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Dietary chenodeoxycholic acid attenuates high-fat diet-induced growth retardation, lipid accumulation and bile acid metabolism disorder in the liver of yellow catfish *Pelteobagrus fulvidraco*

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Abstract

This experiment was conducted to investigate whether dietary chenodeoxycholic acid (CDCA) could attenuate high-fat (HF) diet-induced growth retardation, lipid accumulation and bile acid (BA) metabolism disorder in the liver of yellow catfish *Pelteobagrus fulvidraco*. Yellow catfish (initial weight: 4·40 (SEM 0·08) g) were fed four diets: the control (105·8 g/kg lipid), HF diet (HF group, 159·6 g/kg lipid), the control supplemented with 0·9 g/kg CDCA (CDCA group) and HF diet supplemented with 0·9 g/kg CDCA (HF + CDCA group). CDCA supplemented in the HF diet significantly improved growth performance and feed utilisation of yellow catfish ($P < 0·05$). CDCA alleviated HF-induced increment of hepatic lipid and cholesterol contents by down-regulating the expressions of lipogenesis-related genes and proteins and up-regulating the expressions of lipololysis-related genes and proteins. Compared with the control group, CDCA group significantly reduced cholesterol level ($P < 0·05$). CDCA significantly inhibited BA biosynthesis and changed BA profile by activating farnesoid X receptor ($P < 0·05$). The contents of CDCA, taurochenodeoxycholic acid and glycochenodeoxycholic acid were significantly increased with the supplementation of CDCA ($P < 0·05$). HF-induced elevation of cholic acid content was significantly attenuated by the supplementation of CDCA ($P < 0·05$). Supplementation of CDCA in the control and HF groups could improve the liver antioxidant capacity. This study proved that CDCA could improve growth retardation, lipid accumulation and BA metabolism disorder induced by HF diet, which provided new insight into understanding the physiological functions of BA in fish.

Keywords: **Chenodeoxycholic acid: Lipid metabolism: Bile acid: High-fat diet: Vertebrates**

Lipids, one of the important macronutrients, are necessary for aquatic animals and play a crucial role in fish feed as a non-protein energy source. Recently, high-fat (HF) diet has been widely used in aquaculture due to its protein-saving effect and excellent performance in reducing nitrogen waste[1,2]. However, long-term HF diet causes lipid accumulation in the liver of fish, which leads to liver damage, oxidative stress and metabolic disease, and ultimately increased mortality[3,4]. Meanwhile, HF diet is considered to be the main factor causing fatty liver in cultured fish[2]. Thus, the significance of controlling the balance of the hepatic lipid metabolism is obvious in the prevention of fatty liver and other metabolic diseases. Recently, to improve the adverse effects of long-term HF diet, various feed additives have been applied to aquaculture production processes. For example, dietary sodium butyrate supplementation alleviates the negative influence of HF diet in largemouth bass (*Micropterus salmoides*) by reducing hepatic lipid accumulation and enhancing hepatic antioxidant activity[5]. Choline has been reported to alleviate liver damage caused by HF diet through regulating lipid metabolism, reducing lipid droplet accumulation and suppressing inflammation in juvenile black seabream (*Acanthopagrus schlegelii*)[6]. The study on large yellow croaker (*Larimichthys crocea*) found that supplementation of L-carnitine to HF diet could improve lipid metabolism by promoting fatty acid β-oxidation and inhibiting endoplasmic reticulum stress pathway[7]. Therefore, dietary supplementation with feed

Abbreviations: BA, bile acid; CA, cholic acid; CAT, catalase; CDCA, chenodeoxycholic acid; FBW, final body weight; FXR, farnesoid X receptor; HF, high fat; MDA, malondialdehyde; ROS, reactive oxygen species; SGR, specific growth rate; SHP, short heterodimeric partner; SREBP, sterol regulatory element binding protein; TC, total cholesterol; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; WG, weight gain.

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additives has emerged as a promising strategy to ameliorate the negative effects of HF diet.

Bile acids (BA) are increasingly regarded as complex metabolic integrators and signalling factors[8,9]. Primary BA are synthesised from the cholesterol in the liver through cytochrome P450-mediated oxidation[10]. Then the primary BA are combined with glycine or taurine to form conjugated BA[10,11], and almost all BA conjugates are bound to taurine in fish[12]. They are secreted and stored in the gallbladder until secreted in the intestine, where the intestinal microbiota produces secondary BA through deconjugation, dehydrogenation, exomerism and 7*α*/ *β*-dehydroxylation of conjugated BA[10]. Most of the BA are reabsorbed in the intestine and transported back to the liver *via* enterohepatic circulation[13]. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are two primary BA synthesised in the liver of mammals and fish[10,14]. As one of the main primary BA, CDCA has been shown to be an endogenous farnesoid X receptor (FXR) ligand that plays an important role in regulating lipid and BA metabolism[8,14]. Activated FXR induces short heterodimeric partners (SHP) to inhibit the transcription of cholesterol 7*α*-monooxygenase a1 (CYP7A1) and sterol 12*α*-hydroxylase (CYP8B1) genes, ultimately inhibiting BA biosynthesis[8,9]. FXR is also a regulator of hepatic fatty acid and cholesterol biosynthesis, which mediates the encoding of genes for lipogenic and cholesterol production in a SHP-sterol regulatory element binding protein (SREBP)-dependent manner[15,16]. Additionally, activation of FXR regulates fatty acid *β*-oxidation by activating the *β*-oxidation gene PPAR*α* at the transcriptional level[8,17]. HF diet induces excessive production of reactive oxygen species (ROS), which leads to damage of mitochondrial structure and function, excessive lipid accumulation and ultimately liver damage[18]. Studies pointed out that activation of FXR could reduce the production of ROS by activating transcription of antioxidant-related genes[19,20]. Thus, CDCA is expected to become a new direction for the treatment and prevention of obesity and related diseases.

In recent years, BA have received extensive attention as an additive in aquaculture. Previous studies have shown that BA could improve growth performance and reduce hepatic lipid deposition in fish[21–23]. Dietary BA improved metabolic liver diseases such as fatty liver and immune dysfunction caused by HF or high plant protein diets[22,24,25]. It has been reported that taurocholic acid alleviates excessive hepatic lipid accumulation caused by HF diet *via* regulating BA metabolism in juvenile grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂)[25]. Supplementation of porcine bile extract in HF diet had no effect on growth performance of tiger puffer (*Takifugu rubripes*) but decreased liver lipid accumulation and serum TAG and cholesterol contents[21]. A study on grass carp (*Ctenopharyngodon idella*) reported that supplementation of porcine bile extract in HF diet could improve growth by reducing lipid content and increasing protein synthesis[26]. Among various BA, CDCA, as the most effective natural activator of FXR, plays an important role in regulating lipid metabolism in fish. The positive effect of dietary CDCA supplementation, which could improve growth performance and lipid metabolism, was confirmed in fish[14,27,28]. It has been reported that 900 mg/kg CDCA supplementation in HF diet could inhibit the expression of lipogenesis-related genes and promote the expression of lipolysis genes by activating FXR, and ultimately reduce lipid accumulation in the liver of large yellow croaker[14]. Moreover, dietary 900 mg/kg CDCA supplementation could promote fat digestion and absorption, improve antioxidant capacity and ultimately reduce the adverse effects of HF diet on juvenile largemouth bass[28]. In addition, supplementation of 900 mg/kg CDCA to high replacement of fish oil with soyabean oil could improve the growth performance and lipid deposition of liver in large yellow croaker[27]. Although these studies confirmed that CDCA plays a key role in fish nutrition, the regulatory mechanism of CDCA on liver lipid and BA metabolism in fish fed HF diet remains unclear. Therefore, it is worth exploring the regulatory mechanism of dietary CDCA to alleviate the disorder of lipid and BA metabolism in fish fed HF diet.

Yellow catfish (*Pelteobagrus fulvidraco*), widely farmed as an economic fish in many Asian countries, is used as a good animal model because its genome sequence is known[29]. In yellow catfish, adding too much or too little lipid to the diet could lead to various metabolic disorders, which can affect healthy growth. Studies demonstrated that the suitable feed lipid level of the juvenile yellow catfish is 10–12 %[30–32]. Our recent study found that the growth rate of yellow catfish fed with 6·98 % lipid diet significantly lower than that of yellow catfish fed with 11·3 % lipid diet[33]. When the lipid level in the diet exceeds 12 %, the liver has different degrees of fat degeneration and vesiculation[32]. In generally, raising the lipid content of feed to about 15 % is often used as a HF diet[32–34]. Previous research indicated that feeding yellow catfish with HF diet leads to lipid metabolism disorders, fatty liver and growth retardation[35]. This experiment was conducted to investigate the effects of HF diet supplemented with CDCA on growth performance, antioxidant capacity and lipid and BA metabolism of yellow catfish. Our findings contribute to the understanding of BA physiology and provide new insights into the beneficial effects of CDCA on controlling hepatic lipid metabolism in fish.

Materials and methods

All animal experiments performed in this experiment followed the Huazhong Agricultural University (HZAU) institutional ethical guidelines for the care and use of laboratory animals. At the same time, this experimental protocol was approved by the HZAU Ethics Committee.

Experimental diets

CDCA ($C_{24}H_{40}O_4$, 474-25-9, ≥ 98 % in purity) was purchased from Aladdin Company. Four diets were formulated in this study (online Supplementary Table S1), the control (105·8 g/kg lipid), HF diet (HF group, 159·6 g/kg lipid), control diet supplemented with 0·9 g/kg CDCA (CDCA group, 107·3 g/kg lipid) and HF diet supplemented with 0·9 g/kg CDCA (HF + CDCA group, 160·4 g/kg lipid), respectively. Dietary lipid and CDCA levels were added according to the previous study[14,33]. The feed formulation, processing and storage were similar to our previous reports[33].

Experimental fish, culture management and sampling

Yellow catfish were purchased from a commercial farm. The procedures for yellow catfish feeding and management were similar to our previous study[36]. In brief, 360 yellow catfish (body weight: mean 4·40 (SEM 0·08) g/fish, mixed sex) were randomly stocked in twelve 300-l tanks, 30 fish per tank, after 2 weeks of acclimation. Each treatment had three replicate tanks. Fish were fed to apparent satiation two times each day (08.00 and 16.00 hours, respectively). The feeding experiment lasted for 10 weeks. During the feeding period, water quality was monitored twice a week and followed below: water temperature 27·5–29·4°C, dissolved oxygen ≥ 6·5 mg/l, pH 7·82–7·95 and NH_4-N ≤ 0·05 mg/l.

At the end of the 10 weeks feeding experiment, all fish were fasted for 24 h to avoid the prandial effects. Then, they were euthanised with 100 mg/l/MS-222 solution (Sinopharm Chemical Reagent Co., Ltd., AE1052101) and sampled. Fish from each tank were counted and weighed to calculate their survival, weight gain (WG) and specific growth rate (SGR). Then, nine fish were selected from each tank and their final body weight (FBW), body length, liver weight and visceral mass were measured to calculate condition factor, viscerosomatic index and hepatosomatic index. Liver samples of three fish were randomly collected from each tank and used for oil red O staining and haematoxylin and eosin staining, respectively. The liver tissues of other fish were frozen in liquid N_2 and stored at −80°C for subsequent analysis of antioxidant activity, biochemical indicators, BA concentration and gene and protein levels.

Analysis of the proximate composition of diets

The diets were analysed for their proximate composition, including the contents of DM, crude protein, crude lipid and ash, based on the standard procedures[37]. Briefly, DM was determined by drying feed samples at 105°C for 48 h until constant weight. Crude protein and lipid contents were determined by the Kjeldahl method and Soxhlet ether method, respectively. The ash content was determined by burning the samples in muffle furnace at 550°C for 8 h.

Haematoxylin and eosin and oil red O observation in liver

The histochemical (haematoxylin and eosin and oil red O staining) observations were performed in liver tissue according to our previous protocols[36]. We randomly examined ten fields per sample and then quantified the relative area of lipid droplets in oil red O staining and vacuoles in haematoxylin and eosin staining with Image J (version 1.51, National Institute of Health).

Biochemical indicators

NEFA, TAG, total cholesterol (TC) content and soluble protein concentration were analysed with the corresponding commercial kits (A042-1-1, A110-1-1, A111-1-1, A045-2-1, Nanjing Jiancheng Bioengineering Institute). TAG and TC contents were determined by GPO-PAP and COD-PAP enzymatic methods at 510 nm and 500 nm, respectively. The determination of NEFA was based on the principle that NEFA combines with copper ions to form copper salts of fatty acids soluble in chloroform and was measured at 440 nm of a microplate reader (Tecan infinite M200). Soluble protein concentrations were measured by Bradford assay using bovine serum albumin as the standard.

Enzymatic activity assays

Catalase (CAT), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) contents were analysed with the corresponding commercial kits (A007-1-1, A001-1-2, A015-1-2, A003-1-2, Nanjing Jiancheng Bioengineering Institute). CAT activity was analysed using the molybdenene method at 405 nm. T-SOD was measured using hydroxylamine method at 550 nm. T-AOC was determined at 405 nm by the colorimetric method. MDA content was analysed using the thiobarbituric acid assay at 532 nm.

Quantitative real-time PCR analysis

Total RNA was isolated from liver tissue using the Trizol reagent (TaKaRa) and reverse transcribed into cDNA using a commercial kit (TaKaRa). Quantitative real-time PCR analysis was performed according to our previously published protocols[35]. Eight housekeeping genes *β-actin*, translation elongation factor (*elfa*), ubiquitin-conjugating enzyme (*ubce*), *β*-2-microglobulin (*b2m*), hypoxanthine-guanine phosphoribosyl transferase (*hprt*), TATA-box-binding protein (*tbp*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and tubulin*α*-chain (*tuba*) mRNA expression were determined by the Hieff quantitative real-time PCR 64 SYBR Green Master Mix (Yeasen), and the results were analysed using geNorm software (https://genorm.cmgg.be/) to select the two most stable genes as endogenous controls. The primers for quantitative real-time PCR analysis are presented in online Supplementary Table S2. The mRNA abundances were determined using the $2^{-\Delta\Delta Ct}$ method.

Immunoblotting analysis

According to the protocols in our previous studies[36], we used the immunoblotting to measure the protein levels of Srebp1c, Srebp2, Hmgcr, Ppar*α*, Fxr, Hnf4*α*, Cyp7a1 and Cyp27a1. Briefly, hepatocytes lysates were prepared with the RIPA buffer (Sigma) on ice for 30 min. Forty micrograms of protein from each sample was separated on the 12 % SDS-polyacrylamide gel and subsequently transferred to the polyvinylidene difluoride membranes, blocked with 8 % (w/v) non-fat milk in Tris-HCl buffer solution + Tween (TBST) buffer for 2 h and then washed 3 times with TBST buffer for 4 min each. Afterwards, the membranes were incubated with the primary antibody at 4°C overnight. After that, the membranes were incubated with the secondary antibody for 1 h at room temperature. The specific primary antibodies were followed: anti-SREBP1c (1:1000, ab28481; Abcam), anti-SREBP2 (1:2000, A13049; ABclonal), anti-HMGCR (1:2000, JF0981; HUABIO), anti-PPAR*α* (1:1000, EM1707–71; HUABIO), anti-FXR (1:1000, ab155124; Abcam), anti-HNF4*α* (1:2000, A20865; ABclonal), anti-CYP7A1 (1:1000, A10615; ABclonal), anti-CYP27A1 (1:1000, 14739-1-AP; Proteintech) and anti-GAPDH (1:10 000, 10494-1-AP; Proteintech). Secondary antibodies were HRP-conjugated anti-mouse IgG antibody

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(1:10 000, SA00001-1; Protein Technology) or anti-rabbit IgG antibody (1:10 000, SA00001-2; Protein Technology). Visualisation of protein bands was performed by a Vilber Fusion FX6 Spectra imaging system, followed by quantification using Image-Pro Plus 6.0 software (Media Cybernetics).

Quantitative bile acids analysis

BA composition was analysed in the liver by the ultra-high performance liquid chromatography-tandem mass spectrometry. The method allowed quantitative analysis of forty-eight BA (online Supplementary Table S3). The abbreviations for the forty-eight BA are shown in online Supplementary Table S3. Briefly, we weighed 2·5 mg of liver sample and added 50 μl of 100 ng/ml internal standard and 250 μl of 0·1 M NaOH. After vortexing, the samples were sonicated in the ice-water bath for 15 min. Then, they were incubated at 80°C for 1 h on a drying heater, followed by the addition of 700 μl of 2 % acetonitrile solution, and centrifuged (13 000 rpm/min) at 4°C for 5 min. The column was conditioned with methanol and equilibrated with 2 % acetonitrile solution, and 780 μl sample supernatant was added to the column, washed with 2 % acetonitrile solution, n-hexane, 2 % acetonitrile solution and methanol solution, respectively. The methanol solution was collected and dried with nitrogen and then reconstituted with 700 μl methanol, and the supernatant was taken for the detection on an AB Sciex API 4000 mass spectrometer (AB Sciex). Chromatographic separation was performed *via* a SynergiTM analytical column (100 mm × 3 mm, i.d.; 2·5 μm). The column temperature was 40°C, the flow rate 0·3 ml/min and the injection volume 10 μl. The mobile phase consisted of (a) 0·05 % formic acid and (b) acetonitrile in 0·05 % formic acid. Gradient elution was applied and MS detection was performed in negative mode. Electrospray ionisation was carried out in the form of negative ions with nitrogen as the atomising agent. The heated nebuliser temperature was set at 300°C and the capillary voltage was 4500 V. The AB Sciex API 4000 mass spectrometer operates at unit resolution in multiple reaction monitoring mode. Data acquisition and analysis were performed using the Analyst Software 1.5 and ACD/ChemSketch.

Statistical analysis

GraphPad Prism 8.0 (GraphPad Software Inc.) and software SPSS 19.0 (IBM, Armonk) were used for graphing and data analysis, respectively. Data were presented as mean values with their standard error of the means. Before statistical analysis, the data were analysed for normal distribution and homogeneity of variances using the Shapiro–Wilk test and the Levene test, respectively. Student's *t* test was used to determine statistical significance between two treatments. *P*-value < 0·05 was considered statistically significant.

Results

Growth performance, feed utilisation and morphological parameters

Compared with the control group, HF group significantly increased FCR ($P < 0·05$), and CDCA group significantly increased FBW, WG and SGR ($P < 0·05$) (Table 1). Compared with the HF group, HF + CDCA group significantly increased FBW, WG and SGR but significantly decreased FCR ($P < 0·05$) (Table 1). The differences in survival rate, hepatosomatic index, condition factor and viscerosomatic index were not statistically significant among the four groups ($P \geq 0·05$) (Table 1).

Hepatic histology, histochemistry, TAG, NEFA and total cholesterol contents

Compared with the control group, HF group significantly increased the amounts of cytoplasmic vacuoles and lipid droplets and TAG and NEFA contents ($P < 0·05$), and CDCA supplementation significantly attenuated HF-induced changes of these parameters ($P < 0·05$) (Fig. 1(a)–(f)). There was no significant difference in the amounts of cytoplasmic vacuoles and lipid droplets and TAG and NEFA contents between control group and CDCA group ($P \geq 0·05$). Compared with the control group, HF group significantly increased TC content ($P < 0·05$), and CDCA group significantly decreased TC content ($P < 0·05$). CDCA significantly attenuated HF-induced increase of TC content ($P < 0·05$) (Fig. 1(g)).

Expression of bile acid metabolism-related genes and proteins

To explore the effects of dietary lipid and CDCA supplementation on BA metabolism, the levels of relevant genes and proteins were detected (Fig. 2). Compared with the control group, HF group significantly reduced *fxr* and short heterodimeric partners (*shp*) mRNA levels but increased hepatocyte nuclear factor 4 (*hnf4α*) and liver X receptor (*lxr*) mRNA levels ($P < 0·05$), and CDCA group significantly increased *fxr* and *shp* mRNA levels but reduced *hnf4α* mRNA level ($P < 0·05$) (Fig. 2(a)). CDCA significantly attenuated HF-induced reduction of *fxr* and *shp* mRNA levels and abrogated HF-induced increase of *hnf4α* mRNA level ($P < 0·05$). The *lrh-1* mRNA level showed no significant differences among four groups ($P \geq 0·05$) (Fig. 2(a)).

Compared with the control group, HF group significantly increased sterol 12*α*-hydroxylase (*cyp8b1*), *cyp7a1* and sterol 27-hydroxylase (*cyp27a1*) mRNA levels but reduced *bsep* mRNA level ($P < 0·05$), and CDCA group significantly reduced *cyp7a1* and *cyp27a1* mRNA levels but increased *bsep* mRNA level ($P < 0·05$). CDCA significantly attenuated HF-induced increase of *cyp7a1*, *cyp8b1* and *cyp27a1* mRNA levels and abrogated HF-induced reduction of *bsep* mRNA level ($P < 0·05$) (Fig. 2(b)). The gene level of oxysterol 7-*α*-hydroxylase (*cyp7b1*) showed no significant differences among four groups ($P \geq 0·05$) (Fig. 2(b)).

Furthermore, compared with the control group, HF group significantly decreased the Fxr protein level but increased the Hnf4*α*, Cyp7a1, and Cyp27a1 protein levels ($P < 0·05$) (Fig. 2(c) and (d)), and CDCA group significantly increased the Fxr protein level but decreased the Hnf4*α*, Cyp7a1 and Cyp27a1 protein levels ($P < 0·05$) (Fig. 2(c) and (d)). CDCA significantly attenuated HF-induced down-regulation of Fxr protein level and up-regulation of Hnf4*α*, Cyp7a1 and Cyp27a1 protein levels ($P < 0·05$) (Fig. 2(c) and (d)).

Table 1. Effects of dietary lipid and CDCA supplementation on growth performance and biometric parameters of yellow catfish (Mean values with their standard error of the means)‡

Parameters	Control		HF		CDCA		HF + CDCA	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IBW (g)§	4·41	0·007	4·39	0·005	4·40	0·07	4·41	0·003
FBW (g)‖	16·29	0·86	15·83	0·25	18·31	0·19*	17·06	0·18†
WG (%)¶	269·38	3·34	260·06	5·47	311·32	4·65*	286·64	4·47†
SGR (%)**	3·53	0·10	3·48	0·32	3·74	0·21*	3·62	0·31†
FCR††	1·29	0·04	1·48	0·03*	1·30	0·03	1·36	0·06†
HSI (%)‡‡	1·65	0·03	1·75	0·06	1·69	0·05	1·62	0·03
VSI (%)§§	6·69	0·31	7·37	0·54	7·15	0·51	7·03	0·10
CF (%)‖‖	1·55	0·02	1·54	0·03	1·60	0·03	1·52	0·02
Survival (%)¶¶	96·67	1·92	96·67	1·92	97·78	2·22	97·78	1·11

HF, high-fat diet; CDCA, chenodeoxycholic acid.
* Indicates significant difference when comparing HF or CDCA with the control. $P < 0·05$.
† Indicates significant difference between HF and HF + CDCA, as determined by Student's *t* test. $P < 0·05$.
‡Values are means ± SEM (n 3). *P*-value was calculated by Student's *t* test.
§IBW, initial body weight.
‖FBW, final body weight.
¶WG (weight gain) = (FBW–IBW)/IBW × 100.
** SGR (specific growth rate) = 100 × (Ln FBW–Ln IBW)/feeding days.
††FCR (feed conversion ratio) = dry feed fed (g)/wet weight gain (g).
‡‡HSI (hepatosomatic index) = 100 × (liver weight)/(body weight).
§§VSI (viscerosomatic index) = viscera weight/whole body weight.
‖‖CF (condition factor) = 100 × (live weight, g)/(body length, cm)^3.
¶¶Survival = 100 × final fish number/initial fish number.

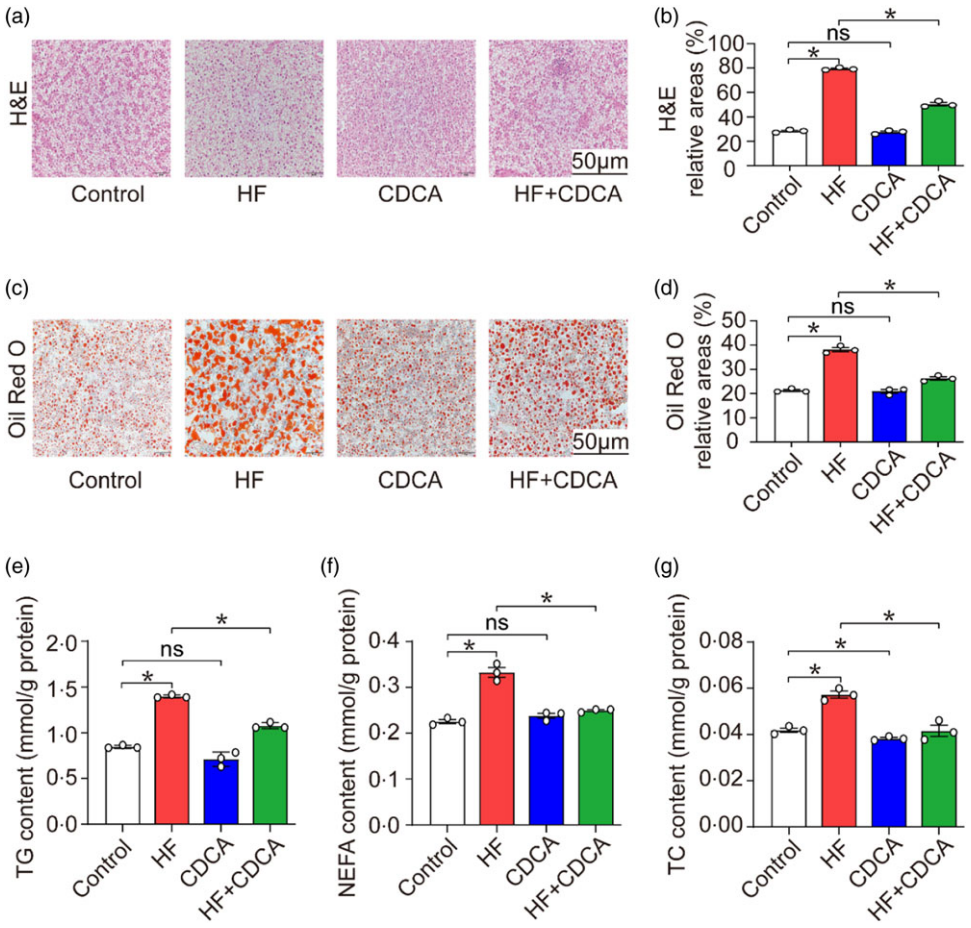

Fig. 1. Effects of dietary lipid and CDCA supplementation on lipid accumulation in the liver of yellow catfish. (a) Representative histology of H&E staining. Scale bar, 50 μm. (B) Relative areas for vacuoles in H&E staining. (c) Representative microphotograph of ORO staining, Scale bar, 50 μm. (d) Relative areas for lipid droplets in ORO staining. (e) TAG content. (f) NEFA content. (g) TC content. Values are means with their standard error of the means, n 3. *P*-value was calculated by Student's *t* test. *$P < 0·05$. ns, not significant. CDCA, chenodeoxycholic acid; H&E, haematoxylin and eosin; HF, high-fat diet; ORO, oil red O; TC, total cholesterol.

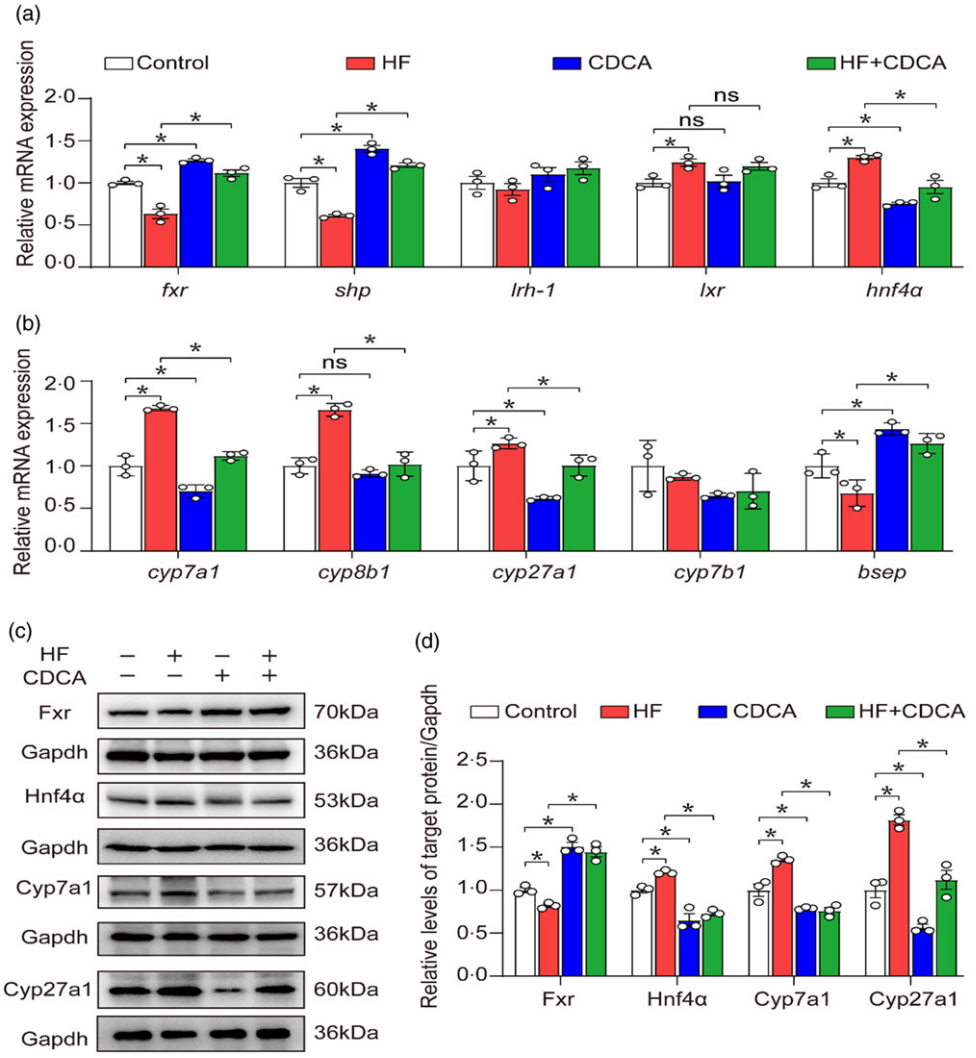

Fig. 2. Effects of dietary lipid and CDCA supplementation on bile acid metabolism in the liver of yellow catfish. (a) The mRNA levels of nuclear receptor genes relevant with bile acid metabolism. (b) The mRNA levels of genes involved in bile acid synthesis and transport. (c), (d) Western blot and protein levels of bile acid metabolism. Values are mean values with their standard error of the means, n 3. P-value was calculated by Student's t test. $^*P < 0{\cdot}05$. ns, not significant. *bsep*, bile salt export pump; CDCA, chenodeoxycholic acid; *cyp27a1*, sterol 27-hydroxylase; *cyp7a1*, cholesterol 7-α-monooxygenase; *cyp7b1*, oxysterol 7-α-hydroxylase; *cyp8b1*, sterol 12α-hydroxylase; *fxr*, farnesoid X receptor; HF, high-fat diet; *hnf4α*, hepatocyte nuclear factor 4; *lrh-1*, liver receptor homolog-1; *lxr*, liver X receptor; *shp*, short heterodimeric partners.

Hepatic bile acid profiles

To explore effects of dietary lipid and CDCA supplementation on the total bile acid pool, we measured the contents of various BA in the liver of yellow catfish. Ten BA were detected in the liver samples (concentration > 0·01 ng/mg). Compared with the control group, HF group significantly increased the contents of CA and TCA, and CDCA group significantly increased the contents of CDCA, TCA, TCDCA, GCDCA and GCA but reduced the GLCA content ($P < 0{\cdot}05$) (Fig. 3(a)). CDCA significantly attenuated the HF-induced increase of CA content ($P < 0{\cdot}05$). CDCA, TCDCA and GCDCA contents in HF + CDCA group were higher than that in the HF group. GDCA and GLCA contents in the HF + CDCA group were lower than that in the HF group (Fig. 3(a)).

Compared with the control group, HF group did not significantly influence total bile acid content ($P \geq 0{\cdot}05$), but CDCA group significantly increased the total bile acid content ($P < 0{\cdot}05$) (Fig. 3(b)). Compared with the control group, HF group increased glycine-conjugated BA content by 7·1 % and correspondingly reduced taurine-conjugated BA content by 7·1 %, and CDCA group reduced glycine-conjugated BA content by 1·84 % and correspondingly increased taurine-conjugated BA content by 1·84 % (Fig. 3(c)). Compared with the HF group, HF + CDCA group decreased the content of glycine-conjugated BA by 6·9 % and correspondingly increased the taurine-conjugated BA content by 6·9 % (Fig. 3(c)).

We then analysed the percentages of different BA in the liver of yellow catfish (Fig. 4). TCA, TCDCA and CA were the three dominant BA among the four treatments. The percentage for TCA was 92·9 %, 86·0 %, 48·6 % and 51·5 % for the control, HF, CDCA, and HF + CDCA groups respectively. The percentage for TCDCA was 2·70 %, 2·53 %, 49·1 % and 43·9 % for the control, HF, CDCA and HF + CDCA groups, respectively. The percentage for

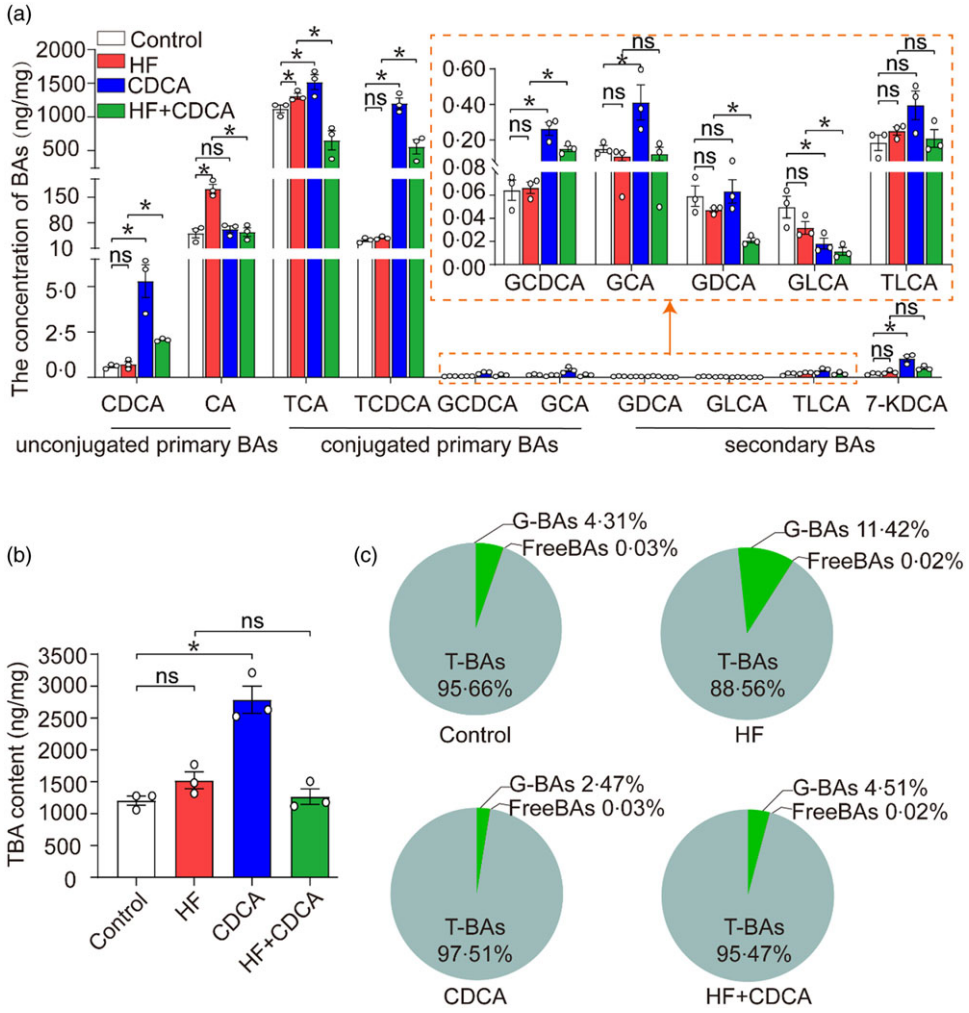

Fig. 3. Effects of dietary lipid and CDCA supplementation on liver bile acid contents of yellow catfish. (a) The content of ten species of bile acids. (b) Total bile acid content. (c) The percent ratio of three types of bile acids to the total bile acid. Values are mean values with their standard error of the means, *n* 3. *P*-value was calculated by Student's *t* test. **P* < 0·05. ns, not significant. 7-KDCA, 7-ketodeoxycholic acid; BA, bile acids; CA, cholic acid; CDCA, chenodeoxycholic acid; G-BA, glycine-conjugated bile acids; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; HF, high-fat diet; TBA, total bile acid; T-BA, taurine-conjugated bile acids; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid.

CA was 4·24 %, 11·36 %, 2·0 % and 4·3 % for the control, HF, CDCA and HF + CDCA groups, respectively.

Hepatic antioxidant response

To investigate the influence of dietary lipid and CDCA supplementation on antioxidants, the enzymes activity was detected (Fig. 5). Compared with the control group, HF group significantly reduced T-SOD, CAT and T-AOC activities ($P < 0·05$) and increased MDA content ($P < 0·05$), and CDCA group significantly increased T-SOD, CAT and T-AOC activities ($P < 0·05$), whereas the differences in MDA content were not significant ($P \geq 0·05$) (Fig. 5). CDCA significantly attenuated HF-induced decrease in the activities of T-SOD, CAT and T-AOC and increase in the MDA content ($P < 0·05$) (Fig. 5).

Expression of lipid metabolism-related genes and proteins

To further investigate the influence of dietary lipid and CDCA supplementation on lipid metabolism, mRNA and protein levels related to lipid metabolism were determined (Fig. 6). Compared with the control group, HF group significantly increased the mRNA levels of *srebp1c*, acetyl-CoA carboxylase (*accα*), sterol regulatory element-binding protein 2 (*srebp2*), fatty acid synthase (*fas*) and 3-hydroxy-3-methylglutaryl co-enzyme A reductase (*hmgcr*) ($P < 0·05$) but reduced *pparα* and *cpt1* mRNA levels ($P < 0·05$), and CDCA group significantly reduced *accα* and *hmgcr* gene levels ($P < 0·05$) but increased *pparα* and *cpt1* mRNA levels ($P < 0·05$) (Fig. 6(a)). CDCA significantly attenuated HF-induced increase of *srebp1c*, *accα*, *fas*, *srebp2* and *hmgcr* mRNA levels and abrogated HF-induced decline of *pparα* and *cpt1* mRNA levels ($P < 0·05$) (Fig. 6(a)). The gene level of adipose TAG lipase (*atgl*) showed no significant differences among four groups ($P \geq 0·05$) (Fig. 6(a)).

Furthermore, compared with the control group, HF group significantly increased Srebp1c, Srebp2 and Hmgcr protein levels ($P < 0·05$) but decreased the Pparα protein level ($P < 0·05$), and CDCA group significantly decreased Srebp1c, Srebp2 and Hmgcr protein levels ($P < 0·05$) but increased Pparα

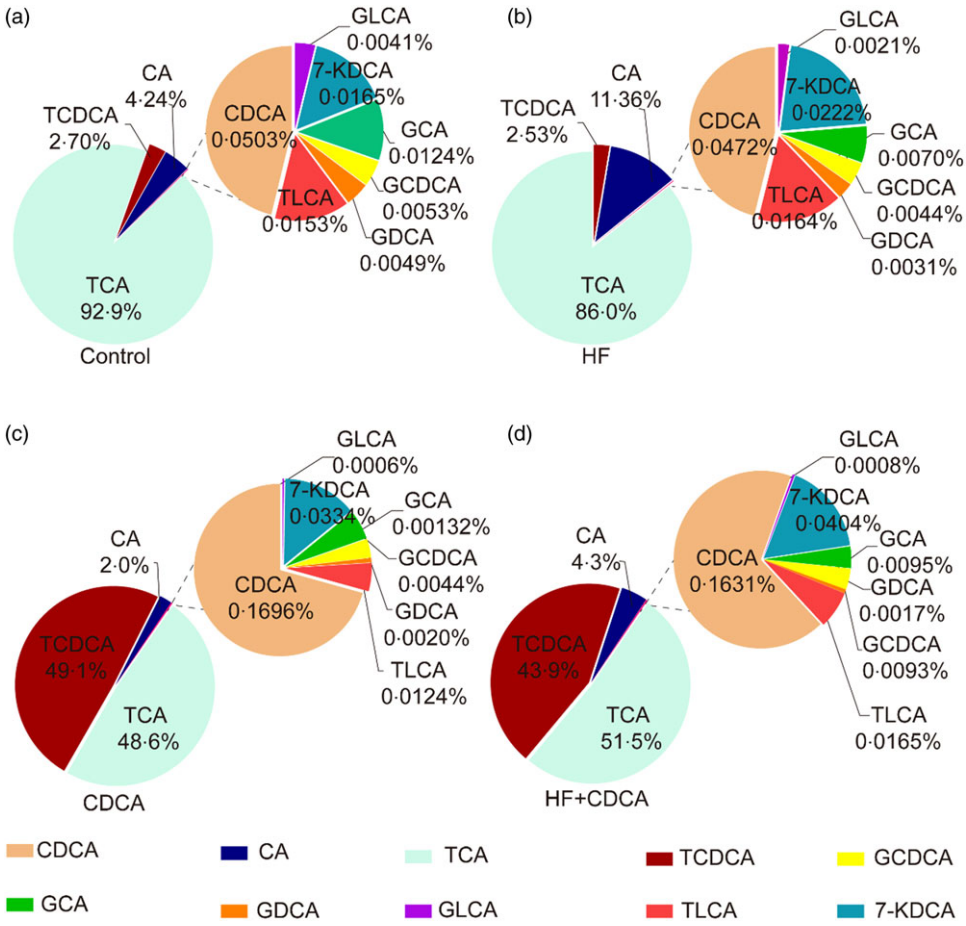

Fig. 4. Effects of dietary lipid and CDCA supplementation on liver bile acid composition profiles of yellow catfish. (a)–(d). Control, HF, CDCA and HF + CDCA, respectively. 7-KDCA, 7-ketodeoxycholic acid; BA, bile acids; CA, cholic acid; CDCA, chenodeoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; HF, high-fat diet; CDCA, chenodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid.

protein level ($P < 0{\cdot}05$). CDCA significantly alleviated HF-induced up-regulation of Srebp1c, Srebp2 and Hmgcr protein levels ($P < 0{\cdot}05$) and abrogated HF-induced down-regulation of Pparα protein level ($P < 0{\cdot}05$) (Fig. 6(b) and (c)).

Discussion

Given that BA play a crucial role in maintaining metabolic homoeostasis, BA have been extensively studied in fish. Our study aimed to investigate the effects of dietary CDCA addition on growth performance, antioxidant capacity, lipid and BA metabolism in yellow catfish. Due to increased costs and limited availability of high-quality protein sources in fishmeal, yellow catfish are intensively farmed and usually fed HF diet, with the aim of reducing the use of protein in feed by increasing dietary lipid level, thereby reducing the cost of farming and reducing the pressure on fishmeal supply[32]. However, excessive lipid in the feed may lead to liver metabolic disorders, ultimately affecting the healthy growth of fish[28,33]. Present study showed no significant difference in FBW, WG and SGR between HF group and control group, suggesting that HF diet did not promote the growth performance of yellow catfish. Similarly, Ling *et al.*[33] found that when the lipid level in the feed increased from 11·3 % to 15·4 %, the growth performance of the yellow catfish did not improve significantly. Zheng *et al.*[31] found that there was no significant difference in the growth of yellow catfish when dietary lipid content was 11·1 % and 15·1 %, while when dietary lipid content was 19·9 %, the growth and survival rate of yellow catfish were significantly reduced. A previous study showed that the growth performance of yellow catfish with a lipid content of 9·04 % in feed was better than that of yellow catfish with a lipid content of 15·81 % in feed[32]. Studies found that feeding HF diet did not promote or even decrease the growth of other fish[21,22,38], due to the reduced feed consumption and utilisation of other nutrients caused by excessive energy intake[1,38]. However, supplementation of CDCA in control and HF groups increased FBW, WG and SGR of yellow catfish, indicating that addition of CDCA improved growth performance. Similar results were found for large yellow croaker[22], largemouth bass[28] and grass carp[39] fed HF diet supplemented with BA. Furthermore, supplementation of CDCA to HF diet alleviated the HF-induced rise in FCR, suggesting that CDCA may improve dietary lipid utilisation in yellow catfish. Similarly, the FCR was significantly

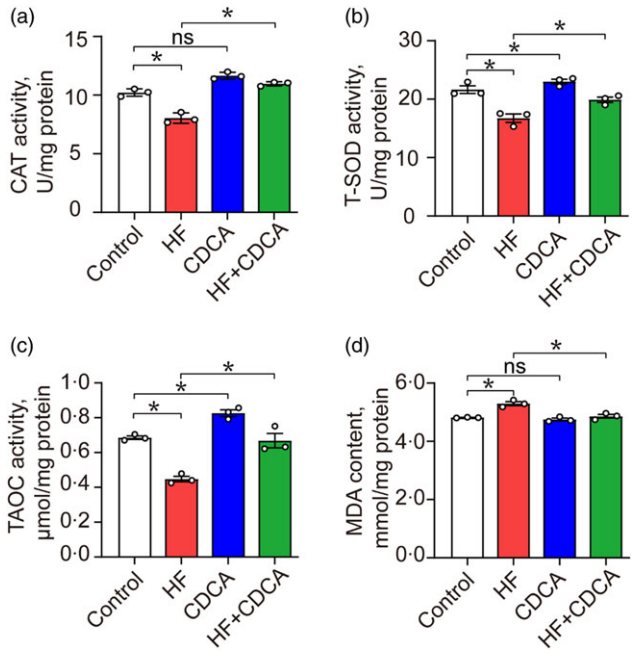

Fig. 5. Effects of dietary lipid and CDCA supplementation on liver antioxidant capacity of yellow catfish. (a) CAT activity. (b) T-SOD activity. (c) T-AOC activity. (d) MDA content. Values are mean values with their standard error of the means, *n* 3. *P*-value was calculated by Student's *t* test. **P* < 0·05. ns, not significant. CAT, catalase; CDCA, chenodeoxycholic acid; HF, high-fat diet; MDA, malondialdehyde; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase.

decreased with supplementation of BA (not mentioned) in HF diet in large yellow croaker[22]. Studies have shown that BA could activate the activity of intestinal lipase, lipoprotein lipase and liver lipase, which may be related to improved growth performance and feed utilisation[22,28,40].

In the present study, HF group increased hepatic lipid droplets, TAG, NEFA and TC contents of yellow catfish compared with the control group, suggesting that fish fed the HF diet possessed higher lipid accumulation. Similarly, a previous study found that HF diet led to an increase in the proportion of lipid droplets and lipid content in the liver of golden pompano (*Trachinotus ovatus*)[41]. In channel catfish (*Ictalurus punctatus*), Desouky *et al.*[42] reported that HF diet significantly increased hepatic TAG and TC contents compared with the control group diet. We found that lipid deposition in the liver of yellow catfish fed a HF diet was reversed by CDCA addition. Similarly, other studies have shown that supplementation with CDCA in HF diet reduced hepatic lipid content in large yellow croaker[14] and largemouth bass[28]. In addition, taurocholic acid sodium in grouper[25] and porcine bile extract in grass carp[26] have been reported to alleviate HF diet-induced liver lipid accumulation. Teodoro *et al.*[43] found that supplemented with CDCA in HF diet had lower lipid accumulation than these fed HF diet in mice. In this study, the decrease of lipid accumulation may be related to the activation of FXR by supplementing CDCA, thereby inhibiting the expression of adipogenic genes and promoting the expression of lipolysis genes. It is suggested that the supplementation of CDCA to HF diet may have a relieving effect on non-alcoholic fatty liver caused by HF diet.

As a BA receptor, FXR regulates genes responsible for maintaining the normal physiological function of the liver by binding to BA, thereby protecting the liver from adverse effects[8,17]. Activated FXR inhibits the transcription of CYP7A1 and CYP8B1 genes through activating SHP[8,9]. Studies suggested that CYP7A1 and CYP8B1 mediate the classical pathway of BA synthesis, thereby controlling BA homoeostasis[8]. In addition, CYP27A1 is a key enzyme in the alternative pathway of BA synthesis[8,9]. Activation of FXR positively induces transcription of the BA transporter BSEP, which transports BA from the liver into the bile ducts[17]. In this study, compared with the control group, HF group down-regulated the mRNA levels of *fxr*, *shp* and *bsep* and protein levels of Fxr and up-regulated the mRNA levels of *hnf4α*, *cyp7a1*, *cyp27a1* and *cyp8b1* and protein levels of Hnf4α, Cyp7a1 and Cyp27a1. These results suggested that HF diet increased the synthesis of BA and inhibited bile flow by inhibiting FXR expression, ultimately reducing the efficiency of enterohepatic circulation and digestion of dietary lipids. CYP7A1 and CYP8B1 are key enzymes in the synthesis of CA from cholesterol in the liver, and their elevation may lead to an increase in the content of hepatic CA in HF diet[44,45]. Du *et al.*[14] reported that HF diet decreased the mRNA abundances of *fxr*, *shp* and *bsep* and reduced the Fxr protein level compared with the control group in the liver of large yellow croaker. Xu *et al.*[25] found that HF diet increased the mRNA abundances of *cyp7b1* and *cyp27a1* and decreased the mRNA abundances of *bsep* compared with the control group in the liver of grouper. We found that CDCA increased the gene expression of *fxr*, *shp* and *bsep* and the protein level of Fxr and decreased the genes and proteins levels of Hnf4α, Cyp7a1 and Cyp27a1. Similarly, a study on grass carp reported that hepatic transcription levels of *cyp7a1*, *cyp7b1* and *cyp27a1* were down-regulated by lithocholic acid supplementation in the normal diet[46]. Yu *et al.*[47] reported that CDCA could activate *fxr* and *bsep* gene expression and repress *cyp7a1* gene expression in mammals. In human hepatocytes, CDCA incubation decreased *cyp7a1* and *hnf4α* mRNA expression and Hnf4α protein level but increased *shp* mRNA expression[48]. We found that supplementation of CDCA to HF diet alleviated the imbalance of BA homoeostasis caused by HF diet. Similarly, Du *et al.*[14] reported that *fxr*, *shp* and *bsep* gene expressions were increased in large yellow croaker fed a HF diet compared with fish fed with the control diet. Liao *et al.*[21] found that CDCA could alleviate HF diet-induced decrease in *fxr* gene expression and increase in *hnf4α* gene expression in tiger puffer. In addition, supplementation of porcine bile extract to the HF diet inhibited the transcription levels of *cyp7a1* and *cyp8b1*, which might be linked to the activation of FXR in common carp (*Cyprinus carpio*)[49]. A study on grouper showed that supplementation of taurocholic acid sodium to HF diet significantly increased the expression of FXR gene and protein and indicated that activation of FXR may be a key step for taurocholic acid sodium to affect BA metabolism[25]. Studies have shown that CDCA inhibits BA *de novo* synthesis by activating hepatic FXR and accelerates hepatic bile excretion, detoxification and recycling to protect hepatocytes from the deleterious consequences of BA[10,15]. Therefore, supplementation of CDCA to HF diet could activate the function of FXR to maintain BA homoeostasis in the liver of yellow catfish.

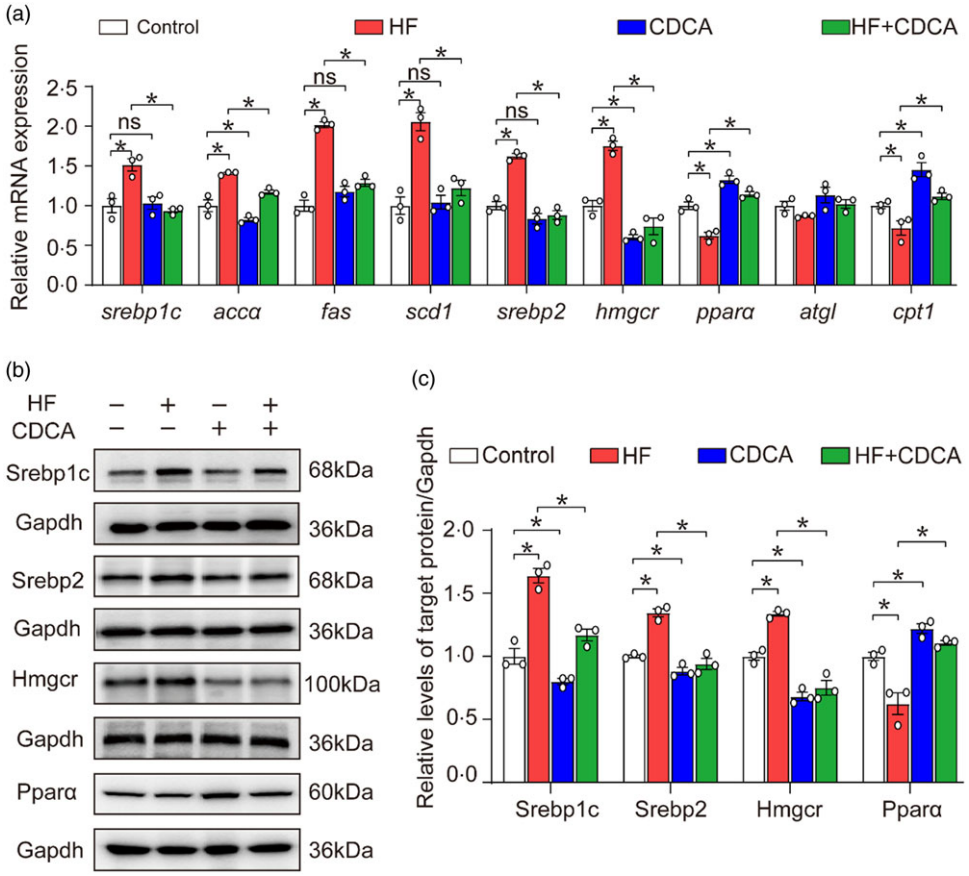

Fig. 6. Effects of dietary lipid and CDCA supplementation on lipid metabolism in the liver of yellow catfish. (a) The mRNA levels of genes in lipid metabolism. (b), (c) Western blot and protein levels of lipid metabolism. Values are mean values with their standard error of the means, *n* 3. *P*-value was calculated by Student's *t* test. $^{*}P < 0{\cdot}05$. ns, not significant. *accα*, acetyl-CoA carboxylase; *atgl*, adipose TAG lipase; CDCA, chenodeoxycholic acid; *cpt1*, carnitine palmitoyltransferase 1; *fas*, fatty acid synthase; HF, high-fat diet; *hmgcr*, 3-hydroxy-3-methylglutaryl co-enzyme A reductase; *scd1*, stearoyl-CoA desaturase1; *srebp1c*, sterol regulatory element binding proteins 1c; *srebp2*, sterol regulatory element binding proteins 2.

To investigate the effects of dietary lipid and CDCA supplementation on hepatic BA composition, we measured BA profiles of yellow catfish livers. In the present study, CDCA addition in control and HF diet increased hepatic CDCA, TCDCA and GCDCA contents. This may be related to the fact that most of the CDCA were reabsorbed by the liver to combine with taurine and glycine to form TCDCA and GCDCA, respectively. Studies have shown that a small fraction of CDCA were converted to LCA by gut microbiota 7*α*-dehydroxyacids, which are 7*α*-rehydroxylated in the liver to reform conjugated CDCA[50]. In tiger puffer, the hepatic TCDCA content was significantly increased by dietary taurine[51]. CDCA and TCDCA (FXR ligand)-mediated BA signalling has beneficial effects in maintaining normal BA and lipid metabolism in the liver[13]. TUDCA attenuates liver toxicity through multiple mechanisms, including stabilising mitochondrial integrity, promoting ATP synthesis and reducing oxidative damage[13]. In addition, conjugated BA play a dominant role in the liver, with taurine-conjugated BA being the highest. Hydrophobic BA are highly toxic, which could damage hepatocytes by inducing plasma membrane damage, mitochondrial damage and oxidative stress[44]. Recent studies suggested that the hydrophobic BA CA disrupts BA homoeostasis and induces metabolic disease in non-alcoholic fatty liver disease[44,45]. In the current study, compared with the control group, HF group enhanced hepatic CA content and proportion of yellow catfish, which may be one of the important factors for the destruction of lipid and BA homoeostasis. In the study of tiger puffer, dietary BA significantly reduced the content of CA in gallbladder[21]. CDCA mitigated the increase in CA content and proportion induced by HF diet, possibly due to the CDCA-activated FXR/SHP pathway interfering with CYP7A1 and CYP8B1 transcription, resulting in reduced cholesterol conversion to CA. Thus, CDCA alters BA distribution and inhibits BA production by activating FXR in the liver of yellow catfish.

Emerging evidence indicates that antioxidant capacity is closely related to lipid metabolism[52,53]. BA have the ability to induce antioxidant defence system to protect the liver from oxidative stress[46]. The CAT, T-SOD and T-AOC are key indicators for evaluating antioxidant capacity, and their main mechanism is to eliminate ROS to reduce damage to the organism[53]. MDA, an important lipid peroxidation end product, is considered as a principal marker of oxidative stress damage[53]. In the present study, HF group reduced T-SOD, CAT and T-AOC activities and increased MDA content compared with the control group, suggesting that the HF diet caused the lower antioxidant levels of yellow catfish. HF diet-induced

damage to the antioxidant system increases ROS production, and the production of excess ROS could disrupt mitochondrial structure and function, which causes lipid accumulation since mitochondria are the main site of fatty acid β-oxidation that negatively affects growth and health[52]. Similar results were found in Chinese perch (*Siniperca chuatsi*)[23], largemouth bass[28] and golden pompano[41] fed HF diet. In this study, we found that supplementation of CDCA in control and HF diets increased the activity of antioxidant enzymes and decreased MDA content, indicating that CDCA had a beneficial effect on the antioxidant capacity of yellow catfish. Similar results have been reported in large yellow croaker[22] and largemouth bass[28], in which dietary CDCA could promote the liver antioxidant capacity. In addition, BA (not mentioned) supplementation in HF diet improved the activities of SOD, CAT and T-AOC along with reduced hepatic MDA content in large yellow croaker[22] and largemouth bass[28]. Previous studies have shown that FXR could regulate the transcription of antioxidant-related genes, including superoxide dismutase and nuclear factor erythroid 2-related factor 2, to maintain cellular redox balance[19,20]. Thus, activation of FXR-mediated transcription of antioxidant enzymes may be the molecular mechanism of CDCA against ROS production. These data indicated that CDCA might alleviate oxidative damage induced by HF diet through FXR-mediated antioxidant defence system.

To further explore the effects of CDCA supplementation on lipid metabolism, the levels of relevant genes and proteins were determined. FXR, as the most potent natural ligand of CDCA, is an important regulator of lipid metabolism[15,54]. SREBP-1 is a key transcription factor that regulates the expression of lipid metabolism-related genes (*fas*, *acc* and *scd1*)[38]. Our study showed that HF group increased *srebp1c*, *accα*, *fas* and *scd1* gene levels and Srebp1 protein level compared with the control group, which may be the main reason for hepatic lipid accumulation. Previous studies have shown that HF diet also increased lipid synthesis gene expression in golden pompano, large yellow croaker and channel catfish compared with control[22,41,42]. In our study, compared with the control group, CDCA group decreased Srebp1c protein level and *accα* gene level. Liu *et al.*[50] reported that CDCA treatment inhibited SREBP1 protein level and *accα* mRNA level compared with the control group in mammals. We found that CDCA alleviated HF-induced increment of *srebp1c*, *accα*, *fas* and *scd1* gene levels, and Srebp1c protein level, which were similar in large yellow croaker[14]. The study reported that dietary BA (not mentioned) could alleviate the increased expression of lipid synthesis genes *srebp1*, *scd1* and *fas* induced by HF diet in large yellow croaker[22]. Further studies have demonstrated that the SREBP1 transcriptional activity was decreased by the activation of FXR *via* up-regulating the gene expression of SHP[15]. Thus, the present results suggested that the supplementation of CDCA reduced liver lipogenesis by activating FXR to reduce SREBP1 transcription in the liver of yellow catfish. SREBP2, a key mediator of cholesterol metabolism, is involved in regulating the expression of cholesterol synthesis rate-limiting enzyme HMGCR[16]. We found that HF group increased *srebp2* and *hmgcr* genes and proteins levels compared with the control group, suggesting that HF diet could promote cholesterol biosynthesis. Moreover, Betancor *et al.*[55] demonstrated that HF diet could promote cholesterol production by activating SREBP2 in Atlantic bluefin tuna (*Thunnus thynnus* L.). In the present study, compared with the control group, CDCA decreased the *hmgcr* mRNA expression and SREBP2 protein level. Studies have shown that CDCA reduced the mRNA levels of *srebp2* and *hmgcr* in mammals[56]. Moreover, we found that CDCA alleviated HF-induced increase of *srebp2* and *hmgcr* genes and proteins levels, which may contribute to the reduction of cholesterol. Similarly, Nilsson *et al.*[57] reported that *hmgcr* gene level was reduced by CDCA treatment in human liver compared with the control group. Previous research has shown that activation of FXR could inhibit SREBP2 expression to reduce cholesterol synthesis[56]. Therefore, it was concluded that the reasons for the decrease in liver cholesterol content may be caused by CDCA-activated FXR negatively regulating SREBP2 to reduce cholesterol biosynthesis. Studies suggested that fatty acid oxidation was an important pathway affecting lipid metabolism[27,58]. PPARα is a key regulator for the lipid metabolism by inducing the expression of fatty acid oxidation genes, such as CPT1[59]. CPT1 is the rate-limiting enzyme of β-oxidation, facilitating the entry of long-chain fatty acids into the mitochondria for the oxidation[59]. In the present study, compared with the control group, HF group decreased PPARα gene and protein level, and *cpt1* mRNA level, suggesting that HF diet inhibited lipolysis. Similar results were also reported in large yellow croaker[14] and black seabream[38]. We found that supplementation of CDCA to control and HF diet significantly increased PPARα gene and protein level, and *cpt1* gene level. Similarly, Du *et al.*[14] reported that the CDCA supplementation to HF diet increased *pparα* and *cpt1* gene levels compared with the control group diet in large yellow croaker. Ding *et al.*[22] reported that the gene expression levels of *pparα* and *cpt1* increased gradually with the increase of dietary BA (not mentioned) levels in large yellow croaker. Studies in Chinese perch[23], grass carp[26] and common carp[49] reported that supplementation of porcine bile extract to HF diet could promote lipolysis by enhancing PPARα. FXR is closely related not only to lipid synthesis but also to lipid degradation. In the liver, activation of FXR could up-regulate the expression of PPARα[8,17,60]. Thus, the present results suggested that the supplementation of CDCA improved fatty acid oxidation by increasing the expression of *pparα via* activating FXR in the liver of yellow catfish. Taken together, our data suggested that CDCA supplementation in HF diet inhibits lipogenesis and promotes lipid oxidation by activating FXR, thereby reducing lipid accumulation in the liver of yellow catfish.

Conclusion

In summary, our study indicated that CDCA supplementation could improve the growth performance and feed utilisation of yellow catfish. Supplementation of CDCA alleviated HF diet-induced hepatic lipid accumulation, low antioxidant capacity and BA metabolism disorders. Furthermore, the supplementation of CDCA to HF diet altered the BA profile of the yellow catfish liver, with increased CDCA, TCDCA and GCDCA contents and decreased CA content. In this study, CDCA-activated FXR

played a leading role in maintaining normal lipid and BA metabolism and enhancing antioxidant activity. These data indicated that CDCA has potential application value in improving liver lipid metabolism, which might provide new ideas for studying the physiological regulation mechanism of CDCA in fish.

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X.-Y. T. and H. Z. conceived and designed the experiments. H. Z. and Y.-C. X. performed the experiments. H. Z. analysed the data and drafted the manuscript. T. Z., D.-G. Z, C.-C. S. and A.-G Y. helped with the data analysis. X.-Y. T. and Z. L. revised the manuscript. X.-Y. T. obtained the fund.

No potential conflict of interest was disclosed.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114523002489

References

1. Boujard T, Gélineau A, Covès D, *et al.* (2004) Regulation of feed intake, growth, nutrient and energy utilisation in European seabass (*Dicentrarchus labrax*) fed high fat diets. *Aquaculture* **231**, 529–545.
2. Luo G, Xu JH, Teng YJ, *et al.* (2010) Effects of dietary lipid levels on the growth, digestive enzyme, feed utilization and fatty acid composition of Japanese sea bass (*Lateolabrax japonicus* L.) reared in freshwater. *Aquacult Res* **41**, 210–219.
3. Rueda-Jasso R, Conceicao LEC, Dias J, *et al.* (2004) Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese sole (*Solea senegalensis*) juveniles. *Aquaculture* **231**, 417–433.
4. Lu KL, Wang LN, Zhang DD, *et al.* (2017) Berberine attenuates oxidative stress and hepatocytes apoptosis *via* protecting mitochondria in blunt snout bream *Megalobrama amblycephala* fed high-fat diets. *Fish Physiol Biochem* **43**, 65–76.
5. Chen W, Gao S, Chang K, *et al.* (2023) Dietary sodium butyrate supplementation improves fish growth, intestinal microbiota composition, and liver health in largemouth bass (*Micropterus salmoides*) fed high-fat diets. *Aquaculture* **564**, 739040.
6. Jin M, Pan T, Tocher DR, *et al.* (2019) Dietary choline supplementation attenuated high-fat diet-induced inflammation through regulation of lipid metabolism and suppression of NFκB activation in juvenile black seabream (*Acanthopagrus schlegelii*). *J Nutr Sci* **8**, e38.
7. Li J, Zhang Z, Kong A, *et al.* (2022) Dietary L-carnitine regulates liver lipid metabolism *via* simultaneously activating fatty acid *β*-oxidation and suppressing endoplasmic reticulum stress in large yellow croaker fed with high-fat diets. *Br J Nutr* **129**, 29–40.
8. Han CY, Kim TH, Koo JH, *et al.* (2009) Farnesoid X receptor as a regulator of fuel consumption and mitochondrial function. *Arch Pharm Res* **39**, 1062–1074.
9. Russell DW (2009) Fifty years of advances in bile acid synthesis and metabolism. *J Lipid Res* **50**, 120–125.
10. Wang L, Sagada G, Wang CY, *et al.* (2023) Exogenous bile acids regulate energy metabolism and improve the health condition of farmed fish. *Aquaculture* **562**, 738852.
11. Ridlon JM, Kang DJ & Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* **47**, 241–259.
12. Hofmann AF, Hagey LR & Krasowski MD (2010) Bile salts of vertebrates: structural variation and possible evolutionary significance. *J Lipid Res* **51**, 226–246.
13. Bilbao-Malavé V, González-Zamora J, de la Puente M, *et al.* (2021) Mitochondrial dysfunction and endoplasmic reticulum stress in age related macular degeneration, role in pathophysiology, and possible new therapeutic strategies. *Antioxidants* **10**, 1170.
14. Du J, Xiang X, Xu D, *et al.* (2021) FXR, a key regulator of lipid metabolism, is inhibited by ER stress-mediated activation of JNK and p38 MAPK in large yellow croakers (*Larimichthys crocea*) fed high fat diets. *Nutrients* **1312**, 4343.
15. Watanabe M, Houten SM, Wang L, *et al.* (2013) Bile acids lower triglyceride levels *via* a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest* **113**, 1408–1418.
16. Chiang JY (2013) Bile acid metabolism and signaling. *Compr Physiol* **3**, 1191–1212.
17. Cariou B (2008) The farnesoid X receptor (FXR) as a new target in non-alcoholic steatohepatitis. *Diabetes Metab* **34**, 685–691.
18. Li J, Wang T, Liu P, *et al.* (2021) Hesperetin ameliorates hepatic oxidative stress and inflammation *via* the PI3K/AKT-Nrf2-ARE pathway in oleic acid-induced HepG2 cells and a rat model of high-fat diet-induced NAFLD. *Food Funct* **12**, 3898–3918.
19. Wang YD, Chen WD, Li C, *et al.* (2015) Farnesoid X receptor antagonizes JNK signaling pathway in liver carcinogenesis by activating SOD3. *Mol Endocrinol* **29**, 322–331.
20. Dong L, Han X, Tao X, *et al.* (2018) Protection by the total flavonoids from Rosa laevigata Michx fruit against lipopolysaccharide-induced liver injury in mice *via* modulation of FXR signaling. *Foods* **7**, 88.
21. Liao ZB, Sun B, Zhang QG, *et al.* (2020) Dietary bile acids regulate the hepatic lipid homeostasis in tiger puffer fed normal or high-lipid diets. *Aquaculture* **519**, 734935.
22. Ding T, Xu N, Liu YT, *et al.* (2020) Effect of dietary bile acid (BA) on the growth performance, body composition, antioxidant responses and expression of lipid metabolism-related genes of juvenile large yellow croaker (*Larimichthys crocea*) fed high-lipid diets. *Aquaculture* **518**, 734768.
23. Zhang Y, Feng H, Liang XF, *et al.* (2022) Dietary bile acids reduce liver lipid deposition *via* activating farnesoid X receptor, and improve gut health by regulating gut microbiota in Chinese perch (*Siniperca chuatsi*). *Fish Shellfish Immunol* **121**, 265–275.
24. Yao T, Gu X, Liang X, *et al.* (2021) Tolerance assessment of dietary bile acids in common carp (*Cyprinus carpio* L.) fed a high plant protein diet. *Aquaculture* **543**, 737012.
25. Xu J, Xie S, Chi S, *et al.* (2022) Protective effects of taurocholic acid on excessive hepatic lipid accumulation *via* regulation of bile acid metabolism in grouper. *Food Funct* **13**, 3050–3062.
26. Zhou JS, Chen HJ, Ji H, *et al.* (2018) Effect of dietary bile acids on growth, body composition, lipid metabolism and microbiota in grass carp (*Ctenopharyngodon idella*). *Aquacult Nutr* **24**, 802–813
27. Du J, Xu H, Li S, *et al.* (2017) Effects of dietary chenodeoxycholic acid on growth performance, body composition and related gene expression in large yellow croaker (*Larimichthys crocea*) fed diets with high replacement of fish oil with soybean oil. *Aquaculture* **479**, 584–590.

28. Yin P, Xie SW, Zhuang ZX, *et al.* (2021) Dietary supplementation of bile acid attenuate adverse effects of high-fat diet on growth performance, antioxidant ability, lipid accumulation and intestinal health in juvenile largemouth bass (*Micropterus salmoides*). *Aquaculture* **531**, 735864.
29. Gong G, Dan C, Xiao S, *et al.* (2018) Chromosomal-level assembly of yellow catfish genome using third-generation DNA sequencing and Hi-C analysis. *GigaScience* **7**, 120.
30. Han Q, Tian Z, Xia W, *et al.* (2005) Optimal dietary lipid requirement of yellow catfish *Pelteobagrus fulvidraco*. *Fish Sci* **24**, 8–11.
31. Zheng K, Zhu X, Han D, *et al.* (2010) Effects of dietary lipid levels on growth, survival and lipid metabolism during early ontogeny of *Pelteobagrus vachelli* larvae. *Aquaculture* **299**, 121–127.
32. Zhu H, Qiang J, He J, *et al.* (2021) Physiological parameters and gut microbiome associated with different dietary lipid levels in hybrid yellow catfish (*Tachysurus fulvidraco*♀× *Pseudobagrus vachellii*♂). *Comp Biochem Physiol Part D Genomics Proteomics* **37**, 100777.
33. Ling SC, Wu K, Zhang DG, *et al.* (2019) Endoplasmic reticulum stress-mediated autophagy and apoptosis alleviate dietary fat-induced triglyceride accumulation in the intestine and in isolated intestinal epithelial cells of yellow catfish. *J Nutr* **149**, 1732–1741.
34. Fei S, Xia Y, Chen Z, *et al.* (2022) A high-fat diet alters lipid accumulation and oxidative stress and reduces the disease resistance of overwintering hybrid yellow catfish (*Pelteobagrus fulvidraco*♀×*P. vachelli*♂). *Aquacult Rep* **23**, 101043.
35. Song YF, Hogstrand C, Ling SC, *et al.* (2020) Creb-Pgc1α pathway modulates the interaction between lipid droplets and mitochondria and influences high fat diet-induced changes of lipid metabolism in the liver and isolated hepatocytes of yellow catfish. *J Nutr Biochem* **80**, 108364.
36. Yang SB, Zhao T, Wu LX, *et al.* (2020) Effects of dietary carbohydrate sources on lipid metabolism and SUMOylation modification in the liver tissues of yellow catfish. *Br J Nutr* **124**, 1241–1250.
37. AOAC (2006) Association of Official Analytical Chemists, Official Methods of Analysis 16th. Arlington, VA: AOAC. 0066–961X.
38. Jin M, Pan T, Cheng X, *et al.* (2019) Effects of supplemental dietary L-carnitine and bile acids on growth performance, antioxidant and immune ability, histopathological changes and inflammatory response in juvenile black seabream (Acanthopagrus schlegelii) fed high-fat diet. *Aquaculture* **504**, 199–209.
39. Peng X, Feng L, Jiang W, *et al.* (2019) Supplementation exogenous bile acid improved growth and intestinal immune function associated with NF-κB and TOR signalling pathways in on-growing grass carp (*Ctenopharyngodon idella*): enhancement the effect of protein-sparing by dietary lipid. *Fish Shellfish Immunol* **92**, 552–569.
40. Jiang M, Wen H, Gou GW, *et al.* (2018) Preliminary study to evaluate the effects of dietary bile acids on growth performance and lipid metabolism of juvenile genetically improved farmed tilapia (*Oreochromis niloticus*) fed plant ingredient-based diets. *Aquacult Nutr* **24**, 1175–1183.
41. Li Y, Liang SS, Shao YR, *et al.* (2021) Impacts of dietary konjac glucomannan supplementation on growth, antioxidant capacity, hepatic lipid metabolism and inflammatory response in golden pompano (*Trachinotus ovatus*) fed a high fat diet. *Aquaculture* **545**, 737113.
42. Desouky HE, Jiang GZ, Zhang DD, *et al.* (2020) Influences of glycyrrhetinic acid (GA) dietary supplementation on growth, feed utilization, and expression of lipid metabolism genes in channel catfish (*Ictalurus punctatus*) fed a high-fat diet. *Fish Physiol Biochem* **46**, 653–663.
43. Teodoro JS, Zouhar P, Flachs P, *et al.* (2014) Enhancement of brown fat thermogenesis using chenodeoxycholic acid in mice. *Int J Obes* **38**, 1027–1034.
44. Li JN & Dawson PA (2019) Animal models to study bile acid metabolism. *Biochim Biophys Acta Mol Basis Dis* **1865**, 895–911.
45. Chun HJ, Shim YJ & Kwon YH (2022) Cholic acid supplementation accelerates the progression of nonalcoholic fatty liver disease to the procarcinogenic state in mice fed a high-fat and high-cholesterol diet. *J Nutr Biochem* **100**, 108869.
46. Xiong F, Wu S, Qin L, *et al.* (2019) Transcriptome analysis of grass carp provides insights into disease-related genes and novel regulation pattern of bile acid feedback in response to lithocholic acid. *Aquaculture* **500**, 613–621.
47. Yu J, Lo JL, Huang L, *et al.* (2002) Lithocholic acid decreases expression of bile salt export pump through farnesoid X receptor antagonist activity. *J Biol Chem* **277**, 31441–31447.
48. Li T, Jahan A & Chiang JY (2006) Bile acids and cytokines inhibit the human cholesterol 7 α-hydroxylase gene *via* the JNK/c-jun pathway in human liver cells. *Hepatology* **43**, 1202–1210.
49. Yang L, Liu M, Zhao M, *et al.* (2023) Dietary bile acid supplementation could regulate the glucose, lipid metabolism, and microbiota of common carp (*Cyprinus carpio* L.) fed with a high-lipid diet. *Aquacult Nutr* **2023**, 9953927.
50. Liu N, Zhao J, Wang J, *et al.* (2016) Farnesoid X receptor ligand CDCA suppresses human prostate cancer cells growth by inhibiting lipid metabolism *via* targeting sterol response element binding protein 1. *Am J Transl Res* **8**, 5118–5124.
51. Xu H, Zhang Q, Kim SK, *et al.* (2020) Dietary taurine stimulates the hepatic biosynthesis of both bile acids and cholesterol in the marine teleost, tiger puffer (*Takifugu rubripes*). *Br J Nutr* **123**, 1345–1356.
52. Noh K, Kim YM, Kim YW, *et al.* (2011) Farnesoid X receptor activation by chenodeoxycholic acid induces detoxifying enzymes through AMP-activated protein kinase and extracellular signal-regulated kinase 1/2-mediated phosphorylation of CCAAT/enhancer binding protein β. *Drug Metab Dispos* **39**, 1451–1459.
53. Zhang DG, Zhao T, Hogstrand C, *et al.* (2021) Oxidized fish oils increased lipid deposition *via* oxidative stress-mediated mitochondrial dysfunction and the CREB1-Bcl2-Beclin1 pathway in the liver tissues and hepatocytes of yellow catfish. *Food Chem* **360**, 129814.
54. Togawa N, Takahashi R, Hirai S, *et al.* (2013) Gene expression analysis of the liver and skeletal muscle of psyllium-treated mice. *Br J Nutr* **109**, 383–393.
55. Betancor MB, Ortega A, de la Gándara F, *et al.* (2019) Performance, feed utilization, and hepatic metabolic response of weaned juvenile Atlantic bluefin tuna (*Thunnus thynnus L.*): effects of dietary lipid level and source. *Fish Physiol Biochem* **45**, 697–718.
56. Kawabe Y, Shimokawa T, Matsumoto A, *et al.* (1995) The molecular mechanism of the induction of the low density lipoprotein receptor by chenodeoxycholic acid in cultured human cells. *Biochem Biophys Res Commun* **208**, 405–411.
57. Nilsson LM, Abrahamsson A, Sahlin S, *et al.* (2007) Bile acids and lipoprotein metabolism: effects of cholestyramine and chenodeoxycholic acid on human hepatic mRNA expression. *Biochem Biophys Res Commun* **357**, 707–711.
58. Pawlak M, Lefebvre P & Staels B (2015) Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* **623**, 720–733.

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59. López-Oliva ME, Garcimartin A & Muñoz-Martínez E (2017) Dietary *α*-lactalbumin induced fatty liver by enhancing nuclear liver X receptor *αβ*/sterol regulatory element-binding protein-1c/PPARγ expression and minimising PPAR*α*/carnitine palmitoyltransferase-1 expression and AMP-activated protein kinase *α* phosphorylation associated with atherogenic dyslipidaemia, insulin resistance and oxidative stress in Balb/c mice. *Br J Nutr* **118**, 914–929.
60. Pineda Torra I, Claudel T, Duval C, *et al.* (2003) Bile acids induce the expression of the human peroxisome proliferator-activated receptor α gene *via* activation of the farnesoid X receptor. *Mol Endocrinol* **17**, 259–272.