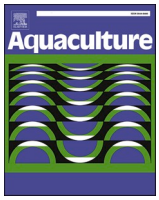
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Different effects of two dietary levels of tea polyphenols on the lipid deposition, immunity and antioxidant capacity of juvenile GIFT tilapia (*Oreochromis niloticus*) fed a high-fat diet

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ABSTRACT

Long-term feeding of fish with a high-fat diet (HFD) causes excess fat deposition and an impairment of immune function. In the present study, we aimed to determine whether dietary tea polyphenols (TPs) would ameliorate the adverse effects of HFD-feeding in GIFT tilapia. Juvenile GIFT tilapias (5.4 ± 0.9 g) were raised in twelve 200-L tanks (three tanks per diet, 20 fish per tank) and fed a control diet (6% fat, 36% protein), an HFD (12% fat, 36% protein), or an HFD supplemented with 50 mg/kg or 200 mg/kg TP for 8 weeks. The fish were hand-fed 5% of their body weight per day in three feeds, and maintained at 28 ± 1 °C under a 14-h light/10-h dark cycle. The fish in each tank were bulk weighed and counted fortnightly, and the daily feed amount was adjusted accordingly. At the end of the trial, the cumulative survival rate was calculated, and the weight gain and feed conversion ratio were calculated according to the bulk weight of fish in each tank. Tissues were collected from nine fish per diet, their organs were weighed, and biochemical and molecular indices were subsequently measured. HFD-feeding significantly increased lipid deposition, reduced cumulative survival from 96% to 75%, reduced hepatic alkaline phosphate activity (AKP) and serum total antioxidant capacity (T-AOC); and reduced the hepatic expression of immunoglobulin M (IgM), transforming growth factor-beta (TGF-β) and glutathione-S-transferase (GST) genes *versus* the control diet. The addition of TPs at 50 or 200 mg/kg both ameliorated the HFD-induced increase in lipid droplets in the liver (50 mg/kg TP from 40.83% to 17.27%; 200 mg/kg TP to 25.33%), and increased the cumulative survival rate of the tilapia. The addition of 50 mg/kg TP had a marked effect increasing cumulative survival to 90%, and increasing the activities of serum acid phosphatase (ACP), T-AOC; and IgM, TGF-β, nuclear factor-κB (NF-κB), superoxide dismutase (SOD), and GST gene expression to the highest level of the HFD-fed groups. The 50 mg/kg TP-containing diet also significantly increased the hepatic expression of carnitine palmitoyltransferase 1 alpha (CPT1α) *versus* the control diet. In contrast, the tilapia fed an HFD supplemented with 200 mg/kg TPs had the lowest expression of adipose triglyceride lipase, hormone-sensitive lipase, CPT1α, fatty acid synthase and acetyl-CoA carboxylase alpha genes of any of the groups, which implies that the lower and higher levels of TP supplementation have differing effects on lipid metabolism. The 200 mg/kg supplement had lower cumulative survival rate (82%), and smaller effects on serum ACP and hepatic AKP activities than the 50 mg/kg dose, and had no significant effect on serum T-AOC or the expression of IgM, TGF-β, GST, or NF-κB genes in the tilapia. These results indicate that the beneficial effects of TPs on the lipid metabolism and health of fish fed an HFD are dose-related. Moreover, they are likely to be largely mediated through lipid catabolism.

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1. Introduction

The use of a high-fat diet (HFD) is very common in modern aquaculture because increasing the dietary lipid content reduces the cost of the diet and increases protein retention in fish by utilizing lipids rather than protein for energy consumption (Watanabe, 1982; Silva et al., 1991; NRC, 1993; Watanabe, 2002). However, excessive dietary fat content may cause an increase in fat deposition (Jobling et al., 1998; Regost et al., 2001) in the fish and impair their feed intake and utilization (Du et al., 2005), leading to poorer growth (Arzel et al., 1994) and flesh quality (Bjerkeng et al., 1997). Moreover, excess fat deposition in fish body can also cause health problems, such as fatty liver, impairments in the immune system, and oxidative stress. For example, feeding an HFD to blunt snout bream (*Megalobrama amblycephala*) for 8 weeks reduces the plasma activities of acid phosphatase (ACP) and lysozyme (LZM) (Chen et al., 2016a). Furthermore, feeding an HFD to turbot (*Scophthalmus maximus*) significantly increases fat deposition and malondialdehyde (MDA) content, and reduces the hepatic activities of catalase (CAT) and superoxide dismutase (SOD) (Jia et al., 2017). Therefore, the effects of various feed additives on lipid metabolism (Shi et al., 2018), the immune system (Zhou et al., 2015; Lu et al., 2019), and stress resistance (Liu et al., 2016) in fish have been studied, as potential means of improving fish production. In particular, the potential effects of these feed additives to ameliorate the adverse effects of HFDs in fish have attracted great interest.

Tea polyphenols (TPs) are polyphenolics that are extracted from tea. Their major components, catechins, including epigallocatechin-3-gallate, epicatechin-3-gallate, epigallocatechin and epicatechin, have antioxidant properties (Frei and Higdon, 2003; Kanwar et al., 2012). Moreover, TPs have also been reported to alter lipid metabolism and improve immune function in mammals (Baeza et al., 2010; Tan et al., 2017; Wang et al., 2018). Recently, the use of TPs as feed additives in aqua-feeds has attracted attention, with TPs being reported to improve growth, flesh quality, non-specific immunity, and oxidative stress resistance in some fish species (Xu et al., 2008; Liu et al., 2012; Long et al., 2017; Liang et al., 2018). Therefore, the potential benefits of using TPs as additives to HFDs are worthy of further investigation.

Genetic improvement farmed tilapia (GIFT tilapia) have been widely cultured in Asia because of their rapid growth and high environmental adaptability (Dey and Gupta, 2000). In the present study, GIFT tilapias were used to test the hypothesis that the use of TPs as a feed additive could ameliorate the adverse effects of an HFD in fish. The growth, cumulative survival rate, body composition, liver histology, immunity, and antioxidant parameters of the GIFT tilapia were assessed after an 8-week feeding trial. We aimed to determine whether TPs have beneficial effects in fish fed an HFD and the mechanisms of any effects of TPs on their lipid metabolism, immunity and antioxidant capacity in fish.

2. Material and methods

2.1. Animal ethics statement

All experiments were conducted under the Guidance of the Care and Use of Laboratory Animals in China. This research was approved by the Committee on the Ethics of Animal Experiments of East China Normal University (F20190101).

2.2. Animals, culture conditions, and dietary experimental design

Experimental juvenile GIFT tilapias were obtained from Nanhai Tongwei Aquatic Technology Co., Ltd. (Foshan, China). The fish were acclimated in an indoor-recirculating experimental system, which was maintained at 28 ± 1 °C, prior to the start of the experiment, during which they were fed a commercial diet (Tongwei. Co Ltd., Chengdu, China) containing 33% protein and 5% lipid three times daily, for 2 weeks. After this, 240 healthy juvenile GIFT tilapias with comparable initial body weights (5.4 ± 0.9 g) were selected and randomly allocated to four diets (three tanks per dietary treatment, 20 fish per tank): a control diet (6% fat, 36% protein; C), an HFD (12% fat, 36% protein; HF), HFD supplemented with 50 mg/kg TPs (high-fat, low-TPs; HFLT), or an HFD supplemented with 200 mg/kg TPs (high-fat, high-TPs; HFHT). The fish in each tank were bulk-weighed before the trial to obtain the initial tank biomass.

During the 8-week feeding trial, the fish were maintained at 28 ± 1 °C in 200-L tanks, with static water, under a 14 h light/10 h dark cycle. The fish in each tank were bulk-weighed fortnightly for recording biomass, and the daily amount of feed provided was adjusted accordingly. The fish were hand-fed with the experimental diets at 5% of their body weight per day, divided into three feeds (at 08:30, 13:00, and 17:30). Every morning, before feeding, the daily feed was weighed precisely and the amount recorded. All the feeds provided were eaten within 15 min of their provision. Feces were siphoned off by using a siphoning tube prior to feeding, and half of the water by volume in each tank was replaced with fresh water every day to maintain optimum water quality. The dissolved oxygen (> 6.2 mg/ L), pH (7.5–7.9), and total ammonia nitrogen (< 0.02 mg/L) of the water were kept at the optimum levels for the survival and growth of tilapia. During the trial, the fish in each tank were checked every day, and any dead fish were collected using a scoop net and weighed to permit the adjustment of the calculated feed conversion ratio (FCR). The total number of fish in each tank was also counted to the calculate the cumulative survival rate (SR, three tanks per diet, $n = 3$).

2.3. Formulation of the experimental diets and supplementation with TPs

The formulations and composition of the experimental diets are listed in Table 1. The main components of the TPs are listed in Table 1 footnote (d). All the ingredients were obtained in dry, ground form. The

Table 1
Formulation and proximate composition of the experimental diets, and the composition of tea polyphenols used in the study[a].

Feed composition (%)	C	HF	HFLT	HFHT
Casein	32	32	32	32
Gelatin	8	8	8	8
Corn starch	30	30	30	30
Soybean oil	6	12	12	12
Vitamin premix[b]	1.2	1.2	1.2	1.2
Mineral premix[c]	1.2	1.2	1.2	1.2
Choline chloride	0.5	0.5	0.5	0.5
Butylated hydroxytoluene (BHT)	0.025	0.025	0.025	0.025
Dimethyl-b-propiothetin (DMPT)	0.1	0.1	0.1	0.1
$Ca(H_2PO_4)_2$	1	1	1	1
Carboxy methyl cellulose CMC	3	3	3	3
Cellulose	16.975	10.975	10.97	10.955
Tea polyphenols[d]	0	0	0.005	0.02
Composition				
Total lipid (%)	6.8	12.5	12.8	12.3
Total protein (%)	36	36	36	36
Moisture (%)	8.8	9	8.9	8.7

[a] Measured according to the National Standard of China-Tea Polyphenols (GB/T 31740.2–2015).

[b] Mixed vitamin (mg or IU/kg): 500,000 IU Vitamin A; 50,000 IU Vitamin D3; 2500 mg Vitamin E; 1000 mg Vitamin K3; 5000 mg Vitamin B1; 5000 mg Vitamin B2; 5000 mg Vitamin B6; 5000 mg Vitamin B12; 25,000 mg Inositol; 10,000 mg Pantothenic acid; 100,000 mg Cholin; 25,000 mg Niacin; 1000 mg Folic acid; 250 mg Biotin; 10,000 mg Vitamin C.

[c] Mixed minerals (g/kg): 469.3 g KH_2PO_4; 314.0 g $CaCO_3$; 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 KI; 0.08 g $CoCl_2{\cdot}6H_2O$; 0.06 g NH_4 molybdate; 0.02 g $NaSeO_3$.

[d] Tea polyphenols (98.12%): the proportion of tea polyphenols is 98.12% and detailed components are 80.06% total catechins; 1.68% free catechin; 6.33% epicatechin; 10.69% epigallocatechin; 21.24% epicatechin gallate; 40.09% epigallocatechin gallate; 0.47% caffeine; 0.35% ach.

ingredients were mixed together, and then oil and water were added. The TPs were dissolved in water before mixing with the other dietary ingredients, based on the listed formulations. Each diet was prepared separately. 2-mm granular feeds were created using a pelleting machine (F-26, SCUT industrial factory, Guangdong, China), and they were air-dried and stored at −20 °C until required (Lu et al., 2019). The TPs used in the present study was in the form of a food-grade product, with a purity of 98.1% (Jining Shengjiade Biological Technology Co. Ltd., China). The quantity of TPs was determined by Folin-Ciocalteu colorimetry and the catechin composition was determined using high-performance liquid chromatography (Chen et al., 2012; Zhao et al., 2014). The proximate composition of the experimental diets was analyzed following standard methods (AOAC, 2005). Total lipid was extracted by the Folch method using chloroform/methanol (2:1, *v/v*) (Folch et al., 1957). Total protein was determined by the Kjeldahl method, using a semi-automatic Kjeldahl System (FOSS, Sweden) after acid digestion. The moisture content was determined by oven-drying the diet samples individually at 105 °C to constant weight (Chen et al., 2016b).

2.4. Sample collection

At the end of the trail period, the fish were fasted overnight, then bulk-weighed to calculate their weight gain (WG), and to calculate FCR (three tanks per diet, $n = 3$). Nine fish per diet (three fish per tank) were randomly sampled, and sacrificed for tissue sample collection, after inducing anesthesia with 20 mg/L tricaine methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, USA). The body weight, length, and weights of the viscera, liver, and adipose tissue of the individual fish were measured and recorded, for the calculation of condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and visceral adipose index (VAI) (n = 3, the mean values for three fish per tank was used as a replicate). Blood samples were collected from the caudal vein of each fish using 2.0 mL syringes (KL Medical, Sichuan, China), then centrifuged at 3000 rpm, 4 °C for 10 min. The serum obtained was immediately aliquoted and stored at −80 °C until further analyzed. A portion of each liver was fixed in 10% neutral-buffered formalin for histological analysis, and the remaining liver tissue was immediately frozen in liquid nitrogen and stored at −80 °C until further analyzed (Pan et al., 2017). The WG, CF, FCR, VSI, HSI, VAI, and SR were calculated using the following formulae:

$$\text{WG}, \% = 100 \times (\text{Final fish weight} - \text{Initial fish weight})/\text{Initial fish weight}$$

$$\text{Condition factor (CF\%)} = 100 \times \text{Final fish weight}/(\text{Final fish length})^3$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed intake in each tank}/(\text{Final tank biomass} + \text{dead fish biomass} - \text{Initial tank biomass})$$

$$\text{VSI}, \% = 100 \times (\text{Weight of viscera}/\text{Body weight})$$

$$\text{HSI}, \% = 100 \times (\text{Liver weight}/\text{Body weight})$$

$$\text{VAI}, \% = 100 \times (\text{Visceral adipose weight}/\text{Body weight})$$

$$\text{SR}, \% = 100 \times (\text{Final fish number}/\text{Initial fish number})$$

2.5. Biochemical parameters

Before the analysis, 100 mg of each liver was weighed out and homogenized in 0.9% normal saline (1:9, *v*/v), then centrifuged at 2000 rpm for 15 min. The supernatants were collected and aliquoted for further analysis. Hepatic and serum triglyceride (TG) concentrations were measured using a commercial kit (Jiancheng Biotech Co. China). The activities of alkaline phosphatase (AKP), acid phosphatase (ACP), glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), lysozyme (LZM), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), and the total antioxidant capacity (T-AOC) of each sample were measured using specific commercial kits (Jiancheng Biotech Co., China), according to the manufacturer's protocols ($n = 9$ fish per diet group).

2.6. Histological analysis

Liver samples from six fish per diet group (two fish per tank, two liver sections per fish) were fixed using 4% paraformaldehyde, then dehydrated and embedded in paraffin wax. Four micron-thick slices were prepared and stained with hematoxylin and eosin or oil red O (Nanjing Jiancheng Bioengineering Institute, China), then examined and photographed using a Nikon Microscope Eclipse Ni (Nikon Corporation, Tokyo, Japan). The relative areas of the lipid droplets in liver sections stained with oil red O were measured by using ImageJ software (Zhang et al., 2019).

2.7. Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from liver samples using Tri Pure Reagent (Aidlab, China), according to the manufacturer's protocol. The quality and quantity of the RNA were determined using a Nano Drop 2000 Spectrophotometer (Thermo, USA). The synthesis of first-strand cDNA was performed using PrimeScript™ RT Master mix (RR047A, Takara, Japan), according to the manufacturer's instructions. Before qRT-PCR assay, the sample cDNA concentrations were equalized based on the measured values. The qRT-PCR analysis of target gene expression was then performed according to the manufacturer's protocol. PCR reaction mixtures with final volumes of 10 μL were prepared, consisting of 5 μL 2 × SYBR qPCR Mixture (Aidlab Biotechnologies Co., Ltd., China), 1 μL each of 2 μM forward and reverse primers, 1 μL diluted cDNA template, and 3 μL nuclease-free water. The PCRs were performed using a CFX96 Real-Time PCR System (Bio-Rad, Richmond, CA) and the following cycling conditions: initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Elongation factor 1 alpha (EF-1α) and β-actin were used as reference genes. All the primers used for the qRT-PCR analyses were specific and were synthesized by Rui Mian Biological Technology (Shanghai, China). The sequences of the primers are listed in Table 2. The relative expression of the target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, and $\Delta Ct = Ct_{target} - (Ct_{EF1\alpha} + Ct_{\beta\text{-actin}})$ / 2 (Livak and Schmittgen, 2001). In each qRT-PCR assay, the diet groups were compared with their corresponding internal control and the cycle numbers were then converted to fold-change values. All the gene expression measurements were made on nine individual fish per diet group ($n = 9$).

2.8. Statistical analyses

All data are expressed as mean ± standard error of the mean (SEM).

Table 2
Primers used for the analysis of mRNA genes expression by qRT-PCR.

Gene name		Sequences (5′ to 3′)	GenBank NO.
ATGL	F	AAAACGTCCTGGTGACCCAGT	XM_003440346
	R	TAGGAGGAATGATGCCACAGTACA	
HSL	F	AACCTGGATGTCCATTTCTGGAAG	FJ601660
	R	TCGGTTTACCTTGACTTGAGTGGA	
CPT1α	F	TTTCCAGGCCTCCTTACCCA	XM_003440552
	R	TTGTACTGCTCATTGTCCAGCAGA	
FAS	F	TCATCCAGCAGTTCACTGGCATT	GU433188
	R	TGATTAGGTCCACGGCCACA	
ACCα	F	TAGCTGAAGAGGAGGGTGCAAGA	XM_005471970
	R	AACCTCTGGATTGGCTTGAACA	
IgM	F	GCTTGACGACTGAGGATTC	KC677037.1
	R	GTTGAAGTGGCTGTTGTGA	
MIF	F	AGCAGAAGCAGGAAGGCGAAGA	XM_003444573.4
	R	CGGTACATCACCTCTGGCAACATT	
NF-κB	F	CGACCACTACCTACACGCTC	XM_019363515.2
	R	GATGTCGTTTGAGGCATCGC	
TGF-β	F	AAGAGGAGGAGGAATACTTTGCCA	NM_001311325.1
	R	GAAGCTCATTGAGATGACTTTGGG	
IL-8	F	TTCTAGATGGATGGCTGCCTTG	XM_019359413.1
	R	CTGTGAAGGCATGGGTGTGGAG	
IL-1β	F	GAGCACAGAATTCCAGGATGAAAG	XM_019365841.1
	R	TGAACTGAGGTGGTCCAGCTGT	
Caspase 3	F	GGAGTGGACGATACAGACGCAAA	NM_001282894.1
	R	TGAAGCTGTGTGACTGGGGCTT	
SOD	F	GCCCACACTTCAATCCCTACAA	XM_003446807.4
	R	GGCTCTCTTCATTTCCTCCTTT	
GSH-Px	F	ACCTTCATTCTCGCTACTCC	NM_001279711.1
	R	GCAGTTCTCCTGATGTCCAA	
GST	F	TAATGGGAGAGGGAAGATGG	NM_001279635.1
	R	CTCTGCGATGTAATTCAGG	
EF1α	F	ATCAAGAAGATCGGCTACAACCCT	KJ123689.1
	R	ATCCCTTGAACCAGCTCATCTTGT	
β-actin	F	AGCCTTCCTTCCTTGGTATGGAAT	KJ126772.1
	R	TGTTGGCGTACAGGTCCTTACG	

ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; CPT1α, carnitine palmitoyltransferase 1α; FAS, fatty acid synthase; ACCα, acetyl-CoA carboxylase α; IgM, immunoglobulin M; MIF, macrophage migration inhibitory factor; NF-κB, nuclear factor-κB; TGF-β, transforming growth factor β; IL-8, interleukin-8; IL-1β, interleukin-1β; Caspase 3, cysteine-aspartic acid protease 3; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione-S-transferase; EF1α, elongation factor 1α; F: forward primer; R: reverse primer.

Statistical comparisons on the measured parameters among the dietary treatments were performed by using one-way analysis of variance (ANOVA). When ANOVA showed significant differences ($P < 0.05$), comparisons among means were determined by Duncan's multiple range test. The Statistical Package for Social Sciences (SPSS version 20.0, IBM, USA) was used for all statistical analyses.

3. Results

3.1. Effects of dietary TPs on growth performance and organ indices

The HFD did not significantly affect WG, whereas the fish in the HFLT group had significantly higher WG than the control fish (Fig. 1A; $P < 0.05$). No significant differences in FCR (Fig. 1B), CF (Fig. 1C), or VSI (Fig. 1E) were found among the four diet groups ($P > 0.05$). The tilapia in the HFLT group, but not in the HFHT group, had significantly lower HSI (Fig. 1D) than those in the HF group ($P < 0.05$).

3.2. Effects of dietary TPs on lipid accumulation and related gene expression

The tilapia fed the HFD had significantly higher VAI than those fed the control diet (Fig. 2A; $P < 0.01$), but no significant differences were observed among the three HFD-fed groups ($P > 0.05$). The tilapia in the HF group had significantly higher TG concentrations in their serum and liver than those in the control group ($P < 0.05$). Interestingly, feeding the tilapia TPs reduced the TG concentrations in the liver (Fig. 2B; $P < 0.05$) and serum (Fig. 2C; $P < 0.01$) to similar levels to those in the fish that were fed the control diet. No significant differences were found in the serum activities of GPT (Fig. 2D) or GOT (Fig. 2E), which are well-known markers of liver injury among the diet groups.

Histochemistry revealed many more lipid droplets (relative area 40.83% ± 7.80%) in the livers of the tilapia fed the HFD alone than in those in the HFLT (relative area 17.27% ± 9.67%) or HFHT (relative area 25.33% ± 9.57%) groups (Fig. 3; $P = 0.00$, $n = 6$). The tilapia fed the TP-supplemented diets (HFLT and HFHT groups) had similar areas of lipid droplets to those fed the control diet (relative areas 20.13% ± 4.99%, n = 6) (Fig. 3; $P > 0.05$). Furthermore, the fish in the HFLT group tended to have a smaller area occupied by lipid droplets than those in the HFHT group, but without a significant difference (Fig. 3; $P > 0.05$).

The relative mRNA expression of genes involved in lipolysis (adipose triglyceride lipase, ATGL; hormone-sensitive lipase, HSL), fatty acid β-oxidation (carnitine palmitoyltransferase 1α, CPT1α) and lipogenesis (fatty acid synthase, FAS; acetyl-CoA carboxylase, ACCα) in the liver is shown in Fig. 4. Feeding the tilapia on HFD alone did not significantly affect the expression of ATGL or HSL, but significantly increased the expression of CPT1α ($P < 0.01$) and reduced the expression of FAS and ACCα ($P < 0.01$) *versus* the fish fed the control diet. The tilapia in the HFLT group showed the same trends as the HF group with respect to the upregulation of CPT1α and the lower expression of FAS and ACCα ($P < 0.01$). However, the fish in the HFHT group showed significantly lower expression of FAS and ACCα than those fed the control diet. Similarly, the HFHT group showed significantly lower HSL and CPT1α gene expression than the HF group; and also showed significantly lower expression of CPT1α than the HFLT group.

3.3. Effects of TPs on cumulative survival rate, immunity enzymes activities and related gene expression

At the end of the 8-week feeding trial, the tilapia in the HF group displayed the lowest cumulative survival rate (75%) of any of the groups, followed by those in the HFHT (82%), HFLT (90%), and control (96%) groups (Fig. 5A). The HFD significantly increased the serum activity of AKP compared with the control diet (Fig. 5B; $P < 0.05$), but interestingly, the HFLT group, but not the HFHT group, had an AKP activity that was similar to that of the control group ($P < 0.05$). No significant differences in the serum ACP (Fig. 5C) or hepatic AKP (Fig. 5E) activities were identified between the HF and control groups ($P > 0.05$). Feeding the tilapia with diets supplemented with TPs increased their serum ACP activity *versus* those fed the HFD alone (Fig. 5C; $P < 0.05$), and interestingly, the HFLT group showed a larger increase in ACP than the HFHT group ($P < 0.05$). Dietary TPs also increased the hepatic AKP activity over fish fed the HFD alone (Fig. 5E), but only those in the HFLT group showed a significant difference ($P < 0.05$). However, the serum LZM (Fig. 5D) and hepatic ACP (Fig. 5F) activities did not differ among the four groups ($P > 0.05$).

The hepatic mRNA expression of genes involved in immunity and inflammation (immunoglobulin M, IgM; macrophage migration inhibitory factor, MIF; nuclear factor-κB, NF-κB; transforming growth factor, TGF-β; interleukin-8, IL-8; and interleukin-1β, IL-1β) and apoptosis (cysteine-aspartic acid protease 3, caspase 3) for the four groups are shown in Fig. 5G. HFD-feeding significantly reduced the mRNA expression of IgM and TGF-β *versus* the control diet ($P < 0.05$). However, the HFLT group showed significantly higher expression of IgM and TGF-β than the HF and HFHT groups ($P < 0.01$), and had an similar expression to the control group. Furthermore, the HFLT group also showed significantly higher expression of NF-κB than the control group ($P < 0.05$). Moreover, the HFHT group showed significant higher expression of IL-1β than the other groups ($P < 0.01$). However, the HFHT group showed significantly lower expression of caspase 3 than the control group ($P < 0.05$). Finally, no significant differences in the expression of MIF or IL-8 were found among the groups. These results

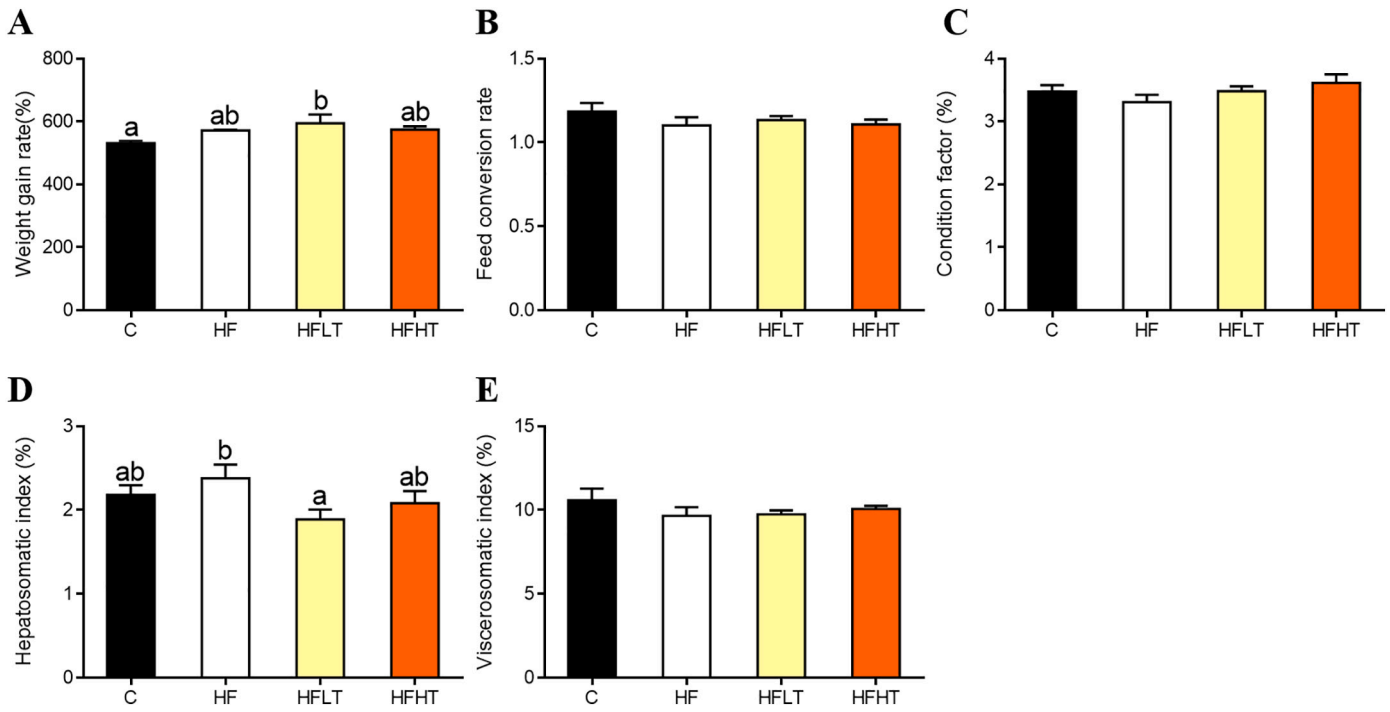


Fig. 1. Effects of dietary TP on growth performance and organ indexes in the GIFT tilapia fed on a high-fat diet. (A) Weight gain; (B) Feed conversion ratio; (C) Condition factor; (D) Hepatosomatic index; (E) Viscerosomatic index. All values are means ± SEM ($n = 3$). Different letters above the bars show significant differences ($P < 0.05$).

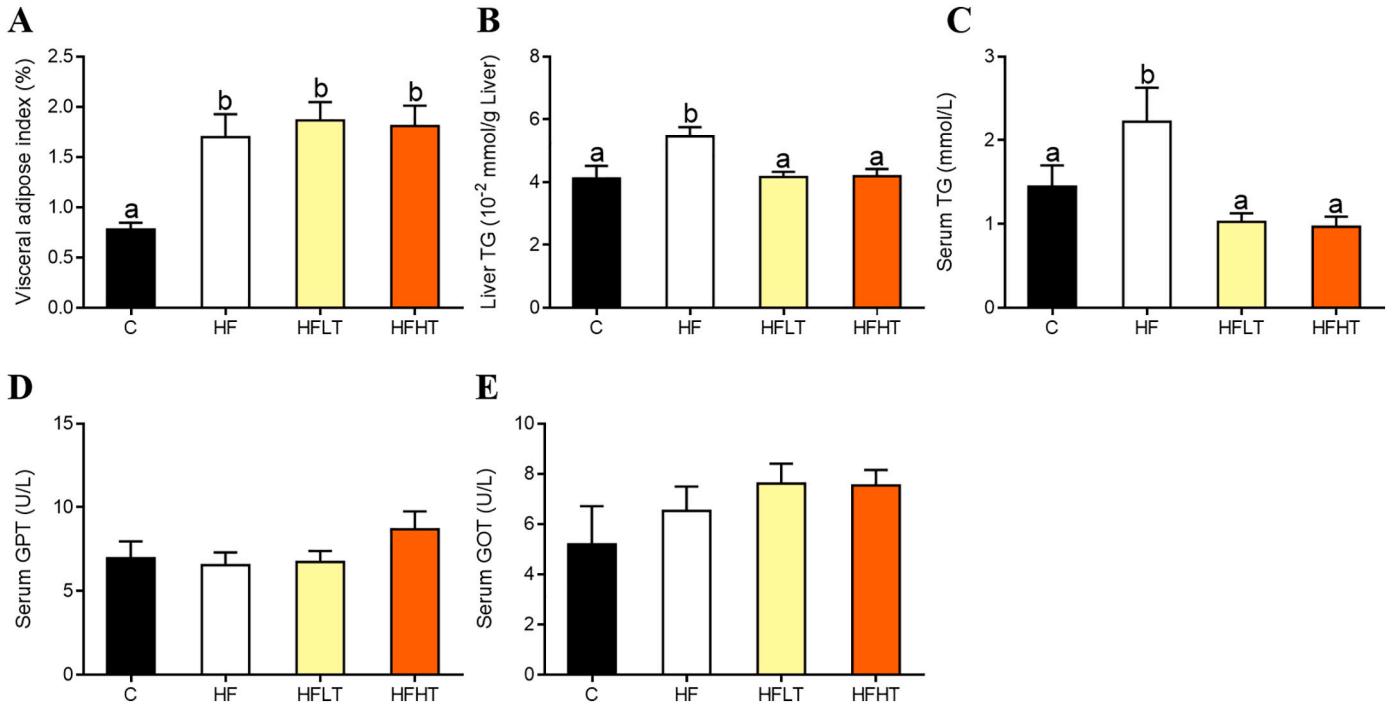


Fig. 2. Effects of dietary TP on lipid accumulation and hepatic function indexes in the GIFT tilapia fed on a high-fat diet. (A) Visceral adipose index; (B) Liver triglyceride (TG); (C) Serum TG; (D) Serum glutamic-pyruvic transaminase (GPT); (E) Serum glutamic-oxalacetic transaminase (GOT). All values are means ± SEM ($n = 9$). Different letters above the bars show significant differences ($P < 0.05$).

suggest that feeding tilapia an HFD impairs their immune function and dietary TP supplementation ameliorates this defect. Moreover, the lower TP dose (50 mg/kg) appears to be more effective in this regard than the higher dose (200 mg/kg).

3.4. Effects of TPs on antioxidant enzymes activities and related genes expression

No significant differences were identified in the activities of T-SOD (Fig. 6A), GSH (Fig. 6B), or CAT (Fig. 6C) among the four groups ($P >$

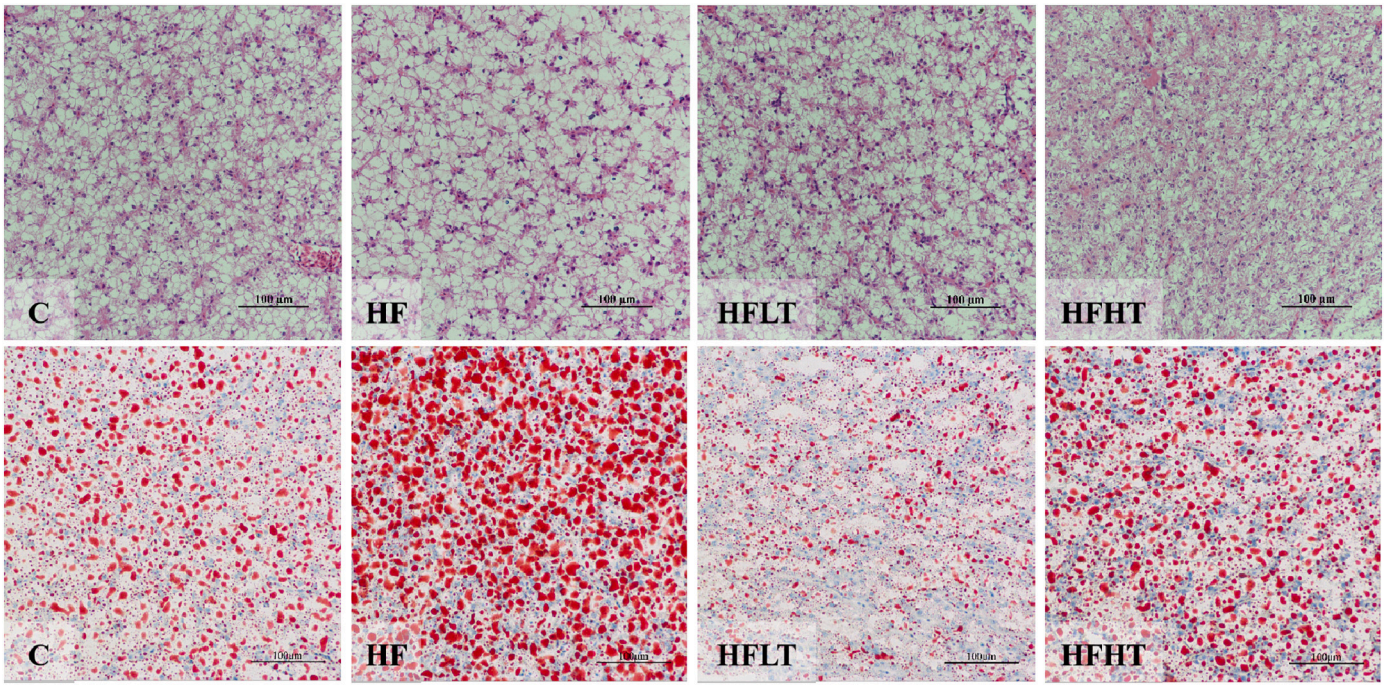


Fig. 3. Effects of dietary TP on histological characteristics of liver in the GIFT tilapia fed on a high-fat diet. The liver slices were treated with HE and Oil Red staining. Scale bars, 100 μm (20×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

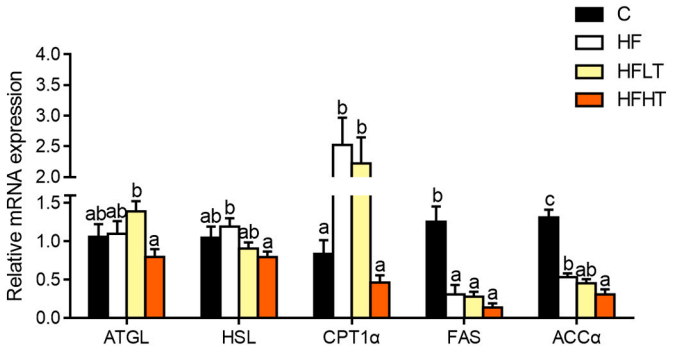


Fig. 4. Effects of dietary TP on expression of genes related to lipid metabolism in the GIFT tilapia fed on a high-fat diet. All values are means ± SEM (n = 9). Different letters above the bars show significant differences (P < 0.05).

0.05). However, HFD-feeding significantly reduced the T-AOC *versus* the control diet (P < 0.05), and the HFLT group showed a higher T-AOC activity, such that it was to that of the control group (Fig. 6D).

The hepatic expression of genes involved in antioxidant mechanisms (SOD, GSH-Px, and GST) is shown in Fig. 6E. HFD-feeding significantly reduced the expression of GST *versus* the control diet (P < 0.05). Furthermore, the HFLT group, but not the HFHT group, showed higher expression of SOD than the control (P < 0.01) and HF groups (P < 0.01); and showed significantly higher GST expression than the HF group (P < 0.01), such that it was similar to that of the control group (P > 0.05). However, there were no significant differences in the expression of GSH-Px among the groups. These results suggest that feeding tilapia an HFD reduces their antioxidant capacity, but that the lower level of TP supplementation is more effective at preventing this impairment than the higher level.

4. Discussion

4.1. Effects of dietary TPs on growth performance and fat deposition of the GIFT tilapia fed an HFD

Some previous studies have shown that the use of TPs as a feed additive in aquaculture improves the growth performance of fish (Long et al., 2015; Liang et al., 2018), but the effects of TPs on the fish fed an HFD have not been investigated. In the present study, the feeding of an HFD containing 12% fat did not significantly improve the growth performance of the tilapia, which is consistent with previous findings in grass carp and yellow catfish (Du et al., 2005; Chen et al., 2016b). Supplementation of the diets with 50 mg/kg TPs significantly increased the weight gain of the HFD-fed tilapia. However, this growth-promoting effect was not significant in the tilapia that were fed a 200 mg/kg TP-supplemented diet. This indicates that the growth stimulatory effect of TP supplementation in fish fed an HFD is dose-dependent and that the lower TP dose has a superior effect to the higher TP dose.

Several previous studies have also shown that appropriate levels of dietary TP supplementation reduce fat deposition in fish. For example, a study conducted in hybrid tilapia (*O. niloticus* × *O. aureus*) showed that a high level of dietary TP supplementation (600 or 800 mg/kg) significantly reduced fat accumulation (Liang et al., 2018), as did a study of. rainbow trout, in which the diet was supplemented with 1000 mg/kg TPs (Xu et al., 2008). Similarly, in the present study, HFD-feeding markedly increased lipid accumulation in the tilapia and initiated a compensatory increase in the expression of lipid catabolism-related genes (ATGL, HSL, and CPT1α) and a compensatory decrease in the expression of lipogenesis-related genes (FAS and ACCα), as was also shown in our previous studies of Nile tilapia (Han et al., 2020; Li et al., 2020a). Furthermore, dietary supplementation with TPs significantly ameliorated the HFD-induced increase in the lipid content of the liver and serum of the tilapia. The lipid-lowering effect of TPs was accompanied by lower expression of lipid metabolism-related genes. Of note, the tilapia supplemented with 50 mg/kg TPs showed significantly higher expression of ATGL and CPT1α than those supplemented, with 200 mg/kg TPs, which suggests that there is more lipolysis and β-oxidation in this

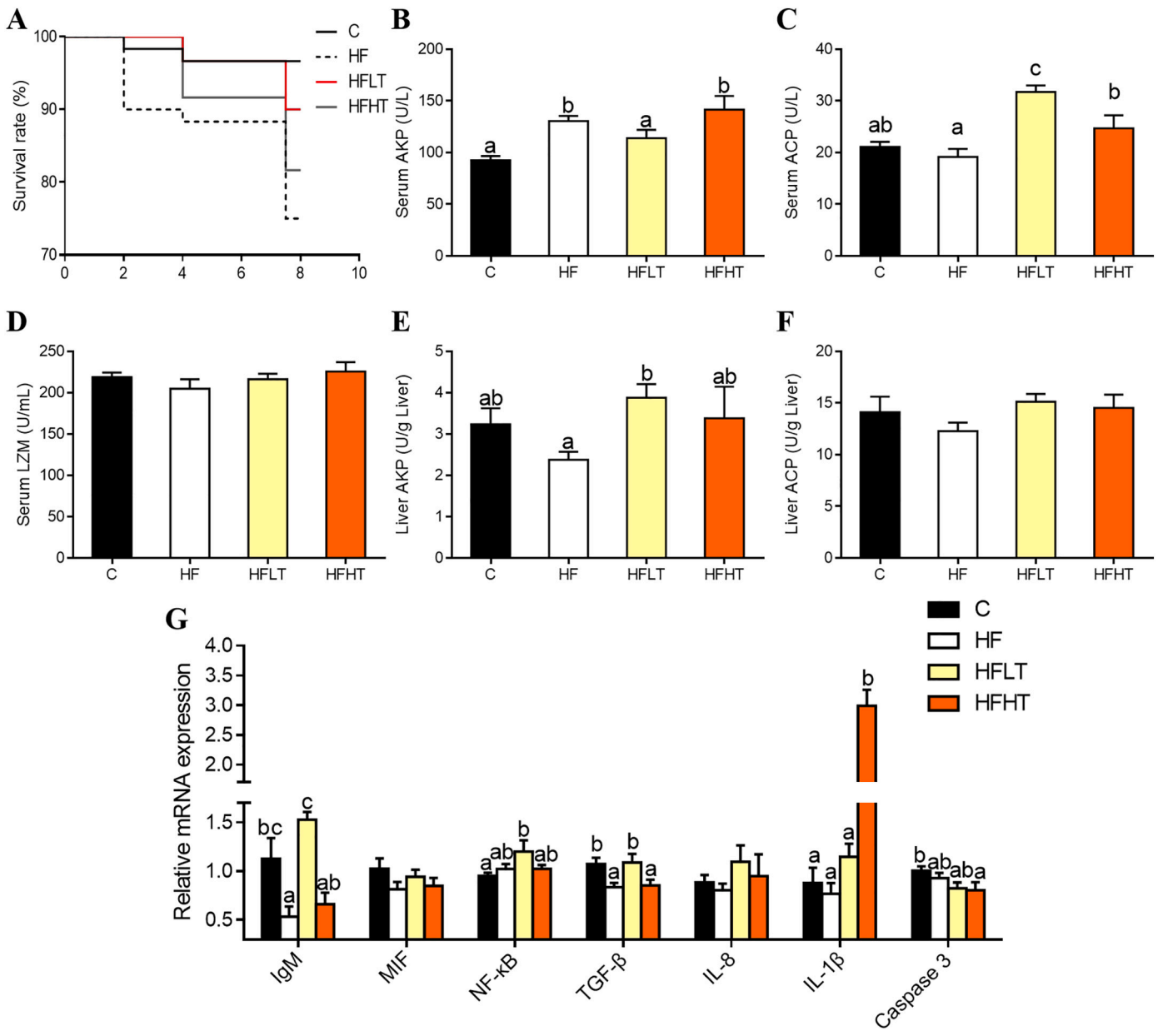


Fig. 5. Influences of TP on cumulative survival rate, immune enzymes activities and expression of related genes in the GIFT tilapia fed on a high-fat diet. (A) cumulative survival rate; (B) Serum alkaline phosphatase (AKP); (C) Serum acid phosphatase (ACP); (D) Serum lysozyme (LZM); (E) Hepatic alkaline phosphatase (AKP); (F) Hepatic acid phosphatase (ACP); (G) The mRNA expression of genes related to inflammation (nuclear factor-kappa B (NF-κB), transforming growth factor beta (TGF-β), interleukin 8 (IL-8), interleukin-1 beta (IL-1β)) and immunity (immunoglobulin M (IgM), macrophage migration inhibitory factor (MIF), cysteine-aspartic acid protease 3 (caspase 3)) in the liver. All biochemical and gene values are means ± SEM (n = 9). Different letters above the bars show significant differences ($P < 0.05$).

group. However, the HFHT group displayed the lowest expression of genes related to lipid catabolism (ATGL, HSL, and CPT1α) and lipogenesis (FAS and ACCα) of the three HFD-fed groups. These results indicate that the lipid-lowering mechanisms of the lower and higher levels of TP supplementation may differ.

Previous studies performed in rats and mature adipocytes showed that TPs activate AMP-activated protein kinase (AMPK), which inhibits ACC activity, thereby reducing the cellular malonyl-CoA concentration, increasing CPT I activity and therefore lipid catabolism, which reduces fat deposition (Ko et al., 2015; Kim et al., 2016; Tan et al., 2017). The lower TP level of supplementation also increased the expression of ATGL and CPT1 and reduced that of ACCα in the present study. We hypothesize that the lipid-lowering effects of the lower level of TP supplementation were likely achieved through the activation of AMPK-induced lipolysis and fatty acid β-oxidation.

Several previous studies have shown that higher levels of dietary TP supplementation tend to inhibit the absorption of lipids in organisms. For instance, the supplementation of an HFD with 1% or 400 mg/kg body weight TPs increased the fecal fatty acid concentrations of rats, which suggests that TPs inhibit the absorption of lipids in the gut, thereby reducing fat storage (Matsumoto et al., 1998; Wu et al., 2016). Furthermore, TPs have been shown to inhibit pancreatic lipase activity, which is important for lipid absorption in the gut, with an *in vitro* IC_{50} of 0.349 μM (Nakai et al., 2005). The results of these studies suggest that the lipid-lowering effects of the higher dietary TP level may be mainly the result of inhibiting lipid absorption in fish, rather than an effect on lipid catabolism, which is likely to be the principal mechanism whereby the lower dietary TP level reduces fat storage. The existence of such

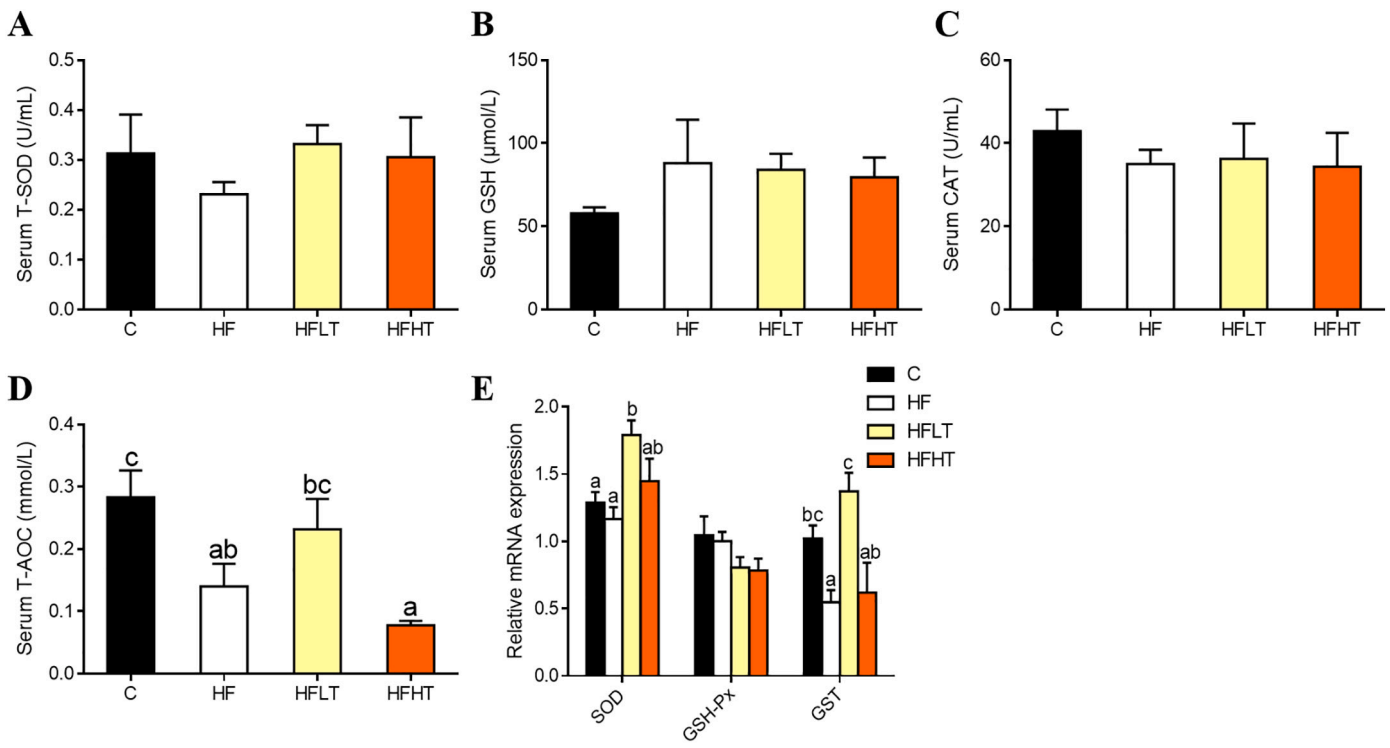


Fig. 6. Influences of TP on antioxidant enzymes activities in serum and relative expression genes in the GIFT tilapia fed on a high-fat diet. (A) Serum total superoxide dismutase (T-SOD); (B) Serum glutathione peroxidase (GSH) (C) Serum catalase (CAT); (D) Serum total antioxidant capacity (T-AOC); (E) Hepatic expression of genes related to oxidative stress (SOD, GSH-Px, glutathione S-transferase (GST)). All values are means ± SEM (n = 9). Different letters above the bars show significant differences ($P < 0.05$).

different mechanisms might also explain the superior growth of the tilapia fed the low level, but not the high level, of TPs in the present study. It may be that the greater energy supply from lipids in this group of fish improved their growth because protein was spared from use for energy generation, as reported previously (Li et al., 2019, 2020b).

4.2. *Effects of TPs on the immunity and inflammation of tilapia fed an HFD*

Excessive dietary fat intake may impair the immune function if fish (Chen et al., 2016a; Jia et al., 2017). For instance, HFD-feeding was shown to reduce the activities of ACP and LZM in blunt snout bream (Chen et al., 2016a) and of LZM in turbot (Jia et al., 2017). In the present study, HFD-feeding reduced the cumulative survival of the tilapia, and reduced the hepatic mRNA expression of IgM, which is the main immunoglobulin type in fish (Tian et al., 2009; Reyes-Becerril et al., 2014). Additionally, HFD-feeding reduced the mRNA expression of TGF-β, which encodes an anti-inflammatory cytokine (Yang et al., 2014). Taken together, these findings may imply that HFD-feeding impairs the immunity of tilapia.

Interestingly, in the present study, dietary TP supplementation, especially at the lower level (50 mg/kg), significantly increased the cumulative survival rate of HFD-fed fish. It also increased the hepatic mRNA expression of IgM, the serum activity of ACP, and the hepatic activity of AKP. Similarly, 50 mg/kg TPs significantly increased the activities of AKP and ACP in the liver and gills of rainbow trout (Xu et al., 2008). Moreover, supplementation of diets with 200–800 mg/kg TPs increased the serum LZM activity of hybrid tilapia and improved their survival after *A. hydrophila* infection (Liang et al., 2018). These studies also suggest that dietary TP supplementation has beneficial effects on the immunity of fish.

Effects of TPs on inflammatory processes have also been reported. Epigallocatechin gallate is one of the major catechin compounds in TPs and has been reported to increase the gene expression of IL-12 and TNF-α and reduce the gene expression of IL-10 in macrophages stimulated by *Legionella pneumophila* infection (Matsunaga et al., 2001). In the present study, supplementation of the HFD with 50 mg/kg TPs increased the expression of the TGF-β gene in the tilapia, probably because it also increased the expression of NF-κB. Consistent with this hypothesis, a study conducted with grass carp head kidney leukocytes showed that IL-1β and TGF-β1 gene expression was mediated by NF-κB signaling (Wei et al., 2015). It should be noted that the higher TP (200 mg/kg) level had similar, but much less marked, effects than the lower TP dose on the serum activity of ACP, and no effects on that of AKP, or the expression of IgM or NF-κB genes in the HFD-fed tilapia. Nevertheless, 200 mg/kg TP supplementation significantly increased the mRNA expression of IL-1β, which is a pro-inflammatory cytokine, the expression of which can be induced by pathogens, endotoxin and reactive oxygen species (Dinarello, 2011). These findings imply that the effects of the lower and higher levels of TP supplementation on immune function also differ.

Immune responses require energy, and therefore, the more marked effects of the lower TP dose on immunity may have relied on its effects to enhance lipid catabolism (fatty acid β-oxidation), to supply more energy. The results of our previous studies of Nile tilapia are consistent with this hypothesis, we found that inhibition of hepatic mitochondrial fatty acid β-oxidation reduced the activities of AKP and ACP (Pan et al., 2017), whereas increasing mitochondrial fatty acid β-oxidation through the activation of PPARα increased the activities of AKP and ACP (Luo et al., 2019). The present data imply that the higher level of TP supplementation dose not stimulate lipid catabolism, unlike the lower level, which might explain the relative lack of effect of the higher level of TP supplementation on the immunity of the tilapia.

4.3. Effects of TPs on oxidative stress resistance in the GIFT tilapia fed an HFD

The di- and/or tri-hydroxyl groups in TPs are thought to have strong antioxidant activities. Therefore, the effects of dietary TPs are thought to be mostly related to their ability to relieve oxidative stress in organisms (Yang et al., 2009). A state of oxidative stress has been widely reported in fish fed an HFD (Jia et al., 2017). Therefore, we aimed to derermine whether dietary TPs would ameliorate HFD-induced oxidative stress in the tilapia. We have shown that HFD-feeding reduces T-AOC activity and GST mRNA expression, which suggests the presence of impaired antioxidant capacity in the HFD-fed fish. As hypothesized, supplementation of the diet of the HFD-fed fish with 50 mg/kg TPs increased the T-AOC activity and mRNA expression of SOD and GST. These results confirm the antioxidant effects of dietary TPs, at least at the lower dose. Dietary supplementation with 200–800 mg/kg TPs also increased the activity of SOD and reduced the serum MDA concentration of hybrid tilapia (Liang et al., 2018), and 25–100 mg/kg dietary TPs was similarly effective in rainbow trout (Xu et al., 2008).

As discussed above, the lower and higher levels of TP supplementation had differing effects on lipid metabolism: the lower level increased lipid catabolism, whereas the higher level may inhibit lipid absorption. As for the immune response, the antioxidant system is also energy-consuming. Therefore, the greater energy supply in the form of fatty acides may explain the superior antioxidant effects of the lower level of TP supplementation. Although few previous studies have investigated the relationship between energy metabolism and antioxidant capacity in fish, Luo et al. (2019) found that the stimulation of fatty acid β-oxidation in Nile tilapia improves their antioxidant capacity, as shown by higher activities of SOD and CAT and a lower MDA concentration in the liver. Consistent with this, Pan et al. (2017) found that the inhibition of fatty acid β-oxidation reduces the activity of GPX in the liver of Nile tilapia. The results of these studies are consistent with our hypothesis that the lower level of TP supplementation improves antioxidant capacity by increasing energy supply in the form of fatty acids. Conversely, because the higher level of TP supplementation did not activate lipid catabolism, the fish in this diet group may have had insufficient energy to support an antioxidant effect. However, the relationship between energy metabolism and antioxidant capacity in fish requires further investigation.

5. Conclusions

We have shown that HFD-feeding significantly increases lipid deposition and caused a significant drop in the cumulative survival rate from 96% to 75%, and that these negative effects can be partially overcome by the addition of TPs at a level of 50 mg/kg for GIFT tilapia, leading to cumulative survival rate of 90%. The 50 mg/kg level of supplementation was more effective than the higher 200 mg/kg level of eupplementation at promoting immune function and oxidative stress resistance in the HFD-fed GIFT fish. This may be because the lower TP concentrations provided more energy by increasing lipid catabolism, as evidenced by the higher expression of CPT1α gene in the livers of the fish. Thus, we have confirmed the beneficial effects of TPs in HFD-fed tilapia, and suggest a connection between the effects of TPs on energy metabolism and their other effects, which should be further investigated in the future.

CRediT authorship contribution statement

Yu-Cheng Qian: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft. **Xue Wang:** Methodology, Data curation, Formal analysis. **Jiong Ren:** Data curation, Formal analysis, Writing - review & editing. **Jie Wang:** Methodology, Data curation, Resources. **Samwel Mchele Limbu:** Formal analysis, Writing - review & editing. **Rui-Xin Li:** Software, Methodology, Data curation. **Wen-Hao Zhou:** Methodology, Data curation. **Fang Qiao:** Software, Validation, Resources. **Mei-Ling Zhang:** Conceptualization, Methodology. **Zhen-Yu Du:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflicts of interests.

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