

The metabolic regulation of fenofibrate is dependent on dietary protein content in male juveniles of Nile tilapia (*Oreochromis niloticus*)

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Abstract

The fenofibrate functions in mammals could be affected by many factors such as dietary nutrient levels and physiological status. However, this phenomenon has not been well studied in fish. The goal of our study was to investigate the effect of dietary protein contents on metabolic regulation of fenofibrate in Nile tilapia. An 8-week experiment was conducted to feed fish with four diets at two protein levels (28 and 38%) with or without the supplementation of fenofibrate (200 mg/kg body weight per d). After the trial, the body morphometric parameters, plasma biochemical parameters and quantitative PCR data were examined. These results showed that fenofibrate significantly reduced the feeding intake and weight gain rate, increased the oxidative stress (increased plasma methane dicarboxylic aldehyde) and liver:body ratio (increased hepatosomatic index) in the low protein (LP)-fed fish. In contrast, fenofibrate exhibited a lipid-lowering (reduced hepatic lipid) effect and up-regulated the expressions of the genes related to lipid catabolism, transport and anabolic metabolism in the high protein (HP)-fed fish. The present study suggested that lipid-lowering effect of fenofibrate would be strengthened in the fish fed with the HP diet containing high energy, but in the fish fed with the LP diet containing low energy, the fenofibrate treatment would cause adverse effects for metabolism. Taking together, our study showed that the metabolic regulation of fenofibrate in Nile tilapia was dependent not only on feed energy content but also on dietary nutrient composition, such as dietary protein and/or lipid levels.

Keywords: Dietary protein level: Fenofibrate: Metabolic regulation: Nile tilapia

Fibrate derivatives are widely used as normolipidaemic or hypolipidaemic drugs for the treatment of hyperlipidaemia, non-alcoholic fatty liver disease and preserving insulin signal transduction^(1,2). As one of the fibrate derivatives and lipid-lowering agents, fenofibrate has been shown to lower the concentration of plasma TAG and cholesterol⁽³⁾. Some mechanisms of the clinical effects of fenofibrate have been identified, including interference with fatty acid (FA) synthesis, stimulation of hepatic FA oxidation, increase in lipoprotein lipolysis, inhibition of cholesterol biosynthesis and decrease of insulin resistance^(4,4). Fenofibrate is also known to activate the transcriptional factor PPAR α , which binds to the peroxisome proliferator response element in the regulatory region of target genes, resulting in hypolipidaemic effects, glucose homeostasis and amino acid metabolism⁽⁵⁾. Many model animal studies have demonstrated significant

lipid-lowering functions of fenofibrate under the high-fat diet (HFD) feeding condition^(6,7). Similarly, fenofibrate has also been reported to lower the hyperinsulinaemia and hyperglycaemia in HFD-induced (C57BL/6) mice⁽⁸⁾. Furthermore, fenofibrate could stimulate the branched-chain amino acid (leucine, isoleucine, and valine) catabolism of rats, resulting in attenuating leucine-stimulated protein synthesis in a low protein (LP) diet⁽⁹⁾. This result suggested that fenofibrate may play an important role in amino acid metabolism and affect the animal growth.

In current aquaculture, due to the popular usage of HFD, the excess fat deposition in farmed fish has been commonly observed to impair fish health and growth⁽¹⁰⁾. Therefore, the regulatory mechanisms and technologies to reduce fat deposition in fish have been an important research area in fish nutritional and physiological studies^(11,12). By using fibrates as an

Abbreviations: CPT, carnitine palmitoyltransferase; FA, fatty acid; FAS, fatty acid synthase; FATP, fatty acid transport protein; FI, feed intake; HFD, high-fat diet; HL, hepatic lipid; HP, high protein; HSI, hepatosomatic index; LP, low protein; MDA, methane dicarboxylic aldehyde; PER, protein efficiency ratio; WGR, weight gain rate.

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efficient lipid-lowering reagent, researchers also found that, as in mammals, fibrates could play lipid-lowering effects in grass carp, yellow catfish and Nile tilapia in HFD-induced fatty fish^(12–14). However, not only HFD, but also excess high protein (HP) diet could result in lipid deposition in fish⁽¹⁵⁾. On the other hand, in the practical dietary formulation in farmed fish, HFD always contains relatively LP content. Therefore, dietary protein content could also affect fat deposition in fish. However, to the best of our knowledge, the metabolic regulatory functions of fibrate in fish at different dietary protein levels have not been fully investigated, which then limited our current understanding of the regulatory mechanisms of fenofibrate functions in fish.

Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species cultured worldwide. A number of studies have indicated that the optimal dietary protein level in Nile tilapia is 30 to 35% during the size of 3.2- to 43.0-g fish^(16,17). To understand and characterise the possible metabolic regulation function of fenofibrate through different dietary protein levels in Nile tilapia, an 8-week feeding experiment was performed to investigate the metabolic effects of fenofibrate on growth, body morphometric parameters, plasma biochemical parameters and selected genes which are related to lipid and protein metabolism at HP (38% protein level) or LP (28% protein level) in the diet. Considering in our earlier study that the lipid-lowering effect of fenofibrate in Nile tilapia was affected by dietary fat levels⁽¹⁴⁾, if the metabolic effects of fenofibrate were also affected by dietary protein level, then it could verify our hypothesis that metabolic regulation of fenofibrate in Nile tilapia was dependent not only on feed energy content but also on dietary nutrient composition, such as dietary protein and/or lipid levels.

Materials and methods

Feeding trial

More than 400 juvenile Nile tilapias (male) were obtained from Guangdong Tilapia Breeding Farm. Before the feeding experiment, all fish were acclimated in three 300-litre tanks for 1 week. Fishes were fed with the commercial diet (Tongwei Feed Group) at 3% of biomass per d. The feeding experiment started after 1-week acclimation of fish. A total of 240 healthy juvenile Nile tilapia with similar body weights (8.02 ± 0.03 g) were selected and randomly distributed into twelve circular fibre glass tanks (300 litres, twenty fish per tank) that were connected as a closed recirculating system containing freshwater in Baiyun experiment base, Guangdong Academy of Agricultural Sciences. This recirculating system has a well-assembled filter system, and it is composed of eight phases of filter materials, including one phase of cotton filter, one phase of foam, two phases of coral stone, two phases of activated charcoal and two phases of biofilms (mainly nitrifying bacteria). During the feeding trial, the cotton filter, foam filter, coral stone and activated charcoal were washed and renewed every week, and this could efficiently maintain water quality and largely reduce the residue of fenofibrate and its metabolites. Two semi-purified basal diets were made from two protein levels (28 and 38%) and one fat level (7%). Each group of feed was either supplemented with fenofibrate (fenofibrate groups) or not (control groups), resulting in four diets in

total. The formulation of each group diet is presented in Supplementary Table S1, and the diet was made as described previously^(12,18). Gross energy values of the diets were calculated based on 23.65, 17.16 and 39.77 kJ/g for protein, carbohydrate (N-free extract) and lipid, respectively⁽¹⁹⁾. Fenofibrate was purchased from the Sigma Chemical Co. and mixed into the corresponding diet by grade mixing protocol, to the final dose of 200 mg fenofibrate/kg body weight per d when the feeding rate was set as 4% body weight. The fish weight from each tank was recorded and weighed every 2 weeks, and the feed supply among different groups was adjusted by the exact fish weight with the fixed feeding rate 4%. Because the fish growth among groups would be different during the trial, the final feed intake (FI) would also be different. During the acclimation and 8-week trial, the fish were fed at 09.00 and 18.00 hours with an equal portion of diet, and all fish could completely eat up all diets within 30 min after each feeding. To limit the possible accumulation of excreted fenofibrate and its metabolites in water, all water was renewed by using stock water daily at 17.00 hours. Water temperature was maintained at $28 \pm 1^\circ\text{C}$ and $\text{NH}_4\text{-N}_2 < 0.01$ mg/l with a 12-h light–dark cycle. The dissolved oxygen and pH during acclimation and the formal trial were 6.17 ± 0.36 mg/l, 7.26 ± 0.35 and 6.11 ± 0.30 mg/l, 7.30 ± 0.33 , respectively.

Growth performance

At the end of 8-week exposure, fish were weighed and the weight gain rate (WGR), FI, protein efficiency ratio (PER), visceral somatic index, mesenteric fat index and hepatosomatic index (HSI) were calculated using the following equations:

$$\text{WGR (\%)} = (\text{final weight} - \text{initial weight}) \times 100 / (\text{initial weight});$$

$$\text{FI (g/ fish)} = \text{feed consumption (g)} / \text{number of fish};$$

$$\text{PER (\%)} = (\text{fish wet weight gain}) \times 100 / (\text{protein intake});$$

$$\text{VSI (\%)} = (\text{viscera weight}) \times 100 / (\text{whole body weight});$$

$$\text{MFI (\%)} = (\text{mesenteric fat weight}) \times 100 / (\text{whole body weight});$$

$$\text{HSI (\%)} = (\text{liver weight}) \times 100 / (\text{whole body weight}).$$

Sampling and samples analysis

At the end of the experiment, all fish were fasted for 24 h, and six fish from each group (two fish \times three experimental units) were euthanised (MS-222-water solution, 20 mg/l). Each fish was used as an individual sample (n 6) to collect blood and tissues for measurements of the components, molecular or biochemical parameters. The blood was taken by heparinised syringes from the caudal vein, and the plasma was separated from the blood via centrifugation at 3000 rpm for 10 min at 4°C . The plasma and tissues were stored at -80°C until analysis. The weight of each individual fish was recorded, and its viscera, liver and mesenteric fat (the fat attached to the organs) were quickly separated with tweezers (the whole process was completed within 2 min) and weighed. The moisture was analysed by drying the samples



to constant weight at 105°C. Protein of whole body and muscle was analysed by Kjeltac™ 8200 (FOSS). The lipid of whole body, liver and muscle were measured by using methanol and chloroform (1:2) as previously described⁽²⁰⁾. The plasma glucose, TAG, HDL, LDL, total protein, total amino acid, NH₄ and methane dicarboxylic aldehyde (MDA) were assessed using the commercial kits (Jiancheng Biotech Co.). NEFA were measured by ELISA kits (YiFei Co.), and the absorbance was measured at 450 nm using the Multiskan Spectrum microplate reader (Thermo Scientific). The experiment was conducted under the Guidance of the Care and Use of Laboratory Animals in China. The procedures were approved by the Committee on the Ethics of Animal Experiments of South China Agricultural University (protocol number: 2017D006).

Determination of residues of fenofibrate or fenofibric acid in culture water

Three 2-litre water samples from the system were collected into 2.5-litre pre-rinsed amber glass bottles with Teflon-lined caps. Upon collection, all samples were immediately transported at 4°C to the laboratory and vacuum filtered through a 0.45-µm glass fibre filter (Millipore). The solid-phase extraction (carried out with Oasis hydrophilic-lipophilic balance cartridges), clean-up and derivatisation were performed as previously described⁽²¹⁾. Determination of the fenofibrate and fenofibric acid was done by GC-MS (7890B-5977B; Agilent). Injection temperature was 275°C and 2 µl was injected splitless for 1 min. A fused-silica capillary column (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm) of DB-35 (Agilent) was used. Helium with a purity of 99.9990 % was used as carrier gas. For GC separation, the temperature program started at 65°C (held for 2 min), set at 30°C/min to 180°C, set at 5°C/min to 300°C and was held isothermally for 12 min. Detector temperature was 200°C. The ion-trap MS was run in the full-scan mode from 80 to 400 m/z. The measurement showed that the water residue of fenofibric acid was very low (63 ng/l), while the fenofibrate was not detected (Supplementary Table S5). Because the residue concentration of fenofibric acid was close to the environmental concentration (0–280 ng/l)⁽²²⁾, and previous report also showed that in zebrafish, 703 µg/l fenofibric acid did not exert any effect on morphometric indices⁽²³⁾, the water residue of fenofibric acid in the present study was not likely to affect the fish that were not fed with fenofibrate diet.

Quantitative real-time PCR analyses

Total RNA was isolated by using an RNAiso Plus Kit (Takara). The quality and quantity of the total RNA were determined by using a NanoDrop 1000 (Thermo Scientific), and only RNA with a 260:280 ratio of >1.9 was used for cDNA synthesis. cDNA of the total RNA from the tissues were synthesised using a PrimeScript™ RT reagent kit with a gDNA Eraser (Perfect Real Time) (Takara). β-Actin and elongation factor 1α (EF1α) were used as the reference genes. Our team cloned partial coding region sequences for these two genes of Nile tilapia and deposited them in GenBank (β-actin: KJ126772, EF1α: KJ123689). All the primers used for quantitative PCR were designed to overlap an intron and were provided in Supplementary Table S2. The different PCR products were

initially checked by sequencing to confirm the nature of the amplicon. Quantitative PCR was conducted by using SYBR Green Premix Ex Taq (Takara) in a CFX96 real-time PCR system (Bio-Rad). Quantitative PCR efficiency was between 98 and 102 % and the correlation coefficient was over 0.97 for each gene. The PCR reaction volume was 25 µl containing 2.0 µl of cDNA template, 12.5 µl of SYBR Premix Ex Taq, 2.0 µl of PCR primers (5 µM) and 6.5 µl of nuclease-free water. PCR reactions were run with the following program: 95°C for 30 s, forty cycles of 95°C for 5 s and 60°C for 20 s. The melting curves of the amplified products were analysed at the end of each PCR. Each PCR run performed in triplicate and negative controls (no cDNA) was conducted. The relative cDNA abundance was estimated using the 2^{-ΔCt} method, thereof, $\Delta Ct = C_{t_{\text{target}}} - (C_{t_{\text{EF1}\alpha}} + C_{t_{\beta\text{-actin}}})/2$.

Statistical analysis

All data were expressed as means and standard deviations and performed using IBM SPSS Statistics 21 (IBM). Two-way ANOVA was used to explore the effects of dietary protein levels, fenofibrate treatment and their interactions. Comparisons between the Control (LP or HP) and Fenofibrate groups were performed using Student's *t* tests.

Results

Growth performance and body composition

To investigate whether fenofibrate would affect the growth performance and body composition of metabolic regulation at different dietary protein levels, related indices were determined. As shown in Figs. 1 and 2, in the LP group, fenofibrate significantly reduced FI, WGR and PER, but significantly increased HSI, with no effect on the visceral somatic index and mesenteric fat index over the 8-week exposure. In the HP group, 8-week fenofibrate treatment did not reduce FI, WGR and PER, but significantly reduced the visceral somatic index, with no effect on HSI and mesenteric fat index. Nevertheless, fenofibrate did not change the whole body protein and lipid concentrations (Fig. 2(a) and (b)), or the protein and lipid concentrations of muscle (Fig. 2(c) and (d)), except a significantly lower hepatic lipid (HL) in the HP group (Fig. 2(e)). There were no interaction effects of the protein content and fenofibrate found in FI, PER, visceral somatic index, mesenteric fat index in the liver, except in the WGR and HSI (Supplementary Table S4), showing WGR and HSI are simultaneously regulated by protein content and fenofibrate supplementation. These results indicated that fenofibrate did play a lipid-lowering effect in liver of the HP group, while having a negative effect on the weight loss and an increase of HSI in the LP group. No mortality in all treatments was detected during the 8-week trial.

Plasma biochemical parameters

Fig. 3 presents the plasma composition of fish from different dietary protein levels with fenofibrate supplement. Firstly, fenofibrate significantly decreased the plasma NEFA, total amino acid and NH₄, but significantly increased TAG and MDA in the LP

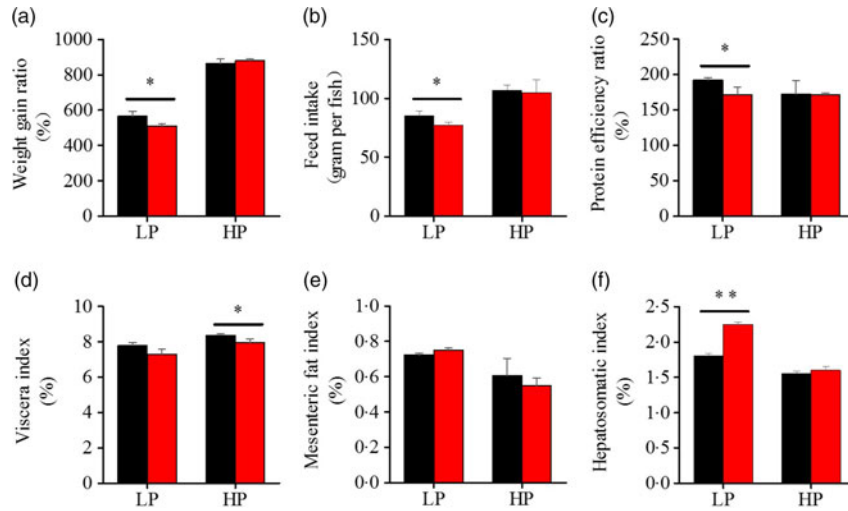


Fig. 1. Effects of fenofibrate (■) compared with control (■) on growth performance in Nile tilapia fed with high-protein (HP) or low-protein (LP) diet. (a) Weight gain rate. (b) Feed intake. (c) Protein efficiency ratio. (d) Visceral somatic index. (e) Mesenteric fat index. (f) Hepatosomatic index. Values are means ($n=3$), with standard deviations represented by vertical bars. Mean values were significantly different: * $P < 0.05$, ** $P < 0.01$ (t test).

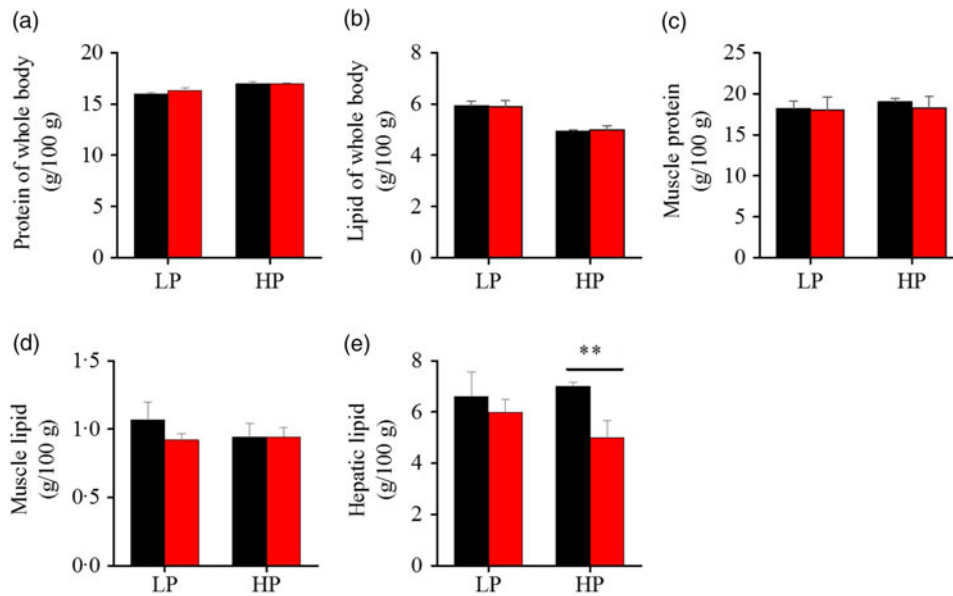


Fig. 2. Effects of fenofibrate (■) compared with control (■) on body composition in Nile tilapia fed with high-protein (HP) or low-protein (LP) diet. (a) Protein of whole body. (b) Lipid of whole body. (c) Muscle protein. (d) Muscle lipid. (e) Hepatic lipid. Values are means ($n=3$), with standard deviations represented by vertical bars. ** Mean values were significantly different ($P < 0.01$; t test).

group (Fig. 3(a) and (b), (f)–(h)). In the HP group, fenofibrate significantly increased the concentrations of plasma total protein, accompanying with a lower MDA (Fig. 3(e), (h)). In addition, fenofibrate significantly decreased LDL in both groups (Fig. 3(d)). The significant interaction between the protein content and fenofibrate was seen in plasma TAG, LDL and MDA (Supplementary Table S4).

mRNA expression of lipid metabolism genes in liver

After fenofibrate treatments (Figs. 4–6), the expressions of lipid metabolism-related genes were highly up-regulated in the HP

group. However, these genes were slightly down-regulated in the LP group. In general, 91.7% of genes in the HP group were significantly changed in contrast to 16.7% of those in the LP group. With respect to the nuclear receptor genes, fenofibrate significantly increased SREBP1c and PPAR β accompanied with a significantly decrease of PPAR γ in the HP group (Fig. 4(b) and 5(b) and (d)). No significant difference in most of genes in the LP group was found. There was no significant change of PPAR α expression in both groups (Fig. 4(a)). With respect to the genes-related FA transport, fenofibrate significantly increased CD36 and fatty acid transport protein 5 (FATP5) in the HP group (Fig. 6(a) and (b)). For genes-related lipoprotein

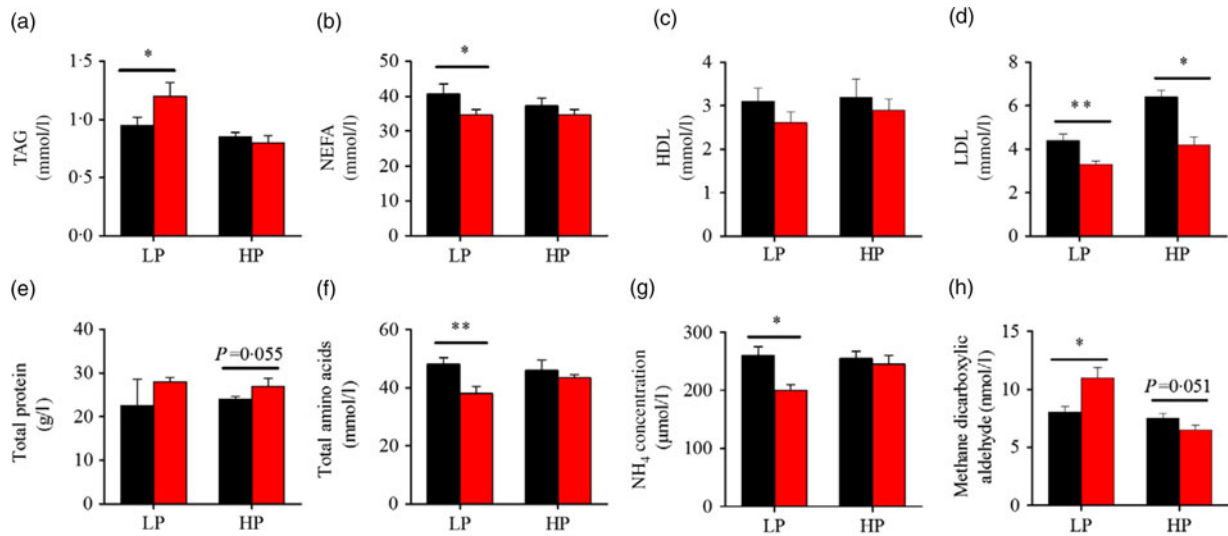


Fig. 3. Effects of fenofibrate (■) compared with control (■) on plasma biochemical parameters in Nile tilapia fed with high-protein (HP) or low-protein (LP) diet. (a) Plasma TAG. (b) Plasma NEFA. (c) Plasma HDL. (d) Plasma LDL. (e) Plasma total protein. (f) Plasma total amino acids. (g) Plasma NH₄. (h) Plasma methane dicarboxylic aldehyde. Values are means (*n* 3), with standard deviations represented by vertical bars. Mean values were significantly different: * *P* < 0.05, ** *P* < 0.01 (*t* test).

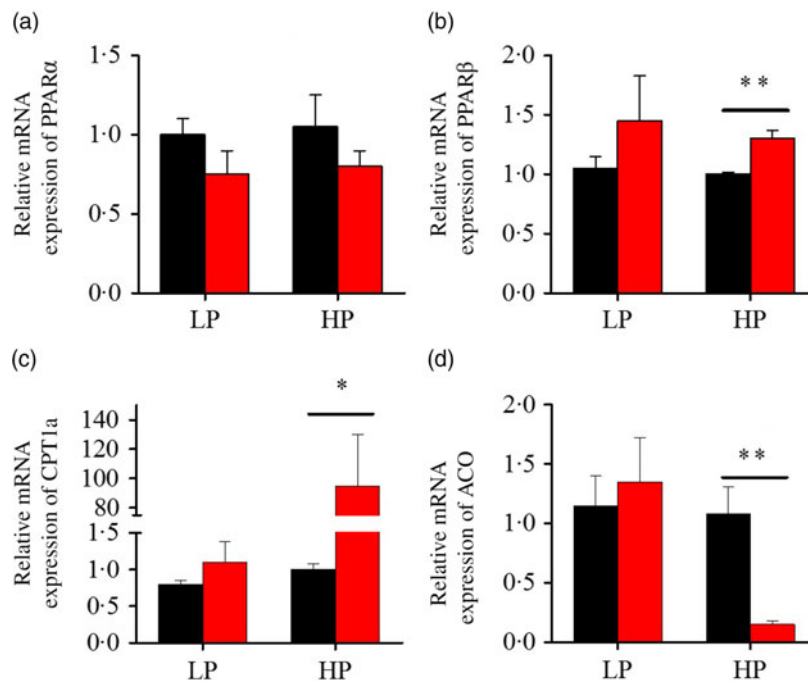


Fig. 4. Effects of fenofibrate (■) compared with control (■) on the mRNA expression of the genes related to lipid catabolism in liver of Nile tilapia fed with high-protein (HP) or low-protein (LP) diet. (a, b) Relative mRNA abundance of PPARα and PPARβ. (c, d) Relative mRNA abundance of carnitine palmitoyltransferase 1a (CPT1a) and acyl-CoA oxidase (ACO), showing the activity of fatty acid β-oxidation. Values are means (*n* 3), with standard deviations represented by vertical bars. Mean values were significantly different: * *P* < 0.05, ** *P* < 0.01 (*t* test).

assembling secretion, fenofibrate significantly decreased microsomal TAG transfer protein and ApoB (Fig. 6(c) and (d)) in both the LP and HP groups. No significantly effect was found in the LP group for genes-related lipid anabolism and catabolism, while fenofibrate significantly increased carnitine palmitoyltransferase 1a (CPT1a) and fatty acid synthase (FAS) accompanied by a decrease of acyl-CoA oxidase

(ACO) and diacylglycerol O-acyltransferase 2α in the HP group (Figs. 4(c) and (d) and 5(a) and (c)). Interaction effects between the protein content and the fenofibrate were observed in the mRNA levels of CPT1α, FAS, SREBP1c, PPARγ, CD36 and FATP5 in liver (Supplementary Table S4), showing protein content and fenofibrate both regulated the lipid synthesis and lipolysis.

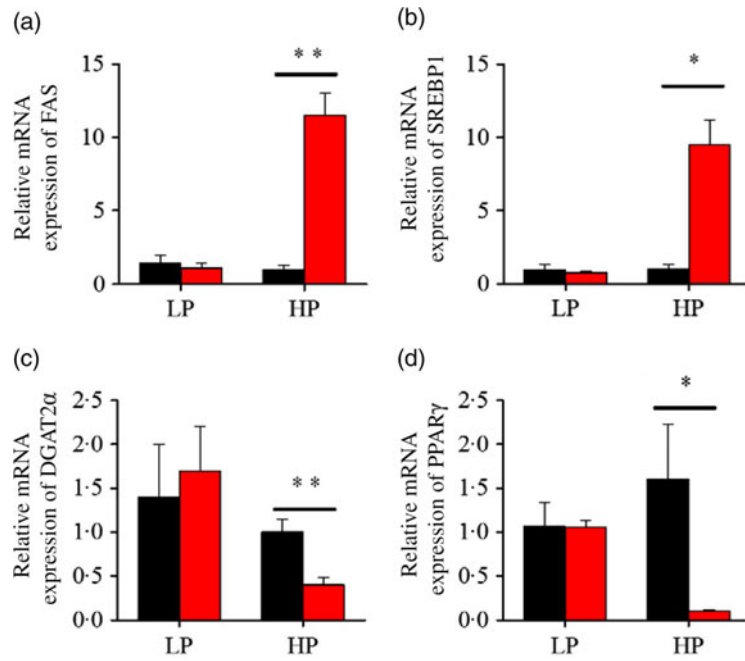


Fig. 5. Effects of fenofibrate (■) compared with control (■) on the mRNA expression of the genes related to lipid anabolism in liver of Nile tilapia fed with high-protein (HP) or low-protein (LP) diet. (a, c) Relative mRNA abundance of fatty acid synthase (FAS) and diacylglycerol O-acyltransferase 2 α (DGAT2 α), showing the activity of fatty acid and TAG synthesis. (b) Relative mRNA abundance of SREBP1c playing an important regulation role in the activity of lipid synthesis. (d) Relative mRNA abundance of PPAR γ . Values are means (n 3), with standard deviations represented by vertical bars. Mean values were significantly different: * $P < 0.05$, ** $P < 0.01$ (t test).

Discussion

Although the effects of dietary lipid content on fenofibrate functions in fish have been previously reported⁽¹⁴⁾, whether the lipid-lowering effect of fenofibrate was dependent on the dietary protein levels is unknown. In the present study, the two-way ANOVA did show many interactions between dietary level and fenofibrate treatment as two factors in HSI, TAG, LDL, MDA and gene's mRNA level of lipid metabolism. Besides, the results indicated that fenofibrate reduced the feeding intake and WGR and increased the oxidative stress and HSI in the LP-fed fish. In contrast, fenofibrate exhibited a lipid-lowering effect as well as up-regulated the genes related to lipid catabolism, transport and anabolic metabolism in the HP-fed fish. These data strongly suggested that the effect of fenofibrate in fish was highly dependent on the dietary protein level.

In the present study, a number of key genes were affected by fenofibrate at different dietary protein levels. CPT1 is an essential and rate-limiting enzyme in the FA β -oxidation. In mammals, CPT1 is encoded by three genes, one being expressed predominantly in the liver (CPT1a) and the second (CPT1b) in the skeletal muscle, and CPT1c is expressed predominantly in the brain and testes⁽²⁴⁾. However, there is relatively little information on the expression and functions of CPT isoforms in fish⁽²⁵⁾. Similarly, the members of the FATP family are also not equally expressed in all tissues, implying tissue-specific regulation of the various subtypes⁽²⁶⁾. In mammals, FATP5 is expressed only in the liver and shows a highest FA transport ability⁽²⁷⁾. However, as most of other genes, the FATP isoforms have not been fully investigated in fish. In the HP group, fenofibrate decreased the HL content and increased the lipid-catabolism-related gene CPT1a

and lipid-transport-related genes CD36 and FATP5, which partially agreed with previous studies on lipid-lowering effect of fibrates in yellow catfish, lactating sows and monkeys^(13,28,29). Nevertheless, fenofibrate also increased FAS and Srebp1c, the genes related to lipid synthesis in the HP group. Such results may be a physiological compensatory mechanism for the lipid-lowering effect of the fenofibrate in the HP group. Consequently, the concentrations of circulating NEFA were maintained relatively constant (Fig. 3(b)). In fact, a previous study in mice showed that increased PPAR α and Srebp1c by fenofibrate treatment collectively ensured proper handling of FA to protect the liver against cytotoxic damage⁽³⁰⁾. Conversely, in the LP group, both the HL content and the genes related to lipid metabolism alteration were abolished, and the WGR was significantly reduced. By taking the plasma biochemical parameters into account, a decreased NH₄ accompanied with decreased total amino acid in the present study implied that not only amino acid catabolism, but also the protein catabolism was both decreased by fenofibrate in the LP group. Thus, further investigation is warranted in protein synthesis in the LP group. Nevertheless, a dose of fenofibrate (200 mg/kg per d) in the present study reduced the FI as well as the PER (Fig. 1(b) and (c)) in the LP group. Therefore, the observed significant difference in WGR could be mostly attributed to the decreased FI and a negative N balance as reported by previous studies in sheep⁽³¹⁾ and mice⁽³²⁾. On the other hand, plasma TAG and MDA were increased, while the expressions of microsomal TAG transfer protein and ApoB, which are two key regulators in the excretion of TAG-enriched lipoprotein (VLDL)⁽³³⁾, were down-regulated in the LP group after fenofibrate treatment. Previous study

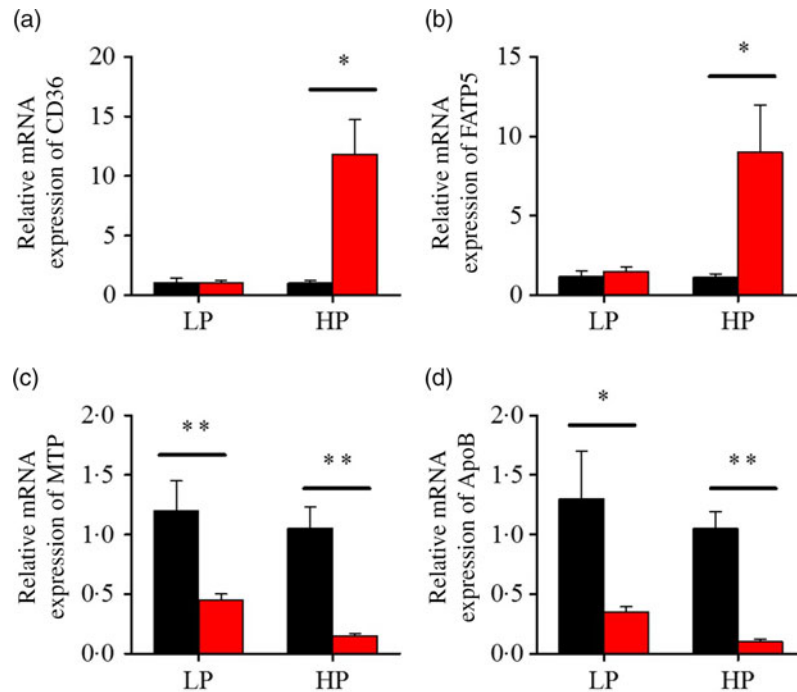


Fig. 6. Effects of fenofibrate (■) compared with control (■) on the mRNA expression of the genes related to lipid transport metabolism in liver of Nile tilapia fed with high-protein (HP) or low-protein (LP) diet. (a, b) Relative mRNA abundance of CD36 and fatty acid transport protein 5 (FATP5), showing the ability of fatty acid uptake. (c, d) Relative mRNA abundance of microsomal TAG transfer protein (MTP) and ApoB, showing the activities of TAG-rich lipoprotein assembling and excretion. Values are means (n 3), with standard deviations represented by vertical bars. Mean values were significantly different: * $P < 0.05$, ** $P < 0.01$ (t test).

on Nile tilapia showed that the time to first spawning was significantly longer when fed at low gross energy (14.6 MJ/kg, 30% protein group) than those fed at 16.7 MJ/kg, 35 and 40% protein groups⁽³⁴⁾. Another study also showed that Nile tilapia fed with low-energy diets (12.56 MJ/kg, 30% protein) exhibited extremely poor growth and feed conversion efficiency with high mortality rates, compared with those fed with moderate- (16.75 MJ/kg, 40% protein) and high-energy (20.93 MJ/kg, 50% protein) diets⁽¹⁹⁾. Furthermore, fenofibrate was reported to exacerbate energy stress and cell death in three human tumour cell lines which had been induced energy stress by 2-deoxy-glucose (a glycolytic inhibitor)⁽³⁵⁾. In agreement with these studies, increasing oxidative stress and HSI could further reflect the energy stress induced by fenofibrate in the low energy intake situation (LP-fed fish) (14.5 MJ/kg, Supplementary Table S1). Therefore, the adverse effect of fenofibrate in the LP group might be related to the low energy intake.

Compared with earlier studies, the lipid-lowering effects, accompanied with the increased lipid-catabolism-related genes expression, were consistent only at the HP or high lipid level, instead of at the LP or low lipid level⁽¹⁴⁾. PPAR α is recognised as a master regulator of lipid catabolism and could be induced by fenofibrate in both fish and mammals^(1,13,36). Interestingly, in the present study, PPAR β expression instead of PPAR α increased significantly, accompanied with a decreased HL content in the HP group after fenofibrate treatment, in contrast to previous studies that specific PPAR α was activated in fish or mammals^(13,37). Previous studies generally used diet with

different fat levels rather than different protein levels, which may partly explain the discrepancy among different studies. In addition, PPAR β was reported to play a role as a transcription repressor-regulator of PPAR α and PPAR γ in 3T3-cells, with the blocked expression of acyl-CoA oxidase⁽³⁸⁾. Similarly, our results showed that PPAR β activation was concomitant with decreasing ACO expression (Fig. 6(a)). Furthermore, CPT1a was induced in the HP group by fenofibrate (Fig. 6(b)), and a previous study showed that CPT1 mRNA expression was highly correlated with the expression of PPAR β in gilthead sea bass white muscle and liver⁽³⁹⁾. In contrast to the hypolipidaemic effect of PPAR α activation mediated by fenofibrate in HFD, PPAR β was more sensitive to fenofibrate activation in the HP-diet-fed fish. On the other hand, dietary fenofibrate addition induced HL depletion only in high-lipid or HP diet rather than low-lipid or LP diets. The HP diet and HFD could be classified as 'high energy' based on the calculation above^(14,19). Furthermore, fenofibrate increased HSI and induced dysfunction of lipid metabolism in the LP group, in contrast to previous studies which found no alteration of hepatic and plasma lipid content in low-fat-diet-fed fish⁽¹⁴⁾. In fact, when the exact dietary energy was calculated, the gross energy of the low-fat feed was much higher than the LP diet, even close to the HP diet⁽¹⁴⁾. Therefore, these results consistently suggested that the lipid-lowering or adverse effect of fenofibrate in Nile tilapia might dependent on the energy intake of fish feed. Nevertheless, fenofibrate only significantly reduced HL contents but compensatorily up-regulated the lipid-anabolism-related genes FAS and Srebp1c expressions in the HP-fed fish. However, there were no compensatory effect was found in fish fed with HFD^(13,14). Thus, these

results indicated that the lipid-lowering effect of fenofibrate in fish might not only related to the feed energy content, but also the dietary nutrient composition.

Conclusion

In the HFD-fed mammals, PPAR α is regarded as an important transcriptional regulator of the genes involved in lipid breakdown and could be activated by fibrates in mammals. In the present study, we demonstrated that the metabolic regulation of fenofibrate was closely related to both dietary protein and energy levels. We also illustrated that fenofibrate activated PPAR β mRNA expression and played a hypolipidaemic effect only under the HP feeding condition. At the LP-diet-feeding situation, Nile tilapia showed no lipid-lowering effect but induced adverse effects.

Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/S0007114519001594>

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L. N. and Z.-Y. D. designed the study. Y. L. and L. N. conducted the research, collected and extracted the data. W. W. performed the statistical analysis. L. N. wrote the draft of the manuscript. Z.-Y. D., W. W., Y. L. and L. C. critically revised the manuscript and approved the final version of manuscript to be submitted. All authors read and approved the final version of the article.

The authors declare that there are no competing interests associated with the paper.

References

- Fabbrini E, Mohammed BS, Korenblat KM, *et al.* (2010) Effect of fenofibrate and niacin on intrahepatic triglyceride content, very low-density lipoprotein kinetics, and insulin action in obese subjects with nonalcoholic fatty liver disease. *J Clin Endocr Metab* **95**, 2727–2735.
- Desager JP, Horsmans Y, Vandenplas C, *et al.* (1996) Pharmacodynamic activity of lipoprotein lipase and hepatic lipase, and pharmacokinetic parameters measured in normolipidaemic subjects receiving ciprofibrate (100 or 200 mg/day) or micronised fenofibrate (200 mg/day) therapy for 23 days. *Atherosclerosis* **124**, S65.
- Juha V, George S, Jean-Claude A, *et al.* (2002) Fenofibrate lowers plasma triglycerides and increases LDL particle diameter in subjects with type 2 diabetes. *Diabetes Care* **25**, 627.
- Black RN, Ennis CN, Young IS, *et al.* (2014) The peroxisome proliferator-activated receptor alpha agonist fenofibrate has no effect on insulin sensitivity compared to atorvastatin in type 2 diabetes mellitus: a randomised, double-blind controlled trial. *J Diabetes Complicat* **28**, 323–327.
- Rakhshandehroo M, Knoch B, Muller M, *et al.* (2010) Peroxisome proliferator-activated receptor alpha target genes. *PPAR Res* **2010**, 393–416.
- Mancini F, Lanni A, Sabatino L, *et al.* (2001) Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats. *FEBS Lett* **491**, 154–158.
- Shin SJ, Lim JH, Chung S, *et al.* (2009) Peroxisome proliferator-activated receptor- α activator fenofibrate prevents high-fat diet-induced renal lipotoxicity in spontaneously hypertensive rats. *Hypertens Res* **32**, 835–845.
- Guerre-Millo M, Gervois P, Raspé E, *et al.* (2000) Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* **275**, 16638–16642.
- Knapik-Czajka M, Gozdzińska A & Jaskiewicz J (2009) Adverse effect of fenofibrate on branched-chain α -ketoacid dehydrogenase complex in rat's liver. *Toxicology* **266**, 1–5.
- Du ZY, Clouet P, Huang LM, *et al.* (2008) Utilization of different dietary lipid sources at high level in herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic fatty acid oxidation. *Aquacult Nutr* **14**, 77–92.
- Guo X, Liang XF, Fang L, *et al.* (2015) Effects of lipid-lowering pharmaceutical clofibrate on lipid and lipoprotein metabolism of grass carp (*Ctenopharyngodon idella* Val.) fed with the high non-protein energy diets. *Fish Physiol Biochem* **41**, 331–343.
- Du ZY, Clouet P, Degrace P, *et al.* (2008) Hypolipidaemic effects of fenofibrate and fasting in the herbivorous grass carp (*Ctenopharyngodon idella*) fed a high-fat diet. *Brit J Nutr* **100**, 1200–1212.
- Zheng JL, Luo Z, Hu W, *et al.* (2015) Dietary fenofibrate reduces hepatic lipid deposition by regulating lipid metabolism in yellow catfish *Pelteobagrus fulvidraco* exposed to waterborne Zn. *Lipids* **50**, 417–426.
- Ning L-J, He A-Y, Lu D-L, *et al.* (2017) Nutritional background changes the hypolipidemic effects of fenofibrate in Nile tilapia (*Oreochromis niloticus*). *Sci Rep* **7**, 41706.
- Ding L, Yongqing M & Fasheng C (1990) Nutritional lipid liver disease of grass carp *Ctenopharyngodon idellus* (C. et V.). *Chin J Oceanol Limnol* **8**, 363–373.
- Abdel-Tawwab M, Ahmad MH, Khattab YAE, *et al.* (2010) Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture* **298**, 267–274.
- Wang KW, Takeuchi T & Watanabe T (1985) Effect of dietary protein levels on growth of *Tilapia nilotica*. *Nippon Suisan Gakkaishi* **51**, 133–140.
- Kobayashi R, Murakami T, Obayashi M, *et al.* (2002) Clofibrac acid stimulates branched-chain amino acid catabolism by three mechanisms. *Arch Biochem Biophys* **407**, 231–240.
- El-Sayed AFM & Teshima SI (1992) Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry. *Aquaculture* **103**, 55–63.
- Lambert P & Dehnel PA (1974) Seasonal variations in biochemical composition during the reproductive cycle of the intertidal gastropod *Thais lamellosa* Gmelin (*Gastropoda*, *Prosobranchia*). *Can J Zool* **52**, 305–318.
- Ternes T, Stumpf M, Schuppert B, *et al.* (1998) Simultaneous determination of antiseptics and acidic drugs in sewage and river water. *Vom Wasser* **90**, 295–309.
- Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. *Water Res* **32**, 3245–3260.
- Madureira TV, Rocha MJ, Cruzeiro C, *et al.* (2012) The toxicity potential of pharmaceuticals found in the Douro River estuary

- (Portugal): evaluation of impacts on fish liver, by histopathology, stereology, vitellogenin and CYP1A immunohistochemistry, after sub-acute exposures of the zebrafish model. *Environ Toxicol Pharmacol* **34**, 34–45.
24. Lavrentyev EN, Matta SG & Cook GA (2004) Expression of three carnitine palmitoyltransferase-I isoforms in 10 regions of the rat brain during feeding, fasting, and diabetes. *Biochem Biophys Res Commun* **315**, 174–178.
 25. Morash AJ & Le MCG (2010) Genome duplication events have led to a diversification in the CPT I gene family in fish. *Am J Physiol Regul Integr Comp Physiol* **299**, R579.
 26. Frohnert B & Bernlohr D (2000) Regulation of fatty acid transporters in mammalian cells. *Progr Lipid Res* **39**, 83–107.
 27. Hirsch D, Stahl A & Lodish HF (1998) A family of fatty acid transporters conserved from mycobacterium to man. *Proc Natl Acad Sci U S A* **95**, 8625–8629.
 28. Gessner DK, Grone B, Rosenbaum S, *et al.* (2015) Treatment of lactating sows with clofibrate as a synthetic agonist of PPARalpha does not influence milk fat content and gains of liters. *BMC Vet Res* **11**, 54.
 29. Hoivik DJ, Qualls CW, Mirabile RC, *et al.* (2004) Fibrates induce hepatic peroxisome and mitochondrial proliferation without overt evidence of cellular proliferation and oxidative stress in cynomolgus monkeys. *Carcinogenesis* **25**, 1757–1769.
 30. Oosterveer MH, Grefhorst A, van Dijk TH, *et al.* (2009) Fenofibrate simultaneously induces hepatic fatty acid oxidation, synthesis, and elongation in mice. *J Biol Chem* **284**, 34036–34044.
 31. Kamalzadeh A, Bruchem JV, Koops WJ, *et al.* (1997) Feed quality restriction and compensatory growth in growing sheep: feed intake, digestion, nitrogen balance and modelling changes in feed efficiency. *Livest Prod Sci* **52**, 209–217.
 32. Carmona M, Louche K, Nibbelink M, *et al.* (2005) Fenofibrate prevents Rosiglitazone-induced body weight gain in ob/ob mice. *Int J Obesity* **29**, 864–871.
 33. Gibbons GF, Wiggins D, Brown A-M, *et al.* (2004) Synthesis and function of hepatic very-low-density lipoprotein. *Biochem Soc Trans* **32**, 59–64.
 34. Abdelfattahm ES & Mamdouh K (2008) Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. *Aquaculture* **280**, 179–184.
 35. Liu H, Kurtoglu M, Leónannicchiarico CL, *et al.* (2016) Combining 2-deoxy-D-glucose with fenofibrate leads to tumor cell death mediated by simultaneous induction of energy and ER stress. *Oncotarget* **7**, 36461–36473.
 36. Ibabe A, Herrero A & Cajaraville MP (2005) Modulation of peroxisome proliferator-activated receptors (PPARs) by PPAR α - and PPAR γ -specific ligands and by 17 β -estradiol in isolated zebrafish hepatocytes. *Toxicol In Vitro* **19**, 725–735.
 37. Casas F, Pineau T, Rochard P, *et al.* (2000) New molecular aspects of regulation of mitochondrial activity by fenofibrate and fasting. *FEBS Lett* **482**, 71–74.
 38. Shi Y, Hon M & Evans RM (2002) The peroxisome proliferator-activated receptor delta, an integrator of transcriptional repression and nuclear receptor signaling. *Proc Natl Acad Sci U S A* **99**, 2613–2618.
 39. Boukouvala E, Leaver MJ, Favre-Krey L, *et al.* (2010) Molecular characterization of a gilthead sea bream (*Sparus aurata*) muscle tissue cDNA for carnitine palmitoyltransferase 1B (CPT1B). *Comp Biochem Physiol B: Biochem Mol Biol* **157**, 189–197.