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Dietary chenodeoxycholic acid inclusion improves carbohydrate utilization and inflammation of largemouth bass (*Micropterus salmoides*) partly mediated by the activation of farnesoid X receptor

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Abstract

This study evaluated the effects of chenodeoxycholic acid (CDCA), a farnesoid X receptor (FXR) potential activator, on growth performance, antioxidant capacity, glucose metabolism, and inflammation in largemouth bass (*Micropterus salmoides*) (initial body weight: 5.45 ± 0.02 g) fed a high-carbohydrate diet. Experimental diets included a positive control (5 % α-starch), a negative control (10 % α-starch), and two diets containing 10 % α-starch supplemented with either 0.05 % or 0.10 % CDCA. After 8 weeks, the high-carbohydrate diet reduced growth performance and increased hepatosomatic and viscerosomatic indexes, which were mitigated by 0.10 % CDCA supplementation. The high-carbohydrate diet also increased hepatic glycogen and crude lipid content, both of which were reduced by 0.10 % CDCA. Furthermore, the high-carbohydrate diet induced oxidative stress, histopathological changes, and reduced liver lysozyme activity, which were ameliorated by CDCA supplementation. Molecular analysis showed that the high-carbohydrate diet suppressed FXR and phosphorylated AKT1 (p-AKT1) protein expression in the liver, downregulated insulin signaling (*ira*, *irs*, *pi3kr1* and *akt1*), gluconeogenesis (*pepck* and *g6pc*), and glycolysis genes (*gk*, *pk* and *pfkl*). CDCA supplementation upregulated *fxr* expression, activated *shp*, enhanced the expression of insulin signaling and glycolytic genes (*gk*, *pk* and *pfkl)*, and inhibited gluconeogenesis. Additionally, CDCA reduced inflammatory markers (*nf-κb* and *il-1β*) and restored anti-inflammatory mediators (*il-10*, *iκb*, and *tgf-β*). In conclusion, 0.10 % CDCA improved carbohydrate metabolism and alleviated liver inflammation in largemouth bass fed a high dietary carbohydrate, partially through FXR activation.

Keywords: Chenodeoxycholic acid; Glycogen deposition; Glucose metabolism; Inflammatory response; Largemouth bass

Abbreviation: CDCA, chenodeoxycholic acid; FXR, farnesoid X receptor; IBW, initial body weight; FBW, final body weight; SGR, specific growth rate; FI, feed intake; CF, condition factor; FCR, feed conversation ratio; HSI, hepatosmatic index; VSI, viscerosomatic index; TP, total protein; MDA, malondialdehyde; CAT, catalase; T-SOD, total superoxide dismutase; T-AOC, total antioxidant capacity; LZM, lysozyme; *fxr*, farnesoid X receptor; *shp*, small heterodimer partner; *irs*, insulin receptor substrate; *ira*, insulin receptor a; *pi3kr1*, phosphoinositide 3-kinase regulatory subunit 1; *akt1*, protein kinase B; *g6pc*, Glucose-6-phosphatase; *pepck*, phosphoenolpyruvate carboxykinase; *fbp1*, fructose bisphosphatase 1; *pk*, pyruvate kinase; *pfkl*, phosphofructokinase liver type; *gk*, glucokinase; *tlr2*, toll-like receptor 2; *nf-κb*, nuclear factor kappa b; *il-1β*, interlenkin-1β; *tnf-α*, tumor necrosis factor α; *il-10*, interlenkin-10; *tgf-β*, transforming growth factor β.

Introduction

Carbohydrates are widely used in aquafeeds due to their ability to enhance the binding between feed ingredients, thereby improving their stability in water, and they are one of the cheapest ingredients in compound feeds [1, 2]. According to Rokey et al. [3], current commercial sinking aquafeeds require a minimum of 10 % carbohydrate, with the requirement for floating aquafeeds being even higher. However, carnivorous fish are generally glucose intolerant, and the recommended carbohydrate level in their diets should not exceed 10 % [4, 5, 6, 7]. In practice, the carbohydrate level in commercial feeds often exceeds the tolerance threshold of carnivorous fish, potentially leading to negative impacts, such as liver abnormalities, impaired glucose metabolism, antioxidant damage, and inflammatory responses [8, 9, 10]. Therefore, further research is crucial to develop effective strategies that enhance growth and improve metabolism to mitigate the negative effects of high dietary carbohydrates.

Chenodeoxycholic acid (CDCA) is a bile acid that fish can synthesize endogenously [11], and is one of the major endogenous bile acids in some teleost species [12]. In aquatic animals, dietary CDCA supplementation has been shown to improve growth, metabolism, antioxidant capacity, and immune function, as demonstrated in species such as large yellow croaker (*Larimichthys crocea*) [13], thinlip mullet (*Liza ramada*) [14] and yellow catfish (*Pelteobagrus fulvidraco*) [15], making it as an exogenous non-additive in aquafeeds. CDCA is a potent ligand that directly activates the farnesoid X receptor (FXR), which plays a critical role in regulating glycolipid metabolism and inflammation [16, 17]. However, Romano et al. [11] reported that excessive CDCA inclusion can disrupt carbohydrate absorption in fish, causing metabolic imbalances and adversely affecting growth performance. Based on previous studies on CDCA supplementation in fish diets [18, 19] (regarding feed formulation and dose-to-weight ratio), this study incorporated CDCA at levels of 0.05 % and 0.10 %. Largemouth bass, known for its limited capacity to utilize carbohydrates, exhibits sustained hepatic glycogen accumulation and compromised health when fed high-carbohydrate diets (≥ 10 %), likely due to dysregulation of glycolysis and gluconeogenesis [8, 22]. In the liver, the negative regulations of FXR on gluconeogenesis are mediated through the inhibitory effects of a small heterodimer partner (SHP) [23, 24, 25]. Additionally, FXR stimulates the phosphorylation of protein kinase B (AKT1) [26, 27], which is a key factor of the insulin pathway [28]. However, the role of CDCA in glucose metabolism in carnivorous fish remains to be further explored. Furthermore, FXR has been shown to negatively regulate NF-κB mediated liver inflammation

[29, 30]. In large yellow croaker (*Larimichthys crocea*) [31], CDCA supplementation in high-soybean-oil diets activated intestinal *fxr*, subsequently downregulated the expression of inflammatory markers, such as tumour necrosis factor-α (*tnf-α*), interleukin 1β (*il-1β*), cyclooxygenase-2 (*cox-2*), and interleukin 6 (*il-6*). These findings suggest that CDCA may regulate inflammation in carnivorous fish through FXR activation.

Largemouth bass is a widely cultured carnivorous freshwater fish species in China, with production reaching 888,030 tons in 2023 [15]. Commercial feeds often contain high levels of carbohydrates to meet processing performance requirements, which seriously threaten the health of this fish species. Previous studies have primarily focused on the role of CDCA in lipid metabolism in fish [16, 19, 33], but its involvement in regulating glucose metabolism remains unclear. Therefore, this study aimed to elucidate the influence of dietary CDCA inclusion on carbohydrate metabolism and the inflammatory response in largemouth bass.

Materials and methods

Experimental diets

Two isonitrogenous (52.74 %) and isolipidic (11.38 %) diets were formulated, including a positive control group (5 % α-starch content, LC) and a negative control group (10 % α-starch content, HC). Another two diets were formulated by supplementing 500 mg/kg (HCC-0.05) and 1000 mg/kg (HCC-0.10) CDCA to the HC group, respectively (Table 1). All low-fat ingredients were crushed to a particle size of less than 178 μm and mixed stepwise according to the recipe before adding the lipid component. Then, all feed ingredients were ground to a particle size of less than 178 μm. An appropriate amount of water (20 % diet weight) was added to the thoroughly mixed feed ingredients, followed by thorough mixing and subsequent extrusion. Complete starch gelatinization was achieved at 120°C, followed by drying the feed at 60°C and storage at -20°C until use.

Experimental procedure

Juvenile fish were obtained from a commercial aquaculture farm (Zhejiang, China), and acclimatized in the culture system for two weeks before the commencement of the formal experiments. The culture system comprised a culture module (24 buckets; Volume 800L), a filtration module (coral stones and mesh sponges), a disinfection module (UV irradiation), and an aeration module with a pipe network. After that, 12 buckets, each containing 360 healthy, morphologically similar fish (5.46 ± 0.03 g) were randomly assigned. The same

experimental diet was fed to each of the three buckets twice daily until apparent saturation (8:00 and 16:00), and the feed consumption was recorded for 8 weeks. The trial was conducted in a recirculating aquaculture system with a daily water exchange rate of 10 %. All tanks were supplied with a continuous flow of aerated water (dissolved oxygen ≥ 6mg/L). The water temperature and pH were maintained at 27 ± 1 ℃ and 7.2 ± 0.2, respectively.

Sample collection

After the culture procedure was completed, fish were anesthetized with eugenol (1:1000; Shanghai Reagent Corp., Shanghai, China) following 24 h of starvation for sampling. All experimental fish in each group were counted and weighed to calculate the survival rate (SR) and specific growth rate (SGR). Body composition analysis was then performed on five randomly selected fish per tank. In addition, twelve fish were selected to measure body weight, length, and condition factor (CF). The viscera and liver from six of these fish were collected for the calculation of viscerosomatic index (VSI) and hepatosomatic index (HSI). Furthermore, a 1 cm^3 sample of liver apex was collected for gene expression and protein quantification analysis. The livers of all dissected fish were photographed for morphological assessment.

Chemical analysis

Moisture content was determined by drying the sample in an oven at 105 °C until a stable weight was achieved. Crude protein content was measured using the Kjeldahl nitrogen method (N × 6.25) with a Kjeldahl nitrogen analyzer. Crude lipid content was determined following the method of Folch et al. (1957) [34], with modifications as described by Peng et al. (2014) [35]. Ash content was determined by placing the sample in a muffle furnace at 550 °C until complete combustion, after which the residue was weighed. Crude fiber content was determined from the defatted powdered sample using the AOAC method (2023) [36]. Nitrogen-free extract (NFE) was calculated as dry matter (DM) minus the sum of crude protein, crude lipid, crude ash, and crude fiber. Glycogen content was determined using the KOH/anthrone method as described by Seifter et al. (1950) [37], which converted glycogen into a detectable form, with results obtained through spectrophotometric analysis.

Hepatic histopathology

Fresh liver tissue was fixed in Bouin's fixative and sent to Servicebio in Shanghai, China for paraffin section preparation. Briefly, the tissue was immersed in Bouin's fixative at a 1:10 volume ratio for 24 h, then dehydrated using increasing concentrations of ethanol (75 % to 100 %). Ethanol was removed with xylene, and the tissue was embedded in paraffin wax. The embedded tissue was sectioned into 5 μm thick slices using a rotary microtome (340E, Thermo Scientific). Sections were stained with hematoxylin and eosin for nuclear and cytoplasmic visualization, then dehydrated, blocked, and images were acquired using a fully automated pathology slide scanner (WS-10, ZhiYue).

Measurement of hepatic biochemical parameters

The liver samples were homogenized with phosphate buffer (1:9, w/w) and centrifuged at 3500 rpm to obtain the supernatant for further testing. The total protein (TP) concentration was quantified using Coomassie Brilliant Blue dye, as described by Bradford (1976) [38], with a commercial kit (A045-2-2). The malondialdehyde (MDA) content was determined using the TBARS test [39] with a commercial kit (A003-1-2). The hydroxylamine technique was used to measure total superoxide dismutase (T-SOD) activity [40] with a commercial kit (A001-1-1). The rate of H_2O_2 breakdown was estimated to determine catalase (CAT) activity [41] using a commercial kit (A007-1-1). Ferrous ion concentration was used to measure total antioxidant capacity (T-AOC) [42] with a commercial kit (A015-1-2). The activity of lysozyme (LZM) was measured using a commercial kit (A050-1-1). All commercial kits were supplied by Nanjing Jiancheng Bioengineering Institute.

RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from liver samples using Trizol Reagent (TransGen Biotech, China) and reverse transcribed to cDNA using the Prime Script™ RT Reagent Kit (Takara, Japan). The primers used for quantifying gene expression are listed in Table 2, with *β-actin* selected as the reference gene. RT-qPCR was performed using a quantitative thermal cycler with the following procedure: 95 °C, 2 min; 40 cycles of 95 °C,10 s; 57 °C, 10 s; 72 °C, 20 s. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method [43].

Western Blot

A 100 mg liver sample was treated with RIPA lysis buffer (Beyotime P0013B, China) and incubated on ice for 40 minutes to lysis the tissue and extract the proteins. The homogenate was then centrifuged (4 °C, 12,000 rpm, 10 min) to obtain the supernatant. Total protein extraction was repeated twice, and the protein concentration was determined using the BCA method (Beyotime P0009, China). The proteins were then treated with SDS-PAGE loading buffer and denatured at 95 °C for 10 minutes. Electrophoresis was performed on a 10 % polyacrylamide gel, followed by protein transfer to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA), which was then blocked with TBST containing 5 % non-fat milk at room temperature for 2 hours. The blocked membrane was incubated overnight at 4 °C with primary antibodies (FXR, AKT1, p-AKT1, and β-ACTIN, all from Cell Signaling Technology, USA). After primary antibody incubation, the membrane was washed five times with TBST buffer, followed by incubation with an enzyme-labeled secondary antibody (Sigma, USA) for 1.5 hours, and then washed again. Finally, the results were analyzed using a ChemiDoc MP Imaging System (Bio-Rad, USA) and Image Lab software (Bio-Rad, USA).

Statistical methods

All data were presented as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) with SPSS 26.0 software, followed by Duncan's multiple range test. Statistical evaluations were performed after testing the data for normality and homoscedasticity. Dunnett's test was applied to compare the data from the HC, HCC-0.05, and HCC-0.10 groups to the LC group. The significant level was set at 5 %.

Results

Growth performance

No significant differences were observed in IBW, SR and CF across all groups ($P > 0.05$). However, increased dietary carbohydrate levels significantly reduced the FBW, SGR, FI, and FCR of cultured fish ($P < 0.05$), while significantly increasing the HSI and VSI ($P < 0.05$). Compared to the NC group, the addition of 0.05 % CDCA had no significant effect on FBW, SGR, HSI, or VSI ($P > 0.05$), but significantly increased the FI and FCR ($P < 0.05$). In contrast, the 0.10 % CDC group showed significant increases in FBW and SGR ($P < 0.05$), while HSI and VSI were significantly decreased.

Body composition

The high-carbohydrate diet increased moisture and crude lipid content and decreased the ash content in the whole body of largemouth bass ($P < 0.05$) (Table 4). Compared to both LC and HC groups, the addition of CDCA significantly reduced the crude lipid content in the whole body ($P < 0.05$) (Table 4). Furthermore, compared to the LC group, the high-carbohydrate diet significantly increased both crude lipid and glycogen content in the liver ($P < 0.05$) (Table 4). However, the inclusion of CDCA in the diet significantly reduced lipid and glycogen accumulation in the liver compared to the HC group ($P < 0.05$) (Table 4). The high-carbohydrate diet significantly reduced liver crude protein content compared to the LC group ($P < 0.05$), but this reduction was significantly mitigated in the HCC-0.10 group compared to the HC group. However, the liver moisture content was not affected by the addition of CDCA ($P > 0.05$). Additionally, no significant differences in muscle moisture content and crude protein content were observed across all experimental groups ($P > 0.05$) (Table 4). The trend in muscle crude lipid content was consistent with that observed in the whole body ($P < 0.05$) (Table 4).

Hepatic histopathology analysis

Simple macroscopic observations of the livers of experimental fish revealed visible differences (Fig. 1). Notably, the livers from the HC group were significantly hypertrophied compared to the LC group, which improved progressively with the addition of CDCA, consistent with the HSI values obtained previously. Additionally, the high-carbohydrate diet caused a slight yellow coloration in the liver, which progressively turned reddish with increasing levels of CDCA inclusion (Fig. 1).

As shown in Fig. 2, the hepatocytes in the LC group were regularly arranged, exhibiting normal cell morphology with only occasional small lipid droplets. In contrast, hepatocytes in the HC group were significantly enlarged, with unclear cell margins, severe swelling, vacuolization, displaced or absent nuclei, and abundant round lipid droplets and inflammatory infiltrates (Fig. 2A, 2B). Following the addition of 0.05 % CDCA, hepatocyte swelling and vacuolization were reduced, and the number of lipid droplets and inflammatory infiltrates decreased. However, the nuclei remained displaced or absent (Fig. 2C). After the addition of 0.10 % CDCA, hepatocyte morphology returned to normal, with only a few swollen cells remaining. The nuclei were largely intact, and lipid droplets nearly disappeared, although a few inflammatory infiltrates were still observed (Fig. 2D).

Hepatic biochemical parameters

Hepatic total protein (TP) content exhibited a significant decrease in response to elevated dietary carbohydrate levels, whereas CDCA supplementation significantly increased TP content ($P < 0.05$) (Table 5). Conversely, hepatic malondialdehyde (MDA) content was significantly higher in the HC group compared to the LC group, showing a dose-dependent decrease with CDCA supplementation (Table 5). Hepatic total superoxide dismutase (T-SOD) and catalase (CAT) activities, and total antioxidant capacity (T-AOC) were all significantly lower in the HC group compared to the LC group ($P < 0.05$) (Table 5). Notably, 0.05 % CDCA supplementation did not affect CAT activity but significantly increased T-SOD and T-AOC activities ($P < 0.05$) (Table 5). However, 0.10 % CDCA supplementation significantly increased all antioxidant enzyme activities ($P < 0.05$) (Table 5). Additionally, the high dietary carbohydrate significantly depressed LZM activity ($P < 0.05$) (Table 5), while 0.10 % CDCA supplementation significantly enhanced LZM activity ($P < 0.05$) (Table 5), restoring it to levels comparable to the positive control group ($P > 0.05$) (Table 5).

Expression of hepatic insulin signaling involved genes and proteins

The results indicated that, compared to the LC group, the expressions of *fxr*, *shp*, insulin receptor substrate (*irs*), phosphatidylinositol 3-kinase 1 (*pi3kr1*), and protein kinase b (*akt1*) were significantly reduced in the HC group ($P < 0.05$) (Fig. 3). The addition of CDCA significantly increased the expression of these genes compared to the HC group ($P < 0.05$) (Fig. 3). Furthermore, the high-carbohydrate diet significantly inhibited the expression of FXR and p-AKT1, with no effect on total AKT1 expression ($P > 0.05$) (Fig. 4). Compared to the HC group, the addition of CDCA significantly activated the expression of FXR and p-AKT1 ($P < 0.05$).

Expression of hepatic glucose metabolism involved genes

As shown in Fig. 5, compared to the LC group, the expressions of phosphofructokinase (*pfkl*), phosphoenolpyruvate carboxylase (*pepck*), glycogen phosphorylase (*g6pc*), glucokinase (*gk*), and phosphorylase (*pk*) were significantly reduced in the HC group ($P < 0.05$) (Fig. 5), while fructose-1,6-bisphosphatase (*fbp1*) expression was significantly elevated ($P < 0.05$) (Fig. 5). Compared to the HC group, the addition of 0.05 % and 0.10 % CDCA significantly increased the gene expression of *gk*, *pk*, and *pfkl* ($P < 0.05$) (Fig. 5), while significantly decreasing the gene expression of *fbp1* ($P < 0.05$) (Fig. 5). However, the

expression of *g6pc* and *pepck* was not affected by CDCA inclusion in the high-carbohydrate group ($P > 0.05$) (Fig. 5).

Expression of hepatic inflammation response involved genes

With the increase of dietary carbohydrate level, the expression of *tlr2*, *nf-κb* and *il-1β* was significantly increased ($P < 0.05$) (Fig. 6), while the expression of *iκb* and *il-10* was significantly decreased ($P < 0.05$) (Fig. 6). Dietary CDCA inclusion significantly reduced the expression of *nf-κb* and *il-1β* ($P < 0.05$) (Fig. 6), while significantly enhancing *iκb*, *il-10* and *tgf-β* expression ($P < 0.05$) (Fig. 6).

Discussion

High-carbohydrate diets have significant negative effects on largemouth bass, including reduced growth performance, hepatic glycogen, and lipid accumulation, as well as subsequent hepatic inflammation [10, 44]. Therefore, this experiment aimed to investigate the effects of dietary CDCA inclusion on growth performance, antioxidant capacity, insulin signaling, glucose metabolism, and the inflammatory response in largemouth bass fed a high-carbohydrate diet. The results showed that a high-carbohydrate diet significantly decreased FI, FCR, FBW, and SGR of largemouth bass, which is consistent with previous studies on largemouth bass [45,46]. However, the inclusion of exogenous bile acids has been demonstrated to improve the feed intake in thinlip mullet (*Liza ramada*) [47]. Similarly, in the present study, the addition of 0.05 % CDCA significantly improved the feed intake, which was partly related to the potential role of CDCA in elevating carbohydrate utilization in largemouth bass. Additionally, as previously reported by Zeng et al. (2015) [48], diets with higher carbohydrate content exhibited greater total energy content, which may induce a protein-sparing effect in fish. It is therefore plausible that the HC group resulted in a lower FCR in largemouth bass compared to the LC group in this experiment. Additionally, feeding largemouth bass a diet including 0.10 % CDCA significantly increased FBW and SGR, suggesting that CDCA inclusion enhances their carbohydrate tolerance. Meanwhile, a high-carbohydrate diet increased the lipid content in the liver, muscle, and whole body of the cultured fish, as evidenced by the numerous aggregated lipid droplets observed in the hepatic histopathology of the HC group. This suggests that largemouth bass cope with a high-carbohydrate diet by converting excess carbohydrates into lipids. CDCA has been shown to improve lipid metabolism [16, 49, 50]. Consistently, we found that adding CDCA to the

high-carbohydrate diet significantly reduced lipid content in the liver, muscle, and whole body of largemouth bass.

Considerable studies have demonstrated that a high-carbohydrate diet can induce oxidative stress and cellular damage in carnivorous fish [51, 52, 53]. The primary cause of oxidative stress is the production of excessive oxygen-free radicals, which interact with various cellular components, damaging cell structure and function. An important target of these oxygen-free radicals is polyunsaturated fatty acids (PUFA) in biological membranes, which can trigger lipid peroxidation, producing lipid peroxides, such as reactive chemical MDA. This process further amplifies the damaging effects of oxygen-free radicals and leads to further cellular damage [54]. Thus, MDA levels reflect the extent of lipid peroxidation and the severity of cellular damage. To counteract oxidative stress, organisms rely on several antioxidant systems, including two key antioxidant enzymes: SOD, which scavenges superoxide radicals, and CAT, which breaks down hydrogen peroxide, thereby reducing the production of hydroxyl radicals [55]. Total antioxidant capacity (T-AOC) reflects the overall level of antioxidant macromolecules, small molecules, and enzymes in the system [56]. In this study, it was found that a high-carbohydrate diet increased liver MDA content while decreasing T-SOD and CAT activities, as well as T-AOC. However, the inclusion of CDCA in the diet had the opposite effect, decreasing MDA levels and increasing T-AOC, T-SOD, and CAT activities. This suggests that CDCA effectively alleviates oxidative stress induced by a high-carbohydrate diet, thereby protecting the liver. Consequently, fewer abnormal hepatocytes were observed in the hepatic histopathology of the HCC-0.10 group.

FXR has been proven to play a direct role in liver glucose homeostasis [57]. In carnivorous fish, excessive carbohydrate intake leads to a sharp increase in postprandial glucose levels [58], but sustained high-carbohydrate consumption has been reported to have no effect on *fxr* expression [22, 59]. In primary rat hepatocytes, D-glucose has been found to increase *fxr* mRNA in a dose and time dependent manner [60]. However, in the present experiment, a significant decrease in *fxr* expression was observed, which requires further investigation. CDCA, a natural and potent activator of FXR [61], has the potential to increase the *fxr* expression included in a high-carbohydrate diet. Activation of *shp* by *fxr* leads to the repression of hepatic gluconeogenesis genes such as *fbp1*, *pepck*, and *g6pc* [24, 25]. However, in this study, *fbp1* expression was significantly up-regulated in the HC group compared to the LC group. This upregulation may be due to the high-carbohydrate diet providing specific substrates that promote *fbp1* synthesis, facilitating cellular adaptation to elevated glucose concentrations,

while gluconeogenesis (*pepck*, *g6pc*) is suppressed. The addition of 0.10 % CDCA significantly reduced FBP1 expression but had no significant effects on *pepck* and *g6pc* expression, indicating an inhibition of gluconeogenesis and supporting the regulatory role of *fbp1*. However, the precise mechanisms underlying the high-carbohydrate-induced upregulation of *fbp1* require further investigation. Additionally, as tauroursodeoxycholic acid has been shown to improve liver and muscle insulin sensitivity and enhance muscle insulin signaling in obese humans [(62)], the present study found CDCA supplementation significantly increased the expression of *ira*, *irs*, *pi3kr1*and *akt1*, which are key genes in the insulin pathway [(29)]. Moreover, the expression of glycolysis-related genes, including *gk*, *pk*, and *pfkl*, was significantly elevated compared to the negative group. These results indicate that CDCA supplementation plays a beneficial role in regulating insulin and glycogen metabolism via *fxr*, enhancing the utilization of dietary carbohydrate by largemouth bass. Moreover, protein expression analysis of FXR, AKT1, and p-AKT1 confirms that CDCA inclusion in the high-carbohydrate diet activates FXR and promotes AKT1 phosphorylation, which may contribute to the regulation of insulin secretion.

The innate immune system in fish serves as the first line of defense against a broad spectrum of pathogens, with LZM activity being a key indicator of immune function [(63)]. Our results confirmed the detrimental effect of a high-carbohydrate diet on liver LZM activity in largemouth bass, but the addition of CDCA ameliorated this negative effect. Furthermore, *tlr2* is a member of the toll receptor family and functions as a pattern recognition receptor that plays a crucial role in innate immunity [(64)]. Histopathological analysis of liver tissue revealed significant inflammatory infiltration induced by the high-carbohydrate diets, which was consistent with the results observed for *tlr2* in this study. Meanwhile, NF-κB is a central transcriptional regulator of inflammatory responses and cell proliferation, and is involved in the inflammatory response induced by high-carbohydrate diets, similar results were obtained in the present experiment [(45, 65, 66)]. Abundant evidence has suggested that FXR can interact with NF-κB to modulate the inflammatory response [(67, 68 69)]. In the present study, the addition of CDCA significantly activated the expression of *fxr* and inhibited the expression of *nf-κb*, which suggested that *fxr* might act on *nf-κb* to inhibit its expression and reduce the inflammatory response. Moreover, dietary CDCA supplementation restored the expression of the anti-inflammatory genes *il-10* and *iκb* which were suppressed by the high dietary carbohydrate diet. The expression of another anti-inflammatory gene, *tgf-β*, was significantly increased with the addition of CDCA, regardless of the dietary carbohydrates level [(70, 71, 72)],

suggesting that CDCA has the potential to restore and enhance the anti-inflammatory response in the liver of carnivorous fish.

Conclusions

In conclusion, the addition of CDCA improved growth performance and liver health, alleviated hepatic glycogen accumulation through FXR-mediated activation of the insulin pathway and inhibition of gluconeogenesis, and reduced inflammatory responses in largemouth bass fed high-carbohydrate diets (Fig. 7).

Ethic Approval

The present experiment strictly followed the requirements of the Animal Care and Use Committee of Shanghai Ocean University.

Conflicts of Interest Statement

The authors declare that there are no conflicts of interest.

Authorship

Wenfei Li: Investigation, Data curation, Formal analysis, Writing-Original Draft; Nihe Zhang: Investigation, Methodology, Data curation; Ning Liu; Investigation, Methodology; Shiwen Chen: Investigation; Ye Gong: Investigation, Methodology, Writing-Review & Editing; Naisong Chen: Conceptualization; Project administration; Songlin Li: Conceptualization, Supervision, Writing-Review & Editing, Funding acquisition.

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References:

1. Sørensen M, Nguyen G, Storebakken T, *et al*. (2010) Starch source, screw configuration and injection of steam into the barrel affect the physical quality of extruded fish feed. *Aquac Res* **41**, 419-432.
2. Romano N & Kumar V (2019) Starch gelatinization on the physical characteristics of aquafeeds and subsequent implications to the productivity in farmed aquatic animals. *Rev Aquacult* **11**, 1271-1284.
3. Rokey G, Plattner B, Souza E (2010) Feed extrusion process description. *Rev Bras Zootecn* **39**, 510-518.
4. Stone DAJ (2003) Dietary Carbohydrate Utilization by Fish. *Rev Fish Sci* **11**, 337-369.
5. Wang J, Li X, Han T, *et al*. (2016) Effects of different dietary carbohydrate levels on growth, feed utilization and body composition of juvenile grouper *Epinephelus akaara*. *Aquaculture* **459**, 143-147.
6. Lin S, Shi C, Mu M, *et al*. (2018) Effect of high dietary starch levels on growth, hepatic glucose metabolism, oxidative status and immune response of juvenile largemouth bass, *Micropterus salmoides*. *Fish Shellfish Immun* **78**, 121-126.
7. Li S, Li Z, Zhang J, *et al*. (2019) The impacts of dietary carbohydrate levels on growth performance, feed utilization, glycogen accumulation and hepatic glucose metabolism in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × E. *lanceolatus* ♂). *Aquaculture* **512**, 734351.
8. Polakof S, Panserat S, Soengas JL, *et al*. (2012) Glucose metabolism in fish: A review. *J Comp Physiol, B* **182**, 1015-1045.
9. Li S, Yin J, Zhang H, *et al*. (2019) Effects of dietary carbohydrate and lipid levels on growth performance, feed utilization, body composition and non-specific immunity of large yellow croaker (*Larimichthys crocea*). *Aquacult Nutr* **25**, 995-1005.
10. Zhang B, Yang H, Nie Q, *et al*. (2024) High dietary wheat starch negatively regulated growth performance, glucose and lipid metabolisms, liver and intestinal health of juvenile largemouth bass, *Micropterus salmoides*. *Fish Physiol Biochem* **26**, 1083-1095.
11. Romano N, Kumar V, Yang G, *et al*. (2020) Bile acid metabolism in fish: disturbances caused by fishmeal alternatives and some mitigating effects from dietary bile inclusions. *Rev Aquacult* **12**, 1792-1817.
12. Hagey LR, Møller PR, Hofmann AF, *et al*. (2010) Diversity of bile salts in fish and amphibians: evolution of a complex biochemical pathway. *Physiol Biochem Zool* **83**, 308-321.

13. 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.
14. Abdel-Tawwab M, Abdel-Latif HM, Basuini MFE, *et al*. (2023) Effects of exogenous bile acids (BAs) on growth, lipid profile, digestive enzymes, and immune responses of thinlip mullet, *Liza ramada*. *Sci Rep-UK* **13**, 22875.
15. Ministry of Agriculture and Rural Affairs China. (2024) China Fishery Statistics Yearbook. China Agriculture Press, Beijing.
16. Zheng H, Xu Y, Zhao T, *et al*. (2023) 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*. *Brit J Nutr* **131**, 1-14.
17. Teodoro JS, Rolo AP & Palmeira CM (2011) Hepatic FXR: key regulator of whole-body energy metabolism. *Trends Endocrin Met* **22**, 458-466.
18. Shaik FB, Prasad DVR, Narala VR. (2015) Role of farnesoid X receptor in inflammation and resolution. *Inflamm Res* **64**, 9-20.
19. Yin P, Xie S, Zhuang Z, *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.
20. Du Y, Wang G, Yu E, *et al*. (2024) Dietary deoxycholic acid decreases fat accumulation by activating liver farnesoid X receptor in grass crap (*Ctenopharyngodon idella*). *Aquaculture* **578**, 740123.
21. Xu X, Chen N, Liu Z, *et al*. (2016) Effects of dietary starch sources and levels on liver histology in largemouth bass, *Micropterus salmoides*. *J Shanghai Ocean Univ* **25**, 61-70.
22. Chen P, Zhu Y, Wu X, *et al*. (2023) Metabolic adaptation to high-starch diet in largemouth bass (*Micropterus salmoides*) was associated with the restoration of metabolic functions via inflammation, bile acid synthesis and energy metabolism. *Brit J Nutr* **129**, 381-394.
23. Wang X, Gong Y, Li W, *et al*. (2024) The beneficial effects of metformin inclusion on growth performance, glucose utilization, antioxidant capacity and apoptosis of largemouth bass (*Micropterus salmoides*) fed with high dietary carbohydrates. *Aquaculture* **588**, 740957.

24. Yamagata K, Daitoku H, Shimamoto Y, *et al*. (2004) Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and foxo1. *J Biol Chem* **279**, 23158-23165.
25. Zhang Y, Lee FY, Barrera G, *et al*. (2006) Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sc. USA* **103**, 1006-1011.
26. Zhang W, Patil S, Chauhan B, *et al*. (2006) Foxo1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression. *J Biol Chem* **281**, 10105-10117.
27. Cipriani S, Mencarelli A, Palladino G, *et al*. (2010) FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (*fa*/*fa*) obese rats. *J Lipid Res* **51**, 771-784.
28. Renga B, Mencarelli A, Vavassori P, *et al*. (2010) The bile acid sensor FXR regulates insulin transcription and secretion. *BBA-Mol Basis Dis* **1802**, 363-372.
29. Caruso MA & Sheridan MA (2011) New insights into the signaling system and function of insulin in fish. *Gen Comp Endocr* **173**, 227-247.
30. Gai Z, Visentin M, Gui T, *et al*. (2018) Effects of farnesoid X receptor activation on arachidonic acid metabolism, Nf-kB signaling, and hepatic inflammation. *Mol Pharmacol* **94**, 802-811.
31. Panzitt K & Wagner M (2021) FXR in liver physiology: Multiple faces to regulate liver metabolism. *Biochim Biophys Acta, Mol Basis Dis* **1867**, 166133.
32. Du J, Xiang X, Li Y, *et al*. (2018) Molecular cloning and characterization of farnesoid X receptor from large yellow croaker (*Larimichthys crocea*) and the effect of dietary CDCA on the expression of inflammatory genes in intestine and spleen. *Comp Biochem Phys B* **216**, 10-17.
33. Zhang Y, Liang XF, He S, et al. (2022) Dietary supplementation of exogenous probiotics affects growth performance and gut health by regulating gut microbiota in Chinese Perch (*Siniperca chuatsi*). Aquaculture **547**, 737405.
34. Folch JMS, M. LM & Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497-509.
35. Peng M, Xu W, Mai K, *et al*. (2014) Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil. *Aquaculture* **433**, 442-449.

36. AOAC (2003) Official methods of analysis (17th ed.). *Association Of Official Analytical Chemists*, Washington.
37. Seifter S, Dayton S & Novic B (1950) The estimation of glycogen with the anthrone reagent. *Arch Biochem* **25**, 191-200.
38. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248-254.
39. Buege JA & Aust SD (1978) Microsomal lipid peroxidation. *Method Enzymol* **52**, 302-310.
40. McCord JM & Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* **244**, 6049-6055.
41. Aebi H (1984) Catalase in vitro. *Method Enzymol* **105**, 121-126.
42. Benzie IF & Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol* **299**, 15-27.
43. Livak KJ & Schmittgen T (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* **25**, 402-408.
44. Hemre G, Mommsen T & Krogdahl Å (2002) Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquacult Nutr* **8**, 175-194.
45. Liu Y, Liu N, Wang A, *et al*. (2021) Resveratrol inclusion alleviated high-dietary-carbohydrate-induced glycogen deposition and immune response of largemouth bass, *Micropterus salmoides*. *Brit J Nutr* **127**, 165-176.
46. Tao J, Gong Y, Chen S, *et al*. (2024). Dietary inclusion of *Clostridium butyricum* cultures alleviated impacts of high-carbohydrate diets in largemouth bass (*Micropterus salmoides*). *Brit J Nutr* **131**, 1308-1325.
47. Abdel-Tawwab M, Abdel-Latif H, Basuini M, *et al*. (2023). Effects of exogenous bile acids (BAs) on growth, lipid profile, digestive enzymes, and immune responses of thinlip mullet, *Liza ramada*. *Sci Rep-UK* **13**, 22875.
48. Zeng L, Lei J, Ai C *et al*. (2015). Protein-sparing effect of carbohydrate in diets for juvenile turbot *Scophthalmus maximus* reared at different salinities. *Chin J Oceanol Limn* **33**, 57-69.

49. 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.
50. Li X, Yao X, Zhang X, *et al*. (2022) Effects of dietary chenodeoxycholic acid supplementation in a low fishmeal diet on growth performance, lipid metabolism, autophagy and intestinal health of Pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immun* **127**, 1088-1099.
51. Li S, Wang A, Li Z, *et al*. (2020) Antioxidant defenses and non-specific immunity at enzymatic and transcriptional levels in response to dietary carbohydrate in a typical carnivorous fish, hybrid grouper (*Epinephelus fuscoguttatus* ♀ × E. *lanceolatus* ♂). *Fish Shellfish Immun* **100**, 109-116.
52. Zhong L, Liu H, Zhang H, *et al*. (2022) High starch in diet leads to disruption of hepatic glycogen metabolism and liver fibrosis in largemouth bass (*Micropterus salmoides*), Which is mediated by the PI3K/Akt signaling pathway. *Front Physiol* **13**, 880513.
53. Xu T, Liu X, Huang W, *et al*. (2023) Effects of dietary carbohydrate levels on growth, metabolic enzyme activities and oxidative status of hybrid snakehead (*Channa maculata* ♀ × *Channa argus* ♂). *Aquaculture* **563**, 738960.
54. Gaweł S, Wardas M, Niedworok E, *et al*. (2004) Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad Lek* **57**, 435-455.
55. Kanter M, Coskun O, Korkmaz A, *et al*. (2004) Effects of Nigella sativa on oxidative stress and β-cell damage in streptozotocin-induced diabetic rats. *Anat Rec, Part A* **279**, 685-691.
56. Ghiselli A, Serafini M, Natella F, *et al*. (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radical Biol Med* **29**, 1106-1114.
57. Duran-Sandoval D, Cariou B, Percevault F, *et al*. (2005) The farnesoid X receptor modulates hepatic carbohydrate metabolism during the fasting-refeeding transition. *J Biol Chem* **280**, 29971-29979.
58. Wilson RP (1994) Utilization of dietary carbohydrate by fish. *Aquaculture* **124**, 67-80.
59. Ge YP, Chen WL, Sun M, *et al*. (2022) Molecular characterization of farnesoid X receptor alpha in *Megalobrama amblycephala* and its potential roles in high-carbohydrate diet-induced alterations of bile acid metabolism. *J Steroid Biochem Mol Biol* **219**, 106065.

60. Duran-Sandoval D, Mautino G, Martin G, *et al*. (2004) Glucose regulates the expression of the farnesoid X receptor in liver. *Diabetes* **53**, 890-898.
61. Song P, Zhang Y & Klaassen CD (2011) Dose-response of five bile acids on serum and liver bile acid concentrations and hepatotoxicty in mice. *Toxicol Sci* **123**, 359-367.
62. Kars M, Yang L, Klein S, *et al*. (2010) Tauroursodeoxycholic acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. *Diabetes* **59**, 1899-1905.
63. Saurabh S & Sahoo PK (2008) Lysozyme: an important defence molecule of fish innate immune system. *Aquac Res* **39**, 223-239.
64. Zarubin T & Han J (2005) Activation and signaling of the p38 MAP kinase pathway. *Cell Res* **15**, 11-18.
65. Karin M & Greten FR (2005) NF-κB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* **5**, 749-759.
66. Tao J, Wang S, Qiu H, *et al*. (2022) Modulation of growth performance, antioxidant capacity, non-specific immunity and disease resistance in largemouth bass (*Micropterus salmoides*) upon compound probiotic cultures inclusion. *Fish Shellfish Immun* **127**, 804-812.
67. Wang YD, Chen WD, Wang M, *et al*. (2008) Farnesoid X receptor antagonizes nuclear factor κB in hepatic inflammatory response. *Hepatology* **48**, 1632-1643.
68. Gadaleta RM, Oldenburg B, Willemsen ECL, *et al*. (2011) Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-κB signaling in the intestine. *Biochimica Et Biophysica Acta* **1812**, 851-858.
69. Bijsmans ITGW, Guercini C, Ramos Pittol JM, *et al*. (2015) The glucocorticoid mometasone furoate is a novel FXR ligand that decreases inflammatory but not metabolic gene expression. *Sci Rep-Uk* **5**, 14086.
70. Letterio JJ & Roberts AB (1998) Regulation of immune responses by TGF-β. *Annu Rev Immunol* **16**, 137-161.
71. Ferreiro DU & Komives EA (2010) Molecular mechanisms of system control of NF-κB signaling by IκB. *Biochemistry-Us* **49**, 1560-1567.
72. Wang T & Secombes CJ (2013) The cytokine networks of adaptive immunity in fish. *Fish Shellfish Immun* **35**, 1703-1718.

73. Liu Y, Liu N, Wang A, *et al*. (2022) Resveratrol inclusion alleviated high-dietary-carbohydrate-induced glycogen deposition and immune response of largemouth bass, *Micropterus salmoides*. *Brit J Nutr* **127**, 165-176.
74. Wang S, Han Z, Turchini G, *et al*. (2022) Effects of dietary phospholipids on growth performance, digestive enzymes activity and intestinal health of largemouth bass (*Micropterus salmoides*) larvae. *Front Immunol* **12**, 827946.
75. Gong Y, Chen S, Wang Z, *et al*. (2024) Dietary lipid sources affect growth performance, lipid deposition, antioxidant capacity and inflammatory response of largemouth bass (*Micropterus salmoides*). *Fish Shellfish Immun* **150**, 109635.
76. Li Y, Zhang J, Xue M, *et al*. (2024) Characterization of the expression stability of largemouth bass (*Micropterus salmoides*) candidate reference genes by qRT-PCR during viral infection. *Comp Immunol Rep* **6**, 200134.
77. Gong Y, Chen S, Wang Z, *et al*. (2024) Dietary lipid sources affect growth performance, lipid deposition, antioxidant capacity and inflammatory response of largemouth bass (*Micropterus salmoides*). *Fish Shellfish Immun* **150**, 109635.

Fig. 1 The livers of largemouth bass fed the experimental diets for 8 weeks.

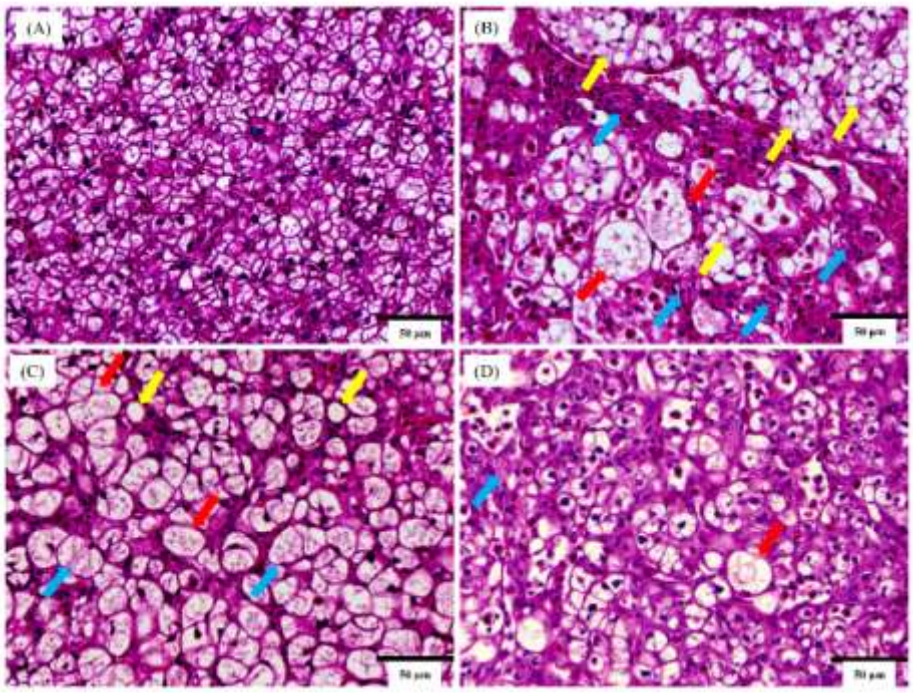

Fig. 2 The morphology analysis of the liver (Bar = 50 μm) of largemouth bass from LC (A), HC (B), HCC-0.05 (C) and HCC-0.10 (D) groups fed the experimental diets for 8 weeks.

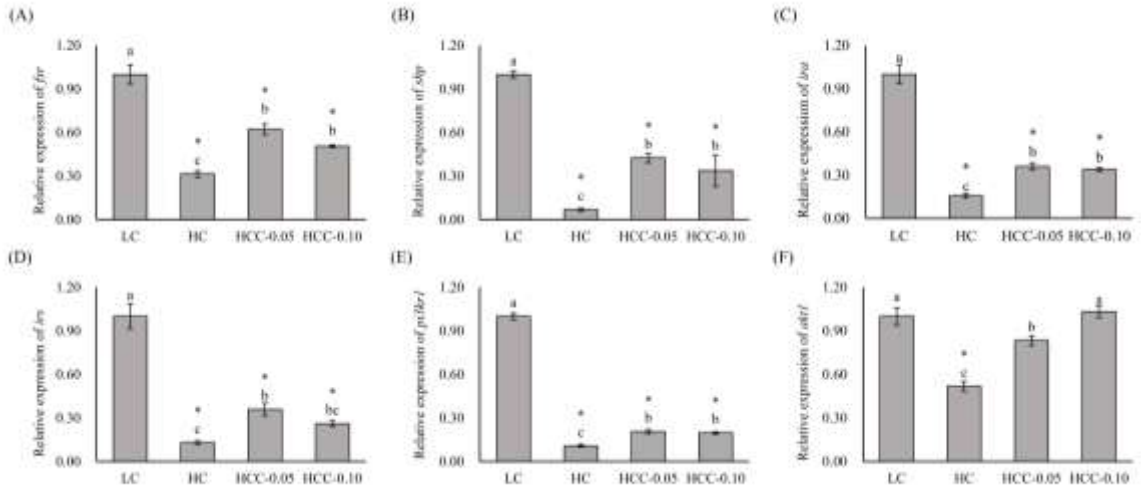

Fig. 3 The expression of genes related to insulin signalling pathway, *fxr* (A), *shp* (B), *irs* (C), ira (D), *pi3kr1* (E), *akt1* (F) in the liver of largemouth bass fed the experimental diets for 8 weeks. Values (mean ± standard error of the mean, SEM) in bars that have the same letter are not significantly different between treatments ($P > 0.05$; Duncan's test, N = 3), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$).

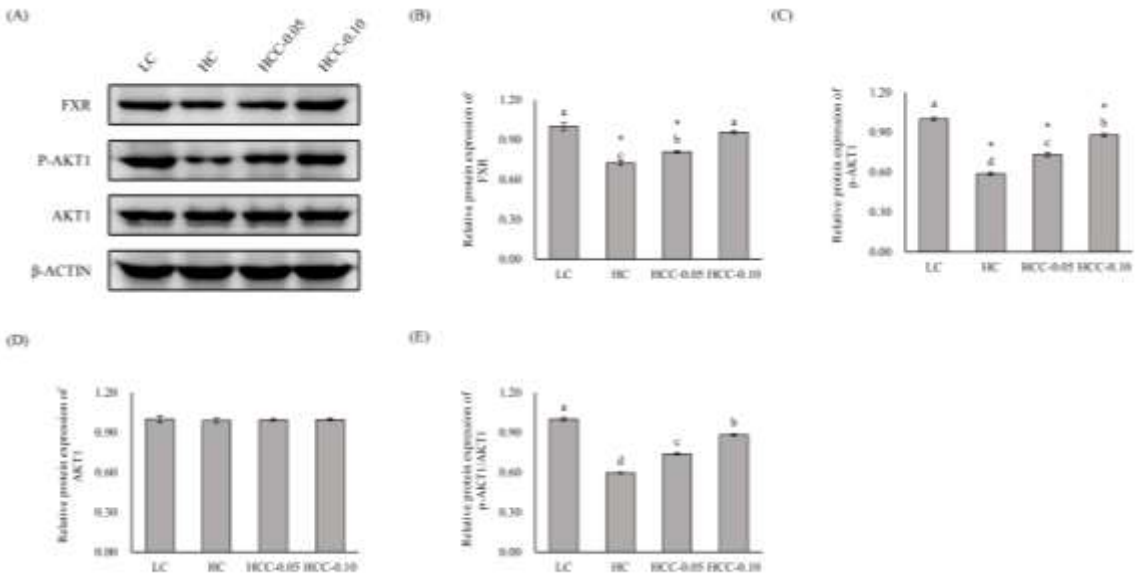

Fig. 4 The expression of FXR, p-AKT1, AKT1 and β-ACTIN protein in the liver of largemouth bass fed the experimental diets for 8 weeks. Values (mean ± standard error of the mean, SEM) in bars that have the same letter are not significantly different between treatments ($P > 0.05$; Duncan's test, N = 3), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$).

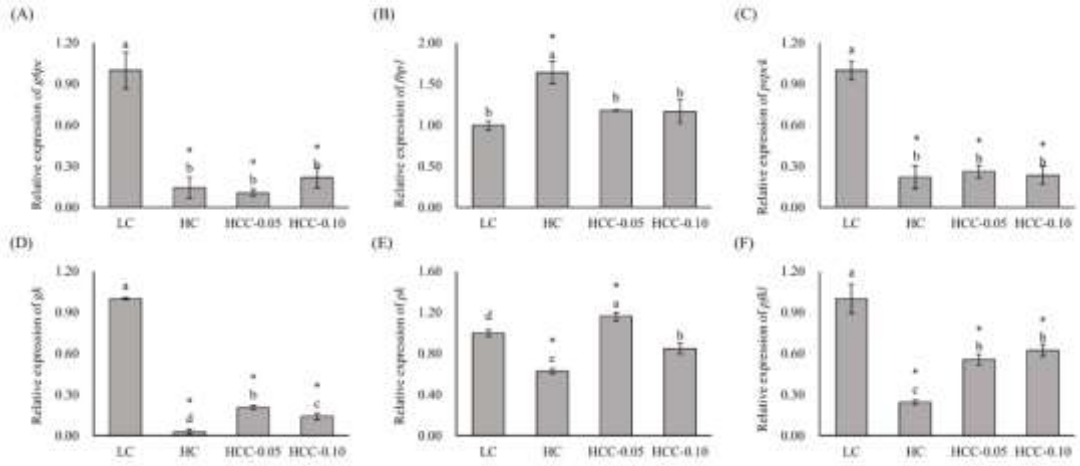

Fig. 5 The expression of genes related to glucose metabolism, *g6pc* (A), *fbp1* (B), *pepck* (C), *gk* (D), *pk* (E) and *pfkl* (F) in the liver of largemouth bass fed the experimental diets for 8 weeks. Values (mean ± standard error of the mean, SEM) in bars that have the same letter are not significantly different between treatments ($P > 0.05$; Duncan's test, N = 3), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$).

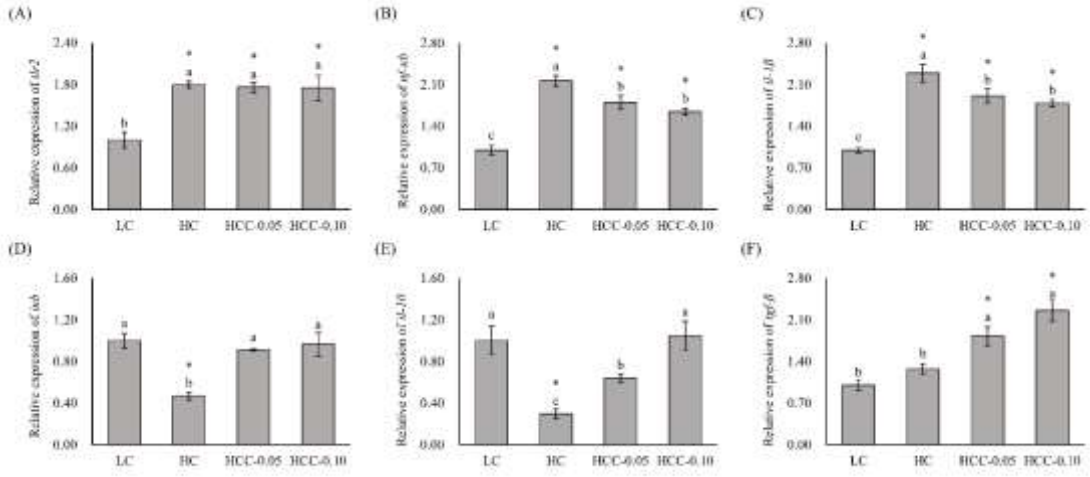

Fig. 6 The expression of genes related to inflammatory response, *tlr2* (A), *nf-κb* (B), *il-1β* (C), *iκb* (D), *il-10* (E), *tgf-β* (F) in the liver of largemouth bass fed the experimental diets for 8 weeks. Values (mean ± standard error of the mean, SEM) in bars that have the same letter are not significantly different between treatments ($P > 0.05$; Duncan's test, N = 3), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$).

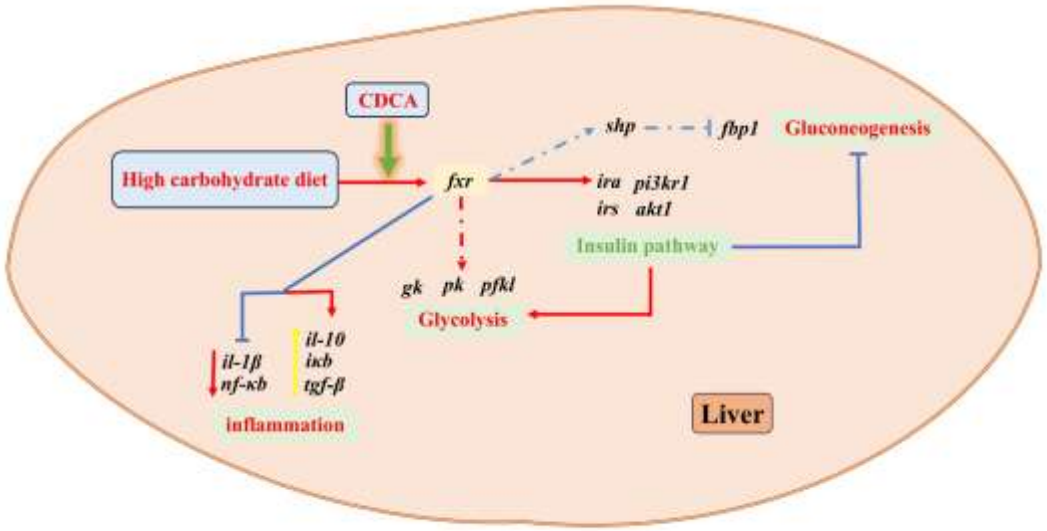

Fig.7 Schematic overview of the mechanisms by which CDCA regulates the hepatic insulin pathway, glucose metabolism, and immune metabolism in largemouth bass via *fxr*.

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

Ingredients	Diets			
	LC	HC	HCC-0.05	HCC-0.10
Fish meal[a]	40.00	40.00	40.00	40.00
Fermented enzymatic soybean meal[a]	11.00	11.00	11.00	11.00
Corn gluten meal[a]	10.00	10.00	10.00	10.00
Shrimp meal[a]	6.00	6.00	6.00	6.00
Blood meal[a]	3.00	3.00	3.00	3.00
Wheat gluten meal[a]	3.00	3.00	3.00	3.00
Beer yeast meal[a]	1.50	1.50	1.50	1.50
Squid paste[a]	2.00	2.00	2.00	2.00
Lecithin oil[a]	2.50	2.50	2.50	2.50
Soybean oil[a]	5.00	5.00	5.00	5.00
$Ca(H_2PO_4)_2$[a]	1.00	1.00	1.00	1.00
Vitamin mixture[b]	1.00	1.00	1.00	1.00
Mineral mixture[c]	1.00	1.00	1.00	1.00
α-Starch[a]	5.00	10.00	10.00	10.00
Microcrystalline cellulose[a]	5.00	0.00	0.00	0.00
Zeolite powder[a]	3.00	3.00	2.95	2.90
Chenodeoxycholic acid[d]	0.00	0.00	0.05	0.10
Proximate analysis (Mean values, % dry weight)				
Crude protein	52.58	52.72	52.78	52.88
Crude lipid	11.35	11.40	11.58	11.18
Ash	15.11	15.22	15.11	15.13
Crude fiber	10.32	5.47	5.21	5.37
Nitrogen free extract	10.64	15.19	15.32	15.44

[a] Supplied by Xinxin Tian'en Aquatic Feed Co., Ltd (Zhejiang, China).

[b] Vitamin Premix (mg/kg diet): vitamin A, 16000 IU; vitamin D_3, 8000 IU; vitamin K_3, 14.72; vitamin B_1, 17.80; vitamin B_2, 48; vitamin B_6, 29.52; vitamin B_{12}, 0.24; vitamin E, 160; vitamin C, 800; niacinamide, 79.20; calcium-pantothenate, 73.60; folic acid, 6.40; biotin, 0.64; inositol, 320; choline chloride, 1500; L-carnitine, 100.

[c] Mineral Premix (mg/kg diet): Cu ($CuSO_4$), 2.00; Zn ($ZnSO_4$), 34.4; Mn ($MnSO_4$), 6.20; Fe ($FeSO_4$), 21.10; I ($Ca(IO_3)_2$), 1.63; Se (Na_2SeO_3), 0.18; Co ($COCl_2$), 0.24; Mg ($MgSO_4 \cdot H_2O$), 52.7.

[d] Supplied by Meryer (Shanghai) Biochemical Technology Co., Ltd. (Shanghai, China).

Table 2. Sequences of the primers used in this study *.

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')	Amplicon size (bp)	Annealing temperatures (℃)	R^2	Efficiency value	GenBank reference or publication
fxr	TAAAAGGCTGCGAAAGAACACC	GGTGGTTGATGTGACCTGTTTGT	103	57	0.9985	0.9302	XM_038705261
shp	TTATTTGTTCTAGGGCTCGC	CTCTTTTGGCTTCTTGTTGG	292	57	0.985	0.9600	XM_038724551
irs	TAGTGGTGGTGTCAGCGGT	GGAGGTGGAAGTAAAGGAT	177	57	0.9801	0.9250	(23)
ira	CCCTTGTATCCCTCTCGTTT	CCAATTTCCTGTTCCTCTCC	164	57	0.9868	0.9834	XM_038717661
pi3kr1	AAGACCTTCCTCATCACGAC	CCTTCCACTACAACACTGCA	154	57	0.9897	0.8837	(23)
akt1	GACTCCTCTCCAGACCCCATG	TTTGCTACGATCACCTCCTTC	191	57	0.9819	0.9868	XM_038729261
g6pc	GCTCAAAGAGAGCGAGGATG	TCCTCTACCATTCGCAATCC	109	57	0.9901	0.9775	(76)
pepck	GGAAACGGCCAACATTCT	GCCAACCAGCAGTTCTCAT	121	57	0.9615	0.9306	(73)
fbp1	GCGATTGGCGAATTTATC	ACTCTGTGACGGCGGGTT	211	57	0.9863	0.9761	(73)
pk	CTCTTTCATCCGCAAAGC	AATTCCCAGGTCACCACG	115	57	0.9945	0.9489	(73)
pfkl	CTGGCTGAGCTCGTAAAG	GTGCCGCAGAAGTCGTTG	179	57	0.9848	0.9271	(73)
gk	GGGTTTTACCTTCTCCTTTC	GGTGGCTACTGTGTCATTCA	190	57	0.9927	0.9720	(73)
tlr2	ACGTGTGTCATGGCGGTGT	CGGGGGCTTCTGTTAGTCC	121	57	0.9913	0.9897	(77)
nf-κb	GCTGGTGTCTGGTTCATT	GCCTCCTCTTCCATCTCT	127	57	0.9886	0.9559	(74)

il-1β	CGTGACTGACAGCAAAAAGAGG	GATGCCCAGAGCCACAGTTC	163	57	0.9809	1.0188	(74)
tnf-α	CTTCGTCTACAGCCAGGCATCG	TTTGGCCACACCGACCTCACC	101	57	0.9934	0.9327	(74)
il-10	CGGCACAGAAATCCCAGAGC	CAGCAGGCTCACAAAATAAACATCT	181	57	0.9865	1.0008	XM_038696021
tgf-β	GCTCAAAGAGAGCGAGGATG	TCCTCTACCATTCGCAATCC	118	57	0.9827	1.0112	(74)
β-actin	CTCTGGGCAACGGAACCTCT	GTGCGTGACATCAAGGAGAAGC	103	57	0.9963	0.9988	(75)

*_fxr_: farnesoid X receptor; _shp_: small heterodimer partner; _irs_: insulin receptor substrate; _ira_: insulin receptor a; _pi3kr1_: phosphoinositide 3-kinase regulatory subunit 1; _akt1_: protein kinase B; _g6pc_: Glucose-6-phosphatase; _pepck_: phosphoenolpyruvate carboxykinase; _fbp1_: fructose bisphosphatase 1; _pk_: pyruvate kinase; _pfkl_: phosphofructokinase liver type; _gk_: glucokinase; _tlr2_: toll-like receptor 2; _nf-κb_: nuclear factor kappa b; _il-1β_: interlenkin-1β ; _tnf-α_: tumor necrosis factor α; _il-10_: interlenkin-10; _tgf-β_: transforming growth factor β.

Table 3. Growth performance of largemouth bass fed the experimental diets for 8 weeks.

Parameters	Diets			
	LC	HC	HCC-0.05	HCC-0.10
IBW (g)	5.44 ± 0.01	5.44 ± 0.01	5.47 ± 0.01	5.47 ± 0.02
FBW (g)	66.28 ± 1.32 ^{a}	47.68 ± 0.87 ^{c*}	46.27 ± 1.00 ^{c*}	54.42 ± 1.62 ^{b*}
SR (%)	100.00	100.00	100.00	100.00
SGR (%/d)	4.46 ± 0.03 ^{a}	3.88 ± 0.04 ^{c*}	3.81 ± 0.04 ^{c*}	4.10 ± 0.06 ^{b*}
FI (%/d)	2.41 ± 0.04 ^{a}	2.05 ± 0.02 ^{c*}	2.15 ± 0.03 ^{b*}	2.07 ± 0.04 ^{bc*}
CF	2.32 ± 0.01	2.29 ± 0.02	2.32 ± 0.01	2.30 ± 0.01
FCR	0.73 ± 0.01 ^{a}	0.64 ± 0.01 ^{c*}	0.68 ± 0.01 ^{b*}	0.67 ± 0.01 ^{b*}
HSI (%)	2.08 ± 0.04 ^{c}	3.80 ± 0.02 ^{a*}	3.75 ± 0.01 ^{a*}	3.45 ± 0.02 ^{b*}
VSI (%)	7.42 ± 0.05 ^{c}	8.51 ± 0.02 ^{a*}	8.48 ± 0.01 ^{a*}	8.30 ± 0.01 ^{b*}

Values (means ± SEM (Standard Error of Mean), N = 3) within a row with a common superscript letter are not significantly different from the other dietary groups ($P > 0.05$), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$).

Survival rate (SR, %) = final fish number/initial fish number × 100;

Specific growth rate (SGR, %/day) = (Ln final body weight - Ln initial body weight) × 100/days;

Feed intake (FI, %/day) = feed consumption (g)/[(initial weight + final weight)/2 × final number of individuals] × 100/days;

Condition factor (CF) = final body weight (g)/length $(cm)^3$ × 100;

Feed conversion ratio (FCR) = Total feed consumption (g DM) / (Final biomass – Initial biomass) (g wet weight);

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

Viscerosomatic index (VSI, %) = viscera weight/final body weight × 100.

Table 4. The whole body, liver proximate composition, and muscle proximate composition (wet weight basis) of largemouth bass fed the experimental diets for 8 weeks.

Parameters	Diets			
	LC	HC	HCC-0.05	HCC-0.10
Whole body				
Moisture (%)	70.90 ± 0.38 ^{b}	73.87 ± 0.08 ^{a*}	74.43 ± 0.77 ^{a*}	72.76 ± 0.85 ^{ab}
Crude protein (%)	18.28 ± 0.28	17.58 ± 0.18	17.23 ± 0.14	17.06 ± 0.55
Crude lipid (%)	4.60 ± 0.12 ^{b}	5.18 ± 0.07 ^{a*}	3.90 ± 0.04 ^{c*}	3.37 ± 0.08 ^{d*}
Ash (%)	3.73 ± 0.12 ^{a}	3.11 ± 0.05 ^{b*}	3.06 ± 0.06 ^{b*}	3.32 ± 0.12 ^{ab*}
Liver				
Moisture (%)	67.46 ± 0.17 ^{b}	70.59 ± 0.18 ^{a*}	70.16 ± 0.42 ^{a*}	69.94 ± 0.14 ^{a*}
Crude protein (%)	9.89 ± 0.11 ^{ab}	9.03 ± 0.20 ^{b}	9.52 ± 0.16 ^{ab}	10.09 ± 0.49 ^{a}
Crude lipid (%)	5.38 ± 0.06 ^{b}	5.74 ± 0.03 ^{a*}	4.72 ± 0.13 ^{c*}	4.87 ± 0.01 ^{c*}
Glycogen (mg/g)	94.92 ± 1.27 ^{c}	130.05 ± 3.34 ^{a*}	110.61 ± 1.24 ^{b*}	106.78 ± 1.58 ^{b*}
Muscle				
Moisture (%)	78.32 ± 0.24	78.72 ± 0.34	78.66 ± 0.16	78.41 ± 0.43
Crude protein (%)	19.29 ± 0.09	18.79 ± 0.09	18.63 ± 0.07	18.82 ± 0.41
Crude lipid (%)	2.02 ± 0.01 ^{bc}	2.28 ± 0.03 ^{a*}	2.05 ± 0.04 ^{b}	1.90 ± 0.04 ^{c}

Values (means ± SEM (Standard Error of Mean), N = 3) within a row with a common superscript letter are not significantly different from the other dietary groups ($P > 0.05$), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$).

Table 5. Hepatic biochemical parameters of largemouth bass fed the experimental diets for 8 weeks.

Parameters	Diets			
	LC	HC	HCC-0.05	HCB-0.10
TP (mg/g·tissue)	105.22 ± 2.09 [a]	53.34 ± 1.08 [c*]	75.79 ± 1.44 [b*]	74.55 ± 3.81 [b*]
MDA (nmol/g tissue)	66.46 ± 4.04 [b]	246.83 ± 25.55 [a*]	202.28 ± 16.38 [a*]	88.89 ± 13.36 [b]
CAT (U/mgprot)	22.48 ± 0.37 [a]	18.64 ± 0.03 [b*]	18.37 ± 0.16 [b*]	24.19 ± 0.98 [a]
T-SOD (U/mgprot)	239.40 ± 9.65 [b]	164.14 ± 10.52 [c*]	273.82 ± 4.46 [a]	257.08 ± 9.50 [ab]
T-AOC (U/mgprot)	0.64 ± 0.01 [a]	0.21 ± 0.01 [c*]	0.52 ± 0.03 [b*]	0.68 ± 0.04 [a]
LZM (U/mgprot)	0.37 ± 0.02 [a]	0.28 ± 0.01 [b*]	0.28 ± 0.01 [b*]	0.35 ± 0.02 [a]

Values (means ± SEM (Standard Error of Mean), N = 3) within a row with a common superscript letter are not significantly different from the other dietary groups ($P > 0.05$), with the asterisks are significantly different compared with that of the LC group (Dunnett's test, $P < 0.05$). TP, total protein; MDA, malondialdehyde; CAT, catalase; T-SOD, total superoxide dismutase; T-AOC, total antioxidant capacity; LZM, lysozyme.