Title

Dietary fenofibrate attenuated high-fat-diet-induced lipid accumulation and inflammation response partly through regulation of *pparα* and *sirt1* in juvenile black seabream (*Acanthopagrus schlegelii*)

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Accepted refereed manuscript of:
Jin M, Zhu T, Tocher D, Luo J, Shen Y, Li X, Pan T, Yuan Y, Betancor M, Jiao L, Sun P & Zhou Q (2020) Dietary fenofibrate attenuated high-fat-diet-induced lipid accumulation and inflammation response partly through regulation of pparα and sirt1 in juvenile black seabream (Acanthopagrus schlegelii). Developmental and Comparative Immunology, 109, Art. No.: 103691.
DOI: https://doi.org/10.1016/j.dci.2020.103691

Keywords: fenofibrate, high-fat diet, inflammation response, *pparα*, *sirt1*

Abstract

An 8-week feeding trail was conducted in *Acanthopagrus schlegelii* with an initial body weight of 8.34±0.01g. Three isonitrogenous diets were formulated, (1) Control:

medium-fat diet (12%); (2) HFD: high-fat diet (18%); (3) HFD+FF: high-fat diet with fenofibrate (0.15%). Liver histological analysis revealed that, compared to HFD, vacuolar fat drops were smaller and fewer in fish fed fenofibrate. Expression of lipid catabolism regulator peroxisome proliferator-activated receptor alpha (*pparα*) was up-regulated by fenofibrate compared with HFD. In addition, fenofibrate significantly increased the expression level of silent information regulator 1 (*sirt1*). Meanwhil e, the expression level of anti-inflammatory cytokine interleukin 10 (*il-10*) in intestine was up-regulated, while pro-inflammatory cytokine interleukin 1β (*il-1β*) in liver and intestine were down-regulated by dietary fenofibrate supplementation. Overall, the present study indicated that fenofibrate reduced fat deposition and attenuated inflammation response caused by HFD partly through a pathway involving regulation of *pparα* and *sirt1*.

1. Introduction

Lipids are the most important dietary energy source for aquatic animals, and can increase dietary protein efficiency and, consequently, high-fat diets have been widely used in aquaculture (Boujard et al., 2004). Within a certain range, increasing dietary lipid/fat level can promote growth performance, spare dietary protein and reduce production costs, as demonstrated in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) (Chou and Shiau, 1996) and hybrid snakehead (*Channa maculata*♀× *Channa argus*♂) (Zhang et al., 2017). However, excessive dietary fat can cause metabolic disorders, fat deposition, endoplasmic reticulum stress and inflammation as reported in blunt snout bream (*Megalobrama amblycephala*), large yellow croaker (*Larmichthys crocea*) and black seabream (*Acanthopagrus schlegelii*) (Cao et al., 2019a; Jin et al., 2019a, b; Wang et al., 2015b). In addition, our previous study revealed that a high fat diet induced lipid accumulation, hepatic steatosis and NF-κB activation, and a resultant inflammatory response in black seabream (Jin et al., 2019b). Recently, studies have demonstrated that dietary fenofibrate supplementation can have functionally important effects on lipid metabolism and the inflammatory response of fish (Luo et al., 2019; Ning et al., 2019). Dietary fenofibrate supplementation appears to impact lipid metabolism through mechanism that can potentially reduce lipid deposition, alleviate hepatic steatosis and attenuate inflammation response.

Fenofibrate belongs to the group of fibrate drugs, which are generally used in the treatment of hypertriglyceridemia and combined hyperlipidemia patients (Packard, 1998). However, in fish, fenofibrate has been shown to also enhance peroxisomal lipid oxidation, reduce liver fatty acid and serum triacylglycerol (TAG) contents, and decrease embryonic yolk sac resorption (Du et al., 2004; Du et al., 2008; Prindiville et al., 2011; Raldúa et al., 2008). It has also been shown that the mechanism whereby fenofibrate can affect lipid metabolism is by activating peroxisome proliferator-activated receptor alpha (PPARα) (Koh et al., 2012). The transcription factor PPARα is one of the nuclear receptor family that, upon activation, regulates the mRNA levels of a series of genes such as acyl-CoA oxidase (ACO) and carnitine palmitoyltransferase I (CPT1) that, in turn, directly regulate lipid metabolism decreasing plasma TAG concentration in mammals (Mandard et al., 2004). Similar results have been reported in fish species, showing that dietary fenofibrate supplementation could modulate lipid deposition by up-regulating the expression of lipid metabolism genes, such as *cpt1a* in yellow catfish (*Pelteobagrus fulvidraco*) (Zheng et al., 2015) and Nile tilapia (*Oreochromis niloticus*) (Ning et al., 2019). Furthermore, Pparα not only affects genes of lipid metabolism, but also impacts inflammatory signaling pathways by directly interacting with nuclear factor kB (NF-κB) to influence mRNA expression levels of inflammatory genes such as interleukin 6 (*il-6*) receptor (Desvergne and Wahli, 1999; Lee et al., 2013; Stienstra et al., 2007). Moreover, studies in mammals indicated that PPARα works in combination with silent information regulator 1 (*sirt1*) to relieve inflammation and metabolic dysregulation to protect heart (Oka et al., 2012; Planavila et al., 2010). Furthermore, Wang et al. (2015a) reported that PPARα suppressed expression of inflammation markers through deacetylation of NF-κB by a SIRT1-mediated mechanism in adipocytes and human vascular endothelial cells. PPARs inhibit the mRNA expression of a number of important genes involved in the inflammatory response including cytokines, cell adhesion molecules and other pro-inflammatory signaling mediators such as interleukin 8 (IL-8), IL-6, interleukin 2 (IL-2), tumor necrosis factor-α (TNFα) and metalloproteases by inhibiting NF-κB,

activation protein-1 (AP1) and signal transducers and activators of transduction (STAT) (Chinetti et al., 2000).

SIRT1, is a highly conserved NAD^+ dependent protein deacetylase, recognized as a master switch in energy homeostasis, which plays an important role in cellular metabolic processes, including stress responses and inflammation (Rodgers and Puigserver, 2007; Wu et al., 2020). A previous study reported that hepatic SIRT1 is an important factor in the regulation of lipid metabolism that is also regulated by transcription factors including PPAR and, as a vital pathway for the regulation of hepatic lipid metabolism, SIRT1 positively regulates PPARα to reduce lipid accumulation in liver (Rodgers and Puigserver, 2007). Furthermore, in mammals, SIRT1 could repress NF-κB signaling that, in turn, greatly attenuated NF-κB-driven inflammation (Salminen et al., 2008; Yao and Rahman, 2012). Accumulating evidence confirmed that PPARα regulated metabolism and inflammation via SIRT1 (Wang et al., 2015a). Meanwhile, as a vital pathway for the regulation of hepatic lipid metabolism, SIRT1 positively regulates PPARα to reduce lipid accumulation in liver (Rodgers and Puigserver, 2007). However, the metabolic regulatory functions of fenofibrate in reducing lipid accumulation and inflammation caused by a high fat diet in fish, and whether it works in association with PPARα and Sirt1 has not been investigated.

Black seabream is an important marine fish species that is cultured commercially on the southeast coast of China, Japan, South Korea and other countries in Southeast Asia. It has been regarded as an excellent species for intensive aquaculture since it exhibits rapid growth, high disease resistance, and can tolerate a wide range of environmental conditions (Jin et al., 2019a,b). Moreover, our previous study verified that feeding black seabream a high fat diet can induce excess lipid deposition and inflammation making it a good experimental model to study the metabolic mechanisms (Jin et al., 2019a,b). The mechanism of the hypolipidemic effect of fenofibrate has been partly revealed in mammals, but little information is available for fish (Du et al., 2008). The activation effects of fibrates vary in different species, and does not always include effects on *ppar* as it does in mammals (Kondo et al., 2007; Luci et al., 2007; Mimeault et al., 2006; Raldúa et al., 2008). Therefore, the present study aimed to explore how fenofibrate supplementation impacts lipid metabolism and inflammatory responses in black seabream fed a high fat diet, and provide further insight into the mechanism of action of dietary fenofibrate in fish.

2. Materials and Methods

Ethics statement

Animal experimentation within the present study was conducted in accordance with the Animal Research Institute Committee guidelines of Ningbo University, China and approved by the Committee of Animal Research Institute, Ningbo University, China.

2.1 Experimental design and diet preparation

Three isonitrogenous (41 % crude protein) diets with two levels of lipid (12 % and 18 % crude lipid) were formulated and termed as (1) control: medium-fat diet (12 %), (2) HFD: high-fat diet (18 %), (3) HFD+FF: high-fat diet with fenofibrate supplement (1.5g/kg dry diet; MCE, USA) (Table 1). Fishmeal, soybean protein concentrate and soybean meal were used as protein sources, with fish oil, palmitic acid and soybean

lecithin used as the main lipid sources. All ingredients were purchased from Ningbo Tech-Bank Feed Co. Ltd., Ningbo, China. The experimental diets were produced according to the method described in detail previously (Jin et al., 2019a,b). Briefly, the ground ingredients were mixed in a Hobart type mixer and cold-extruded pellets produced (F-26, Machine factory of South China University of Technology) with pellet strands cut into uniform sizes (2 mm and 4 mm diameter pellets) (G-250, Machine factory of South China University of Technology). Pellets were steamed for 30 min at 90 °C, and then air-dried to approximately 10 % moisture, sealed in vacuum-packed bags and stored at − 20 °C until used in the feeding trial.

Insert Table 1 here.

2.2 Feeding trial and experimental conditions

Juvenile black seabream (initial weight 8.34 ± 0.01 g) were obtained from a local commercial hatchery at Xiangshan Bay, Ningbo, China. Prior to the start of the experiment, black seabream juveniles were acclimated to the experimental facilities and fed a commercial diet (45 % protein, 12 % crude lipid, Ningbo Tech-Bank Corp.). The feeding trial was carried out with a completely randomized design. A total of 270 black seabream juveniles were randomly allocated to 9 floating net cages (1.5 m × 1.5 m × 2.0 m) with triplicate cages for each of the three dietary treatments. Fish were hand-fed twice daily at 07:00 and 17:00 over 8 weeks, with the amount of feed being 6 % of body weight, which was adjusted according to feeding recommendations for growth stages. During the experimental period, seawater conditions including temperature (26.6 - 30.7 °C), salinity (22.53 - 27.86 g/L), dissolved oxygen (4.7 - 6.8 mg/L) and pH (8.0 - 8.1 mg/L) were measured with YSI Proplus (YSI, Yellow Springs, Ohio, USA).

2.3 Samples collection

At the end of the feeding trial, fish were sampled 24 h after the last feed, all fish were euthanized (MS-222 at 10 mg/L). All fish in each cage were individually weighed and counted to determine final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE) and survival. Three fish from each cage (9 per treatment) were pooled (n = 3) and used for analyzing the proximal composition of whole body, where three fish (nine per treatment) were used to determine morphological parameters including condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI) and intraperitoneal fat (IPF) ratio (n=9). Liver, muscle and intestine samples were also collected from these fish and stored at −80 °C until analysis. Fresh liver tissues were collected into 4 % formaldehyde from one fish per tank for histological analyses. Blood samples were taken from the caudal vasculature of 10 fish per cage by using non-heparinized syringes (2 ml) for serum biochemical indices.

2.4 Proximate composition analysis

The moisture, crude lipid, crude protein and ash contents of feeds and whole fish body

samples were determined by AOAC (2006) methods. Moisture content was measured by drying the samples to a constant weight at 105 ℃. Crude lipid was extracted via the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Crude protein (N × 6.25) was determined according to the Dumas combustion method with a protein analyzer (FP-528, Leco, USA) and ash content was measured using a muffle furnace at 550 ℃ for 8 h. Protein productive value (PPV) and lipid retention (LR) were determined based on the measured crude protein and lipid contents of diet and fish.

2.5 Assay of serum and liver biochemical indices

Blood was stored at 4 °C for 24 h and then serum collected by centrifugation at 956 g for 10 min at 4 °C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, and triacylglycerol (TAG) and cholesterol (CHOL) contents in serum were assayed using an automatic biochemistry analyzer (VITALAB SELECTRA Junior Pros, Netherlands). TAG and CHOL contents in liver were assayed using a diagnostic reagent kit purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.6 Histological analysis of liver

Fresh liver tissue was fixed with 4% paraformaldehyde before paraffin sections were prepared (Servicebio, Hangzhou, China). Briefly, after fixation for at least 24 h, tissue samples were trimmed appropriately in a fume hood before being dehydrated in ethanol with concentration increasing incrementally from 75 % to 100 %. Liver samples were then embedded in paraffin and sliced into sections of 4 μm using a microtome. They were stained with haematoxylin and eosin (H&E) and Oil Red O, and images were acquired under a microscope (Nikon Eclipse CI, Tokyo, Japan).

2.7 Total RNA extraction, reverse transcription and real-time PCR

Total RNA in the liver and intestine were extracted by the TRIzol method, and quality and quantity of isolated RNA assessed by 1.0 % agarose gel electrophoresis and spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, USA). The cDNA was prepared from 1000 ng of DNAase-treated RNA and synthesized using PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Takara). The housekeeping gene β-actin was used as reference gene after confirming its stability across the experimental treatments. Specific primers for the candidate genes *pparα*, *atgl*, *cpt1a*, *accα*, *fas*, *srebp-1*, *tnfα*, *il-1β*, *nf-κb*, *tgfβ-1*, *il-10*, *sirt1* used for qPCR were designed by Primer Premier 5.0 (Table 2). Amplification was performed using a quantitative thermal cycler (Lightcycler 96, Roche, Switzerland). The qPCR assays were performed in a total volume of 20 μL, containing 0.4 μL of each primer, 10 μL of 2×ChamQ SYBR qPCR Green Master Mix (Vazyme), 0.8 μL of 1/8 diluted cDNA and 8.4 μL DEPC-water. The thermal-cycling conditions used for qPCR were as follows: 95 °C for 2 min, followed by 45 cycles of 95 °C for 10 s, 58 °C for 10 s and 72 °C for 20 s. Standard curves were generated using six different dilutions (in triplicate) of the cDNA samples, and the amplification efficiency was analyzed using the equation $E=10^{(-1/Slope)}-1$[40]. The amplification efficiencies of all genes were approximately equal and ranged from 87 to 109 %. In the present study, all the gene expression data were presented as relative gene expression with regards to the expression values in fish fed the high fat diet (HFD)

(reference group). The expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001).

Insert Table 2 here.

2.8 Calculations

The parameters were calculated as follows:

Weight gain (WG, %) = 100× ((final body weight – initial body weight) / initial body weight)

Specific growth ratio (SGR, % day^{-1}) = 100 × ((Ln final body weight (g) – Ln initial body weight) (g) / days)

Feed efficiency (FE) = weight gain (g, wet weight) / feed consumed (g, dry weight);

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

Protein productive value (PPV, %) =100× (final body weight × final body protein-initial body weight ×initial body protein)/ (feeding diets × diet protein)

Lipid retention (LR, %) = (final body weight × final body lipid-initial body weight × initial body Lipid) / (feeding diets × diet lipid)

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

Viscerosomatic index (VSI, %) = 100 × (visceral weight / wet body weight)

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

2.9 Statistical analysis

Results are presented as means and SEM (number of replicates as indicated). The relative gene expression results (qPCR analyses) were expressed as mean normalized ratios corresponding to the ratio between the copy number of the target gene and the copy number of the reference gene, β-actin. The homogeneity of variances (Levene's test) were checked prior to one-way analysis of variance (ANOVA) followed by Tukey's HSD test at a significance level of $P \leq 0.05$ (IBM SPSS Statistics 20).

3 Results

3.1 Growth performance, feed utilization, survival and lipid content

No statistical differences in final body weight (FBW), weight gain (WG), feed efficient (FE), specific growth rate (SGR) or survival were found among treatments (Table 3). However, the lowest values of protein productive value (PPV) and lipid retention (LR) were recorded in fish fed the HFD and HFD+FF diets, respectively. The lipid contents of whole body and liver were both significantly increased in fish fed the HFD diet compared to fish fed the other diets. Dietary fenofibrate supplementation significantly reduced the lipid content of whole body compared to fish fed HFD. Intraperitoneal fat ratio (IPF) and hepatosomatic index (HSI) were also significantly reduced by dietary fenofibrate supplementation. However, no significant differences were found in muscle lipid content among treatments (Fig. 1).

Insert Table 3 here.

Insert Figure 1 here

3.2 Serum and hepatic biochemical indices

There were no significant differences in serum TAG and CHOL concentrations among treatments (Fig. 2). The highest activities of AST and ALT were found in fish fed diet HFD, and AST activity was significantly reduced by dietary fenofibrate supplementation (Fig. 2). Fish fed the HFD diet showed significantly higher TAG content in liver, whereas diet had no significant effect on hepatic CHOL concentration (Fig. 3).

Insert Figure 2 here.
Insert Figure 3 here.

3.3 Hepatic histological analysis

The results of staining liver sections with hematoxylin and eosin (H & E) and Oil Red O are presented in Figs. 4 and 5, respectively. In the control group fed MFD, the distribution of hepatocytes was regular, the arrangement was relatively tight and most of the nuclei were in the middle of cells (Fig. 4A). In fish fed HFD, the hepatocytes were mostly swollen and nuclei were offset to the edge of the cell, or difficult to distinguish, and many vacuolar lipid drops of varying size were observed (Fig. 4B). Compared with fish fed HFD, the shape of hepatocytes were more regular and fewer vacuolar fat drops were observed in fish fed fenofibrate (Fig. 4C). The above results were confirmed by Oil Red O staining with red stained lipid droplets significantly more numerous in fish fed HFD compared to the control group (Figs. 5A & B). Furthermore, the amount of red fat droplets in the fenofibrate group showed a decreasing trend and were smaller compared to fish fed HFD (Fig. 5C).

Insert Figure 4 here.
Insert Figure 5 here.

3.4 Regulatory factor, lipogenesis and lipolysis pathway key markers

Compared to fish fed the HFD treatment, the expression level of *sirt1* in liver was significantly up-regulated in fish fed the diet with fenofibrate supplementation. In the lipolysis pathway, the mRNA levels of *pparα* and *cpt1a* were significantly up-regulated in fish fed HFD+FF compared to the control group (Fig. 6). There were no significant differences in the expression levels of *accα*, *fas* or *srebp-1* in livers of fish fed HFD compared to fish fed the control diet, MFD. However, the hepatic expression levels of *accα*, *fas* and *srebp-1* were significantly up-regulated by dietary fenofibrate compared to fish fed both the other diets (Fig. 7).

Insert Figure 6 here.
Insert Figure 7 here.

3.5 Inflammatory markers

In liver, the expression levels of *tnfα*, *il-1β* and *nf-κb* were significantly higher in fish fed HFD than in fish fed the control diet. There were no significant differences in the liver expression levels of anti-inflammatory cytokine *il-10* and *tgfβ-1* among treatments (Fig. 8). In intestine, fish fed HFD showed a significantly lower expression level of *il-10* than fish fed the other treatments. On the contrary, the expression level of *il-1β* was highest in fish fed HFD and down-regulated by dietary fenofibrate supplementation. Although a similar tendency was recorded for the expression levels of *tnfα* and *nf-κb*, there were no statistical differences among the three treatments (Fig. 9).

Insert Figure 8 here.
Insert Figure 9 here.

4 Discussion

Various mechanisms have been identified in mammals for the functional effects of dietary fibrates including increasing lipoprotein lipolysis, stimulating mitochondria and inducing fatty acid oxidation in liver, and reducing fatty acid and very low-density lipoprotein production (Kondo et al., 2007; Luci et al., 2007; Mimeault et al., 2006; Raldúa et al., 2008). The fibrate drugs clofibrate and fenofibrate have been tested in aquatic animal feeds, with studies showing that they had no effects on growth performance in various fish species, such as grass carp (*Ctenopharyngodon idellal Val*.) (Du et al., 2008; Guo et al., 2015), mosquitofish (*Gambusia holbrooki*) (Nunes et al., 2004) or rainbow trout (*Oncorhynchus mykiss*) (Du et al., 2004). However, in contrast, dietary fenofibrate supplementation increased and reduced growth performance in yellow catfish and Nile tilapia, respectively (Zheng et al., 2015; Ning et al., 2019). In the present study, black seabream fed HFD with fenofibrate did not significantly affect FBW, WG, SGR, FE or survival, consistent with the results obtained in grass carp, mosquitofish and rainbow trout. The different inconsistent results with yellow catfish or Nile tilapia might be attributed to different nutritional background and experimental conditions, as the tilapia were fed a low protein level with fenofibrate, and the catfish were cultured in Zn-exposed water, while black seabream in the present study were fed a high fat diet (HFD) in normal water. Overall therefore, the present results indicated that, in black seabream fed HFD, fenofibrate had no impact on growth performance.

Previous studies reported that whole body and muscle lipid contents were increased in black seabream fed a high fat diet (Jin et al., 2019a, b; Pan et al., 2018). In the present study, whole body and hepatic lipid contents as well as IPF and VSI were significantly higher in fish fed HFD than in fish fed the other two diets. Supplementing the HFD diet with fenofibrate significantly reduced IPF and VSI as well as lipid contents of whole body and liver. Similar results were obtained in grass carp (Guo et al., 2015), which indicated that dietary fenofibrate can reduce body fat reserves compared to levels in fish fed a high fat diet. Interestingly, it had also been shown in mammals that obesity in mice caused by a high fat diet can be reversed by fenofibrate treatment (Srivastava et al., 2006). Black seabream fed dietary fenofibrate supplementation had lower HSI than fish fed HFD, which was consistent with the results of fenofibrate treatment in Nile tilapia (Luo et al., 2019), which might be explained by the fact that high fat diets caused lipid accumulation and liver enlargement, while fenofibrate reduced hepatic lipid content and size. Moreover, the histopathological analyses further confirmed the lipid-lowering effect of fenofibrate in black seabream. The results showed that fish fed HFD showed liver damage, such as lysed nuclei and large cysts observed in H & E staining. Furthermore, Oil Red O staining was consistent with H & E staining, with almost all the cytoplasmic volume in hepatocytes of fish fed HFD being filled with a large amount of red-stained lipid droplets. Similar results were obtained in blunt snout bream (*Megalobrama amblycephala*) fed a high fat diet (Dai et al., 2019). The present study also revealed that dietary fenofibrate was beneficial in reducing hepatic lipid accumulation caused by a high fat diet, which was consistent with results observed previously in grass carp (Du et al., 2008), Nile tilapia (Ning et al., 2019) and rat (Dai et al., 2016; Lu et al., 2015). This has demonstrated that dietary fenofibrate could prevent or mitigate the histopathological alterations in key tissues such as liver induced by feeding high fat

diets. Overall, the data obtained in the present study indicated that dietary fenofibrate supplementation can have beneficial lipid-lowing effects in black seabream fed high energy (fat) diets.

Fenofibrate, as a known agonist of PPARα, has been used commonly as a clinical drug for modifying blood lipids in humans (Dai et al., 2016). The reported mechanism was that fenofibrate reduced plasma CHOL and TAG through inhibition of cholesterol synthesis and regulation of liver lipoprotein synthesis, and increasing fatty acid oxidation through a *ppar*-dependent mechanism (Du et al., 2008). Although, serum and hepatic TAG and CHOL were not significantly reduced after fenofibrate treatment in the present study, there was a trend of decreasing TAG contend in both serum and liver in fish fed the diet supplemented with fenofibrate compared to fish fed HFD. These results were consistent with previous studies in blunt snout bream (Cao et al., 2019b; Dai et al., 2018; Zhou et al., 2019) and common carp (*Cyprinus carpio*) (Abasubong et al., 2018) fed high fat diets. The aminotransferase enzymes, AST and ALT, play important roles in fish as in mammals and are used generally as key indicators of liver function and cellular damage (Ma et al., 2018). Exactly, cellular damaged with an increasing of AST and ALT in blood to cause hepatic steatosis and injury occurred (Cheng and Kong, 2010; Takeuchi-Yorimoto et al., 2013). In the present study, the highest levels of AST and ALT activities were found in fish fed HFD, and AST activity was significantly decreased after fenofibrate treatment, which demonstrated that dietary fenofibrate supplementation could mitigate the liver and tissue damage in fish fed a high fat diet.

In order to further elucidate the molecular mechanisms underpinning the effects of fenofibrate on lipid metabolism and accumulation, mRNA expression levels of several related genes were determined. In the present study, no differences were found in the expression levels of *pparα* and *cpt1a* between fish fed the HFD and control diets. Similar results were obtained in blunt snout bream (Dai et al., 2018). These results suggested that these fish species were not able to establish a complete response and self-protection measures for adapting to a high dietary fat intake. As a *pparα* agonist, fenofibrate up-regulated the expression of *pparα* in mammals such as hamsters (Guo et al., 2001) and mice (Harano et al., 2006), and in fish including yellow catfish (Zheng et al., 2015) as well as hepatocytes of Nile tilapia (Ning et al., 2016). Another fibrate drug, clofibrate, induced *pparα* expression hepatocytes of Atlantic salmon (*Salmo salar*) (Ruyter et al., 1997). Meanwhile, as a key regulator of lipid metabolism, *pparα* induced the expression of multiple genes involved in fatty acid β-oxidation and up-regulated expression of *cpt1a* (Guo et al., 2015). Interestingly, the present study showed that the mRNA expression level of *pparα* was increased and, in addition, *atgl* and *cpt1a* expression levels were upregulated in liver of fish fed HFD supplemented with fenofibrate. These results were largely in agreement with a previous study in Nile tilapia and any differences could be attributed to species-specific effects (Luo et al., 2019). In SIRT1-knockdown mice, it was reported that PPARα as well as β-oxidation gene expression in downstream signaling pathways were down-regulated (Rodgers et al., 2007). Furthermore, the expression level of PPARα was up-regulated in SIRT1 overexpression mice (Rodgers and Puigserver, 2007) indicating that SIRT1 can regulate lipid metabolism in mammals by positive regulation of PPARα. In the present study, the expression level of *sirt1* was greatly up-regulated by dietary fenofibrate supplementation.*SREBP-1* is a key modulator of lipogenesis regulating lipogenic genes such as *accα*, *accβ* and *fas* (Amemiya-Kudo et al., 2002; Minghetti et al., 2011; Rho et al., 2005) and, in the present study, the hepatic expression levels of *accα*, *fas* and *srepb-1* were all up-regulated by dietary fenofibrate supplementation. Similar results were

observed in yellow catfish exposed to waterborne Zn and fed a diet supplemented with 0.15 % fenofibrate for 8 weeks (Zheng et al., 2015). This may suggest that lipogenesis may also be enhanced in fish fed fenofibrate and, therefore, we speculate that it might be a normal homeostatic mechanism to maintain lipid balance, although further studies are required.

A previous study concluded that *pparα* is not merely a regulator of lipid metabolism, but also has powerful inflammation-reducing capabilities (Zambon et al., 2006). Moreover, Wang et al. (2015a) reported that the inhibitory effect of fenofibrate on TNF-α-induced apoptosis was enhanced by knockdown of SIRT1 in vascular adventitial fibroblasts (VAF), whereas cell apoptosis in VAFs was decreased by overexpression of SIRT1. In mammals, it was reported that PPARα could suppress nuclear transcription factor NF-κB, which is a transcription factor promoting inflammation as, once NF-κB is activated, it will increase gene expression of proinflammatory markers such as IL-1β, TNFα and COX2 (Pahl, 1999). Furthermore, high fat diets cause lipid deposition, which may lead to endoplasmic reticulum stress (ERS) and accelerate the release of cytokines like *tnfα* and *il-1β* (Jin et al., 201a, b). In the present study, compared with fish fed HFD, the expression levels of *nf-κb*, *il-1β* and *tnfα* in liver and intestine of fish fed dietary fenofibrate all showed decreasing trends, albeit not statistically significant with *nf-κb* and *tnfα.* However, a recent study in fish demonstrated that dietary fenofibrate supplementation reduced the expression levels of *nf-κb* and *tnfα* genes to attenuate inflammation responses (Luo et al., 2019). Additionally, the present study showed that the anti-inflammatory cytokine *il-10* was up-regulated in liver of fish fed dietary fenofibrate compared with fish fed HFD. Hence, the present study revealed that the high fat diet HFD could induce inflammation, meanwhile, *sirt1* regulated *pparα* activation to improve anti-inflammatory ability through up-regulating expression levels of anti-inflammatory cytokines and down-regulating proinflammatory cytokine genes.

Conclusion

In conclusion, the present study has provided further insight into the pathway whereby dietary fenofibrate attenuates lipid accumulation, hepatic steatosis and inflammatory responses caused by high fat diets. Our findings demonstrated that dietary fenofibrate supplementation decreased aminotransferase activities and regulated *sirt1* and activated *ppara*, consequently, increased the downstream expression of key lipolytic and anti-inflammatory genes, which could significantly improve the health status of fish (Fig. 10). Dietary fenofibrate also exhibited positive impacts on liver histology changes with no negative impacts on growth performance or feed utilization of black seabream fed a high fat diet.

Insert Figure 10 here.

Author Contributions

Q. C. Z., M.J. and Z. T. T. conceived and designed the research; Z. T. T. and L. J. X. conducted the research; Z. T. T. and M.J. performed the statistical analysis; M.J. and Z. T. T. contributed reagents/materials/analysis tools. T. T. Z., M. J., L. J. X. T. T. P., Y. D. S. and Y. Y. make fish diets. T. T. Z., M. J., L. J. X., T. T. P., Y. D. S., Y. Y., P. S. and L. F. J. collected samples. Z. T. T. and M.J. Wrote the paper: M.J., T.T.Z., D.R.T. and M.B.B. All authors contributed to manuscript revision, read and approved the subm itted version.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (Grant No. 31802303), Natural Science Foundation of Ningbo (Grant No. 2018A610343), National Key R & D Program of China (2018YFD0900400), Key Research Program of Zhejiang Province of China (No. 2018C02037), Zhejiang Aquaculture Nutrition & Feed Technology Service Team (ZJANFTST2017-2), the Open Fund of Zhejiang Provincial Top Key Discipline of Aquaculture in Ningbo University and K. C. Wong Magna Fund in Ningbo University.

Conflict of Interest

The authors have declared that no competing interests exist.

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

Ingredient (%)	Diets		
	Control	HFD	HFD+FF
Peruvian fishmeal[a]	26.00	26.00	26.00
Soybean protein concentrate[a]	10.00	10.00	10.00
Soybean meal	20.00	20.00	20.00
Wheat flour[a]	25.05	25.05	25.05
Palmitic acid[b]	0.00	6.00	6.00
Soybean oil	8.00	8.00	8.00
Soybean lecithin[a]	1.00	1.00	1.00
Vitamin premix[a]	0.50	0.50	0.50
Mineral premix[a]	2.00	2.00	2.00
Choline chloride	0.30	0.30	0.30
Fenofibrate[c]	0.00	0.00	0.15
$Ca(H_2PO_4)_2$	1.00	1.00	1.00
Cellulose	6.15	0.15	0.00
Sum	100.00	100.00	100.00
Proximate composition (%)			
Dry matter	89.84	90.17	89.36
Crude protein	40.79	41.72	41.74
Crude lipid	12.22	17.78	17.62
Ash	9.29	8.94	8.81

[a] All the ingredients were obtained from Ningbo Tech-Bank Feed Co. Ltd., China.
[b] Palmitic acid: 97 % of total fatty acids as palmitic acid methyl ester, Shanghai Yiji Chemical Co., Ltd., China.
[c] Fenofibrate was obtained from MCE.

Table 2. Primers for real-time quantitative PCR for lipid catabolism genes, inflammation related genes, regulation fctor gene and *β-actin* of black seabream (*Acanthopagrus schlegelii*)

Gene	Nucleotide sequence (5' –3')	Size (bp)	GenBank reference or Publication	Function
pparα [1]	F: ACGACGCTTTCCTCTTCCC R: GCCTCCCCCTGGTTTATTC	183	KX066234	Lipolysis pathway
atgl [2]	F: GCATCCAGTTCACCCTCAC R: TTTGCCTCATCTTCATCGC	241	KX078570	Lipolysis pathway
cpt1a [3]	F: TGCTCCTACACACTATTCCCA R: CATCTGCTGCTCTATCTCCCG	203	KX078572	Lipolysis pathway
accα [4]	F: AGTAGCCTGATTCGTTGGT R: GATTGAGGAGTCTGTTCGC	154	KX066238	Lipogenesis pathway
fas [5]	F: AAGAGCAGGGAGTGTTCGC R: TGACGTGGTATTCAGCCGA	213	KX066240	Lipogenesis pathway
srebp-1[6]	F: TGGGGGTAGGAGTGAGTAG R: GTGAAGGGTCAGTGTTGGA	247	KX066235	Lipogenesis pathway
tnfα [7]	F: GTCCTGCTGTTTGCTTGG R: AATGGATGGCTGCCTTGG	154	AY335443	Pro-inflammation cytokine
il-1β [8]	F: CATCTGGAGGCGGTGAA R: CGGTTTTGGTGGGAGGA	231	JQ973887	Pro-inflammation cytokine
nf-κb [9]	F: AGCCCAAGGCACTCTAGACA R: GTTCTGGGCAGCTGTAGAGG	154	MK922543	Nuclear transcription factor
il-10[10]	F: CCCAGATAGAAGCCCAGGAT R: AAACGATGATTTGGACACAGC	105	MK922542	Anti-inflammation cytokine
tgfβ-1 [11]	F: GGGTTTCCAACTTCGGC R: TTGTGTCCGTGGAGCGT	209	Xue et al. (2008)	Anti-inflammation cytokine
sirt1 [12]	F: TGGATGAAACTGTAGGAACC R: ACAACAATGGACTGGGAA	238	MN871952	Metabolic sensor
β-actin	F: ACCCAGATCATGTTCGAGACC R: ATGAGGTAGTCTGTGAGGTCG	-	Jiao et al. (2006)	Housekeeping gene

[1] *pparα*, peroxisome proliferators-activated receptor alpha;[2]*atgl*, adipose triglyceride lipase;[3] *cpt1a*, carnitine palmitoyltransferase 1A; [4]*accα*, acetyl-CoA carboxylase alpha; [5]*fas*, fatty acid synthase;

[6]*srebp-1*, sterol regulator element-binding protein-1 ; [7] *tnfα*, tumor necrosis factor alpha; [8] *il-1β*, interleukin 1 beta; [9]*nf-κb*, nuclear factor-kappa b; [10]*il-10*, interleukin-10; [11] *tgfβ-1*, transforming growth factor beta-1; [12]*sirt1*, silent information regulator.

Table 3. Growth response, feed utilization and biometric indices of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks.

	Diets		
	Control	HFD	HFD+FF
IBW[1] (g)	8.36±0.01	8.34±0.01	8.33±0.00
FBW[2] (g)	39.55±1.06	35.92±1.71	36.39±0.84
WG[3] (%)	373.33±12.13	330.53±21.03	336.69±10.07
SGR[4] (% day $^{-1}$)	2.73±0.04	2.56±0.09	2.59±0.04
FE[5]	0.61±0.02	0.53±0.03	0.55±0.02
Survival (%)	98.89±1.11	91.11±2.94	98.89±1.11
PPV (%)[6]	30.80±0.69^{b}	26.43±1.15^{a}	27.75±0.46^{ab}
LR (%)[7]	35.59±0.97^{b}	28.37±2.95^{ab}	26.77±1.40^{a}
VSI (%)[8]	9.28±0.28^{a}	10.46±0.04^{b}	9.47±0.25^{a}
HSI (%)[9]	2.79±0.03^{b}	2.47±0.01^{b}	1.91±0.14^{a}
IPF (%)[10]	3.11±0.06^{a}	3.94±0.07^{c}	3.49±0.05^{b}

Data are reported as the mean and SEM ((n = 3 for IBW, FBW, SGR, FE, Survival and PPV; n = 9 for CF, VSI, HSI and IPF). Values in the same column with different superscripts are significantly different ($P < 0.05$).

[1] IBW, initial body weight; [2] FBW, final body weight; [3] WG, Weight gain;[4] SGR, Specific growth ratio;[5] FE, Feed efficiency; [6] PPV, Protein productive value; [7] LR, Lipid retention; [8] VSI, Viscerosomatic index; [9] HSI, Hepatosomatic index; [10] IPF, Intraperitoneal fat ratio.

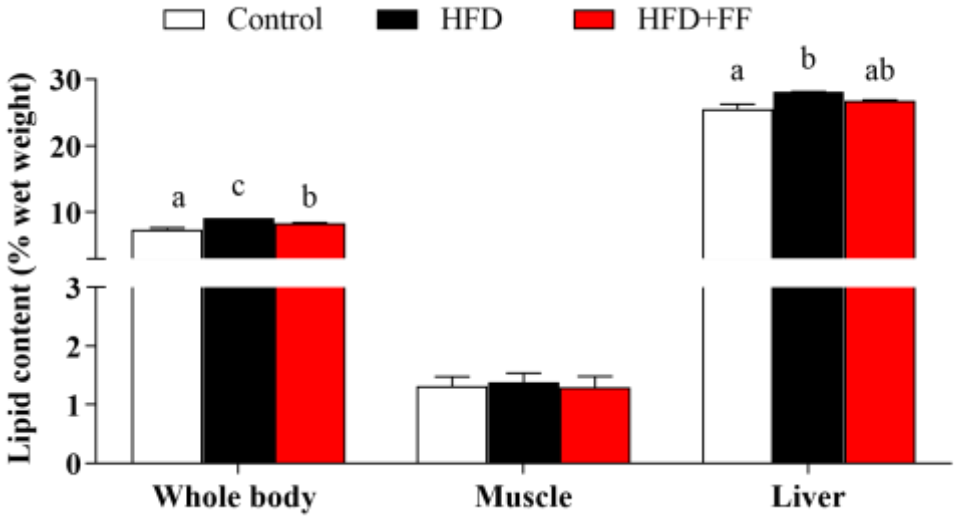


Figure 1. Whole body, muscle and liver lipid content of the juvenile black seabream (*Acanthopagrus schlegelii*) (% wet weight) fed the experimental diets for 8 weeks. Data are reported as means and SEM (n = 3). Values in the same line with different superscripts are significantly different ($P < 0.05$).

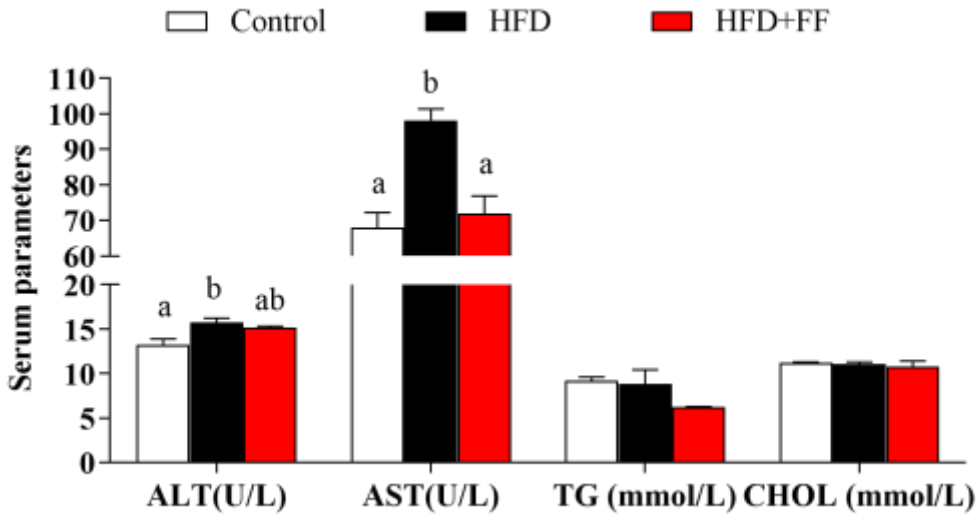
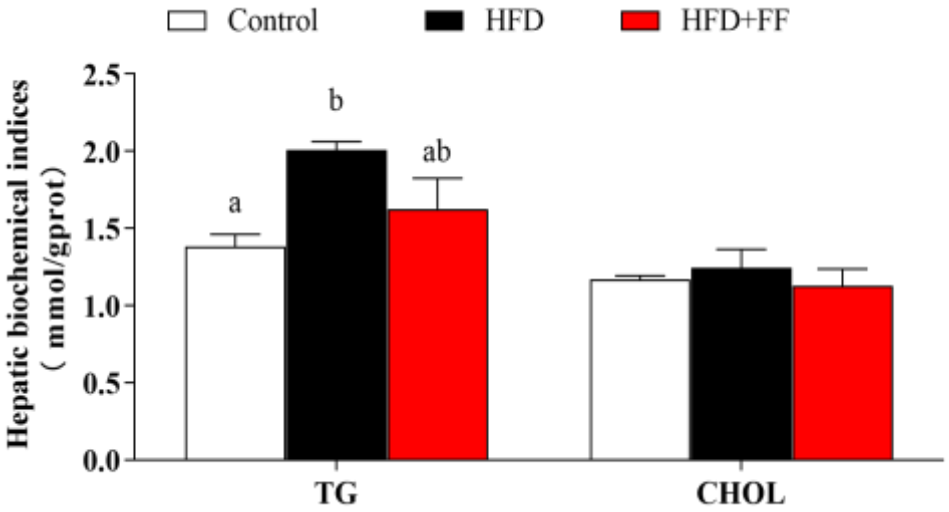


Figure 2. Serum parameters of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks. Data are reported as the mean and SEM of three replicates. Values in the same line with different superscripts are significantly different (P <0.05). ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; CHOL, cholesterol.

Figure 3. Hepatic biochemical indices of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks. Data are reported as means and SEM (n = 3). Values in the same line with different superscripts are significantly different ($P < 0.05$). TG, triglyceride; CHOL,



cholesterol.

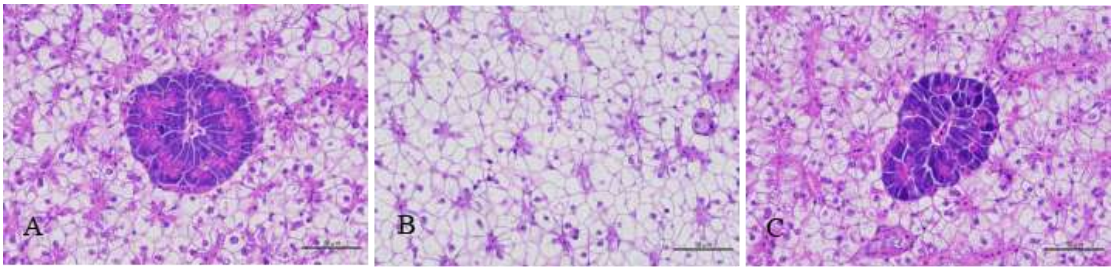


Figure 4. Paraffin section of liver in juvenile black seabream (*Acanthopagrus schlegelii*). The liver section was stained with H&E to enhance the contrast (400X). (A) Paraffin section of liver in Control group; (B) Paraffin section of liver in HFD group; (C) Paraffin section of liver in HFD+FF group.

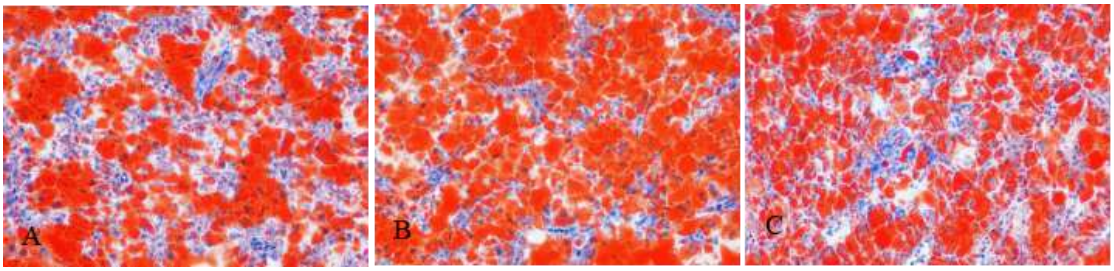
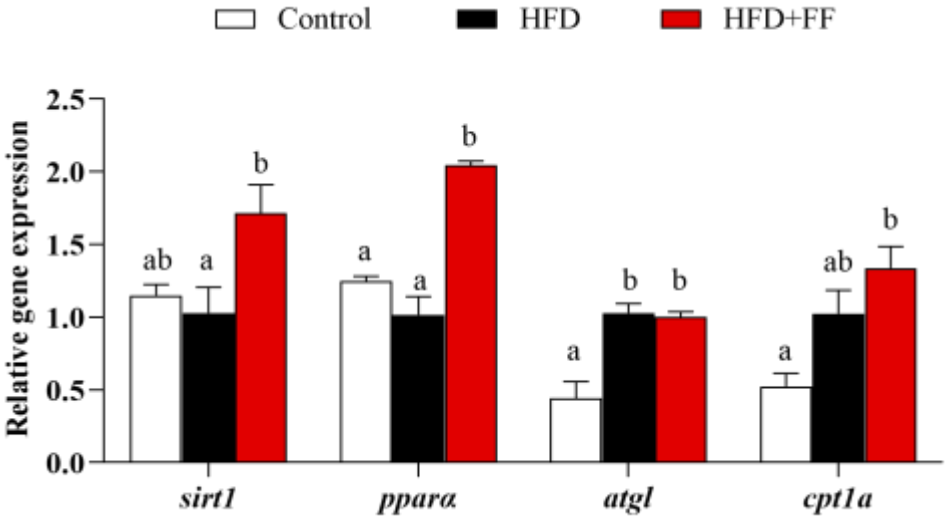


Figure 5. Paraffin section of liver in juvenile black seabream (*Acanthopagrus schlegelii*). The liver section was stained with Oil Red O to enhance the contrast (400X). (A) Paraffin section of liver in Control group; (B) Paraffin section of liver in HFD group; (C) Paraffin section of liver in HFD+FF group.

Figure 6. Regulatory factor and lipid catabolism gene expression in liver of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks. Data are represented as the mean ± SEM of three replicates (n = 3). Values in the same row with different letters are significantly different ($P<0.05$). The gene expression of the positive control diet group (dietary HFD) was set at 1. *sirt1*, silent information regulator; *pparα*, peroxisome proliferators-activated receptor alpha; *atgl*,



adipose triglyceride lipase; *cpt1a*, carnitine palmitoyltransferase 1A.

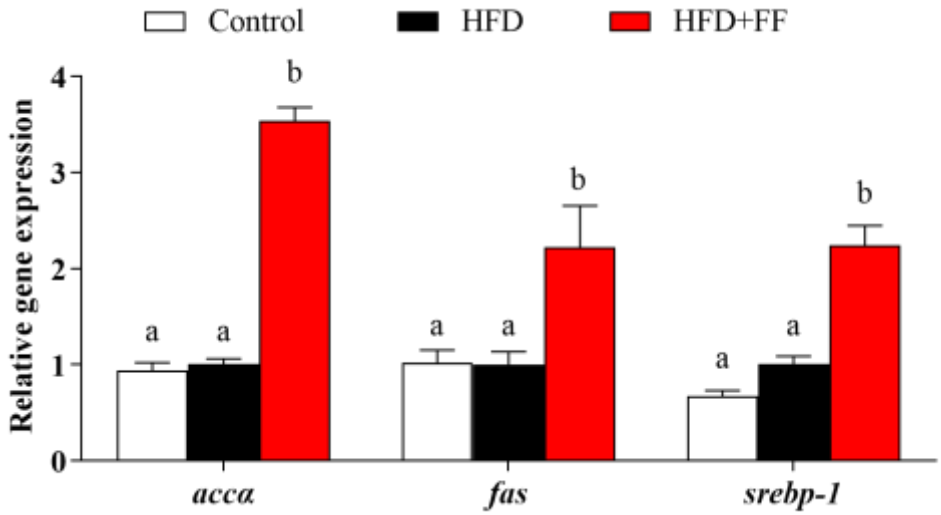
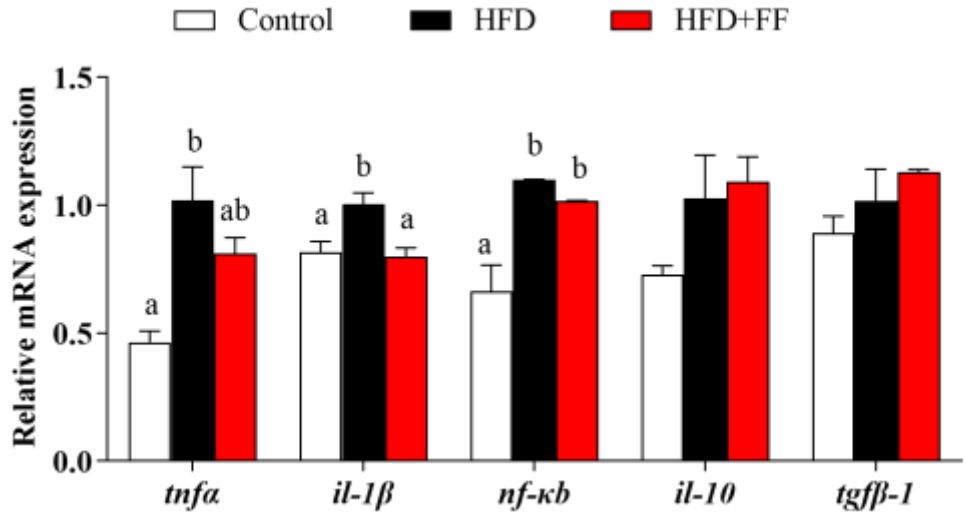


Figure 7. lipid anabolism gene expression in liver of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks. Data are represented as the mean ± SEM of three replicates (n = 3). Values in the same row with different letters are significantly different (P<0.05). The gene expression of the positive control diet group (dietary HFD) was set at 1. *accα*, acetyl-CoA carboxylase alpha; *fas*, fatty acid synthase; *srebp-1*, sterol regulatory element binding protein-1.

Figure 8. Inflammation gene expression in liver of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks. Data are represented as the mean ± SEM of three replicates (n = 3). Values in the same row with different letters are significantly different (P<0.05).



The gene expression of the positive control diet group (dietary HFD) was set at 1.

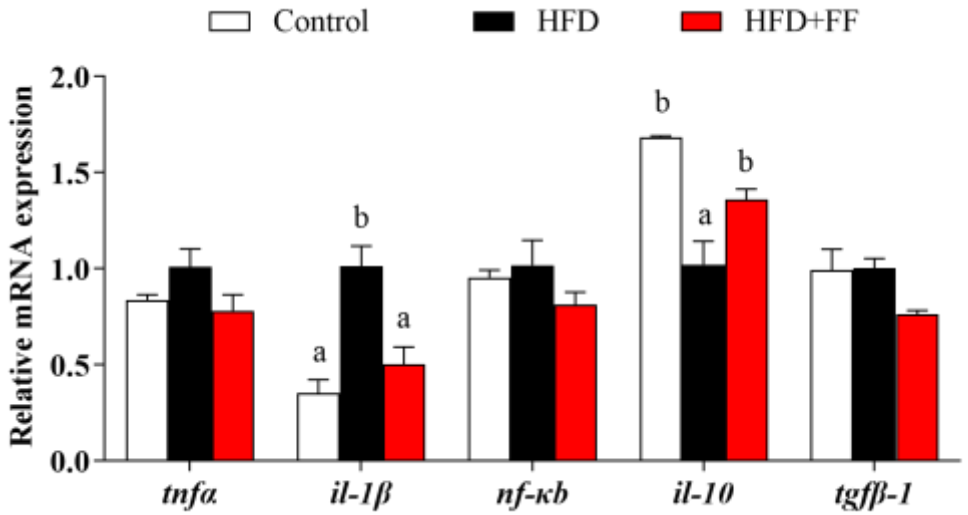


Figure 9. Inflammation gene expression in intestinal of juvenile black seabream (*Acanthopagrus schlegelii*) fed the experimental diets for 8 weeks. Data are represented as the mean ± SEM of three replicates (n = 3). Values in the same row with different letters are significantly different ($P<0.05$). The gene expression of the positive control diet group (dietary HFD) was set at 1.

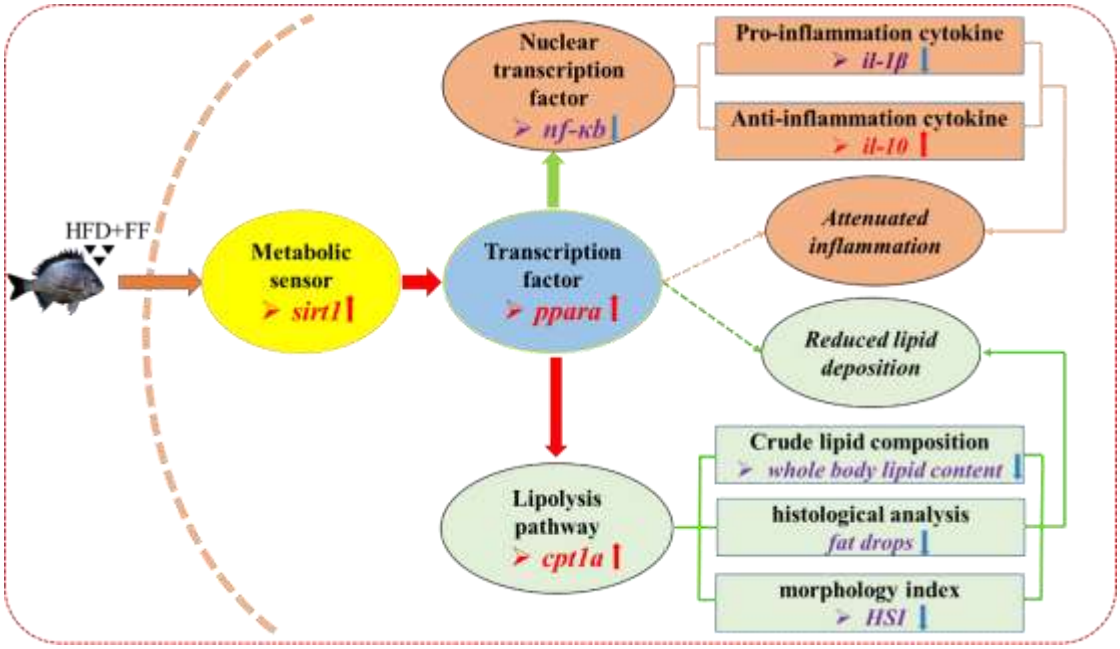


Figure 10. Proposed pathway of dietary fenofibrate attenuation of HFD-induced lipid accumulation, hepatic steatosis and inflammatory response in black seabream (*Acanthopagrus schlegelii*). Red arrows represent increase/up-regulation, blue arrows represent decrease/down-regulation.