Aquaculture Reports 24 (2022) 101139

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Dietary supplementation of L-carnitine relieved detrimental impacts of a high-fat diet in juvenile *Trachinotus ovatus*

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ARTICLE INFO

Keywords:
Trachinotus ovatus
L-carnitine
High-fat diet
Growth performance
Antioxidation
Anti-inflammation

ABSTRACT

In recent years, the dramatically high lipid level has been used in fish feeds, resulting in low survival rates of fish and huge economic losses. Based on these issues, a six-week feeding experiment was conducted to investigate whether diet supplements with L-carnitine can be used to relieve detrimental impacts on growth performance, hepatic lipid accumulation, antioxidant, anti-inflammatory and non-specific immune status, as well as intestinal morphology of Trachinotus *ovatus,* fed on a high-fat diet. Three isonitrogenous diets were formulated to either include or exclude high-fat and L-carnitine (lipid positive (LP): 130 g kg^{-1} lipid, lipid negative (LN): 210 g kg^{-1} lipid, lipid negative with L-carnitine (LNC): 210 g kg^{-1} lipid with 0.6 g kg^{-1} L-carnitine). Results indicated that the growth performance and mid-intestine villi length of T. *ovatus* in the LN group were significantly lower than that of the LP group ($p < 0.05$), while significantly higher growth performance and better mid-intestine morphology of fish in the LNC group was found compared to the LN group ($p < 0.05$). mRNA expression levels of hepatic genes associated with the inflammatory and non-specific immune statue of *T. ovatus* in the LN group were significantly higher compared with the LP group ($p < 0.05$), while that of the LNC group was significantly lower compared with the LN group ($p < 0.05$). Hepatic triglyceride content and lipid droplets in the LN group were significantly elevated, compared with the LP group ($p < 0.05$). On the contrary, diet supplemented with L-carnitine could mitigate hepatic lipid accumulation in fish caused by a high-fat diet. Regarding antioxidant states, higher malondialdehyde content in the LN group ($p > 0.05$) and significantly higher levels in the LNC group ($p < 0.05$) were found, compared with the LP group, while no statistical difference was found between the LN and LNC groups ($p > 0.05$). In conclusion, L-carnitine can relieve the detrimental impact on growth performance of T. *ovatus* exposed to a high-fat diet treatment by reducing hepatic lipid accumulation and improving intestinal morphology, anti-inflammatory and non-specific immune status, but could not mitigate oxidative pressure.

1. Introduction

Compared with other farmed animals, fish prefer a higher protein content in their daily diet (Harpaz, 2005). However, dietary protein is expensive and part of proteins are used to provide energy rather than for physiological functions (Wilson, 2003). Along with the development of the fish industry, dietary protein reduction has exerted increasing pressure over past ten years. Regarding energy provision, carbohydrates and lipids are generally regarded as more ideal resources than proteins due to their relatively lower cost. In many carnivorous fish, lipids are the common choice for providing energy due to the lower carbohydrate utilization capacity (Council, 2011). To reduce the dietary fish meal used and control the aquaculture budget, the dramatically high lipid level (35% or more) had been used in fish diet in recent years (Harpaz, 2005). Excessive dietary lipid level may cause hepatic and abdominal fat accumulation and metabolic imbalance, which leads to a reduction of growth performance and survival rate of fish (Du et al., 2008).

L-carnitine, located between the inner and outer mitochondria membranes, plays a significant role in β-oxidation by assisting the translocation of long-chain fatty acids (Bueno et al., 2005). L-carnitine is generally regarded as an ideally bioactive additive for fish feeds. On the one hand, it can reduce the whole-body and tissue lipid accumulation

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https://doi.org/10.1016/j.aqrep.2022.101139
Received 23 February 2022; Received in revised form 8 April 2022; Accepted 17 April 2022
Available online 23 April 2022

and enhance the growth performance of fish (Dias et al., 2001). On the other hand, L-carnitine can achieve the lower oxidative pressure in *Oreochromis niloticus* fed with high-fat diet by exerting the antioxidant property (Guzmán-Guillén et al., 2017). Therefore, this additive may be able to relieve detrimental impacts of Trachinotus ovatus caused by a high-fat diet treatment.

T. ovatus, belongs to *Carangidae* (*Trachinotus*) and contents highly nutritional value, which has become one of most prevalent sea aquaculture species in South China in recent years (Liu et al., 2019; Tan et al., 2018). On the one hand, higher and higher fat level was used in fish feeds, resulting in low survival rates of fish and huge economic losses. However, limited solutions are available to relieve detrimental impacts of T. *ovatus* fed on a high-fat diet. On the other hand, high-fat diet treatment is generally used as an ideally experimental model to investigate the inflammation, oxidation, and lipid metabolism of animals. Therefore, the present study aimed to determine whether dietary L-carnitine supplementation could relieve detrimental impacts exerted on growth performance, hepatic lipid accumulation, antioxidant, inflammatory and non-specific immune statue, as well as intestinal morphology of juvenile *T. ovatus* caused by a high-fat diet treatment. These results may provide a theoretical basis to formulate proper high-fat diets for *T. ovatus*.

2. Materials and methods

2.1. Fish breeding and management

Experimental fish were obtained and cultured at the feeding station of the Chinese Academy of Fishery Science (Lingshui, China). Before feeding, juvenile *T. ovatus* were cultured in sea cages and fed the LP diet for acclimating for two weeks, and then fish were starved for 1 day. Thereafter, 240 fish (initial body weight: 7.28 ± 0.05 g) were stochastically divided into 12 sea cages (1 × 1 × 2 m). Fish were fed twice per day (9:00 and 16:00) until they stopped eating to prevent wastage (Liu et al., 2018). The feeding experiment was lasted for 6 weeks. The weight of the diet fed, as well as the number and weight of the dead fish in each cage were recorded.

2.2. Experimental feeds

In this study, three isonitrogenous (40% approximately) diets were formulated by either supplementing or not supplementing with high-fat and L-carnitine (Sigma C0158) (Table 1):

lipid positive group (LP): 130 g kg^{-1} lipid

lipid negative group (LN): 210 g kg^{-1} lipid

lipid negative with L-carnitine group (LNC): 210 g kg^{-1} lipid with 0.6 g kg^{-1} L-carnitine.

The LP and LN groups were shared control groups to our previous study (Fang et al., 2021a).

Dietary lipid levels designed in three experimental groups were based on the manuscript of Fang et al. (2021b). The L-carnitine concentration used in the LNC group was based on our previous results (unpublished manuscript). Briefly, four experimental diets were formulated including 120 g kg^{-1} lipid approximately with 0, 0.2 g kg^{-1}, 0.4 g kg^{-1}, 0.6 g kg^{-1} L-carnitine respectively. After 8 weeks diets treatment, the 0.6 g kg^{-1} L-carnitine treatment group obtained the highest growth performance of *T. ovatus* compared to other groups. Diets were prepared and processed according to the method prescribed by Zhao et al. (2020).

2.3. Sample collection

After the feeding experiment, fish were starved for 1 day. Thereafter, the total weight and number of fish in each cage were recorded. Seven fish were stochastically selected in each cage and anesthetized (MS-222, Sigma, USA) for sampling: four fish were kept in ice to inspect whole-body compositions. Other three fish were euthanized and then livers were isolated and frozen in liquid nitrogen immediately for determining oxidative parameters and gene expressions (3 livers from 3 fish were combined in one tube). Meanwhile, the same section of liver and mid-intestine of fish from each group were separated and fixed in 4% para-formaldehyde for morphology detection.

All procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH). All research protocols were approved by the Experimental Animal Ethics Committee of Sun-Yat San University.

Table 1
Ingredients and proximate compositions of three experiment diets.

Ingredients (g kg^{-1})	LP	LN	LNC
Fish meal	320	320	320
Soybean meal	220	220	220
Microcrystalline cellulose	70.6	0.6	0
Wheat four	107.4	107.4	107.4
Krill meal	30	30	30
Peanut meal	100	100	100
Fish oil	80	150	150
Soya lecithin	20	20	20
$Ca(H_2PO_4)_2$	20	20	20
Pre-vitamin[a]	10	10	10
Pre-mineral[b]	10	10	10
Choline	5	5	5
DL-Met	3	3	3
Lys-HCL (99%)	4	4	4
L-carnitine[c]	0	0	0.6
sum	1000	1000	1000
Nutrient levels (%)[d]			
Moisture	9.22	9.48	9.04
Crude protein	40.13	40.26	40.43
Crude lipid	13.58	20.42	20.26

[a] Vitamin premix provides the following per kg of diet: VB1 25 mg, VB2 45 mg, pyridoxine HCl 20 mg, VB12 0.1 mg, VK3 10 mg, inositol 800 mg, pantothenic acid 60 mg, niacin acid 200 mg, folic acid 20 mg, biotin 1.20 mg, retinal acetate 32 mg, cholecalciferol 5 mg, α-tocopherolα 120 mg, ascorbic acid 2000 mg, choline chloride 2500 mg, ethoxyquin 150 mg, wheat middling 14.012 g.
[b] Mineral premix provides the following per kg of diet: NaF 2 mg, KI 0.8 mg, $CoCl_2{\cdot}6H_2O$ 50 mg, $CuSO_4{\cdot}5H_2O$ 10 mg, $FeSO_4{\cdot}H_2O$ 80 mg, $ZnSO_4{\cdot}H_2O$ 50 mg, $MnSO_4{\cdot}H_2O$ 60 mg, $MgSO_4{\cdot}7H_2O$ 1200 mg, $Ca(H_2PO_4)_2{\cdot}H_2O$ 3000 mg, NaCl 100 mg, zeolite 15.447 g.
[c] L-carnitine: Sigma C0158.
[d] Measured values.

2.4. The detection of fish body composition

The crude protein, crude lipid and moisture content of whole fish body and diets were detected using the AOAC method (Horwitz, 2010). The crude protein content was detected using the Kjeldahl method (1030-Auto-analyzer, Tecator, Höganäs, Sweden); crude lipid content was determined using the Soxhlet extraction method (Soxtec System HT6, Tecator, Sweden). The moisture content was analyzed by drying the sample in the ventilating oven at 105 °C until the weight was stable.

2.5. Detections of hepatic oxidative parameters and triglyceride

Livers were homogenized and then centrifuged based on the method described by Zhao et al. (Zhao et al., 2020b). Supernatants were collected for analyzing hepatic oxidative parameters, including superoxide dismutase (SOD) (A001-1), total antioxidant capacity (T-AOC) (A015-2-1), malondialdehyde (MDA) (A003-1) and glutathione (GSH) (A006-2). In addition, hepatic triglyceride (TG) (A110-1-1) was measured as well. Analysis methods were conducted as instructed by the manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Histological observations of liver and mid-intestine

The stain and observation methods were used on the liver and mid-intestine, which were conducted as described in our previous manuscript (Zhao et al., 2020b). In brief, tissue sections (mid-intestine and liver, 0.5 μm thick) were stained using hematoxylin and eosin. Then, mid-intestinal and hepatic morphology were observed under a microscope (Olympus CKX41 microscope, Tokyo, Japan). The intestinal villi length and goblet cell number were detected using the method described by Zhao et al. (Zhao et al., 2020b). Briefly, the villi length and the goblet cell number are respectively equating to the average value of randomly selected eight villus length and the average value of the goblet cell number randomly selected eight villus per slide.

2.7. RNA extraction and gene expression analysis

Total RNA was extracted from the liver using Trizol® reagent (Invitrogen, USA) according to the manufacturer's instruction. 1% agarose gel electrophoresis and spectrophotometer (NanoDrop 2000, Thermo Fisher, United States) were used to assess RNA quality and quantity, respectively. Then, cDNA was synthesized using a PrimeScript RT reagent kit (Takara, Japan), based on the manufacturer's instruction. Real-time PCR for the target genes were performed using SYBR® Premix Ex TaqTM II (Takara, Japan) and quantified on the LightCycler 480 (Roche Applied Science, Basel, Switzerland).

Primers used in the present study are presented in the Table 2. The *β-actin* was used as a housekeeping gene for RNA expression analysis (Xie et al., 2020). The relative mRNA expression of target genes was determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.8. Statistical analysis

Results in the present study were presented as mean ± standard error (SE). Data were checked for normality and homogeneity of variance in the software of SPSS 22.0 (Chicago, USA) and then followed by one-way analysis of variance (ANOVA) with Duncan's test. $p < 0.05$ was regarded to indicate a significant difference between groups.

Table 2
Sequences of primers used for real-time quantitative PCR.

Gene name	Primer sequence (5′−3′)	Reference
β-actin F	TACGAGCTGCCTGACGGACA	(Xie et al., 2020)
β-actin R	GGCTGTGATCTCCTTCTGCA	
nrf2 F	TTGCCTGGACACAACTGCTGTTAC	(Xie et al., 2020)
nrf2 R	TCTGTGACGGTGGCAGTGGAC	
gsh-px F	GCTGAGAGGCTGGTGCAAGTG	(Xie et al., 2020)
gsh-px R	TTCAAGCGTTACAGCAGGAGGTTC	
cat F	GGATGGACAGCCTTCAAGTTCTCG	(Xie et al., 2020)
cat R	TGGACCGTTACAACAGTGCAGATG	
keap1 F	CAGATAGACAGCGTGGTGAAGGC	(Xie et al., 2020)
keap1 R	GACAGTGAGACAGGTTGAAGAACTCC	
ho-1 F	AGAAGATTCAGACAGCAGCAGAACAG	(Xie et al., 2020)
ho-1 R	TCATACAGCGAGCACAGGAGGAG	
mn-sod F	CCTCATCCCCCTGCTTGGTA	(Xie et al., 2020)
mn-sod R	CCAGGGAGGGATGAGAGGTG	
p65 F	CGTGAGGTCAGCGAGCCAATG	(Xie et al., 2020)
p65 R	ATGTGCCGTCTATCTTGTGGAATGG	
il-1β F	CGGACTCGAACGTGGTCACATTC	(Xie et al., 2020)
il-1β R	AATATGGAAGGCAACCGTGCTCAG	
iκb F	GCTGGTCCATTGCCTCCTGAAC	(Xie et al., 2020)
iκb R	GTGCCGTCTTCTCGTACAACTGG	
ikk F	CCTGGAGAACTGCTGTGGAATGAG	(Xie et al., 2020)
ikk R	ATGGAGGTAGGTCAGAGCCGAAG	
myd88 F	AATACCTTGACAGCGATGCCTG	(Xie et al., 2019)
myd88 R	GTGCAAGGCCTGGTGTAATCA	
hsp70 F	TTGAGGAGGCTGCGCACAGCTTGTG	(Zhao et al., 2020a)
hsp70 R	ACGTCCAGCAGCAGCAGGTCCT	
irf3 F	GCTGGGATCCGCTTAGTCTACA	(Xie et al., 2019)
irf3 R	GCCCAGCTTGTCCAGGATG	
irf7 F	CGAATACACCAACCGCATCCT	(Xie et al., 2019)
irf7 R	AGCTTTCTTGGGTCTGGGTCA	

3. Results

3.1. Growth performance

The growth performance of *T. ovatus* fed either with or without high-fat and L-carnitine is presented in Table 3. Significantly lower growth performance parameters (final body weight (FBW), weight gain rate (WGR) and specific growth rate (SGR)) were obtained in the LN group than in the LP group and LNC groups ($p < 0.05$), while no significant differences were found between the LP and LNC groups ($p > 0.05$). Diet supplementation with L-carnitine had no impact on the survival rate (SR) and feed conversion ratio (FCR) of *T. ovatus* among three experimental groups ($p > 0.05$).

3.2. Whole-body composition

Whole-body compositions of *T. ovatus* fed either with or without a high-fat and L-carnitine diet are presented in Table 4. The crude lipid content of *T. ovatus* in the LN group was significantly higher, compared with that in the LP group ($p < 0.05$), while that of the LNC group was significantly lower, compared with the LN group ($p < 0.05$). Significantly lower contents of crude protein were found in the LN and LNC groups, compared with the LP group ($p < 0.05$), while no statistical difference was found between the LNC group and the LN group ($p > 0.05$). Diet supplementation with L-carnitine was unable to alter the moisture content of the whole fish in either group ($p > 0.05$).

3.3. Hepatic oxidative parameters

The hepatic oxidative parameters of *T. ovatus* fed either with or without a high-fat and L-carnitine diet is presented in Fig. 1. Significantly higher levels of T-SOD and GSH were found in the LN group, compared with the LP and LNC groups ($p < 0.05$). A higher level of MDA was found in the LN group ($p > 0.05$) and LNC group ($p < 0.05$), compared with the LP group, while no statistical difference was found between the LN and LNC groups ($p > 0.05$). Diet supplementation with L-carnitine had no effect on the hepatic T-AOC of *T. ovatus* in either group ($p > 0.05$).

3.4. mRNA expression of genes related to immunity

Diet supplementation with L-carnitine had a significant impact on the Nrf2 - Keap1 pathway (Fig. 2). mRNA expression levels of related genes of *T. ovatus* in the LN group, including *nrf2*, keap1, *m*n-sod, *gsh-px*, ho-1 and *cat*, were significantly higher than in the LP and LNC groups

Table 3
Growth performances and feed utilization of *T. ovatus* fed dietary supplementation of L-carnitine for 42 days.

	LP	LN	LNC
IBW	7.24 ± 0.07	7.29 ± 0.08	7.33 ± 0.06
WGR	123.54 ± 10.7^{a}	81.81 ± 9.2^{b}	150.25 ± 18.69^{a}
SGR	1.91 ± 0.11^{a}	1.42 ± 0.12^{b}	2.17 ± 0.17^{a}
SR	96 ± 2.92	91 ± 1.87	90 ± 2.89
FCR	1.47 ± 0.03	1.44 ± 0.02	1.43 ± 0.05

IBW (g per fish): initial body weight.
Weight gain rate (WGR, %) = 100 × (final body weight – initial body weight) / initial body weight
Specific growth rate (SGR, % day-1): 100 × (Ln final fish weight - Ln initial fish weight) / the experimental duration in days.
Survival rate (SR) (%) = 100 * (final number of fish) / (initial number of fish)
Feed conversion ratio (FCR) = dry diet fed / wet weight gain
Values are mean ± SE (n = 4). Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 4
Whole-body compositions of *T. ovatus* fed dietary supplementation of L-carnitine for 42 days.

	LP	LN	LNC
Moisture (%)	72.59 ± 0.56	70.88 ± 1.07	70.8 ± 0.36
Crude lipid (%, DWB)	25.51 ± 0.29[a]	35.43 ± 0.69[c]	31.2 ± 0.45[b]
Crude protein (%, DWB)	56.76 ± 0.78[b]	51.97 ± 1.00[a]	52.05 ± 0.70[a]

DWB: Dry weight basis.
Values are mean ± SE (n = 4). Means in the same row with different superscripts are significantly different ($p < 0.05$).

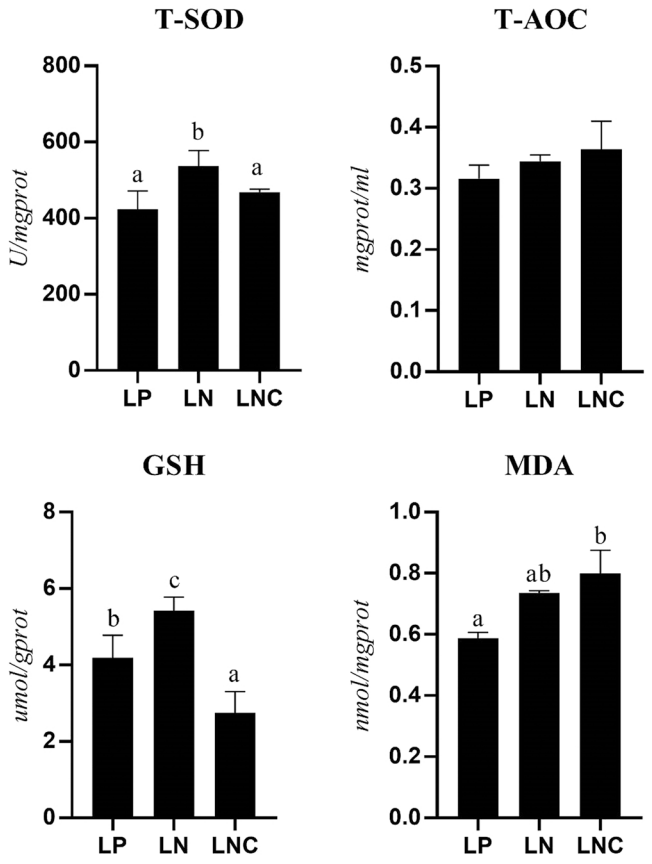

Fig. 1. Hepatic antioxidant parameters of *T. ovatus* fed dietary supplement L-carnitine for 6 weeks. The small letters indicated significant differences at $p < 0.05$, Values are mean ± SE (n = 4). T-SOD, total superoxide dismutase; MDA, malondialdehyde; T-AOC, total antioxidant capacity; GSH, glutathione.

($p < 0.05$). At the same time, no significant difference was found between the LP and LNC groups ($p > 0.05$).

mRNA expression levels of NF-κB pathway related genes of *T. ovatus* fed either with or without a high-fat and L-carnitine, as shown in Fig. 3. mRNA expression levels of associated genes in fish liver in the LNC group, including *p65*, *il-1β*, ikk, and *iκb*, were significantly lower than that of the LN group ($p < 0.05$). Meanwhile, mRNA expression levels of ikk and *iκb* in the LNC group were significantly lower than in the LP group ($p < 0.05$).

mRNA expression levels of non-specific immunity related genes of *T. ovatus* fed either with or without a high-fat and L-carnitine diet are shown in Fig. 4. Hepatic transcription levels of *hsp70*, *irf3*, *irf7* and *myd88* of *T. ovatus* in the LN group were significantly higher, compared with the LP group ($p < 0.05$), while that of the LNC group were significantly lower, compared with the LN group ($p < 0.05$) and no statistical difference was found in the LP group ($p > 0.05$).

3.5. Hepatic and intestinal morphology

The hepatic morphology and TG content of *T. ovatus* fed either with or without a high-fat and L-carnitine diet are shown in Fig. 5. *T. ovatus* in the LN group had more lipid droplets, compared with the LP group, while that of the LNC group was lower, compared with the LN group. At the same time, significantly higher hepatic TG content was found in the LN group, compared with the LP group ($p < 0.05$), while that of the LNC group was significantly lower, compared with the LN group ($p < 0.05$) and no statistical difference was found in the LP group ($p > 0.05$).

Intestinal morphology and related parameters of *T. ovatus* fed either with or without a high-fat and L-carnitine diet are shown in Fig. 6 and Table 5, respectively. The highest villi length was found in the LNC group ($p < 0.05$). No statistical difference in the number of goblet cells was found between groups ($p > 0.05$).

4. Discussion

Dietary lipids play a significant role in fish growth as they not only can provide considerable energy compared with carbohydrates and protein (Yan et al., 2015), but also possess various physiological functions, such as the regulation of hormones (Havel, 2004), transmission of nerve impulses (Bezine et al., 2018), and transport fat-soluble nutrients (German and Dillard, 2006). A high-fat diet has been widely used in aquaculture in recent years, but excess of dietary lipids would show the inhibition effect on growth performance of aquatic animals (Trenzado et al., 2018). In the present study, the 210 g kg^{-1} lipid diet treatment group showed lower growth performance of *T. ovatus* than the 130 g kg^{-1} lipid diet treatment group, indicating that a high-fat diet inhibits the growth performance of fish. This result is in agreement with conclusions reported by Zhou et al. (2020), Wang et al. (2005), and Yin et al. (2021). There are two significant reasons why a high-fat diet affects the growth performance of fish. Firstly, excessive dietary lipids during a short period making the fish reaches satiety quickly, and thus reduces food intake, which will affect the intake of other nutrients, such as proteins and minerals (Li et al., 2016). In addition, a high-fat diet may cause hepatic fat deposition in fish, which will lead to metabolic disorders and reduce anti-stress capacity (Du et al., 2005; Lee et al., 2002). As a valuable and green feed additive, L-carnitine is widely used in agriculture, especially in aquaculture. The most crucial role of L-carnitine is promoting the growth performance of fish. The present study demonstrated that the growth performance of *T. ovatus* fed on a high-fat diet supplemented with 0.6 g kg^{-1} L-carnitine was significantly higher, compared with the LN group, while no statistical difference was found between the LP and LNC groups, indicating that L-carnitine could relieve the negative impact on growth performance caused by a high-fat diet treatment. This result is probably due to L-carnitine can increase the β-oxidation capacity of fish (Becker et al., 1999). Conversely, Gaylord and Gatlin Iii (2000), Chatzifotis et al. (1997) and Jin et al. (2019) found that diet supplementation with L-carnitine was unable to alter the growth performance of fish. Different results in these manuscripts might be associated with fish species, fish initial size, feeding environment and dietary lipid level. The growth performance of fish is closely related to the intestinal morphology because the intestine plays a vital role in nutrient absorption and immune barrier (Gao et al., 2013; Vizcaíno et al., 2014). Specifically, intestinal villus can increase the contact area between the intestine and chyme, so its length reflects the absorption capacity of fish to some extent (Al-Fataftah and Abdelqader, 2014; Chen and Wang, 2013; Emami et al., 2012). In the present study, significantly higher villus length was found in the LNC group, compared with the LN and LP groups, indicating that diet supplementation with L-carnitine could mitigate the suppression effect of intestine development caused by a high-fat diet. Therefore, supplementation of 0.6 g kg^{-1} L-carnitine can improve the growth performance of *T. ovatus* fed on a 210 g kg^{-1} lipid diet, which is correlated with the better intestine morphology.

In the present study, significantly higher lipid but lower protein

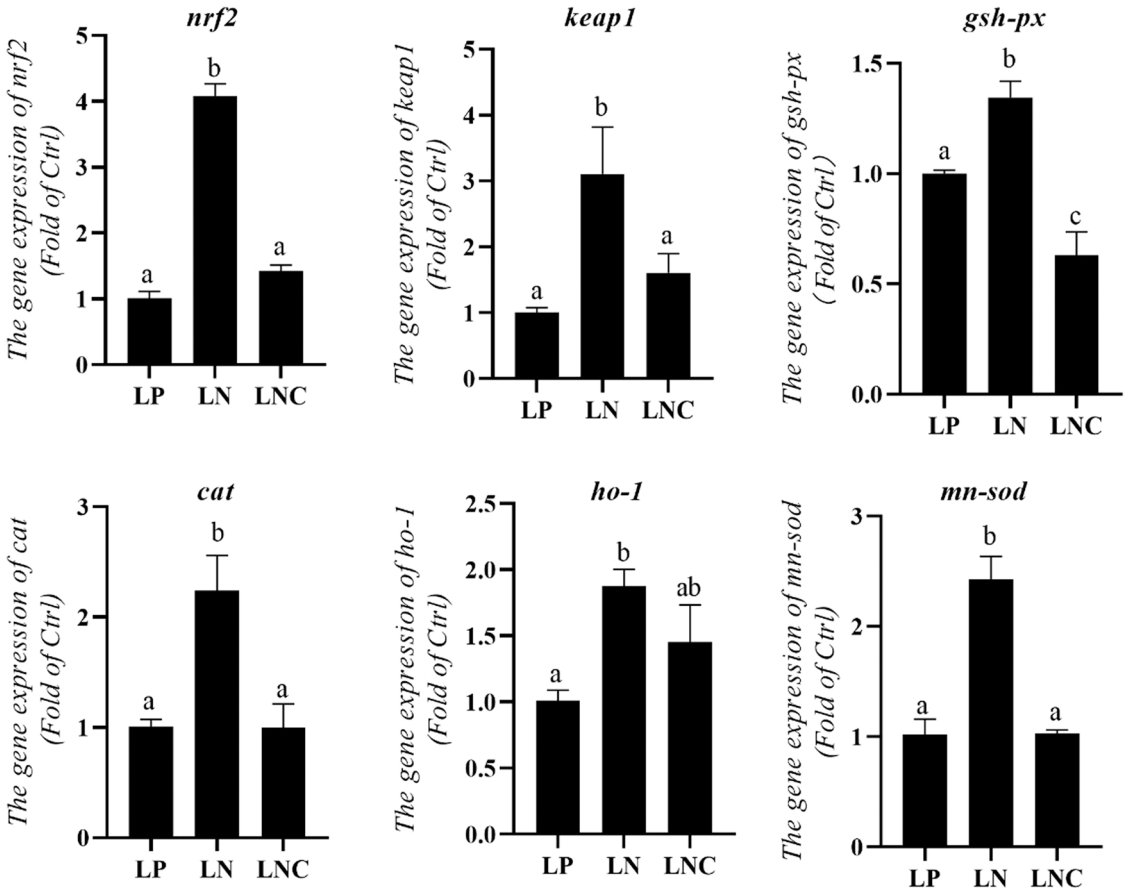

Fig. 2. Hepatic mRNA expression levels of Nrf2-keap1 pathway related genes of *T. ovatus* fed dietary supplementation of L-carnitine for 6 weeks. The small letters indicated significant differences at $p < 0.05$. Values are mean ± SE (n = 4).

content of whole fish were found in the high-fat diet treatment group, compared with the LP group, which were in agreement with manuscripts published by Chatzifotis et al. (2010), Ding et al. (2010), and Song et al. (2009). Meanwhile, a lower level of whole-body lipid content was found in the LNC group, compared with the LN group, which is in agreement with the results reported by Ji et al. (1996), Keshavanath and Renuka (1998), and Mohseni et al. (2008). In addition, fewer hepatic vacuoles, reflecting lipid droplets (Li et al., 2019), and lower hepatic TG content, were found in the LNC group, compared with the LN group. These results indicated that diet supplementation with L-carnitine may be associated with improving fatty acids utilization of the fish (Harpaz, 2005), and thus decreasing whole-body and hepatic lipid deposit.

Previous manuscripts demonstrated that a high-fat diet would lead to the production of reactive oxygen species (ROS) (Kesh et al., 2013; Ruggiero et al., 2011). Excessive ROS would attack cells and thus expose cells to oxidative stress (Lesser, 2006). Correspondingly, cells would scavenge ROS by activating the antioxidant system (Lesser, 2006). Among them, T-SOD, T-AOC, and GSH play a crucial role in scavenging ROS (Lesser, 2006). In the present study, higher levels of hepatic T-SOD and GSH in the LN group, compared with the LP group, indicating that *T. ovatus* responds to oxidative stress induced by a high-fat diet by enhancing antioxidative enzyme activities. On the other hand, L-carnitine has been reported to exert an antioxidative function and can protect cells from oxidative stress (Binienda and Ali, 2001; Brown, 1999; Kremser et al., 1995). In the present study, lower levels of hepatic T-SOD and GSH in the LNC group, compared with the LN group, indicating that L-carnitine can act as an antioxidative substance for scavenging ROS, resulting in without more T-SOD and GSH in the LNC group. The Nrf2-ARE pathway plays a significant role in responding to oxidative stress. Specifically, Under inactive conditions, Nrf2 is fixed by Keap1 in the cytoplasm and maintains a dynamic equilibrium between degradation ubiquitination and synthesis (Alfieri et al., 2013). On the contrary, when cells face oxidative stress, Nrf2 would separate from Keap1 and then translocate into the nucleus (Xie et al., 2019). Meantime, Nrf2 forms a dimer with Maf protein and subsequently integrates with antioxidant response elements (ARE) to upregulate downstream antioxidative genes (Klaassen and Reisman, 2010). In the present study, the mRNA expression levels of genes associated with the Nrf2-ARE pathway (*nrf2*, keap1, mn-sod, gsh-px, ho-1 and cat) were significantly higher in the LN group, compared with the LP and LNC groups. These results corresponded with the T-SOD and GSH trend, indicating that *T. ovatus* responds to oxidative pressure caused by a high-fat diet via activating the Nrf2-ARE pathway. At the same time, L-carnitine can act as an antioxidative substance and assist the scavenging of ROS, and eventually without expressing more antioxidant genes. On the other hand, the lipid peroxidation product, MDA, reflects the oxidative stress condition of cells attacked by ROS (Cheng et al., 2015; Tan et al., 2016), and was significantly elevated in the LNC group, compared with the LP group, and no statistical differences in the LN group. These results indicate that although L-carnitine can help with the scavenging of ROS, it still could not alter the oxidative stress in *T. ovatus* fed on a high-fat diet. This phenomenon has also been reported in *Acanthopagrus schlegelii* (Jin et al., 2019). Overall, a diet supplemented with 0.6 g kg^{-1} L-carnitine could not mitigate oxidative pressure in *T. ovatus* caused by a 210 g kg^{-1} lipid diet.

Previous studies demonstrated that a high-fat diet could induce an inflammatory response in fish (Varga et al., 2011), and our present study confirmed this conclusion. The NF-κB pathway plays a vital role in responding to inflammatory response in cells. Briefly, When cells are free from environmental pressure, the p65/p50 heterodimer was

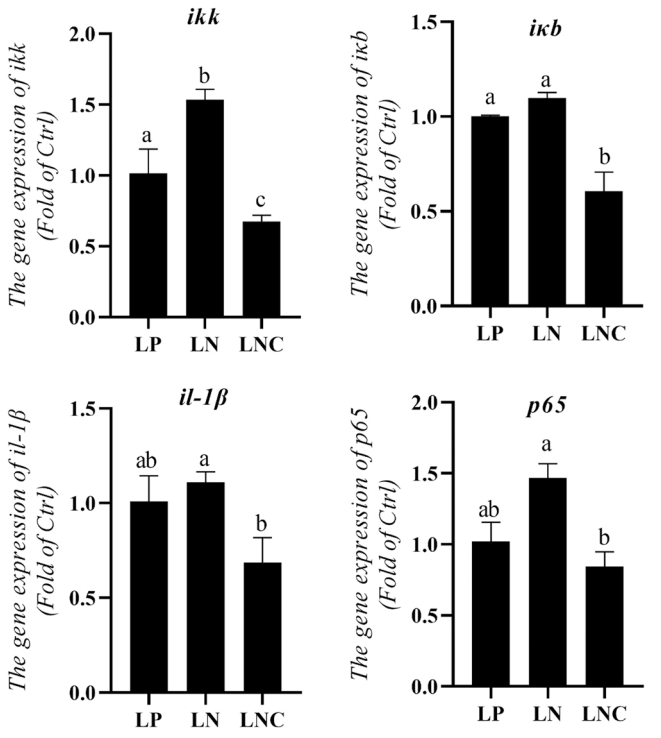

Fig. 3. Hepatic mRNA expression levels of NF-kB pathway related genes of *T. ovatus* fed dietary supplementation of L-carnitine for 6 weeks. The small letters indicated significant differences at $p < 0.05$. Values are mean ± SE (n = 4).

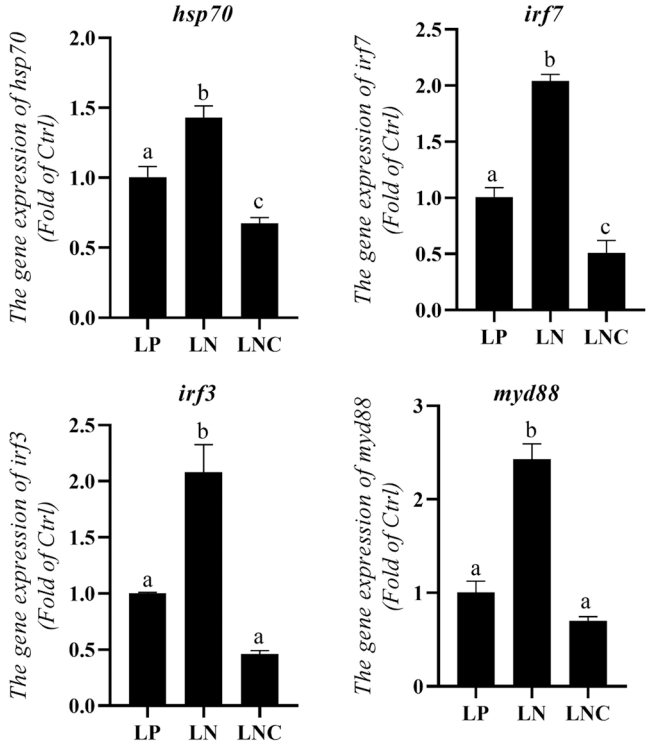

Fig. 4. Hepatic mRNA expression levels of non-specific immunity related genes of *T. ovatus* fed dietary supplementation of L-carnitine for 6 weeks. The small letters indicated significant differences at $p < 0.05$. Values are mean ± SE (n = 4).

inactive due to binding to the inhibitory protein, IkB to form a trimeric complex in the cytoplasm (Ghosh and Karin, 2002; Hayden and Ghosh, 2004). On the contrary, if cells were exposed to environmental stresses, IKK kinase would be activated and induce the phosphorylation of IκB protein and thus dissociate it from the trimeric complex, resulting in the translocation of NF-κB protein into the nucleus, which would activate transcriptions of downstream inflammatory-related genes (Ghosh and Karin, 2002; Hayden and Ghosh, 2004), such as pro-inflammatory cytokines, IL-1β (Dinarello, 2000, 1996). In the present study, the elevated trend in the RNA expression of *iκb*, *il-1β*, and *p65*, and the significantly higher expression level of *ikk* in LN group, compared with the LP group, indicated that a high-fat diet induces an inflammatory response. Previous studies demonstrated that a high-fat diet would lead to oxidative damage of animals (Veeramani et al., 2017; Zhong et al., 2020), which might correlate with the upregulation of NF-κB pathway. In the present study, significantly lower levels of *iκb*, *il-1β*, *p65* and *ikk* were found in the LNC group, compared with the LN group. These results indicated that L-carnitine is associating with the anti-inflammatory property in *T. ovatus* fed on a high-fat diet. Previous manuscripts also showed that L-carnitine could improve the anti-inflammatory capacity of aquatic animals (Mahalanobish et al., 2019; Suliman et al., 2018). Without a doubt, more research needs to be performed in the future to clarify this mechanism.

The non-specific immunity is generally regarded as the first line of defensive involved in eliminating the invasion of pathogenic microorganisms (Meylan et al., 2006). The toll-like receptor (TLR) family is considered a crucial regulatory system in non-specific immunity, which plays an essential role in the inflammation, regulation, survival, and proliferation of immune cells (Barton and Medzhitov, 2003). Cells recognize pathogenic microorganisms by ascertaining conserved pathogen-associated molecular patterns (PAMP) (Kaisho and Akira, 2001). Among them, when organisms are exposed to the invasion of pathogenic microorganisms, pattern recognition receptors (PRR) are activated. Then, activated PRR regulates two downstream defensive signals, the MyD88 - dependent TLR pathway and the TRIF - dependent pathway (Kim et al., 2018; Medzhitov, 2001). In the MyD88 - dependent TLR pathway, MyD88 interacts with corresponding IL-1R-associated kinases (IRAK) (Krishnan et al., 2007; Wesche et al., 1997), while in the TRIF - dependent pathway, the TRIF adapter stimulates transcription factors and cascades in the pathway, such as IRF3 and IRF7 (Takeda and Akira, 2005). These two pathways increase levels of type I IFNs and inflammatory cytokines (Takeda and Akira, 2005). Previous studies showed that *hsp70* was highly expressed when cells were exposed to various environmental pressures, such as microbial invasion, heat, heavy metal, and hypoxia (Yamashita et al., 2010). In the present study, RNA expression levels of *myd88*, *irf3*, *irf7*, and *hsp70* in the LN group were significantly higher in the LP and LNC groups. These results indicate that a high-fat diet might be associated with the invasion of pathogenic microorganisms, while L-carnitine plays an essential role in resistance against pathogenic microorganisms and mitigate the innate immunity response caused by a high-fat diet by regulating the MyD88-dependent TLR pathway and the TRIF-dependent pathway.

5. Conclusion

In conclusion, L-carnitine can relieve the detrimental impact on the growth performance of T. *ovatus* exposed to a high-fat diet treatment by reducing hepatic lipid accumulation and improving intestinal morphology, anti-inflammatory and non-specific immune status, but could not mitigate oxidative pressure. Therefore, supplementation of 0.6 g kg^{-1} of L-carnitine is recommended for T. *ovatus* fed on a 210 g kg^{-1} lipid diet.

CRediT authorship contribution statement

FHH and NJ designed the study. **FHH** carried out the experiment,

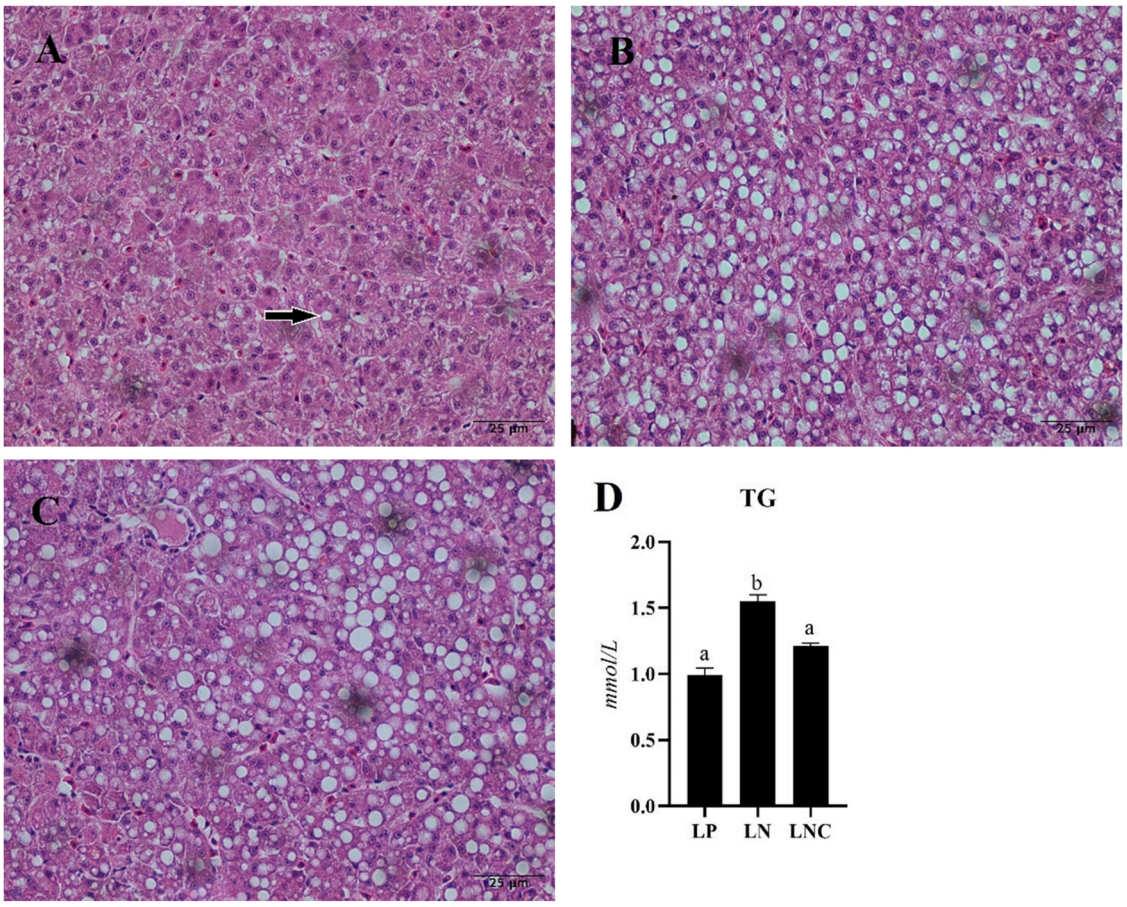

Fig. 5. Light microscopy observation of hepatic morphology of *T. ovatus* fed dietary supplement L-carnitine (A: LP group, B: LN group, C: LNC group) for 6 weeks. Scale bars in pictures A, B, C are 25 μm. The arrow in picture A means hepatic lipid droplet. Fig. D means hepatic triglyceride (TG) contents among groups. Values are mean ± SE (n = 4).

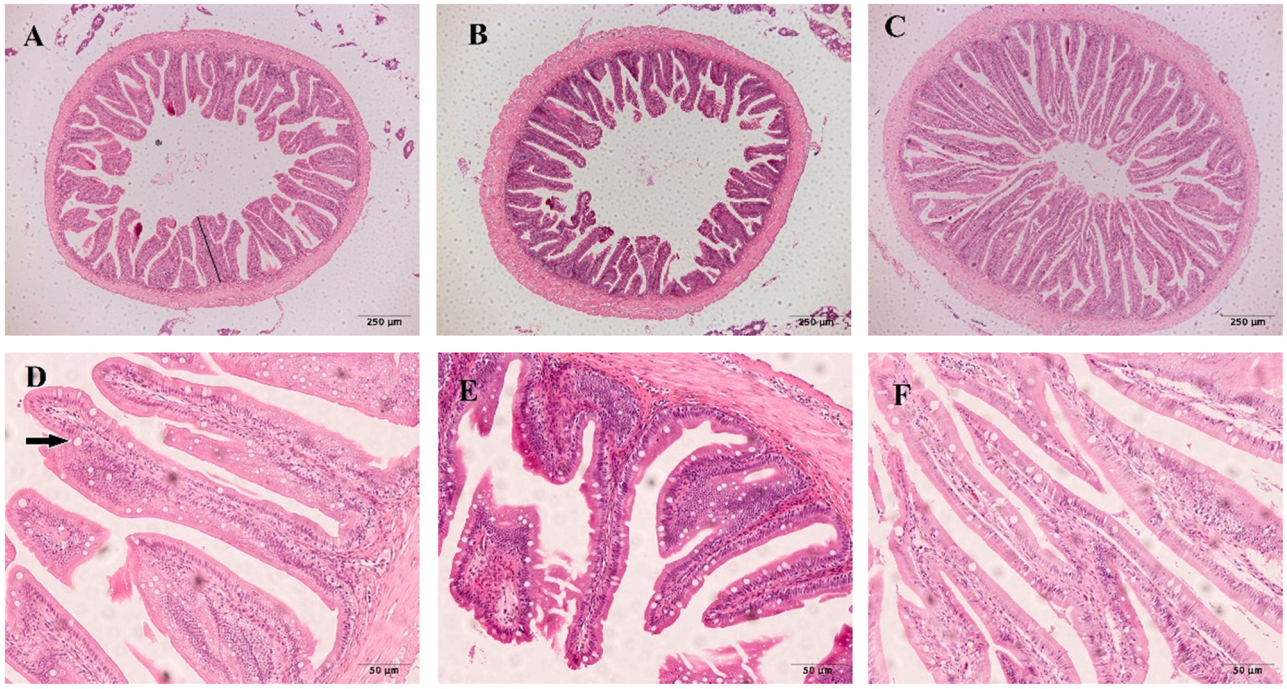

Fig. 6. Light microscopy observation of mid-intestine morphology of *T. ovatus* fed dietary supplement L-carnitine (A, D: LP group, B, E: LN group, C, F: LNC group) for 6 weeks. Scale bars in pictures A, B, C are 250 μm, while scale bars in pictures D, E, F are 50 μm, respectively. Locations of the black line and arrow in pictures (A) and (D) points to villi length and goblet cell, respectively.

Table 5
Mid-intestine morphology of *T. ovatus* fed dietary supplementation of L-carnitine for 42 days.

	LP	LN	LNC
Villi length (μm)	365.19 ± 6.47[a]	278.58 ± 8.9[b]	580.98 ± 29.12[c]
Goblet cell (number per villi)	23.5 ± 0.87	23.25 ± 0.63	23 ± 0.71

Values are mean ± SE. Means in the same row with different superscripts are significantly different ($p < 0.05$).

analyzed data and wrote the manuscript.

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.

Data availability

The experiment data in this study are available from the corresponding author based on reasonable request.

Acknowledgments

This research was supported by Project of National Natural Science Foundation of China (31872580, 32172982), and Project of Science and Technology of Guangdong Province (2021B0202050002), and Project of Science and Technology of Guangdong Province (2019B110209005), and Guangdong Provincial Special Fund for Morden Agriculture Industry Technology Innovation Teams (2019KJ143).

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