± 0.41^{b}	4.23 ± 0.28^{bc}	4.26 ± 0.26^{c}	3.49 ± 0.41^{b}

FAS = fatty acid synthetase; ACC = acetyl-CoA carboxylase; LPL = lipoprotein lipase; HSL = hormone-sensitive triglyceride lipase; CPT-1 = carnitine palmitoyl transferase 1.
$^{a, b, c}$ Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively.

HDL-C was increased, which facilitated the removal of excess TC from blood and tissue cells to regulate the metabolic balance of lipids. It indicated that the fish liver was most capable of transporting TC when fish ingested diets with 0.1% or 0.15% lysophospholipids. Elevated serum TG and Glu might be due to the fact that lysophospholipids promoted the digestion and absorption of feed by largemouth bass, thus increased the transfer of lipid and glucose in blood for utilization by the organism.

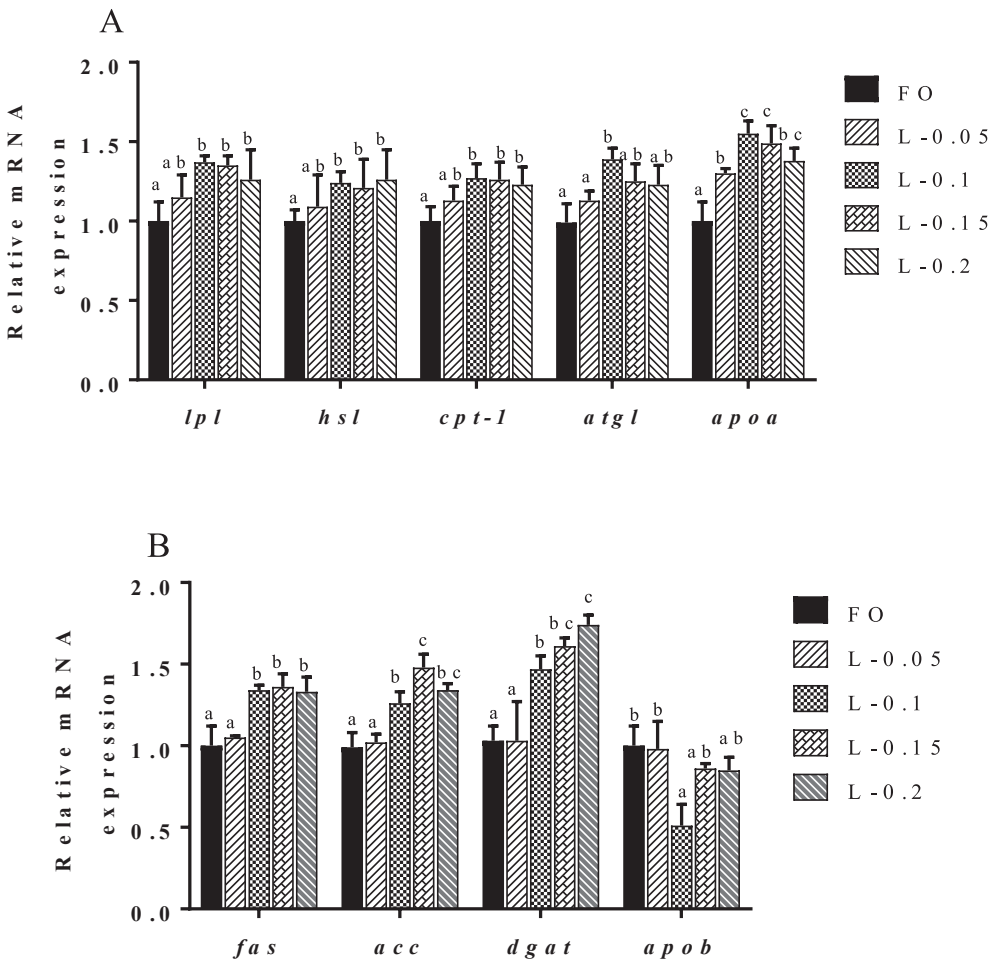

Fig. 4. Hepatic mRNA expression of genes in largemouth bass fed experimental diets. (A) Genes related to lipid catabolism, (B) genes related to lipid anabolism. Bars with different letters differ significantly. FO, L-0.05, L-0.1, L-0.15, and L-0.2 indicate largemouth bass fed a basal diet without lysophospholipid, low-fat diets with 0.5%, 1%, 1.5%, and 2% lysophospholipids, respectively. *lpl* = lipoprotein lipase; *hsl* = hormone-sensitive triglyceride lipase; *cpt-1* = carnitine palmitoyl transferase 1; *atgl* = adipose triglyceride lipase; *apoa* = apolipoprotein A; *fas* = fatty acid synthetase; *acc* = acetyl-CoA carboxylase; *dgat* = diacylglycerol acyltransferase; *apob* = apolipoprotein B.

The activities of serum AST and ALT were not influenced by the diets, which reflected the fish livers were healthy compare to the FO group (Chen and Li, 2014). The combined results of the hepatic sections stained by H&E and Oil red O showed that less lipid droplets in the liver of fish fed lysophospholipids above 0.05% compare to the fish fed FO diet. This confirmed the fish liver was healthy with the normal physiological function. Lysophospholipids reduced the lipid content of the whole fish and liver, and increased the body protein content in channel catfish (Liu et al., 2019) and broilers (Ge et al., 2019), which were consistent with the results of this experimental study. These indicate that lysophospholipids could improve the utilization of dietary lipid by fish, increase lipid circulation and reduce lipid deposition in largemouth bass, thus lower protein consumption as energy and improving protein deposition in the body.

The two distinctly different hydrophilic and lipophilic regions in the lysophospholipids molecule make its surface activity stronger and with higher capacity to emulsify lipids than those of the regular phospholipids (Zhang, 2007). The chemical structure of lysophospholipids plays an important role in the transport of lipids, as it contributes to dissolution and absorption of the lipid, and reduces the lipid contents in the liver to prevent fatty liver (Li et al., 2019; Tan et al., 2020). The results of this experiment showed that the addition of lysophospholipids increased the activities of lipid metabolism-related enzymes FAS, ACC, LPL, HSL and CPT-1 and up-regulated the expression of lipid metabolism-related genes, as well as those observed in turbot (Li et al., 2019). According to the serum lipid contents and hepatic histology data, lipid catabolism was stronger than lipid synthesis, which provided more energy to be used for growth. Similar to the present study, lysophospholipids were found to promote the utilization of palm oil in tiger shrimp (*P. monodon*) fed the diet with reduced amount of fish oil (Khan et al., 2018). Although the largemouth bass cannot use carbohydrate very well, in this study, the activities of GK, PEPCK and G-6-PDH in the liver also increased with the addition of lysophospholipids. G-6-PDH, a key enzyme in the pentose phosphate pathway, could produce NADPH (nicotinamide adenine dinucleotide phosphate) involved in fatty acid synthesis. The results suggested adding lysophospholipids in diet with reducing 1% fish oil could promote the conversion of sugars to lipid and thus improve the supply of energy.

5. Conclusion

Based on the present experimental, lysophospholipids could save protein and greatly exert the efficiency of dietary lipid through promoting the activity of intestinal digestive enzymes and liver glycolipid metabolizing enzymes. The recommended addition level of lysophospholipids was 0.1% in the feed with reducing 1% fish oil, which helps to reduce hepatic lipid accumulation and then promote growth of largemouth bass.

Author contributions

Mingxiao Che: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original Draft; **Ziye Lu**: Investigation, Formal analysis; **Liang Liu**: Methodology, Resources; **Ning Li**: Methodology, Resources; **Lina Ren**: Methodology, Re-sources; **Shuyan Chi**: Conceptualization, Methodology, Resources, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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