Received: 16 August 2018 | Revised: 10 December 2018 | Accepted: 18 December 2018
DOI: 10.1111/anu.12881

ORIGINAL ARTICLE

WILEY Aquaculture Nutrition

Effects of α-lipoic acid on growth performance, body composition, antioxidant profile and lipid metabolism of the GIFT tilapia (*Oreochromis niloticus*) fed high-fat diets

Fangning Xu[1] | **Chang Xu**[2] | **Shusheng Xiao**[1] | **Maixin Lu**[3] | **Samwel M. Limbu**[1,4] | **Xiaodan Wang**[1] | **Zhenyu Du**[1] | **Jian G. Qin**[5] | **Liqiao Chen**[1]

[1]Laboratory of Aquaculture Nutrition and Environmental Health, School of Life Sciences, East China Normal University, Shanghai, China

[2]Department of Aquaculture, College of Marine Sciences, Hainan University, Haikou, Hainan, China

[3]Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, Guangdong, China

[4]Department of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, Dar es Salaam, Tanzania

[5]College of Science and Engineering, Flinders University, Adelaide, South Australia, Australia

Correspondence
Liqiao Chen and Xiaodan Wang, Laboratory of Aquaculture Nutrition and Environmental Health, School of Life Sciences, East China Normal University, Shanghai, China.
Emails: lqchen@bio.ecnu.edu.cn; xdwang@bio.ecnu.edu.cn

Funding information
China Agriculture Research System-46 (CARS-46).; Major State Basic Research Development Program of China (973 Program), Grant/Award Number: 2014CB138600)

Abstract

The wide use of lipid as a non-protein energy substitute has led to lipid metabolic problems in cultured tilapia. Therefore, studies that reduce the effects of high-fat diets in genetically improved farmed tilapia (GIFT) are required. This study evaluated the optimum level and effects of dietary α-lipoic acid (α-LA) on growth performance, body composition, antioxidant capacity and lipid metabolism of GIFT tilapia. The basal diet (120 g/kg lipid) was supplemented with six concentrations of α-LA at 0 (control), L300, L600, L900, L1200 and L2400 mg/kg diet to make the experimental diets, which were fed to GIFT tilapia juveniles (initial body weight: 0.48 ± 0.01 g) for 8 weeks. The weight gain of fish improved significantly in the L300 than other dietary treatments. The intraperitoneal fat index and lipid content of fish fed on the L2400 diet decreased significantly than those fed on the control diet. The activities of superoxide dismutase and glutathione peroxidase (GSH-Px) in serum and liver were significantly higher in fish fed on the L300 diet than the control. The reduced GSH content of fish fed on the L300 in serum and liver was significantly higher than those fed on control diet. The malondialdehyde content in serum and liver was significantly lower in L300 than in the control. The adipose triglyceride lipase gene was significantly up-regulated in fish fed on the L2400, but the diacylglycerol acyltransferase 2 gene was down-regulated in adipose. The liver-type fatty acid-binding protein gene in the liver was significantly up-regulated in fish fed on the L300 and L600 diets. Moreover, the acyl-coenzyme A oxidase gene in liver was significantly up-regulated in fish fed on the L300, L600, L900 and L1200 diets. Polynomial regression analysis indicated that 439–528 mg/kg α-LA is an appropriate dosage in high-fat diet to improve growth performance and relieve lipid oxidative damage by accelerating lipid catabolism and reducing lipid synthesis in GIFT tilapia.

KEYWORDS
antioxidant status, growth performance, high-fat diet, lipid metabolism, *Oreochromis niloticus*, α-lipoic acid

1 | INTRODUCTION

Lipoic acid (LA) also known as 5-(1, 2-dithiolan-3-yl) pentanoic acid is a common coenzyme in eukaryotes and prokaryotes (Gorąca et al., 2011). The LA is a water- and lipid-soluble molecule with a capacity to pass through cell membranes (Longaray-Garcia et al., 2013) and contains two oxidized or reduced thiol groups. The oxidized form is defined as lipoic acid, and the reduced form of LA is known as dihydrolipoic acid (DHLA) (Gorąca et al., 2011). The DHLA interacts predominantly with reactive oxygen species (ROS), although the oxidized form of LA can inactivate free radicals (Packer, Kraemer, & Rimbach, 2001).

The α-LA and DHLA are used as antioxidants to improve fish health and immunity (Navari-Izzo, Quartacci, & Sgherri, 2002). A study conducted on catfish species (*Corydoras paleatus*) indicated that dietary α-LA improved the detoxification and antioxidant capacity and reduced oxidative stress parameters (Monserrat et al., 2008). The α-LA has also been used as a chemo-protectant against oxidative damage in white shrimp (*Litopenaeus vannamei*) (Lobato et al., 2013). In grass carp (*Ctenopharyngodon idellus*), α-LA protected n-3 HUFAs against lipid peroxidation to promote its deposition in fish and enhanced the activity of antioxidant enzymes via mediating NF-E2-related nuclear factor 2 and Kelch-like ECH-associated protein 1 (Nrf2-Keap1) signalling pathways (Shi et al., 2017). Moreover, supplementation of α-LA in the diet also improved growth performance of hybrid tilapia (Xiong, Zhang, & Liu, 2012).

The level of α-LA to improve growth performance, fish health, immunity and antioxidants varies among different species. In juvenile abalone (*Haliotis discus hannai*), the optimal level of dietary α-LA (709 mg/kg) promoted growth and stimulated antioxidative defence capacity (Zhang et al., 2010). However, excessive α-LA supplementation affects negatively animal growth and antioxidative capacity (Kütter, Romano, Ventura-Lima, Tesser, & Monserrat, 2014). Therefore, it is necessary to determine the optimal α-LA dose in the diet to maximize its benefits on growth enhancement and antioxidant capacity and minimizes its pro-oxidant toxic effect (Enamorado et al., 2015; Kütter et al., 2014).

Studies in mammals show that the α-LA plays a role as a therapeutic agent for obesity and dyslipidaemia using different mechanisms (Butler, Hagen, & Moreau, 2009; Xu et al., 2012). The α-LA exerted an anti-obesity effect by suppressing the hypothalamic AMP-activated protein kinase (AMPK) activity in C_2C_{12} myotubes (Kim et al., 2004). Moreover, α-LA activated both Sirtuin 1 and adipose triacylglycerol lipase (ATGL), increased AMPK and acetyl-CoA carboxylase phosphorylation and suppressed fatty acid synthase protein production (Chen, Kang, Wang, & Lee, 2012; Kim et al., 2004). The α-LA supplementation reduced intracellular lipid accumulation by inducing ATGL expression and enhanced the forkhead box O1 (FOXO1)/ATGL pathway in HepG2 cells (Kuo, Lin, Chen, & Lee, 2012). Dietary supplementation of α-LA also decreased blood and liver triglycerides in sn-glycerol-3-phosphate acyltransferase-deficient mice (Schüller et al., 2018). Furthermore, dietary LA inhibited liver lipogenic gene expression, decreased hepatic TG secretion, but stimulated the clearance of TG-rich lipoproteins (Butler et al., 2009). These studies indicate that α-LA may provide beneficial effects in alleviating high-fat-induced metabolic disturbances in different animals.

Tilapia species constitute the second most important group of fish in global aquaculture due to their fast growth rate, disease resistance, good meat quality and marketability. The availability of the complete genomic information on tilapia has enabled scientists to improve further their productivity in aquaculture (Ng & Romano, 2013; Oliveira et al., 2014). The genetically improved farmed tilapia (GIFT tilapia, *Oreochromis niloticus*) through selective breeding has been used as a strain of fish stock in aquaculture (Boonanuntanasarn et al., 2018; Ng & Romano, 2013; Oliveira et al., 2014; Xie et al., 2017). Despite its wide use, little has been done to reduce effectively its costly dietary protein and nitrogen pollution in its culture systems (Oliveira et al., 2014). Provision of adequate energy by using dietary lipid or carbohydrate can minimize the use of costly protein and increase feed efficiency (He et al., 2015; Lim, Yildirim-Aksoy, Li, Welker, & Klesius, 2009; Ma et al., 2018; Pan et al., 2017; Xie et al., 2017). However, the wide use of lipid as a non-protein energy substitute has led to hyperlipidaemia and hypercholesterolaemia in cultured tilapia (Huang, Lin, Lee, Liu, & Pan, 2016). Therefore, studies that reduce the effects of high-fat diets in GIFT tilapia are required to ensure its fast growth rate and health.

In this study, GIFT tilapia were fed on high-fat diets supplemented with different concentrations of dietary α-LA for 8 weeks to evaluate the optimal level and examine the role of α-LA in lipid metabolism. The results of this study provide new insights for improving growth and health of GIFT tilapia fed on high-fat diets.

2 | MATERIALS AND METHODS

2.1 | Ethical consideration

This research project for using aquatic animals was approved by the Animal Ethics Committee of East China Normal University, and all experiments were conducted according to the protocols and procedures of the Laboratory Animal Management Ordinance of China.

2.2 | Experimental diets

Six high-fat practical diets containing 120 g/kg total lipid were formulated with different concentrations of α-LA (0, 300, 600, 900, 1,200 and 2,400 mg/kg diet), abbreviated as L0 (control), L300, L600, L900, L1200 and L2400, respectively. The α-LA used in this study was a racemic mixture of R-LA (dextro isomer) and S-LA (laevo isomer). Corn oil and corn starch were the main lipid and carbohydrate sources, respectively. Fishmeal, soybean meal and wheat middling were the main protein sources. Butylated hydroxytoluene was added in oil at a concentration of 0.5 g/kg as the antioxidant to avoid lipid oxidation in the diets, as described in our previous study (Xu et al., 2018). The feed ingredients were ground and sieved through an 80-μm mesh. All dry ingredients were finely ground and mixed separately for each diet before adding oil. Deionized water (100 ml/

TABLE 1 Ingredient formulation and proximate composition (g/kg dry basis) of the six experimental diets fed to GIFT tilapia

	Experimental diets[a]					
Ingredients (g/kg diet)	L0	L300	L600	L900	L1200	L2400
Fishmeal	80	80	80	80	80	80
Soybean meal	454	454	454	454	454	454
Cornstarch	250	250	250	250	250	250
Wheat middling	50	50	50	50	50	50
Maize oil	100	100	100	100	100	100
Dicalcium phosphate	10	10	10	10	10	10
Vitamin mix[b]	5	5	5	5	5	5
Mineral mix[c]	5	5	5	5	5	5
Celufil	16	16	16	16	16	16
Carboxymethyl cellulose	30	30	30	30	30	30
α-lipoic acid	0	0.3	0.6	0.9	1.2	2.4
Proximate composition						
Moisture	97.0	94.6	92.3	96.4	91.6	92.3
Crude protein	306.8	302.2	304.5	302.0	307.6	305.2
Crude lipid	120.0	123.5	125.4	122.7	121.0	124.1
Ash	62.5	64.1	63.4	64.1	64.6	62.8

Notes. GIFT: genetically improved farmed tilapia; α-LA: α-lipoic acid.

[a] Experimental diets: control diet without α-LA (L0) and diets containing 300, 600, 900, 1,200 and 2,400 mg/kg α-LA (L300–L2400). [b] Vitamin mix provided the following vitamins (mg or IU/kg diet): 500,000 I.U. (international units) vitamin A; 50,000 I.U. vitamin D3; 2,500 mg vitamin E; 1,000 mg vitamin K3; 5,000 mg vitamin B1; 5,000 mg vitamin B2; 5,000 mg vitamin B6; 5,000 μg vitamin B12; 25,000 mg inositol; 10,000 mg pantothenic acid; 100,000 mg choline; 25,000 mg niacin; 1,000 mg folic acid; 250 mg biotin; 10,000 mg vitamin C. [c] Mineral mix provided the following minerals (g/kg diet): 314.0 g $CaCO_3$; 469.3 g KH_2PO_4; 147.4 g $MgSO_4 \cdot 7H_2O$; 49.8 g NaCl; 10.9 g Fe (II) gluconate; 3.12 g $MnSO_4 \cdot H_2O$; 4.67 g $ZnSO_4 \cdot 7H_2O$; 0.62 g $CuSO_4 \cdot 5H_2O$; 0.16 g KJ; 0.08 g $CoCl_2 \cdot 6H_2O$; 0.06 g NH_4 molybdate; 0.02 g $NaSeO_3$.

kg diet) was added to dissolve the mixture of the ingredients and then wet-extruded into 2.5-mm-diameter pellets using a double helix plodder (F-26, SCUT industrial factory, Guangdong, China). The resulting pellets were dried by blowing air at room temperature until reaching <100 g/kg moisture. Pellets were sieved into various sizes (16, 14, 10 and 6 mesh sieves) and stored at −20°C until needed for use. The ingredients and proximate composition of the six experimental diets are given in Table 1.

2.3 | Experimental fish and management procedure

About 1,000 GIFT tilapia were purchased from Hainan Progift Aqua-Tech Co. Ltd, Dingan, Hainan Province, China. After 8-day acclimation in plastic tanks (330 L) in the Biological Station of East China Normal University, 630 fish (0.48 ± 0.01 g) with relatively similar sizes were assigned randomly into 18 aquariums. The experiment consisted of six dietary treatments each consisting of three replicates (35 fish in each replicate). During the experiment, the fish were hand-fed twice daily at 09:00 and 17:00 at a feeding rate of 4% body weight for 8 weeks. The weight of fish in each treatment was measured on a weekly basis, and the diet quantity was adjusted accordingly. Uneaten feeds were removed by siphoning after 1.5 hr postfeeding. The daily water exchange rate was 50% of the aquarium volume. The incoming freshwater was aerated by using air stones from air pumps thoroughly before entering the water recirculation system. Dead fish were removed, and their weight was recorded. During the whole trial, the water quality conditions were maintained at 28 ± 2°C, 7.5–7.9 pH, and at a photoperiod of 12-hr light/12-hr dark. Dissolved oxygen level was >6.0 mg/L, and ammonia-N was <0.02 mg/L.

2.4 | Sample collection

After 8 weeks, all fish were fasted for 24 hr, collected by using a scoop net, counted and individually weighed. A sample of seven fish was collected randomly from each treatment and anaesthetized by using MS222 (20 mg/L) (tricaine methanesulfonate; Western Chemicals, Inc., Ferndale, WA, USA). They were dissected aseptically, and blood, liver and intraperitoneal fat samples were collected. Four out of the seven fish were used for body weight, blood, liver and intraperitoneal fat determination, and the other three were stored at −20°C for whole-body composition analysis. Blood was collected from the caudal vasculature by puncture by using 2-ml syringes (Klmedical, China) and centrifuged at 4°C and 956 *g* for 10 min. Serum and all other samples were stored at −80°C for further biochemical and molecular analysis.

TABLE 2 Primers used for the analysis of mRNA gene expressions by RT-PCR

Gene	Position	Sequence (5′–3′)	Length	Accession no.
ATGL	Forward	AAAACGTCCTGGTGACCCAGT	104	XM_003440346
	Reverse	TAGGAGGAATGATGCCACAGTACA		
DGAT2	Forward	GCTTGAATTCTGTCACCCTGAAGA	106	XM_003458972
	Reverse	ACCTGCTTGTAGGCGTCGTTCT		
L-FABP	Forward	ACTATCGGACAGGAGGCTGAACTA	98	XM_003446092
	Reverse	TTCTTCAGGGTGGTCTTCAGCTT		
CPT1α	Forward	TTTCCAGGCCTCCTTACCCA	102	XM_003440552
	Reverse	TTGTACTGCTCATTGTCCAGCAGA		
ACO	Forward	AGTCCCACTGTGAGCTCCATCAA	108	KF918710
	Reverse	CAGACCATGGCAGTTTCCAAGA		
β-actin	Forward	AGCCTTCCTTCCTTGGTATGGAAT	102	KJ126772
	Reverse	TGTTGGCGTACAGGTCCTTACG		

Note. ACO: acyl-coenzyme A oxidase; ATGL: adipose triglyceride lipase; CPT1α: carnitine palmitoyl transferase 1α; DGAT2: diacylglycerol acyltransferase 2; L-FABP: liver-type fatty acid-binding protein; RT-PCR: real-time quantitative PCR.

2.5 | The proximate composition analysis of whole fish and diets

The proximate composition of the whole fish body and the diets were analysed following the standard methods (AOAC, 1995). The moisture was analysed by drying to a constant weight at 105°C. The crude protein content was measured by the Kjeldahl method (8200, Kjeltec, Foss, Sweden). The total lipid content was extracted by using a chloroform/methanol mixture and a 0.37 mol/L KCl solution and quantified by gravimetry using the vacuum drying oven (DZF-6050; Jinghong, Ltd., Shanghai, China) (Folch, Ascoli, Lees, Meath, & Lebaron, 1951). Samples were carbonized completely on a heating plate (TR-30A, SuDa, China) at 330°C for 30 min and then incinerated in a muffle furnace (PCD-E3000 Serials, Peaks, Japan) at 550°C for 6 hr to measure the ash content.

2.6 | Biochemical analysis

Malondialdehyde (MDA; Cat. No. A003-1), superoxide dismutase (SOD; Cat. No. A001-1), glutathione peroxidase (GSH-Px; Cat. No. A005), reduced glutathione (GSH; Cat. No. A006-2), triglycerides (TG; Cat. No. A110-1), non-esterified free fatty acids (NEFA; Cat. No. A042-2) and total protein (Cat. No. A045-2) were measured by using specific commercial assay kits by following manufacturer's instructions (Jiancheng, Bioengineering Institute, Nanjing, China).

2.7 | Calculations

The growth performance, feed utilization, survival rate and organ indices parameters were calculated using the following formulae:

$$\text{Weight gain (WG, \%)} \\ = (\text{final weight} - \text{initial weight})/\text{initial weight} \times 100;$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) \\ = (\text{Ln final weight} - \text{Ln initial weight})/\text{number of days} \times 100;$$

$$\text{Feed conversion ratio (FCR)} \\ = \text{feed intake}/(\text{final fish weight} - \text{initial fish weight});$$

$$\text{Survival (\%)} = (\text{final fish number}/\text{initial fish number}) \times 100;$$

$$\text{Hepatosomatic index (HSI, \%)} = (\text{liver weight}/\text{body weight}) \times 100;$$

$$\text{Intraperitoneal fat index (IPF, \%)} \\ = (\text{intraperitoneal fat weight}/\text{body weight}) \times 100$$

2.8 | Isolation of RNA, synthesis of cDNA and quantitative real-time PCR

Total RNA from liver and adipose tissues was isolated by using the TRIzol reagent (RN0101; Aidlab, China) according to the manufacturer's protocol. The quality and quantity of total RNA were estimated using the NanoDrop 2000 spectrophotometer (Thermo, Wilmington, DE, USA). The RNA was reversely transcribed using the PrimeScript™ RT Reagent Kit (RR047A, Takara, Japan) at 42°C for 2 min to remove genomic DNA and 37°C for 15 min plus 85°C for 5 s to complete reverse transcription. Before the real-time quantitative PCR (RT-PCR), the concentration of cDNA was unified. The RT-PCR was performed in a final volume of 10 μl containing 5 μl of 2 × Ultra SYBR Mixture (CW0957, KangWei, China), 0.4 μl of forward and reverse primer mixture specific for genes, 1 μl of diluted synthesized cDNA template and 3.6 μl nuclease-free water with the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The RT-PCR was conducted in the CFX96 real-time PCR system (Bio-Rad, Richmond, CA, USA). Each gene per treatment was run in five copies, and β-actin was used as the internal control. We conducted a preliminary genes verification experiment using tissues from the experimental fish and found that the expression of β-actin was not affected by α-LA. To ensure specificity on intended genes, the primers for RT-PCR were designed to span an intron. The primer sequences used for the RT-PCR analysis of genes are listed in Table 2. The cycle time (C_t) values of different treatments were compared to their corresponding internal control and then converted to fold change values by comparing to the control group (120 g/kg lipid diet without α-LA) and calculated by the $2^{-\Delta\Delta C_t}$ method.

2.9 | Histological analysis

Three fish liver tissues from each treatment were used for histological analysis. The tissues were fixed by using 4% paraformaldehyde and dehydrated in ethanol in a concentration gradient from 70% to 100% (Betancor et al., 2015). Thereafter, the samples were infiltrated by xylol and embedded in paraffin. Vertical sections of 5 μm thickness were cut, and the slides were deparaffinized in xylol and rehydrated in a graded alcohol series before the specimens were stained with haematoxylin and eosin. Subsequently, the sections were dehydrated in ethanol and xylol again and mounted with neutral resins. The sections of each slide were analysed and photomicrographed using a light microscope (model BX51; Olympus, Tokyo, Japan) (Sun, Du, Liu, Dahms, & Wang, 2018).

2.10 | Statistical analyses

Results are presented as means ± standard error (*SE*), and all data were tested for normality by using the Kolmogorov–Smirnov test and homogeneity of variances by using Levene's test before statistical analysis. Thereafter, the data were subjected to one-way analysis of variance followed by Newman–Keuls test for specific comparisons among groups. Polynomial regression analysis was used to assess the optimum inclusion levels of dietary α-LA supplementation based on weight gain (WG), SOD activity in liver and mRNA expression of liver-type fatty acid-binding protein (L-FABP). All statistical analyses were performed by using the SPSS Statistics 19.0 software (IBM, Armonk, NY, USA). Results with p value < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Growth, survival and feed utilization

The results showed that α-LA in the high-fat diet had significant effects on final body weight (FBW), WG, SGR and IPF ($p < 0.05$; Table 3). The FBW, WG and SGR for fish fed on L300 were significantly higher than those fed on the control and other dietary treatments ($p < 0.05$), except those fed on L600 diet ($p > 0.05$). The FCR increased significantly in fish fed on L2400 diet compared to

TABLE 3 Growth performance, feed utilization and survival of GIFT tilapia fed diets containing diverse α-LA concentrations

Diets	L0	L300	L600	L900	L1200	L2400
IBW (g)	0.48 ± 0.01^{a}	0.47 ± 0.01^{a}	0.48 ± 0.01^{a}	0.48 ± 0.01^{a}	0.47 ± 0.01^{a}	0.48 ± 0.01^{a}
FBW (g)	$8.75 \pm 0.53^{b,c}$	10.79 ± 0.19^{a}	$9.6 \pm 0.16^{a,b}$	$9.12 \pm 0.57^{b,c}$	$9.17 \pm 0.47^{b,c}$	7.72 ± 0.13^{c}
WG (%)	$1,734.20 \pm 88.10^{b,c}$	$2,194.74 \pm 11.18^{a}$	$1,909.79 \pm 58.91^{b}$	$1,782.76 \pm 122.67^{b,c}$	$1,843.90 \pm 123.21^{b,c}$	$1,505.65 \pm 45.67^{c}$
SGR (%/day)	$5.19 \pm 0.09^{b,c}$	5.6 ± 0.01^{a}	$5.36 \pm 0.05^{a,b}$	$5.23 \pm 0.12^{b,c}$	$5.29 \pm 0.12^{a,b,c}$	4.96 ± 0.05^{c}
FCR	1.08 ± 0.09^{b}	1.04 ± 0.05^{b}	$1.14 \pm 0.03^{a,b}$	$1.17 \pm 0.03^{a,b}$	$1.17 \pm 0.02^{a,b}$	1.32 ± 0.03^{a}
HSI (%)	1.27 ± 0.04^{a}	1.03 ± 0.05^{b}	1.03 ± 0.03^{b}	1.07 ± 0.04^{b}	0.99 ± 0.03^{b}	1.03 ± 0.03^{b}
IPF (%)	0.38 ± 0.03^{a}	$0.31 \pm 0.06^{a,b}$	$0.22 \pm 0.04^{a,b}$	$0.24 \pm 0.04^{a,b}$	$0.23 \pm 0.03^{a,b}$	0.14 ± 0.01^{b}
Survival (%)	89.52 ± 5.04^{a}	87.62 ± 4.15^{a}	85.71 ± 1.65^{a}	88.57 ± 4.36^{a}	88.57 ± 5.95^{a}	93.33 ± 2.52^{a}

Notes. Values are means ± *SE*. Values with different superscript letters within a row are significantly different ($p < 0.05$).

FBW: final body weight; GIFT: genetically improved farmed tilapia; IBW: initial body weight; α-LA: α-lipoic acid.

Weight gain (WG, %) = (final weight − initial weight)/initial weight × 100;

Specific growth rate (SGR, %/day) = 100 × (Ln finial weight − Ln initial weight)/number of days;

Feed conversion ratio (FCR) = feed intake/(final fish weight − initial fish weight);

Hepatosomatic index (HSI, %) = (liver weight/body weight) × 100;

Intraperitoneal fat index (IPF, %) = (intraperitoneal fat weight/body weight) × 100

Survival (%) = (final fish number/initial fish number) × 100;

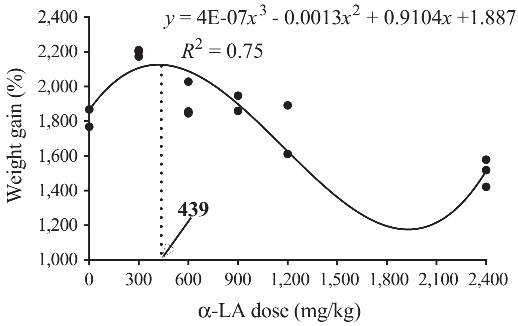

FIGURE 1 Polynomial regression of the effect of dietary α-lipoic acid (α-LA) supplementation levels on weight gain (WG)

the control diet ($p < 0.05$). The HSI decreased significantly in all α-LA diets compared to the control ($p < 0.05$). The IPF of fish fed on L2400 diet was significantly lower than those fed on the control diet ($p < 0.05$). The survival did not vary among all groups ($p > 0.05$). The optimum α-LA to obtain maximum growth performance was estimated as 439 mg/kg based on WG (Figure 1).

3.2 | Whole-body proximate composition

The moisture content of GIFT tilapia fed on L2400 was significantly higher than that in the control ($p < 0.05$; Table 4). The crude protein content increased significantly in GIFT tilapia fed on L600, L900, L1200 and L2400 compared to those fed on the control and L300 diets ($p < 0.05$). The content of crude lipid in GIFT tilapia decreased significantly in L2400 compared to the control ($p < 0.05$). Dietary α-LA addition significantly increased the ash content in treated GIFT tilapia compared to the control ($p < 0.05$).

3.3 | Serum biochemical parameters

The SOD activity increased significantly in GIFT tilapia fed on L300 diet compared to the control diet ($p < 0.05$: Table 5). The GSH-PX activity increased significantly in GIFT tilapia fed on L300 diet compared to the control and L2400 diets ($p < 0.05$). The GSH content increased significantly in GIFT tilapia fed on L300 compared to the control ($p < 0.05$). However, the MDA content of GIFT tilapia fed on L300 decreased significantly compared to those fed on all α-LA diets and the control diet ($p < 0.05$). The NEFA content increased significantly in GIFT tilapia fed on L1200 and L2400 than in the control diet ($p < 0.05$; Table 5).

3.4 | Liver biochemical parameters

The activity of SOD increased significantly in GIFT tilapia fed on L300 than those fed on the control, L900, L1200 and L2400 diets ($p < 0.05$). The activity of GSH-Px increased significantly in GIFT tilapia fed on L300 and L600 compared to the control diet ($p < 0.05$). Dietary α-LA addition induced GSH accumulation in GIFT tilapia fed on L300 and L600 diets compared to the control diet ($p < 0.05$). Dietary α-LA addition effectively decreased MDA content of GIFT tilapia fed on L300 diet compared to the control diet ($p < 0.05$). The TG content in the liver decreased significantly in GIFT tilapia fed on dietary α-LA treatments compared to the control diet ($p < 0.05$; Table 6). The maximum activity of SOD was reached at 460 mg/kg α-LA level (Figure 2).

3.5 | mRNA expressions of lipid metabolism genes

The mRNA expressions of lipid metabolism genes in the liver and adipose of GIFT tilapia fed on the diets supplemented with different α-LA contents are presented in Figure 3a,b, respectively. The mRNA expression of adipose triglyceride lipase (ATGL) showed an increasing tendency with increasing dietary α-LA addition (Figure 3b). The ATGL mRNA expression increased significantly in adipose of fish fed on L2400 diet compared to the control ($p < 0.05$). The mRNA expression of diacylglycerol acyltransferase 2 (DGAT2) gene was down-regulated in a dose-dependent manner with increasing dietary α-LA addition in adipose. The DGAT2 mRNA expression was significantly lower in fish fed on L2400 diet compared to the control diet ($p < 0.05$). The tendency of mRNA expressions of ATGL and DGAT2 genes in liver was similar to that in adipose, but there was no significant difference between dietary α-LA levels ($p > 0.05$). The mRNA expression of L-FABP in the liver of fish fed on L300 and L600 diets was significantly up-regulated compared to those fed on the control diet ($p < 0.05$). The mRNA expression of acyl-coenzyme A oxidase (ACO) gene in the liver of fish fed on L300, L600, L900 and L1200 diets was significantly up-regulated compared to the control diet ($p < 0.05$). The tendency of mRNA expression of ACO in adipose was similar to that in the liver although there was no significant difference among dietary α-LA levels ($p > 0.05$). The mRNA expression of carnitine palmitoyl transferase 1α (CPT1α) gene was up-regulated in fish fed on L300 diet compared to the control diet but decreased with the increase of α-LA addition in diets in both the liver and adipose tissues ($p > 0.05$). The maximum mRNA expression of L-FABP was reached at 528 mg/kg α-LA inclusion level (Figure 4).

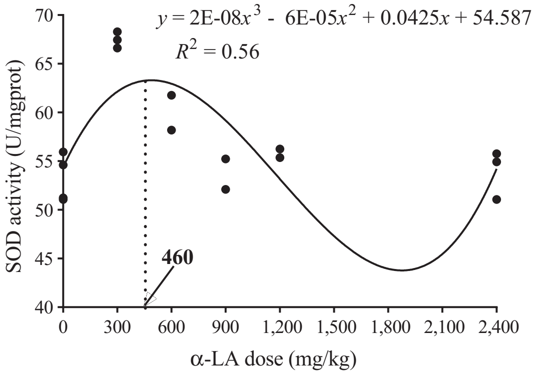

FIGURE 2 Polynomial regression of the effect of dietary α-lipoic acid (α-LA) supplementation levels on superoxide dismutase activity (SOD)

TABLE 4 Proximate composition of GIFT tilapia fed on diets containing diverse α-LA concentrations

Diets	L0	L300	L600	L900	L1200	L2400
Moisture (g/kg)	771.15 ± 6.67^{b}	778.29 ± 3.15^{a,b}	779.23 ± 4.51^{a,b}	784.22 ± 2.94^{a,b}	787.42 ± 3.71^{a,b}	789.86 ± 3.05^{a}
Crude protein (g/kg)	585.14 ± 15.06^{b}	594.54 ± 3.23^{b}	668.78 ± 19.16^{a}	686.09 ± 23.78^{a}	686.55 ± 14.21^{a}	643.24 ± 10.05^{a}
Crude lipid (g/kg)	173.48 ± 20.80^{a}	127.9 ± 8.18^{a,b}	145.13 ± 10.11^{a,b}	128.69 ± 3.18^{a,b}	123.59 ± 10.92^{a,b}	109.4 3 ± 7.15^{b}
Ash (g/kg)	151.52 ± 10.10^{b}	177.6 ± 1.83^{a}	189.02 ± 2.67^{a}	184.59 ± 7.23^{a}	177.22 ± 1.22^{a}	188.07 ± 4.46^{a}

Notes. Values are means ± *SE*. Values with different superscript letters within a row are significantly different ($p < 0.05$).
GIFT: genetically improved farmed tilapia; α-LA: α-lipoic acid.

TABLE 5 Serum biochemical parameters of GIFT tilapia fed diets containing diverse α-LA concentrations

Diets	L0	L300	L600	L900	L1200	L2400
SOD (U/ml)	38.94 ± 9.40^{b}	85.09 ± 8.62^{a}	71.15 ± 2.2^{a,b}	63.81 ± 7.47^{a,b}	62.14 ± 6.53^{a,b}	58.4 ± 0.56^{a,b}
GSH-PX (U)	203.11 ± 16.70^{b}	360.72 ± 21.56^{a}	316.17 ± 15.83^{a,b}	289.34 ± 25.94^{a,b}	253.89 ± 31.15^{a,b}	221.32 ± 45.66^{b}
GSH (μmol/L)	32.63 ± 3.94^{b}	61.02 ± 6.13^{a}	52.97 ± 4.23^{a,b}	48.31 ± 7.63^{a,b}	44.92 ± 2.54^{a,b}	42.94 ± 3.95^{a,b}
MDA (nmol/ml)	5.43 ± 0.25^{a,b}	3.21 ± 0.14^{c}	4.26 ± 0.35^{b}	4.81 ± 0.29^{a,b}	5.25 ± 0.25^{a,b}	5.76 ± 0.58^{a}
NEFA (mmol/L)	0.17 ± 0.02^{b}	0.22 ± 0.01^{a,b}	0.24 ± 0.01^{a,b}	0.26 ± 0.02^{a,b}	0.29 ± 0.02^{a}	0.32 ± 0.05^{a}

Notes. Values are means ± *SE*. Values with different superscript letters within a row are significantly different ($p < 0.05$).
GIFT: genetically improved farmed tilapia; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; NEFA: non-esterified free fatty acids; SOD: superoxide dismutase; α-LA: α-lipoic acid.

TABLE 6 Liver biochemical parameters of GIFT tilapia fed diets containing diverse α-LA concentrations

Diets	L0	L300	L600	L900	L1200	L2400
SOD (U/mgprot)	53.21 ± 1.22^{b}	66.71 ± 0.89^{a}	59.88 ± 2.91^{a,b}	56.21 ± 2.7^{b}	56.25 ± 0.89^{b}	53.92 ± 1.45^{b}
GSH-PX (U/mgprot)	113.85 ± 9.22^{b}	179.50 ± 10.82^{a}	171.52 ± 7.77^{a}	151.89 ± 15.71^{a,b}	146.23 ± 4.12^{a,b}	134.39 ± 20.35^{a,b}
GSH (μmol/gprot)	11.65 ± 2.23^{b}	21.85 ± 2.17^{a}	18.78 ± 0.51^{a}	17.42 ± 1.13^{a,b}	16.86 ± 1.06^{a,b}	16.24 ± 0.98^{a,b}
MDA (nmol/mgprot)	0.84 ± 0.12^{a}	0.42 ± 0.09^{b}	0.50 ± 0.06^{a,b}	0.62 ± 0.01^{a,b}	0.65 ± 0.02^{a,b}	0.71 ± 0.03^{a,b}
TG (mmol/gprot)	121.65 ± 15.53^{a}	68.75 ± 5.73^{b}	62.74 ± 5.41^{b}	54.14 ± 11.16^{b}	52.36 ± 2.23^{b}	45.52 ± 3.99^{b}

Notes. Values are means ± *SE*. Values with different superscript letters within a row are significantly different ($p < 0.05$).
GIFT: genetically improved farmed tilapia; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; SOD: superoxide dismutase; TG: triglycerides; α-LA: α-lipoic acid.

3.6 | Histology of liver tissues

There were substantial empty spaces corresponding to fat droplets in the liver of fish in the control diet, but less empty spaces were found in L900 diet and least empty spaces in L2400 diet (Figure 5a–c).

4 | DISCUSSION

To the best of our knowledge, this study was the first to examine the role of α-LA on lipid metabolism in GIFT tilapia fed on high-fat diet. In this study, FBW, WG and SGR were increased significantly when α-LA was added to the diet to a maximum of 300 mg/kg compared to the control, beyond which they showed a decreasing tendency. Based on WG, the maximum growth of GIFT tilapia was reached at 439 mg/kg α-LA level. These results are similar to those obtained in Plata pompano (*Trachinotus marginatus*), in which the dietary supplementation of α-LA between 316.4 and 524 mg/kg increased growth rate, but growth and feed intake were retarded and reduced at 890 or 1,367 mg/kg LA supplementation, respectively (Kütter, Monserrat, Primel, Caldas, & Tesser, 2012). Similarly, in abalone the WG increased with the increasing amount of α-LA content in the diet to the highest value of 800 mg/kg dietary α-LA supplement (Zhang et al., 2010). However, in abalone growth reduction occurred when the dose of α-LA supplementation reached 1,600 and 3,200 mg LA kg^{-1} (Zhang et al., 2010) compared to 2,400 mg LA kg^{-1} obtained in the present study, indicating species dose-dependent effects. We relate the increased growth rate in GIFT tilapia in our study to acceleration of lipid utilization to allow more energy allocation for growth due to the α-LA supplementation in the diets.

Excessive lipid intake and accumulation can increase the risk of oxidative damage. As an "ideal antioxidant," α-LA can directly scavenge ROS and regenerate endogenous antioxidants, such as glutathione, vitamins E and vitamins C (Gorąca et al., 2011). In this study, α-LA in diets increased significantly the activities of SOD and GSH-Px. This is similar to the results obtained in grass carp; whereby, α-LA increased significantly the activities of SOD both in the liver

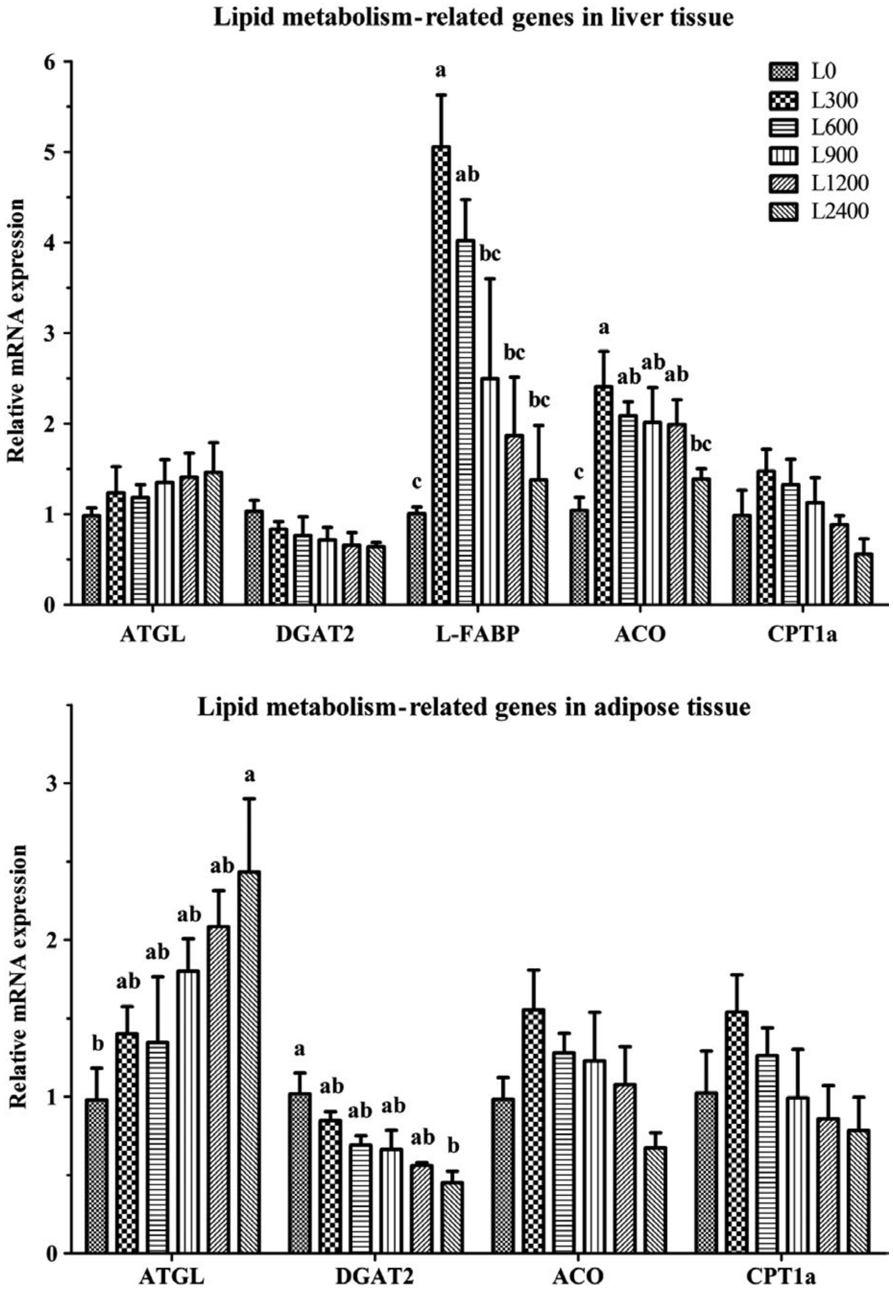

FIGURE 3 Transcriptional changes of lipid metabolism-related genes in liver and in adipose tissues. (a) Liver tissue, (b) adipose tissue. All values are means ± *SEM*. ^{a,b,c}Values on bars without a common superscript letter are significantly different ($p < 0.05$) (a indicated the highest value). Values on bars that show nothing are not significantly different ($p > 0.05$)

and muscle (Shi et al., 2017). Similarly, in the Chinese mitten crab (*Eriocheir sinensis*) fed on a α-LA-supplemented diet, the activities of SOD and GSH-PX were increased significantly in hepatopancreas (Xu et al., 2018). In the present study, the contents of GSH in both serum and liver increased significantly in GIFT tilapia fed the diets supplemented with α-LA. Similarly, in common carp (*Cyprinus carpio*) the GSH content was significantly higher in the intestine, liver and muscle of fish fed on α-LA for 2–3 weeks (Enamorado et al., 2015). The increased antioxidant capacity in the GIFT tilapia fed on α-LA diets, particularly levels below 500 mg/kg, is due to its ability to reduce ROS production.

The MDA content is a marker of oxidative stress as a result of lipid peroxidation of polyunsaturated fatty acids (Draper & Hadley, 1990). In this study, the content of MDA decreased significantly in GIFT tilapia fed on L300 and L600 diets, indicating that oxidative stress can be relieved by appropriate supplement of α-LA in the diet. The decreased MDA content in GIFT tilapia fed on L300 and L600 diets is due to higher antioxidant capacity resulting from increased enzymes activities (SOD, GSH-Px) and non-enzymatic antioxidants concentration (GSH). The enhanced antioxidant capacity enabled fish to cope with environmental or biological stress (Kütter et al., 2014). In rats, a high-fat diet (21.45%) induced dyslipidaemia (Yang, Li, Shi,

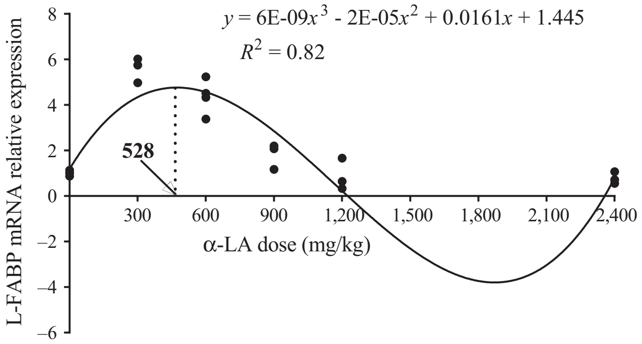

FIGURE 4 Polynomial regression of the effect of dietary α-lipoic acid (α-LA) supplementation levels on mRNA expression of liver-type fatty acid-binding protein (L-FABP)

& Le, 2008), which supports our claim that α-LA can decrease oxidative stress by increasing free radical scavenger enzymes. However, in abalone, high α-LA doses in diets (1,600–3,200 mg LA kg^{-1}) decreased antioxidant capacity (Zhang et al., 2010). Therefore, it seems that the antioxidant capacity induced by α-LA is closely related to the supplement dosage in the diet. We argue that α-LA exerts antioxidant properties by catalysing the toxic free oxides into a non-toxic (water and hydrogen peroxide) form by using SOD and ends with detoxification of dismutase toxic hydrogen peroxide into a non-toxic form by GSH-PX (Ramesh, Thilagavathi, Rathika, & Poopal, 2018). In addition, GSH-Px could connect with the GSH enzyme and convert it to the oxidized glutathione (GSSG) form to protect from cellular and neuronal damage (Ramesh et al., 2018). The results suggest that supplementing α-LA in diets for GIFT tilapia at a level of 460 mg/kg increases the antioxidant capacity and reduces the negative effects of high-fat diets.

In the present study, the contents of crude lipid and TG in the liver decreased significantly in GIFT tilapia fed on diets supplemented with α-LA. The optimum level of α-LA for accelerating lipid catabolism and reducing lipid synthesis in GIFT tilapia was determined as 528 mg/kg in the diet. Similarly, research in rats showed that total hepatic cholesterol and total lipid decreased significantly when α-LA was supplemented in the diet (Seo, Ha, & Kim, 2012). Dietary α-LA supplementation up-regulated mRNA expression of ATGL which is a rate-limiting enzyme in lipolysis (Li, Sang, Zhang, Li, & Chen, 2018; Sathyanarayan, Mashek, & Mashek, 2017). In the present study, dietary α-LA accelerated lipolysis in a dose-dependent manner by up-regulating the mRNA expression of ATGL. Similarly, α-LA ameliorated effectively intracellular lipid accumulation and induced ATGL expression through the FOXO1/ATGL pathway in HepG2 cells (Kuo et al., 2012). Moreover, in a study on C2C12 myotubes, α-LA increased ATGL protein production in mice fed a high-fat diet (Wang, Li, Guo, Chan, & Guan, 2010). The DGAT2 is an isoform of DGAT and is most highly expressed in the liver and adipose tissues. The final and rate-limiting step in the de novo triglyceride synthesis is catalysed by acyl-CoA where the DGAT2 is a vital gene (Hung, Carreiro, & Buhman, 2017; Paland, Gamliel-Lazarovich, Coleman, & Fuhrman, 2014; Wang, Airola, & Reue, 2017). In this study, mRNA expression of DGAT2 was down-regulated by dietary α-LA supplement, suggesting a weakened lipid synthesis in a dose-dependent manner. Moreover, the mRNA expression of L-FABP in the liver was up-regulated significantly by dietary α-LA. The FABP binds to long-chain fatty acids (LCFAs) and certain other lipids. Particularly, L-FABP can bind fatty acids and transport them to the mitochondria or peroxisomes, where they are metabolized via β-oxidation and provide energy for tubular epithelial cells (Gonçalves et al., 2015; Xu, Xie, Shao, Ni, & Mou, 2015). Therefore, α-LA supplementation in diets can reduce lipid content by up-regulating lipolysis as well as down-regulating lipogenesis, which is consistent with other studies in rats (Butler et al., 2009; Kuo et al., 2012). In rats, α-LA reduced TG in blood and liver by inhibiting liver lipogenic gene expression (e.g., sn-glycerol-3-phosphate acyltransferase-1 and diacylglycerol O-acyltransferase-2), lowering hepatic TG secretion and stimulating clearance of TG-rich lipoproteins (Butler et al., 2009). The histological sections of the liver in treated fish with decreased vacuole cells also demonstrate the role of α-LA in promoting lipolysis. The present study illustrates that adding dietary α-LA at an appropriate content can inhibit lipogenesis and induce lipolysis.

The β-oxidation in fatty acids (FA) is an essential pathway in ATP production and is primarily facilitated by the mitochondrial trifunctional protein. The CPT1 is one of the regulator enzymes, which catalyses the transfer of LCFA from CoA to carnitine for translocation across the mitochondrial inner membrane and is well recognized as the primary rate-controlling step in fatty acid oxidation (Pan et al., 2017; Sousa et al., 2005). The ACO is the enzyme associated with intracellular oxidation of FA in fat cells and is also the initial enzyme of

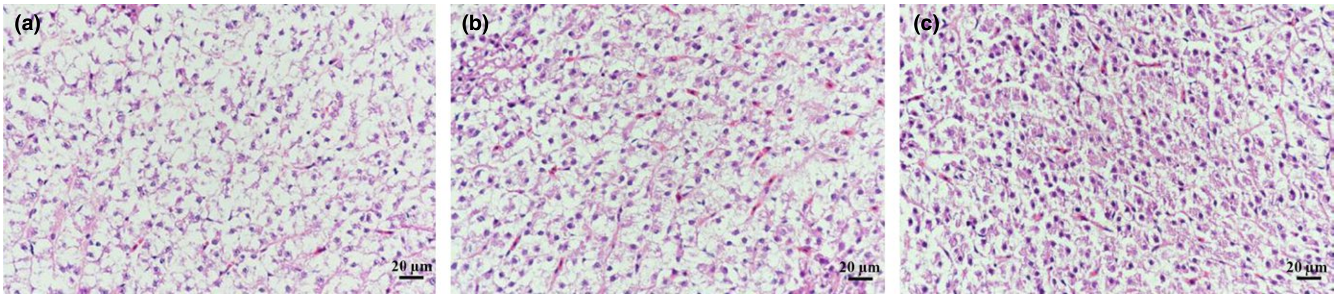

FIGURE 5 Histological characteristics of liver (40×). (a) L0, (b) L900, (c) L2400

the β-oxidation system in peroxisomes (Liu et al., 2016). Similarly, in this study, a proper supplementation of α-LA in the diet can enhance FA β-oxidation in peroxisomes.

In our study, a proper concentration of α-LA in the diet can accelerate lipolysis and β-oxidation. Similarly, research in dyslipidaemia rats showed that α-LA can prevent high-fat diet-induced dyslipidaemia by increasing the expression of genes related to β-oxidation (Yang et al., 2008). Although high-dose α-LA addition in diet can up-regulate lipolysis and down-regulate lipogenesis, it may interfere with the transfer and β-oxidation of FA. This may explain why high-dose α-LA addition decreased lipid content but did not favour fish growth in this study. Moreover, TG content in liver decreased in comparison with the control due to α-LA supplement in diets, whereas NEFA content in serum increased. A high dose of α-LA addition in diets caused transfer deficiency and reduced β-oxidation, leading to NEFA accumulation in serum. This study shows that appropriate addition of dietary α-LA can enhance FA β-oxidation in mitochondria, yet a high dose of α-LA may retard this process.

5 | CONCLUSION

The α-LA supplementation in the diet improved significantly the growth performance and antioxidant capacity in GIFT tilapia fed high-fat diets. The addition of α-LA in the diet can accelerate lipolysis and fatty acid β-oxidation and control lipid synthesis. Considering the overall responses in growth performance, antioxidant status and lipid metabolism, the level of 439–528 mg/kg dietary α-LA addition is a recommended dose in the diet for GIFT tilapia fed on high-fat diets.

ACKNOWLEDGEMENTS

This research was supported by the Major State Basic Research Development Program of China (973 Program) (No. 2014CB138600) and China Agriculture Research System-46 (CARS-46).

ORCID

Fangning Xu https://orcid.org/0000-0002-1465-4748
Maixin Lu https://orcid.org/0000-0003-1502-0656
Jian G. Qin https://orcid.org/0000-0003-2448-8058
Liqiao Chen https://orcid.org/0000-0003-0138-7828

REFERENCES

AOAC (1995). *Official methods of analysis of association of official analytical chemists (AOC)* (16th ed.). Arlington, VA: AOC International.

Betancor, M. B., Sprague, M., Sayanova, O., Usher, S., Campbell, P. J., Napier, J. A., ... Tocher, D. R. (2015). Evaluation of a high-EPA oil from transgenic *Camelina sativa* in feeds for Atlantic salmon (*Salmo salar* L.): Effects on tissue fatty acid composition, histology and gene expression. *Aquaculture*, *444*, 1–12. https://doi.org/10.1016/j.aquaculture.2015.03.020

Boonanuntanasarn, S., Kumkhong, S., Yoohat, K., Plagnes-Juan, E., Burel, C., Marandel, L., & Panserat, S. (2018). Molecular responses of Nile tilapia (*Oreochromis niloticus*) to different levels of dietary carbohydrates. *Aquaculture*, *482*, 117–123. https://doi.org/10.1016/j.aquaculture.2017.09.032

Butler, J. A., Hagen, T. M., & Moreau, R. (2009). Lipoic acid improves hypertriglyceridemia by stimulating triacylglycerol clearance and downregulating liver triacylglycerol secretion. *Archives of Biochemistry and Biophysics*, *485*, 63–71. https://doi.org/10.1016/j.abb.2009.01.024

Chen, W. L., Kang, C. H., Wang, S. G., & Lee, H. M. (2012). α-Lipoic acid regulates lipid metabolism through induction of sirtuin 1 (SIRT1) and activation of AMP-activated protein kinase. *Diabetologia*, *55*, 1824–1835. https://doi.org/10.1007/s00125-012-2530-4

Draper, H. H., & Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, *186*, 421–431. https://doi.org/10.1016/0076-6879(90)86135-I

Enamorado, A. D., Martins, A. C., Flores, J. A., Tesser, M. B., Caldas, S. S., Primel, E. G., & Monserrat, J. M. (2015). Biochemical responses over time in common carp *Cyprinus carpio* (Teleostei, Cyprinidae) during fed supplementation with α-lipoic acid. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology*, *188*, 9–16. https://doi.org/10.1016/j.cbpa.2015.05.023

Folch, J., Ascoli, I., Lees, M., Meath, J. A., & Lebaron, N. (1951). Preparation of lipide extracts from brain tissue. *Journal of Biological Chemistry*, *191*, 833–841.

Gonçalves, F. L., Soares, L. M., Figueira, R. L., Simões, A. L., Gallindo, R. M., & Sbragia, L. (2015). Evaluation of the expression of I-FABP and L-FABP in a necrotizing enterocolitis model after the use of *Lactobacillus acidophilus*. *Journal of Pediatric Surgery*, *50*, 543–549. https://doi.org/10.1016/j.jpedsurg.2014.07.007

Gorąca, A., Huk-Kolega, H., Piechota, A., Kleniewska, P., Ciejka, E., & Skibska, B. (2011). Lipoic acid – biological activity and therapeutic potential. *Pharmacological Reports*, *63*, 849–858. https://doi.org/10.1016/S1734-1140(11)70600-4

He, A. Y., Ning, L. J., Chen, L. Q., Chen, Y. L., Xing, Q., Li, J. M., ... Du, Z. Y. (2015). Systemic adaptation of lipid metabolism in response to low- and high-fat diet in Nile tilapia (*Oreochromis niloticus*). *Physiological Reports*, *3*, e12485. https://doi.org/10.14814/phy2.12485

Huang, S. C., Lin, J. J., Lee, M. F., Liu, Y. C., & Pan, B. S. (2016). Freshwater clam extracts alleviate dyslipidaemia of tilapia fed a high-fat diet as an animal model. *Journal of Functional Foods*, *25*, 559–567. https://doi.org/10.1016/j.jff.2016.06.017

Hung, Y. H., Carreiro, A. L., & Buhman, K. K.. (2017) Dgat1 and Dgat2 regulate enterocyte triacylglycerol distribution and alter proteins associated with cytoplasmic lipid droplets in response to dietary fat. *Biochimica Et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1862*, 600–614. https://doi.org/10.1016/j.bbalip.2017.02.014

Kim, M. S., Park, J. Y., Namkoong, C., Jang, P. G., Ryu, J. W., Song, H. S., ... Park, I. S. (2004). Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nature Medicine*, *10*, 727–733. https://doi.org/10.1038/nm1061

Kuo, Y. T., Lin, T. H., Chen, W. L., & Lee, H. M. (2012). Alpha-lipoic acid induces adipose triglyceride lipase expression and decreases intracellular lipid accumulation in HepG2 cells. *European Journal of Pharmacology*, *692*, 10–18. https://doi.org/10.1016/j.ejphar.2012.07.028

Kütter, M. T., Monserrat, J. M., Primel, E. G., Caldas, S. S., & Tesser, M. B. (2012). Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae). *Aquaculture*, *368–369*, 29–35. https://doi.org/10.1016/j.aquaculture.2012.09.010

Kütter, M. T., Romano, L. A., Ventura-Lima, J., Tesser, M. B., & Monserrat, J. M. (2014). Antioxidant and toxicological effects elicited by alpha-lipoic acid in aquatic organisms. *Comparative Biochemistry & Physiology Part C: Toxicology & Pharmacology*, *162*, 70–76. https://doi.org/10.1016/j.cbpc.2014.03.008

Li, S., Sang, C., Zhang, J., Li, Z., & Chen, N. (2018). Molecular cloning, expression profiling of adipose triglyceride lipase (ATGL) and forkhead box O1 (FoxO1), and effects of dietary carbohydrate level on their expression in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). *Aquaculture*, *492*, 103–112. https://doi.org/10.1016/j.aquaculture.2018.03.062

Lim, C., Yildirim-Aksoy, M., Li, M. H., Welker, T. L., & Klesius, P. H. (2009). Influence of dietary levels of lipid and vitamin E on growth and resistance of Nile tilapia to *Streptococcus iniae* challenge. *Aquaculture*, *298*, 76–82. https://doi.org/10.1016/j.aquaculture.2009.09.025

Liu, Z. Y., Jiang, Y. P., Li, L., You, L. L., Wu, Y., Xu, B., … Wu, J. C. (2016). Silencing of ACO decreases reproduction and energy metabolism in triazophos-treated female brown plant hoppers, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). *Pesticide Biochemistry & Physiology*, *128*, 76–81. https://doi.org/10.1016/j.pestbp.2015.09.014

Lobato, R. O., Nunes, S. M., Wasielesky, W., Fattorini, D., Regoli, F., Monserrat, J. M., & Ventura-Lima, J. (2013). The role of lipoic acid in the protection against of metallic pollutant effects in the shrimp *Litopenaeus vannamei* (Crustacea, Decapoda). *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology*, *165*, 491–497. https://doi.org/10.1016/j.cbpa.2013.03.015

Longaray-Garcia, M., Flores, J. A., Külkamp-Guerreiro, I. C., Guterres, S. S., Pereira, T. C., Bogo, M. R., & Monserrat, J. M. (2013). Modulation of antioxidant and detoxifying capacity in fish *Cyprinus carpio* (Cyprinidae) after treatment with nanocapsules containing lipoic acid. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology*, *165*, 468–475. https://doi.org/10.1016/j.cbpa.2013.02.004

Ma, Q., Li, L.-Y., Le, J.-Y., Lu, D.-L., Qiao, F., Zhang, M.-L., … Li, D.-L. (2018). Dietary microencapsulated oil improves immune function and intestinal health in Nile tilapia fed with high-fat diet. *Aquaculture*, *496*, 19–29. https://doi.org/10.1016/j.aquaculture.2018.06.080

Monserrat, J. M., Lima, J. V., Ferreira, J. L., Acosta, D., Garcia, M. L., Ramos, P. B., … Amado, L. L. (2008). Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *148*, 287–292. https://doi.org/10.1016/j.cbpc.2008.06.011

Navari-Izzo, F., Quartacci, M. F., & Sgherri, C. (2002). Lipoic acid: A unique antioxidant in the detoxification of activated oxygen species. *Plant Physiology and Biochemistry*, *40*, 463–470. https://doi.org/10.1016/S0981-9428(02)01407-9

Ng, W. K., & Romano, N. (2013). A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture*, *5*, 220–254. https://doi.org/10.1111/raq.12014

Oliveira, M. M. D., Ribeiro, T., Orlando, T. M., Oliveira, D. G. S. D., Drumond, M. M., Freitas, R. T. F. D., & Rosa, P. V. (2014). Effects crude protein levels on female Nile tilapia (*Oreochromis niloticus*) reproductive performance parameters. *Animal Reproduction Science*, *150*, 62–69. https://doi.org/10.1016/j.anireprosci.2014.08.006

Packer, L., Kraemer, K., & Rimbach, G. (2001). Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrition*, *17*, 888–895. https://doi.org/10.1016/S0899-9007(01)00658-X

Paland, N., Gamliel-Lazarovich, A., Coleman, R., & Fuhrman, B. (2014). Urokinase-type plasminogen activator (uPA) stimulates triglyceride synthesis in Huh7 hepatoma cells via p38-dependent upregulation of DGAT2. *Atherosclerosis*, *237*, 200–207. https://doi.org/10.1016/j.atherosclerosis.2014.09.003

Pan, H., Li, L. Y., Li, J. M., Wang, W. L., Limbu, S. M., Degrace, P., … Du, Z. Y. (2017). Inhibited fatty acid β-oxidation impairs stress resistance ability in Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, *68*, 500–508. https://doi.org/10.1016/j.fsi.2017.07.058

Ramesh, M., Thilagavathi, T., Rathika, R., & Poopal, R. K. (2018). Antioxidant status, biochemical and hematological responses in a cultivable fish *Cirrhinus mrigala* exposed to an aquaculture antibiotic Sulfamethazine. *Aquaculture*, *491*, 10–19. https://doi.org/10.1016/j.aquaculture.2018.02.046

Sathyanarayan, A., Mashek, M. T., & Mashek, D. G. (2017). ATGL promotes autophagy/lipophagy via SIRT1 to control hepatic lipid droplet catabolism. *Cell Reports*, *19*, 1–9. https://doi.org/10.1016/j.celrep.2017.03.026

Schüller, Á. K., Mena Canata, D. A., Hackenhaar, F. S., Engers, V. K., Heemann, F. M., Putti, J. S., … Benfato, M. S. (2018). Effects of lipoic acid and n-3 long-chain polyunsaturated fatty acid on the liver ovariectomized rat model of menopause. *Pharmacological Reports*, *70*, 263–269. https://doi.org/10.1016/j.pharep.2017.10.006

Seo, E. Y., Ha, A. W., & Kim, W. K. (2012). α-Lipoic acid reduced weight gain and improved the lipid profile in rats fed with high fat diet. *Nutrition Research and Practice*, *6*, 195–200. https://doi.org/10.4162/nrp.2012.6.3.195

Shi, X. C., Jin, A., Sun, J., Yang, Z., Tian, J. J., Ji, H., … Du, Z. Y. (2017). α-lipoic acid ameliorates n-3 highly-unsaturated fatty acids induced lipid peroxidation via regulating antioxidant defenses in grass carp (*Ctenopharyngodon idellus*). *Fish & Shellfish Immunology*, *67*, 359–367. https://doi.org/10.1016/j.fsi.2017.06.036

Sousa, U. L., De, J., Koss, M. D., Marion, F., Anja, G., Scheeder, M. R. L., … Monika, L. (2005). CPT1alpha over-expression increases long-chain fatty acid oxidation and reduces cell viability with incremental palmitic acid concentration in 293T cells. *Biochemical and Biophysical Research Communications*, *338*, 757–761. https://doi.org/10.1016/j.bbrc.2005.10.016

Sun, M., Du, X. L., Liu, J. Q., Dahms, H. U., & Wang, L. (2018). Histological analysis of oogenesis and ovarian development of the freshwater crab *Sinopotamon henanense*. *Tissue and Cell*, *53*, 37–43. https://doi.org/10.1016/j.tice.2018.05.009

Wang, H., Airola, M. V., & Reue, K. (2017). How lipid droplets "TAG" along: Glycerolipid synthetic enzymes and lipid storage. *Biochimica Et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids*, *1862*, 1131–1145. https://doi.org/10.1016/j.bbalip.2017.06.010

Wang, Y., Li, X., Guo, Y., Chan, L., & Guan, X. (2010). α-Lipoic acid increases energy expenditure by enhancing adenosine monophosphate–activated protein kinase–peroxisome proliferator-activated receptor- coactivator-1 signaling in the skeletal muscle of aged mice. *Metabolism*, *59*, 967–976. https://doi.org/10.1016/j.metabol.2009.10.018

Xie, D., Yang, L., Yu, R., Chen, F., Lu, R., Qin, C., & Nie, G. (2017). Effects of dietary carbohydrate and lipid levels on growth and hepatic lipid deposition of juvenile tilapia, *Oreochromis niloticus*. *Aquaculture*, *479*, 696–703. https://doi.org/10.1016/j.aquaculture.2017.07.013

Xiong, D., Zhang, X., & Liu, X. (2012). Effects of anethole trithione and lipoic acid on growth performance and antioxidant ability of *Oreochromis niloticus* × *O. aureus* (In Chinese with English Abstract). *China Feed*, *23*, 28–31. https://doi.org/10.15906/j.cnki.cn11-2975/s.2012.23.010.

Xu, C., Li, E., Liu, S., Huang, Z., Qin, J., & Chen, L. Q. (2018). Effects of α-lipoic acid on growth performance, body composition, antioxidant status and lipid catabolism of juvenile Chinese mitten crab *Eriocheir sinensis* fed different lipid percentage. *Aquaculture*, *484*, 286–292. https://doi.org/10.1016/j.aquaculture.2017.09.036

Xu, J., Yang, W., Deng, Q., Huang, Q., Yang, J. E., & Huang, F. (2012). Flaxseed oil and α-lipoic acid combination reduces atherosclerosis risk factors in rats fed a high-fat diet. *Lipids in Health and Disease*, *11*, 148. https://doi.org/10.1186/1476-511x-11-148

Xu, Y., Xie, Y., Shao, X., Ni, Z., & Mou, S. (2015). L-FABP: A novel biomarker of kidney disease. *Clinica Chimica Acta*, *445*, 85–90. https://doi.org/10.1016/j.cca.2015.03.017

Yang, R. L., Li, W., Shi, Y. H., & Le, G. W. (2008). Lipoic acid prevents high-fat diet-induced dyslipidemia and oxidative stress: A microarray analysis. *Nutrition*, *24*, 582–588. https://doi.org/10.1016/j.nut.2008.02.002

Zhang, W., Chen, Q., Mai, K., Xu, W., Wang, X., & Liufu, Z. (2010). Effects of dietary α-lipoic acid on the growth and antioxidative responses of juvenile abalone *Haliotis discus hannai* Ino. *Aquaculture Research*, *41*, e781–e787. https://doi.org/10.1111/j.1365-2109.2010.02592.x

How to cite this article: Xu F, Xu C, Xiao S, et al. Effects of α-lipoic acid on growth performance, body composition, antioxidant profile and lipid metabolism of the GIFT tilapia (*Oreochromis niloticus*) fed high-fat diets. *Aquacult Nutr.* 2019;00:1–12. https://doi.org/10.1111/anu.12881