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 diet-induced growth retardation, lipid accumulation, and bile acid metabolism disorder in the liver of yellow catfish *Pelteobagrus fulvidraco*. Yellow catfish (initial weight: 4.40 ± 0.08 g) were fed four diets: the control (105.8 g kg\(^{-1}\) lipid), high fat diet (HF group, 159.6 g kg\(^{-1}\) lipid), the control supplemented with 0.9 g kg\(^{-1}\) CDCA (CDCA group) and high fat diet supplemented with 0.9 g kg\(^{-1}\) CDCA (HF+CDCA group). CDCA supplemented in the high fat diet significantly improved growth performance and feed utilization of yellow catfish (*P* < 0.05). CDCA alleviated HF-induced increment of hepatic lipid and cholesterol contents by down-regulating the expressions of lipologenation-related genes and proteins and up-regulating the expressions of lipololysis-related genes and proteins. Compared to the control group, CDCA group significantly reduced cholesterol level (*P* < 0.05). CDCA significantly inhibited bile acid biosynthesis and changed bile acid profile by activating farnesoid X receptor (FXR) (*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 control and HF groups could improve the liver antioxidant capacity. This study proved that CDCA could improve growth retardation, lipid accumulation, and bile acid metabolism disorder induced by high fat diet, which provided new insight into understanding the physiological functions of bile acids in fish.

**Keywords:** Chenodeoxycholic acid; Lipid metabolism; Bile acid; High fat diet; Vertebrates
Abbreviations: ACCα: acetyl-CoA carboxylase; ATGL, adipose triglyceride lipase; B2M, beta-2-microglobulin; BAs, bile acids; BSEP, bile salt export pump; CAT, catalase; CDCA, chenodeoxycholic acid; CF, condition factor; CPT1, carnitine palmitoyltransferase 1; CYP27A1, Sterol 27-hydroxylase; CYP7A1, cholesterol 7-alpha-monooxygenase; CYP7B1, oxysterol 7-α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; ELFA, translation elongation factor; FAS, fatty acid synthase; FBW, final body weight; FXR, farnesoid X receptor; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; G-BAs, glycine-conjugated bile acids; H&E, hematoxylin and eosin; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; HNF4α, hepatocyte nuclear factor 4; HPRT, hypoxanthine-guanine phosphoribosyl transferase; HSI, hepatosomatic index; IBW, initial body weight; LRH-1, liver receptor homolog-1; LXR, liver X receptor; MDA, malondialdehyde; NEFA, nonesterified fatty acids; ORO, oil red O; PPARα, peroxisome proliferators-activated receptor α; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase 1; SGR, specific growth rate; SHP, short heterodimeric partners; SREBP1c, sterol regulatory element binding proteins 1c; SREBP2, sterol regulatory element binding proteins 2; T-AOC, total antioxidant capacity; TBA, total bile acid; T-BAs, taurine-conjugated bile acids; TBP, TATA-box-binding protein; TC, total cholesterol; TG, triglyceride; T-SOD, total superoxide dismutase; TUBA, tubulin alpha chain; UBCE, ubiquitin-conjugating enzyme; UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry; VSI, viscerosomatic index; WG, weight gain;
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Introduction

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 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 high fat 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, high fat 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 high fat diet, various feed additives have been applied to aquaculture production processes. For example, dietary sodium butyrate supplementation alleviates the negative influence of high fat 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 high fat 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 high fat diet could improve lipid metabolism by promoting fatty acid β-oxidation and inhibiting endoplasmic reticulum stress pathway \((7)\). Therefore, dietary supplementation with feed additives has emerged as a promising strategy to ameliorate the negative effects of high fat diet.

Bile acids \((BAs)\) are increasingly regarded as complex metabolic integrators and signaling factors \((8,9)\). Primary bile acids are synthesized from the cholesterol in the liver through cytochrome P450-mediated oxidation \((10)\). Then the primary bile acids are combined with glycine or taurine to form conjugated bile acids \((10,11)\), and almost all bile acid 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 bile acids through deconjugation, dehydrogenation, exomerism and 7α/β-dehydroxylation of conjugated bile acids \((10)\). Most of the BAs 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 bile acids synthesized in the liver of mammals and fish \((10,14)\). As one of main primary
bile acid, CDCA has been shown to be an endogenous farnesoid X receptor (FXR) ligand that plays an important role in regulating lipid and bile acid metabolism (8,14). Activated FXR induces short heterodimeric partners (SHP) to inhibit the transcription of cholesterol 7α-monoxygenase a1 (CYP7A1) and sterol 12α-hydroxylase (CYP8B1) genes, ultimately inhibiting bile acid 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 (SREBPs)-dependent manner (15,16). Additionally, activation of FXR regulates fatty acid β-oxidation by activating the β-oxidation gene peroxisome proliferator-activated receptor alpha (PPARα) at the transcriptional level (8,17). High fat 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, BAs have received extensive attention as an additive in aquaculture. Previous studies have shown that BAs could improve growth performance and reduce hepatic lipid deposition in fish (21-23). Dietary BAs improved metabolic liver diseases such as fatty liver and immune dysfunction caused by high fat or high plant protein diets (22,24,25). It has been reported that taurocholic acid alleviates excessive hepatic lipid accumulation caused by high fat diet via regulating bile acid metabolism in juvenile grouper (Epinephelus fuscoguttatus♀ × E. lanceolatus♂) (25). Supplementation of porcine bile extract in high fat diet had no effect on growth performance of tiger puffer (Takifugu rubripes), but decreased liver lipid accumulation and serum triglyceride (TG) and cholesterol contents (21). A study on grass carp (Ctenopharyngodon idella) reported that supplementation of porcine bile extract in high fat diet could improve growth by reducing lipid content and increasing protein synthesis (26). Among various BAs, 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 were confirmed in fish (14,27,28). It has been reported that 900 mg kg⁻¹ CDCA supplementation in
high fat 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\(^{-1}\) CDCA supplementation could promote fat digestion and absorption, improve antioxidant capacity, and ultimately reduce the adverse effects of high fat diet on juvenile largemouth bass \(^{(28)}\). In addition, supplementation 900 mg kg\(^{-1}\) CDCA to high replacement of fish oil with soybean 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 bile acid metabolism in fish fed high fat diet remains unclear. Therefore, it is worth exploring the regulatory mechanism of dietary CDCA to alleviate the disorder of lipid and bile acid metabolism in fish fed high fat diet.

Yellow catfish (\textit{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\% to 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 high fat diet \(^{(32-34)}\). Previous research indicated that feeding yellow catfish with high fat diet leads to lipid metabolism disorders, fatty liver, and growth retardation \(^{(35)}\). This experiment was conducted to investigate the effects of high fat diet supplemented with CDCA on growth performance, antioxidant capacity, and lipid and bile acid metabolism of yellow catfish. Our findings contribute to the understanding of bile acid physiology and provide new insights into the beneficial effects of CDCA on controlling hepatic lipid metabolism in fish.
Materials & 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

Chenodeoxycholic acid (CDCA) (C_{24}H_{40}O_{4}, 474-25-9, ≥ 98% in purity) was purchased from Aladdin company (Shanghai, China). Four diets were formulated in this study (Table S1), the control (105.8 g kg^{-1} lipid), high fat diet (HF group, 159.6 g kg^{-1} lipid), control diet supplemented with 0.9 g kg^{-1} CDCA (CDCA group, 107.3 g kg^{-1} lipid), and high fat diet supplemented with 0.9 g kg^{-1} CDCA (HF+CDCA group, 160.4 g kg^{-1} lipid), respectively. Dietary lipid and CDCA levels were added according to 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 (Wuhan, China). The procedures for yellow catfish feeding and management were similar to our previous study (36). In brief, 360 yellow catfish (body weight: 4.40 ± 0.08 g/fish, mean ± SEM, 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 (8:00 and 16:00, 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 ℃, dissolved oxygen ≥6.5 mg L^{-1}, pH 7.82-7.95, NH_{4}-N ≤0.05 mg L^{-1}.

At the end of the 10 weeks feeding experiment, all fish were fasted for 24 h to avoid the prandial effects. Then, they were euthanized with 100 mg L^{-1} 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 (CF), viscerosomatic index (VSI), and hepatosomatic index (HSI). Liver samples of three fish were randomly collected from each tank and used for oil red O (ORO) staining and hematoxylin and eosin.
(H&E) staining, respectively. The liver tissues of other fish were frozen in liquid nitrogen and stored at -80°C for subsequent analysis of antioxidant activity, biochemical indicators, bile acid concentration, and gene and protein levels.

Analysis of the proximate composition of diets

The diets were analyzed for their proximate composition, including the contents of dry matter, crude protein, crude lipid, and ash, based on the standard procedures \(^{(37)}\). Briefly, dry matter was determined by drying feed samples at 105 °C for 48 hours 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.

H&E and ORO observation in liver

The histochemical (H&E and ORO 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 ORO staining and vacuoles in H&E staining with Image J (version 1.51, National Institute of Health, Bethesda, Maryland, USA).

Biochemical indicators

Non-esterified fatty acid (NEFA), TG, total cholesterol (TC) content, and soluble protein concentration were analyzed with the corresponding commercial kits (A042-1-1, A110-1-1, A111-1-1, A045-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). TG 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, Switzerland). 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) content were analyzed with the corresponding commercial kits (A007-1-1, A001-1-2, A015-1-2, A003-1-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). CAT activity was analyzed using the molybdenine 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 analyzed using the thiobarbituric acid assay at 532 nm.

**Quantitative Real-Time PCR (qPCR) analysis**

Total RNA was isolated from liver tissue using the Trizol reagent (TaKaRa, Japan) and reverse transcribed into cDNA using a commercial kit (TaKaRa, Japan). qPCR analysis was performed according to our previously published protocols \(^{(35)}\). Eight housekeeping genes \(\beta\)-actin, translation elongation factor (elfa), ubiquitin-conjugating enzyme (ubce), beta-2-microglobulin (b2m), hypoxanthine-guanine phosphoribosyl transferase (hpmt), TATA-box-binding protein (thp), glyceraldehyde-3-phosphate dehydrogenase (gapdh), and tubulin alpha chain (tubu) mRNA expression were determined by the Hieff qPCR 64 SYBR Green Master Mix (Yeasen, Shanghai, China), and the results were analyzed using geNorm software (https://genorm.cmgg.be/) to select the two most stable genes as endogenous controls. The primers for qPCR analysis are presented in 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, Ppara, Fxr, Hnf4a, Cyp7a1, and Cyp27a1. Briefly, hepatocytes lysates were prepared with the RIPA buffer (Sigma, USA) on ice for 30 min. 40 μg protein from each sample were separated on the 12% SDS-polyacrylamide gel and subsequently transferred to the polyvinylidene difluoride (PVDF) membranes, blocked with 8% (w/v) non-fat milk in TBST buffer for 2 h, 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, USA), anti-SREBP2 (1:2000, A13049; ABclonal, Wuhan, China), anti-HMGCR (1:2000, JF0981; HUABIO, Hangzhou, China), anti-PPARα (1:1000, EM1707-71; HUABIO, Hangzhou, China), anti-FXR (1:1000, ab155124; Abcam, USA), anti-HNF4α (1:2000, A20865; ABclonal, Wuhan, China), anti-CYP7A1 (1:1000, A10615; ABclonal, Wuhan, China), anti-CYP27A1 (1:1000, 14739-1-AP; Proteintech, Wuhan, China),
and anti-GAPDH (1:10000, 10494-1-AP; Proteintech, Wuhan, China). Secondary antibodies were HRP-conjugated anti-mouse IgG antibody (1:10000, SA00001-1; Protein Technology, Wuhan, China) or anti-rabbit IgG anti-body (1:10000, SA00001-2; Protein Technology, Wuhan, China). Visualization 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**

BAs composition were analyzed in the liver by the ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The method allowed quantitative analysis of 48 BAs (Table S3). The abbreviations for the 48 BAs were shown in Table S3. Briefly, we weighed 2.5 mg of liver sample, added 50 μL of 100 ng mL⁻¹ 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⁻¹) 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, then reconstituted with 700 μL methanol, and the supernatant was taken for the detection on an AB Sciex API 4000 mass spectrometer (AB Sciex, Germany). 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⁻¹, 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 ionization was carried out in the form of negative ions with nitrogen as the atomizing agent. The heated nebulizer 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., USA) and software SPSS 19.0 (IBM, Armonk, NY, USA) were used for graphing and data analysis, respectively. Data were presented as mean ± standard error of the mean (SEM). Before statistical analysis, the data were analyzed 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 utilization, and morphological parameters**

Compared to the control, HF group significantly increased FCR (*P* < 0.05), and CDCA group significantly increased FBW, WG, and SGR (*P* < 0.05) (Table 1). Compared to 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, HSI, CF, HSI, and VSI were not statistically significant among the four groups (*P* ≥ 0.05) (Table 1).

**Hepatic histology, histochemistry, TG, NEFA and TC contents**

Compared to the control, HF group significantly increased the amounts of cytoplasmic vacuoles and lipid droplets, TG and NEFA contents (*P* < 0.05), CDCA supplementation significantly attenuated HF-induced changes of these parameters (*P* < 0.05) (Fig. 1A-F). There was no significant difference in the amounts of cytoplasmic vacuoles and lipid droplets, TG and NEFA contents between control group and CDCA group (*P* ≥ 0.05). Compared to the control, HF group significantly increased TC content (*P* < 0.05), CDCA group significantly decreased TC content (*P* < 0.05). CDCA significantly attenuated HF-induced increase of TC content (*P* < 0.05) (Fig. 1G).

**Expression of bile acid metabolism-related genes and proteins**

To explore the effects of dietary lipid and CDCA supplementation on bile acid metabolism, the levels of relevant genes and proteins were detected (Fig. 2). Compared to the control, 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), CDCA group significantly increased *fxr* and *shp* mRNA levels, but reduced *hnf4α*...
mRNA level \((P < 0.05)\) (Fig. 2A). CDCA significantly attenuated HF-induced reduction of \(fxr\) and \(shp\) mRNA levels, and abrogated HF-induced increase of \(hnf4a\) mRNA level \((P < 0.05)\). The \(lrh-1\) mRNA level showed no significant differences among four groups \((P \geq 0.05)\) (Fig. 2A).

Compared to the control, HF group significantly increased sterol 12α-hydroxylase \((cyp8b1)\), \(cyp7a1\), and sterol 27-hydroxylase \((cyp27a1)\) mRNA levels, but reduced \(bsep\) mRNA level \((P < 0.05)\), 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. 2B). The gene level of oxysterol 7-α-hydroxylase \((cyp7b1)\) showed no significant differences among four groups \((P \geq 0.05)\) (Fig. 2B).

Furthermore, compared to the control, HF group significantly decreased the \(Fxr\) protein level, but increased the \(Hnf4\alpha\), \(Cyp7\alpha1\), and \(Cyp27\alpha1\) protein levels \((P < 0.05)\) (Fig. 2C,D), CDCA group significantly increased the \(Fxr\) protein level, but decreased the \(Hnf4\alpha\), \(Cyp7\alpha1\), and \(Cyp27\alpha1\) protein levels \((P < 0.05)\) (Fig. 2C,D). CDCA significantly attenuated HF-induced downregulation of \(Fxr\) protein level, and upregulation of \(Hnf4\alpha\), \(Cyp7\alpha1\), and \(Cyp27\alpha1\) protein levels \((P < 0.05)\) (Fig. 2C,D).

**Hepatic BAs profiles**

To explore effects of dietary lipid and CDCA supplementation on the total bile acid (TBA) pool, we measured the contents of various BAs in the liver of yellow catfish. Ten BAs were detected in the liver samples \((\text{concentration} > 0.01 \text{ ng mg}^{-1})\). Compared with the control, HF group significantly increased the contents of CA and TCA, CDCA group significantly increased the contents of CDCA, TCA, TCDDA, GCDCA, and GCA, but reduced GLCA content \((P < 0.05)\) (Fig. 3A). CDCA significantly attenuated the HF-induced increase of CA content \((P < 0.05)\). CDCA, TCDDA, and GCDCA contents in HF+CDCA group were higher than that in HF group. GDCA and GLCA contents in HF+CDCA group were lower than that in HF group (Fig. 3A).

Compared to the control, HF group did not significantly influence TBA content \((P \geq 0.05)\), but CDCA group significantly increased the TBA content \((P < 0.05)\) (Fig. 3B). Compared with the control, HF group increased glycine-conjugated BAs (G-BAs) content by 7.1% and
correspondingly reduced taurine-conjugated BAs (T-BAs) content by 7.1%, and CDCA group reduced G-BAs content by 1.84% and correspondingly increased T-BAs content by 1.84% (Fig. 3C). Compared with the HF group, HF+CDCA group decreased the content of G-BAs by 6.9% and correspondingly increased the T-BAs content by 6.9% (Fig. 3C).

We then analyzed the percentages of different BAs in the liver of yellow catfish (Fig. 4). TCA, TCDCA, and CA were the three dominant BAs 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 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 to the control, HF group significantly reduced T-SOD, CAT, and T-AOC activities (P < 0.05), and increased MDA content (P < 0.05), CDCA group significantly increased T-SOD, CAT, and T-AOC activities (P < 0.05), whereas the differences MDA content were not significant (P ≥ 0.05) (Fig. 5). CDCA significantly attenuated HF-induced decrease the activities of T-SOD, CAT, and T-AOC, and increase of 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, 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 coenzyme A reductase (hmgcr) (P < 0.05), but reduced ppara and cpt1 mRNA levels (P < 0.05), CDCA group significantly reduced accα and hmgcr gene levels (P < 0.05), but increased ppara and cpt1 mRNA levels (P < 0.05) (Fig. 6A). CDCA significantly attenuated HF-induced increase of srebp1c, accα, fas, srebp2, and hmgcr mRNA levels, and abrogated HF-induced decline of ppara and cpt1 mRNA levels (P < 0.05) (Fig. 6A). The gene level of adipose triglyceride lipase (atgl) showed no significant differences.
among four groups ($P \geq 0.05$) (Fig. 6A).

Furthermore, compared with the control, HF group significantly increased Srebp1c, Srebp2, and Hmgcr protein levels ($P < 0.05$), but decreased the Ppara protein level ($P < 0.05$). CDCA group significantly decreased Srebp1c, Srebp2, and Hmgcr protein levels ($P < 0.05$), but increased Ppara protein level ($P < 0.05$). CDCA significantly alleviated HF-induced upregulation of Srebp1c, Srebp2, and Hmgcr protein levels ($P < 0.05$), and abrogated HF-induced downregulation of Ppara protein level ($P < 0.05$) (Fig. 6B,C).

Discussion

Given that BAs play a crucial role in maintaining metabolic homeostasis, BAs have been extensively studied in fish. Our study aimed to investigate the effects of dietary CDCA addition on growth performance, antioxidant capacity, lipid and bile acid 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 high fat 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 high fat 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 high fat diet did not promote or even decrease the growth of other fish (21,22,38), due to the reduced feed consumption and utilization 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\textsuperscript{(22)}, largemouth bass\textsuperscript{(28)}, and grass carp\textsuperscript{(39)} fed high fat diet supplemented with BAs. Furthermore, supplementation of CDCA to high fat diet alleviated the HF-induced rise in FCR, suggesting that CDCA may improve dietary lipid utilization in yellow catfish. Similarly, the FCR was significantly decreased with supplementation of BAs (not mentioned) in high fat diet in large yellow croaker\textsuperscript{(22)}. Studies have shown that BAs could activate the activity of intestinal lipase, lipoprotein lipase and liver lipase, which may be related to improved growth performance and feed utilization\textsuperscript{(22,28,40)}.

In the present study, HF group increased hepatic lipid droplets, TG, NEFA, and TC contents of yellow catfish compared with the control, suggesting that fish fed the high fat diet possessed higher lipid accumulation. Similarly, a previous study found that high fat diet led to an increase in the proportion of lipid droplets and lipid content in the liver of golden pompano\textsuperscript{(Trachinotus ovatus)}\textsuperscript{(41)}. In channel catfish\textsuperscript{\textit{(Ictalurus punctatus)}}, Desouky et al.\textsuperscript{(42)} reported that high fat diet significantly increased hepatic TG and TC contents compared with control diet. We found that lipid deposition in the liver of yellow catfish fed a high fat diet was reversed by CDCA addition. Similarly, other studies have shown that supplementation with CDCA in high fat diet reduced hepatic lipid content in large yellow croaker\textsuperscript{(14)} and largemouth bass\textsuperscript{(28)}. In addition, taurocholic acid sodium in grouper\textsuperscript{(25)} and porcine bile extract in grass carp\textsuperscript{(26)} have been reported to alleviate high fat diet-induced liver lipid accumulation. Teodoro et al.\textsuperscript{(43)} found that supplemented with CDCA in high fat diet had lower lipid accumulation than these fed high fat 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 high fat diet may have a relieving effect on non-alcoholic fatty liver caused by high fat diet.

As a bile acid receptor, FXR regulates genes responsible for maintaining the normal physiological function of the liver by binding to BAs, thereby protecting the liver from adverse effects\textsuperscript{(8,17)}. Activated FXR inhibits the transcription of CYP7A1 and CYP8B1 genes through activating SHP\textsuperscript{(8,9)}. Studies suggested that CYP7A1 and CYP8B1 mediate the classical pathway of bile acid synthesis, thereby controlling bile acid homeostasis\textsuperscript{(8)}. In addition, CYP27A1 is a key enzyme in the alternative pathway of bile acid synthesis\textsuperscript{(8,9)}. Activation of
FXR positively induces transcription of the bile acid transporter BSEP, which transports BAs from the liver into the bile ducts \(^{(17)}\). In this study, compared with the control, HF group down-regulated the mRNA levels of \textit{fxr}, \textit{shp}, and \textit{bsep} and protein levels of Fxr, and up-regulated the mRNA levels of \textit{hnf4a}, \textit{cyp7a1}, \textit{cyp27a1}, and \textit{cyp8b1} and protein levels of Hnf4a, Cyp7a1, and Cyp27a1. These results suggested that high fat diet increased the synthesis of bile acids 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 high fat diet \(^{(44,45)}\). Du et al. \(^{(14)}\) reported that high fat diet decreased the mRNA abundances of \textit{fxr}, \textit{shp}, and \textit{bsep}, and reduced the Fxr protein level compared with the control in the liver of large yellow croaker. Xu et al. \(^{(25)}\) found that high fat diet increased the mRNA abundances of \textit{cyp7b1} and \textit{cyp27a1}, and decreased the mRNA abundances of \textit{bsep} compared with control in the liver of grouper. We found that CDCA increased the mRNA expression of \textit{fxr}, \textit{shp}, and \textit{bsep}, and the protein level of Fxr, and decreased the mRNA and protein levels of Hnf4α, Cyp7a1, and Cyp27a1. Similarly, a study on grass carp reported that hepatic transcription levels of \textit{cyp7a1}, \textit{cyp7b1}, and \textit{cyp27a1} were downregulated by lithocholic acid supplementation in the normal diet \(^{(46)}\). Yu et al. \(^{(47)}\) reported that CDCA could activate \textit{fxr} and \textit{bsep} gene expression, and repress \textit{cyp7a1} gene expression in mammals. In human hepatocytes, CDCA incubation decreased \textit{cyp7a1} and \textit{hnf4a} mRNA expression and Hnf4a protein level, but increased \textit{shp} mRNA expression \(^{(48)}\). We found that supplementation of CDCA to high fat diet alleviated the imbalance of bile acid homeostasis caused by high fat diet. Similarly, Du et al. \(^{(14)}\) reported that \textit{fxr}, \textit{shp}, and \textit{bsep} gene expressions were increased in large yellow croaker fed a high fat diet compared with fish fed with control diet. Liao et al. \(^{(21)}\) found that CDCA could alleviate high fat diet-induced decrease in \textit{fxr} gene expression, and increase in \textit{hnf4a} gene expression in tiger puffer. In addition, supplementation of porcine bile extract to the high fat diet inhibited the transcription levels of \textit{cyp7a1} and \textit{cyp8b1}, which might be linked to the activation of FXR in common carp (\textit{Cyprinus carpio}) \(^{(49)}\). A study on grouper showed that supplementation of taurocholic acid sodium to high fat diet significantly increased the expression of FXR gene and protein, indicated that activation of FXR may be a key step for taurocholic acid sodium to affect bile acid metabolism \(^{(25)}\). Studies have shown that CDCA...
inhibits BAs *de novo* synthesis by activating hepatic FXR, and accelerates hepatic bile excretion, detoxification, and recycling to protect hepatocytes from the deleterious consequences of BAs \(^{(10,15)}\). Therefore, supplementation of CDCA to high fat diet could activate the function of FXR to maintain bile acid homeostasis in the liver of yellow catfish.

To investigate the effects of dietary lipid and CDCA supplementation on hepatic BAs composition, we measured bile acid profiles of yellow catfish livers. In the present study, CDCA addition in control and high fat 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 7α-dehydroxyacids, which are 7α-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 bile acid signaling has beneficial effects in maintaining normal bile acid and lipid metabolism in the liver \(^{(13)}\). TUDCA attenuates liver toxicity through multiple mechanisms, including stabilizing mitochondrial integrity, promoting ATP synthesis, and reducing oxidative damage \(^{(13)}\). In addition, conjugated bile acids play a dominant role in the liver, with taurine-conjugated bile acids being the highest. Hydrophobic BAs are highly toxic, which could damage hepatocytes by inducing plasma membrane damage, mitochondrial damage, and oxidative stress \(^{(44)}\). Recent studies suggested that the hydrophobic bile acid CA disrupts bile acid homeostasis and induces metabolic disease in nonalcoholic fatty liver disease \(^{(44,45)}\). In the current study, compared with the control, 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 bile acid homeostasis. In the study of tiger puffer, dietary BAs significantly reduced the content of CA in gallbladder \(^{(21)}\). CDCA mitigated the increase in CA content and proportion induced by high fat 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 bile acid distribution and inhibits bile acid production by activating FXR in the liver of yellow catfish.

Emerging evidence indicates that antioxidant capacity is closely related to lipid metabolism \(^{(52,53)}\). BAs have the ability to induce antioxidant defense 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 to the control, suggesting that the high fat diet caused the lower antioxidant levels of yellow catfish. High fat 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 high fat diet. In this study, we found that supplementation of CDCA in control and high fat 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, BAs (not mentioned) supplementation in high fat 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 high fat diet through FXR-mediated antioxidant defense 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, acca, fas, and scd1 gene levels and Srebp1 protein level compared with the control, which may be the main reason for hepatic lipid accumulation. Previous studies have shown that high fat 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, CDCA group decreased Srebplc protein level and \textit{acc\alpha}\ gene level. Liu et al. \(^{(50)}\) reported that CDCA treatment inhibited SREBP1 protein level and \textit{acc\alpha}\ mRNA level compared with the control in mammals. We found that CDCA alleviated HF-induced increment of \textit{srebplc}, \textit{acc\alpha}, \textit{fas}, and \textit{scd1}\ gene levels, and Srebplc protein level, which were similar in large yellow croaker \(^{(14)}\). The study reported that dietary BAs (not mentioned) could alleviate the increased expression of lipid synthesis genes \textit{srebpl}, \textit{scd1}, and \textit{fas}\ induced by high fat diet in large yellow croaker \(^{(22)}\). Further studies have demonstrated that the SREBP1 transcriptional activity was decreased by the activation of FXR \textit{via} upregulating 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 \textit{HMGCR} \(^{(16)}\). We found that HF group increased \textit{srebpl2} and \textit{hmgcr}\ genes and proteins levels compared with the control, suggesting that high fat diet could promote cholesterol biosynthesis. Moreover, Betancor et al. \(^{(55)}\) demonstrated that high fat diet could promote cholesterol production by activating SREBP2 in Atlantic bluefin tuna \textit{(Thunnus thynnus \textit{L.})}. In the present study, compared with the control, CDCA decreased the \textit{hmgcr}\ mRNA expression and SREBP2 protein level. Studies have shown that CDCA reduced the mRNA levels of \textit{srebpl2} and \textit{hmgcr}\ in mammals \(^{(56)}\). Moreover, we found that CDCA alleviated HF-induced increase of \textit{srebpl2} and \textit{hmgcr}\ genes and proteins levels, which may contribute to the reduction of cholesterol. Similarly, Nilsson et al. \(^{(57)}\) reported that \textit{hmgcr}\ gene level was reduced by CDCA treatment in human liver compared to control. 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 \(^{(27,58)}\). PPAR\(\alpha\) 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 \(\beta\)-oxidation, facilitating the entry of long-chain fatty acids into the mitochondria for the oxidation \(^{(59)}\). In the present study, compared with the
control, HF group decreased PPARα gene and protein level, and cpt1 mRNA level, suggesting that high fat 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 high fat diet significantly increased PPARα gene and protein level, and cpt1 gene level. Similarly, Du et al. (14) reported that the CDCA supplementation to high fat diet increased ppara and cpt1 gene levels compared with control diet in large yellow croaker. Ding et al. (22) reported that the gene expression levels of ppara and cpt1 increased gradually with the increase of dietary BAs (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 high fat 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 upregulate 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 ppara via activating FXR in the liver of yellow catfish. Taken together, our data suggested that CDCA supplementation in high fat 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 utilization of yellow catfish. Supplementation of CDCA alleviated high fat diet-induced hepatic lipid accumulation, low antioxidant capacity, and bile acid metabolism disorders. Furthermore, the supplementation of CDCA to high fat diet altered the BAs 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 bile acid 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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Authors’ contributions

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 and provided funding.

Declaration of Competing Interest

No potential conflict of interest was disclosed.

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References


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.


Table 1. Effects of dietary lipid and CDCA supplementation on growth performance and biometric parameters of yellow catfish.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HF</th>
<th>CDCA</th>
<th>HF+CDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBW (g)(^b)</td>
<td>4.41 ± 0.007</td>
<td>4.39 ± 0.005</td>
<td>4.40 ± 0.07</td>
<td>4.41 ± 0.003</td>
</tr>
<tr>
<td>FBW (g)(^c)</td>
<td>16.29 ± 0.86</td>
<td>15.83 ± 0.25</td>
<td>18.31 ± 0.19(^*)</td>
<td>17.06 ± 0.18(^#)</td>
</tr>
<tr>
<td>WG (%)(^d)</td>
<td>269.38 ± 3.34</td>
<td>260.06 ± 5.47</td>
<td>311.32 ± 4.65(^*)</td>
<td>286.64 ± 4.47(^#)</td>
</tr>
<tr>
<td>SGR (%)(^e)</td>
<td>3.53 ± 0.10</td>
<td>3.48 ± 0.32</td>
<td>3.74 ± 0.21(^*)</td>
<td>3.62 ± 0.31(^#)</td>
</tr>
<tr>
<td>FCR(^f)</td>
<td>1.29 ± 0.04</td>
<td>1.48 ± 0.03(^*)</td>
<td>1.30 ± 0.03</td>
<td>1.36 ± 0.06(^#)</td>
</tr>
<tr>
<td>HSI (%)(^g)</td>
<td>1.65 ± 0.03</td>
<td>1.75 ± 0.06</td>
<td>1.69 ± 0.05</td>
<td>1.62 ± 0.03</td>
</tr>
<tr>
<td>VSI (%)(^h)</td>
<td>6.69 ± 0.31</td>
<td>7.37 ± 0.54</td>
<td>7.15 ± 0.51</td>
<td>7.03 ± 0.10</td>
</tr>
<tr>
<td>CF(%i)</td>
<td>1.55 ± 0.02</td>
<td>1.54 ± 0.03</td>
<td>1.60 ± 0.03</td>
<td>1.52 ± 0.02</td>
</tr>
<tr>
<td>Survival (%)(^j)</td>
<td>96.67 ± 1.92</td>
<td>96.67 ± 1.92</td>
<td>97.78 ± 2.22</td>
<td>97.78 ± 1.11</td>
</tr>
</tbody>
</table>

\(^a\)Values are means ± SEM (n = 3). P-value was calculated by Student’s t test. \(^*\) \(^\#\) P < 0.05.

\(^*\)Indicates significant difference when comparing HF or CDCA to control; \(^\#\) indicates significant difference between HF and HF+CDCA, as determined by Student’s t test.

\(^b\)IBW: Initial body weight.

\(^c\)FBW: Final body weight

\(^d\)WG (weight gain) = (FBW - IBW) / IBW × 100.

\(^e\)SGR (specific growth rate) = 100 × (Ln FBW - Ln IBW) / feeding days.

\(^f\)FCR (feed conversion ratio) = dry feed fed (g) / wet weight gain (g).

\(^g\)HSI (hepatosomatic index) = 100 × (liver weight) / (body weight).

\(^h\)VSI (viscerosomatic index) = viscera weight / whole body weight.

\(^i\)CF (condition factor) = 100 × (live weight, g) / (body length, cm)\(^3\).

\(^j\)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 Oil Red O staining, Scale bar, 50 μm. D. Relative areas for lipid droplets in Oil Red O staining. E. TG content. F. NEFA content. G. TC content. Values are means ± SEM., n=3. *$P$-value was calculated by Student’s $t$ test. *$P < 0.05$. ns, not significant. CDCA, chenodeoxycholic acid; H&E, hematoxylin and eosi; HF, high fat diet; NEFA, nonesterified fatty acid; ORO, oil red O; TG, triglyceride; 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 means ± SEM., n=3. *P < 0.05. ns, not significant. bsep, bile salt export pump; CDCA, chenodeoxycholic acid; cyp27a1, sterol 27-hydroxylase; cyp7a1, cholesterol 7-alpha-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.
Fig 3. Effects of dietary lipid and CDCA supplementation on liver bile acid contents of yellow catfish. A. The content of 10 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 means ± SEM., n=3. P-value was calculated by Student’s t test. *P < 0.05. ns, not significant. 7-KDCA, 7-ketodeoxycholic acid; BAs, bile acids; CA, cholic acid; CDCA, chenodeoxycholic acid; G-BAs, glycine-conjugated bile acids; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeloxycholic acid; GLCA, glyco-lithocholic acid; HF, high fat diet; TBA, total bile acid; T-BAs, taurine-conjugated bile acids; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid.
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; BAs, bile acids; CA, cholic acid; CDCA, chenodeoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; HF, high fat; CDCA, chenodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid.
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 means ± SEM, 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.
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 means ± SEM., n=3. P-value was calculated by Student’s t test. *P < 0.05. ns, not significant. accα, acetyl-CoAcarboxylase; atgl, adipose triglyceride lipase; CDCA, chenodeoxycholic acid; cpt1, carnitine palmitoyltransferase 1; fas, fatty acid synthase; HF, high fat diet; hmgcr, 3-hydroxy-3-methylglutaryl coenzyme A reductase; ppara, peroxisome proliferators-activated receptor α; scd1, stearoyl-CoA desaturase1; srebp1c, sterol regulatory element binding proteins 1c; srebp2, sterol regulatory element binding proteins 2.