Benfotiamine Ameliorates High Carbohydrate Diet-Induced Hepatic Oxidative Stress, Inflammation and Apoptosis in *Megalobrama Amblycephala*

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Research

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Abstract

Background: The impairment of immunity induced by high-carbohydrate diet is closely associated with the development of glucose metabolic disorders. In the study of diabetes, benfotiamine can prevent β-cell dysfunction by inhibiting inflammation, thereby improving insulin resistance. However, information regarding the effects of this substance on aquatic animals is extremely scarce.

Methods: A 12-week nutritional research was conducted to evaluate the influences of benfotiamine on the growth performance, oxidative stress, inflammation and apoptosis in *Megalobrama amblycephala* (45.25 ± 0.34 g) fed high-carbohydrate (HC) diets. Six experimental diets were formulated, containing a control diet (30% carbohydrate, C), a HC diet (43% carbohydrate), and the HC diet supplemented with four graded benfotiamine levels (0.7125 (HCB1), 1.425 (HCB2), 2.85 (HCB3), and 5.7 (HCB4) mg/kg).

Results: HC diet intake remarkably decreased daily growth coefficient (DGC), growth rate per metabolic body weight (GR_{MBW}), feed intake (FI), liver antioxidant enzymes activities, sirtuin-1 (SIRT1) protein expression as well as liver mRNA levels of SIRT1, nuclear factor erythroid 2-related factor 2 (Nrf2), catalase (CAT), manganese superoxide dismutase (Mn-SOD), interleukin10 (IL10) than those of the control group, but the opposite was true for plasma activities of alanine transaminase (AST) and aspartate aminotransferase (ALT), and contents of interleukin 1β (IL1β) and interleukin 6 (IL6), liver contents of malondialdehyde (MDA), and mRNA levels of kelch-like ECH associating protein 1 (Keap1), nuclear factor kappa B (NF-κB), tumour necrosis factor α (TNF α), IL1β, IL6, Bax, Caspase 3, Caspase 9 and P53. As with benfotiamine supplementation, HCB2 diet remarkably increased DGC, GR_{MBW}, liver antioxidant enzymes activities, SIRT1 protein expression as well as liver mRNA levels of SIRT1, Nrf2, CAT, Mn-SOD, IL10 and Bcl2, while the opposite was true for plasma activities of AST and ALT, and contents of IL1β and IL6, liver MDA contents as well as mRNA levels of Keap1, NF-κB, TNF α, IL1β, IL6, Bax, Caspase 3, Caspase 9 and P53.

Conclusion: Benfotiamine at 1.425 mg/kg can improve the growth performance and alleviate the oxidative stress, inflammation and apoptosis of *M. amblycephala* fed HC diets through the activation of the SIRT1 pathway.

1. Introduction

Carbohydrates (CHO) are the most economical energy source for animals including fish, due to their abundance and relatively low price [1]. However, most fish species (especially carnivorous ones) have limited ability to utilize dietary carbohydrates for energy purposes compared with mammals [2, 3]. This inadequate ability is characterized by prolonged postprandial hyperglycemia after a glucose loading or the intake of high-carbohydrate diets and even impaired growth [1]. To date, the basis for this apparent glucose intolerance in fish still remain obscure, although several hypotheses have been proposed, including a poor inhibition of postprandial gluconeogenesis, a weak ability of glucose to act as insulinotropins compared with amino acids, relatively low numbers of insulin receptors, etc [1-3]. Recently, researchers used several approaches such as metabolomics and transcriptomics to evaluate diet-induced metabolic syndromes in fish. Results from these studies suggested the glucose intolerance in fish is closely implicated in the impairment of immunity induced by high-carbohydrate diet [4-6]. However, the underlying mechanisms remain poorly understood, thus deserving our special attention.

Until now, numerous experimental studies showed that the direct link between inflammation and glucose intolerance [7, 8]. In fact, the long-term postprandial hyperglycemia caused by excess intakes of dietary carbohydrates can trigger oxidative stress in tissues, which is reflected by the production of superoxide radical anions [9]. Overproduction of superoxide radical anions can stimulate the generation of multiple pro-inflammatory cytokines (like tumour necrosis factor α (TNF α), interleukin 1β (IL1β) and interleukin 6 (IL6)) by enhancing the activation of pro-inflammatory transcription factors, such as nuclear factor kappa B (NF-κB) and toll like receptors (TLRs), thereby resulting in inflammation [10,11]. The increased pro-inflammatory cytokines then induce insulin insensitivity of tissues by blocking the activation of some key proteins that can mediate the conduction of insulin signaling pathway, thus resulting in glucose intolerance [12-14]. Moreover, multiple pro-

inflammatory cytokines, such as TNF α and IL6, can also trigger apoptosis by up-regulating transcription of the pro-apoptotic genes, such as Bcl2 family member Bax, thereby accelerating the programmed cell death [15, 16]. This is further exacerbates glucose intolerance. These results all showed that inflammation plays an important role in glucose intolerance. Recently, researchers on fish indicated the pronounced glucose intolerance is strongly associated with the low postprandial supervision of metabolic sensor that can regulate glucose metabolism and inflammatory response [1, 17]. Among them, silent information regulator 1 (SIRT1), a conserved NAD^+-dependent histone deacetylase, has attracted considerable attention. Generally, the activated SIRT1 can regulate multiple biological processes including glucose metabolism, inflammation and apoptosis, such as 1) the enhancement of glucose uptake in peripheral tissues [18]; 2) the suppression of NF-κB pro-inflammatory pathway [19]; 3) the inhibition of P53 pro-apoptotic pathway [20]. However, the aforementioned findings are mainly derived from mammals. Relevant information in aquatic animals is still quite limited. Recent evidence from studies in fish indicated that high-carbohydrate (HC) diets can cause chronic inflammation accompanied by the generation of pro-inflammatory cytokines, thus increasing the metabolic burden of liver [4, 21, 22]. This indicated a close link between inflammation and glucose metabolism in fish. However, the relevant physiological basis is still barely understood until now. Considering this, the biochemical and molecular investigation of these aspects will undoubtedly facilitate better understanding of the carbohydrates utilization by fish.

Benfotiamine is a thiamine prodrug with higher bioavailability and absorption than thiamine [23]. At present, it is commonly used as a food supplement for the treatment of type 2 diabetes mellitus (T2DM) to improve glucose homeostasis via enhancing the glycolytic capabilities, promoting insulin synthesis and increasing glucose oxidation in mitochondria [24]. In addition, it also has potent anti-oxidant, anti-inflammatory, anti-apoptotic and anti-carcinogenic properties [25, 26]. In the study of diabetes, benfotiamine has been shown to prevent β-cell dysfunction by inhibiting inflammation, thereby improving insulin resistance in tissues [27]. However, information regarding the effects of this substance on aquatic animals is extremely scarce. Recently, our study had confirmed that long-term administration of benfotiamine significantly improved the glucose homeostasis of *Megalobrama amblycephala* (an herbivorous freshwater carp) fed with a HC diet (43% carbohydrate levels) [28]. For example, dietary benfotiamine significantly decreased plasma glucose levels of this fish fed HC diet [28, 29]. However, the underlying mechanisms are barely understood. Here, we speculated that benfotiamine may benefit the glucose homeostasis of this species through the improvement of immune function. Bearing this in mind, our current research was conducted to investigate the effects of dietary benfotiamine on the growth performance, oxidative stress, inflammation and apoptosis of juvenile *M. amblycephala* fed with a HC diet. The findings obtained here can serve to provide information regarding the impact of HC diet on immune response in fish, and promote the development of nutritional strategies for improving the carbohydrate utilization by aquatic animals.

2. Material And Methods

2.1. Ethics statement

Animal experimentation within the present study was conducted in accordance with the Animal Care and Use guidelines of Nanjing Agricultural University (Nanjing, China) with the permissions obtained (permit number: SYXK (Su) 2011–0036).

2.2. Benfotiamine and the experimental diets

Benfotiamine with a purity of at least 98% was supplied by Xian Reain Biomedical Company (Xian, China). Six isonitrogenous and isolipidic diets with two levels of carbohydrate (30% and 43% carbohydrate) were prepared, containing a control diet (30% carbohydrate, C), a HC diet (43% carbohydrate), and the HC diet containing graded levels of benfotiamine [0.7125 (HCB1), 1.425 (HCB2), 2.85 (HCB3) and 5.7 (HCB4) mg/kg, respectively] (Table 1). Fish meal, soybean meal, rapeseed meal, and cottonseed meal were used as protein sources, with fish oil and soybean oil used as the main lipid sources. Corn starch was the main carbohydrate source. Microcrystalline cellulose was used to compensate for the carbohydrate levels required.

The experimental diets were produced by the method described in detail previously [30]. Briefly, dry ingredients were grounded, weighed, then mixed with oils. An appropriate amount of water was added to produce dough. The dough was later pelleted using a laboratory pellet machine (MUZL 180, Jiangsu Muyang Group Co., Ltd., Yangzhou, China) and dried in a ventilated oven at 30 °C. After drying, the diets were broken up and sieved into proper pellet size. All diets were stored at −20 °C in plastic-lined bags until use.

2.3. Feeding trial and experimental conditions

M. amblycephala were obtained from the National Fish Hatchery Station at Yangzhou (Jiangsu, China). The experiment was performed in a re-circulating aquaculture system in the laboratory. The system includes an inlet conduit, a tank, a water treatment unit, a clarifier, and an outlet conduit. The tank is in fluid communication with the inlet conduit to receive water displaced from the inlet conduit. The volume of each tank is 300 L. Prior to the start of the experiment, fish were acclimated to the experimental facilities and fed a commercial diet (32% protein, 6% lipids, and 33% carbohydrates) for 2 weeks. Then, 360 fish (average weight: 45.25 ± 0.34 g) were allocated to 24 indoor tanks (300 L volume) at a number of 15 fish per tank. Fish were hand-fed thrice daily (07:00, 12:00, and 17:00 h) for 12 weeks. During the experimental period, photoperiod 12: 12 h (dark: light), water conditions including temperature (27.4 ± 0.6 ℃), pH (7.4–7.5) and dissolved oxygen (>5.1 mg/L).

2.4. Samples collection

After the 12-week feeding trial, fish were starved for 24 h prior to sampling. All the fish in each tank were counted and weighed. Then, 4 fish from each tank were anesthetized by the diluted MS-222 (100 mg/L). Blood was put into heparinized tubes [28, 29]. Also, liver samples were collected from these fish, and then snap frozen in liquid nitrogen and stored at −80 °C until analysis.

2.5. Analysis of proximate composition and plasma and liver biochemical indices

Moisture, crude lipid, crude protein, ash, gross energy and crude fiber contents of feeds were assayed by AOAC (2006) methods [31]. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma were assayed according to Habte-Tsion et al (2016) [32]. Plasma concentrations of interleukin 1β (IL 1β) and interleukin 6 (IL 6) were measured using cytokine-specific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (R and D Systems, USA, no. MTA00B, D6050 and DCP00). Liver activities of total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC) and catalase (CAT) were assayed as previously described by Zhou et al. (2013) [33] and Aebi (1984) [34], respectively. Liver malondialdehyde (MDA) content was assayed according to Zhao et al. (2016) [35] with thiobar-bituric acid.

2.6. Analysis of western blot (WB) and RT-PCR

Total protein was extracted from liver (about 3 g) by the RIPA lysis buffer (Beyotime, China). Then, western blot analysis was performed by our previous methods [30]. Rabbit antibody SIRT1 and β-actin were purchased from Proteintech (13161-1-AP, United States) and Boster (BM3873, China), respectively. Anti-rabbit antibody was purchased from Cell Signaling Technology (#7074, United States). The signals of WB are quantitatively assayed by ImageJ 1.44p (National Institutes of Health, Bethesda, USA).

Total RNA extraction and cDNA synthesis in liver (about 2 g) were performed by our previous studies [28, 29, 36]. Briefly, total RNA was extracted by Tri Pure Reagent (Aidlab, China). Then, the quality and quantity of RNA were assayed by using Nanodrop 2000 and electrophoresis, respectively. The mRNA levels were assayed for some target genes containing SIRT1, Nrf2 (nuclear factor erythroid 2-related factor 2), Keap1 (kelch-like ECH associating protein 1), CAT (catalase), Cu/Zn-SOD (copper/zinc superoxide dismutase), Mn-SOD (manganese superoxide dismutase), NF-κB, TNF α, IL1β, IL6, IL8 (interleukin 8), IL10, P53, Bcl2, Bax, Caspase 3 and Caspase 9. The relative mRNA levels were calculated by a housekeeping gene (EF1α) using $2^{-\Delta\Delta CT}$ method.

2.7. Statistical analysis

The results in this study were presented as means ± S.E.M. (standard error of the mean). The normality of distribution and the homogeneity of variances were checked prior to one-way analysis of variance (ANOVA) followed by Tukey's HSD test (IBM SPSS Statistics 22.0). Differences with P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Growth performance and feed utilization

No statistical differences in feed intake per metabolic body weight (FI_{MBW}) and feed efficiency ratio (FER) were found among treatments (Table 3). Daily growth coefficient (DGC), growth rate per metabolic body weight (GR_{MBW}) and feed intake (FI) of the HC group were significantly ($P < 0.05$) lower than those of the C group ($P < 0.05$). DGC, GR_{MBW} and FI increased significantly ($P < 0.05$) as benfotiamine levels increased from 0 to 1.425 mg/kg, but decreased significantly ($P < 0.05$) with further increasing levels.

3.2. Plasma and liver biochemical indices

Plasma activities of AST and ALT, and levels of IL1β and IL6 as well as hepatic MDA contents of the HC group were significantly ($P < 0.05$) higher than those of the C group, while the opposite was true for liver T-AOC, SOD and CAT activities (Table 4). Dietary benfotiamine supplementation led to a decrease of plasma AST and ALT activities, and IL1β and IL6 levels as well as hepatic MDA contents, but the opposite was true for liver T-AOC, SOD and CAT activities. In addition, the lowest values of AST, ALT, IL1β and MDA, as well as the highest values of SOD and CAT were found in the HCB2 group.

3.3. Liver protein expression of SIRT1 and mRNA levels of SIRT1

The protein expression of SIRT1 and mRNA levels of SIRT1 were significantly ($P < 0.05$) lower in fish fed HC diet than in fish fed the control diet (Figure 1). As for the HC groups, their values were significantly ($P < 0.05$) increased in fish fed the diet with benfotiamine supplementation.

3.4. The transcriptions of antioxidant-related genes in liver

No statistical difference ($P > 0.05$) was observed in the transcriptions of Cu/Zn-SOD among all the treatments (Figure 2). The mRNA levels of Nrf2, CAT and Mn-SOD of the HC group were significantly ($P < 0.05$) lower than those of the C group, but the opposite was true for Keap1 expression. The HC2 diets significantly ($P < 0.05$) increased the mRNA levels of Nrf2, CAT and Mn-SOD, but the opposite was true for Keap1.

3.5. The transcriptions of inflammation-related genes in liver

No statistical difference ($P > 0.05$) was observed in the mRNA levels of IL8 among all the treatments (Figure 3). The mRNA levels of NF-κB, TNF α, IL1β and IL6 of the HC group were significantly ($P < 0.05$) higher than those of the C group, but the opposite was true for IL10. The HC2 diets significantly ($P < 0.05$) decreased the mRNA levels of NF-κB, TNF α, IL1β and IL6, but the opposite was true for IL10 expression.

3.6. The transcriptions of apoptosis-related genes in liver

The mRNA levels of P53, Bcl2, Bax, Caspase 3 and Caspase 9 of the HC group were higher than those of the C group, but no statistical difference was observed in Bcl2 mRNA levels ($P > 0.05$) (Figure 4). The HC2 diets significantly ($P < 0.05$) decreased the expression of P53, Bax, Caspase 3 and Caspase 9, but the opposite was true for Bcl2 expression.

4. Discussion

In the present study, the DGC, GR_{MBW} and FI of the HC group were all significantly lower than those of the C group, which indicating an retarded growth and an low feed consumption in *M. amblycephala* fed with a HC diet. These results might be attributed to the following facts: (1) the intake of high-carbohydrate diets can cause persistent hyperglycemia, which is considered as a physiological stress response, thus negatively affecting the growth performance of fish [2]; (2) high-carbohydrate levels in diet can decrease feed palatability, and increase animal satiety, as might consequently result in a decline of feed consumption [38]. In fact, a significant high level of plasma glucose has been found in *M. amblycephala* fed HC diet [28, 29]. Then, DGC and GR_{MBW} were significantly increased by dietary benfotiamine supplementation with increasing level up to 1.425 mg/kg, which suggesting a beneficial effect of benfotiamine at 1.425 mg/kg on the growth of *M. amblycephala* fed with HC diet. According to previous study, optimal levels of thiamine (the analog of benfotiamine) can enhance the activities of intestinal digestive enzymes of Jian carp (*Cyprinus carpio* var. Jian), thus leading to an increase in the feed efficiency [40]. This might improves the growth performance of fish. Additionally, benfotiamine administration has been demonstrated to reduce the metabolic damage induced by hyperglycemia in mammals by the inhibition of inflammation, and the decrease of advanced glycation end products (AGEs) formation [24, 27]. This is possible that a similar mechanism exists in fish. However, the underlying mechanisms are poor understood. In order to characterize the corresponding mechanisms, molecular investigations were performed in certain groups (namely the C, HC and HCB2).

In this study, HC diet intake led to an increase of AST, ALT, IL1β, IL6 and MDA, but the opposite trend was true for the activities of T-AOC, SOD and CAT in the liver. These results suggested high dietary carbohydrates induced an inflammation coupled with low antioxidant ability in the liver of *M. amblycephal*. This result was supported by the following facts: (1) the pro-inflammatory cytokines containing IL 1β and IL 6 are the sensitive indicators of host response to inflammation [41]; (2) the protective effects against oxidative damage could be directly reflected by the activities of some antioxidant enzymes containing SOD, CAT and T-AOC in fish as in mammals [42]. According to previous study, this result may be partly due to that high-carbohydrate intake can inevitably result in the prolonged postprandial hyperglycemia, which in turn stimulates the overproduction of superoxide radical anions via increasing the activities of nicotinamide adenine dinucleotide phosphate oxidase, and inducing mitochondrial membrane hyperpolarization [43]. Then, overproduction of superoxide radical anions can induce intracellular chronic oxidative and inflammatory stresses characterized by decreasing liver SOD, CAT and T-AOC activities, as well as increasing plasma IL1β and IL6 levels [44, 45], thus damaging liver metabolic functions. As for the HC groups, dietary supplementation of benfotiamine significantly decreased the values of AST, ALT, IL1β, IL6 and MDA, but the opposite trend was true for the activities of T-AOC, SOD and CAT in the liver. This result indicated benfotiamine supplementation significantly enhanced antioxidant capacity, but inhibited inflammation in the liver of *M. amblycephala* fed with HC diet. According to previous studies, these results may be due to that the administration of benfotiamine could promote insulin synthesis and secretion, thereby accelerating glucose disposal in peripheral insulin target tissues, as might accordingly mitigate hyperglycemia-induced intracellular oxidative and inflammatory stresses [46, 47]. In addition, the lowest values of AST, ALT, IL1β and MDA, as well as the highest values of SOD and CAT were found in HCB2 group. This result indicated that benfotiamine supplementation at 1.425 mg/kg could effectively enhance the antioxidant capacity, but inhibit inflammation in the liver of *M. amblycephala* fed with HC diet.

SIRT1 is a highly conserved nicotinamide adnine dinucleotide (NAD^+) dependent protein deacetylase, which recognized as a critical regulator in the cellular response to the metabolic, inflammatory, and oxidative stresses [48-50]. In this study, the intake of HC diets down-regulated hepatic SIRT1 protein and mRNA levels compared with the control group. According to previous study, the intake of HC diets can inhibit the activity of pyruvate dehydrogenase complex, thereby resulting in a decrease of NAD^+ content [51]. This might inevitably reduces SIRT1 protein and mRNA levels. As for the HC groups, the supplementation of benfotiamine at 1.425 mg/kg significantly up-regulated SIRT1 protein and mRNA levels. Previous study showed that benfotiamine could increase adenosine triphosphate (ATP)/adenosine monophosphate (AMP) ratio via the following reaction: thiamine + ATP = thiamine diphosphate (ThDP) + AMP, thereby activating AMP-activated protein kinase (AMPK) [52]. Subsequently, AMPK activation could increase SIRT1 activity by promoting the generation of NAD^+ [53]. This might be reflected by up-regulating the protein and mRNA levels of SIRT1.

According to previous studies, the activated SIRT1 could improve the antioxidant capacity of organisms by enhancing the activity of oxidative stress regulator-Nrf2 [54, 55]. In the present study, hepatic Nrf2, CAT and Mn-SOD mRNA levels of the HC group were significantly lower than those of the control group, but the opposite was true for Keap1 mRNA levels. This result was in line with the antioxidant enzymes (SOD and CAT) activities in the liver, indicating that the intake of HC diets reduced hepatic antioxidant capacity of *M. amblycephala*. This may be attributed to that the intake of HC diets could inhibit the conduction of Nrf2 antioxidant response element signaling pathway, which might correspondingly down-regulates the activities and mRNA levels of Nrf2-modulated antioxidant enzymes (such as, SOD and CAT) [56, 57], thus resulting in a decrease of hepatic antioxidant capacity. This was further supported by that high Keap1 mRNA levels was found in HC group, since Keap1 can decrease the activity of Nrf2 by mediating the ubiquitination [58]. As for the HC groups, dietary supplementation of benfotiamine significantly up-regulated the mRNA levels of Nrf2, CAT and Mn-SOD, but the opposite was true for Keap1 mRNA levels. This may be due to that the activated SIRT1 could enhance the activities of Nrf2 by inhibiting the Keap1-mediated ubiquitination, thus up-regulating the mRNA levels of Nrf2-modulated antioxidant enzymes [59, 60].

Furthermore, the activated SIRT1 can inhibit high glucose-induced inflammation by suppressing NF-κB pro-inflammatory pathway, thus enhancing the glucose homeostasis [61]. However, such information is mainly derived from mammals. In this study, hepatic NF-κB, TNF α, IL1β and IL6 mRNA levels of the HC group were significantly higher than those of the control group, but the opposite was true for IL 10 mRNA levels. These results suggested that the intake of HC diets induced hepatic inflammation of *M. amblycephala*. According to previous study, the activation of NF-κB induced by high-glucose could stimulate the generation of multiple pro-inflammatory cytokines containing TNF α, IL1β and IL6, thus resulting in the inflammation of organisms [62]. In addition, excessive pro-inflammatory cytokines could also reduce insulin sensitivity in tissues by disturbing the conduction of insulin signaling pathway [63], thereby further aggravating glucose metabolism dysfunction. As for the HC groups, HCB2 significantly down-regulated the mRNA levels of NF-κB, TNF α, IL1β and IL6, but the opposite was true for IL 10. These results suggested that dietary benfotiamine supplementation alleviated hepatic inflammation induced by the intake of HC diets. According to previous studies, this result might be attributed to the following facts: (1) the activated SIRT1 by benfotiamine can deacetylate lysine 310 in the p65 subunit of NF-κB, thereby blocking NF-κB mediated pro-inflammatory pathways [64]; and (2) the activated SIRT1 can enhance the synthesis of anti-inflammatory cytokine IL10, which is beneficial to the mitigation of the inflammatory stress [65].

Previous studies indicated that high glucose levels can induce pancreatic β-cell apoptosis, thus aggravating the glucose metabolic dysfunction in tissues [66, 67]. The activated SIRT1 can inhibit high glucose-induced apoptosis by the suppression of P53 pro-apoptotic pathway [68]. However, such information in fish is still barely understood. In this study, the mRNA levels of P53, Bax, Caspase 3 and Caspase 9 of the HC group were all significantly higher than those of the control group, which indicating HC diet intake might induce apoptosis in the liver of *M. amblycephala*. This might be due to the fact that P53 activation by high glucose level could increase the activity of Bax, which further activates caspase 9 and caspase 3, thus resulting in apoptosis [15, 48]. As for the HC groups, HCB2 significantly down-regulated the mRNA levels of P53, Bax, Caspase 3 and Caspase 9, but the opposite was true for Bcl2 mRNA levels. According to previous studies, the activated SIRT1 could inhibit the activity of P53 by the deacetylation at Lys310, which in turn restrains the activation of P53 downstream target genes containing Bax, thereby reducing cell apoptosis [15, 16, 49, 69].

5. Conclusions

In summary, our findings demonstrated that dietary supplementation of benfotiamine could attenuate high-carbohydrate induced hepatic oxidative stress, inflammation and apoptosis in *M. amblycephala*, via the SIRT1 activation, the increase of the activities of the Nrf2-modulated antioxidant enzymes coupled with the down-regulation of the transcriptions of the NF-κB-mediated pro-inflammatory cytokines and P53-mediated pro-apoptotic genes. These results might benefit the improvement of glucose metabolism of *M. amblycephala* fed HC diets. In addition, fish offered 1.425 mg/kg benfotiamine have the best growth performance.

6. Declarations

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author Contributions

CX, X-FL, and W-BL conceived and designed the experiments. CX analyzed the data. X-FL, CX and H-JS performed the experiments and contributed reagents, materials, and analysis tools. CX wrote the paper. All authors contributed to the revision of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

7. References

1. Kamalam BS, Medale F, Panserat S. Utilisation of dietary carbohydrates in farmed fishes, new insights on influencing factors, biological limitations and future strategies. Aquaculture. 2017;467: 3–27.
2. Hemre GI, Mommsen TP, Krogdahl A. Carbohydrates in fish nutrition, effects on growth, glucose metabolism and hepatic enzymes. Aquac Nutr. 2002;8: 175–194.
3. Enes P, Panserat S, Kaushik S, Oliva-Teles A. Nutritional regulation of hepatic glucose metabolism in fish. Fish Physiol Biochem. 2002;35: 519–539.
4. Miao LH, Lin Y, Pan WJ, Huang X, Ge XP, Ren MC, et al. Identification of differentially expressed microRNAs associate with glucose metabolism in different organs of blunt snout bream (*Megalobrama amblycephala*). Int J Mol Sci. 2017;18: 1161–1176.
5. Prathomya P, Prisingkorn W, Jakovli´c I, Deng FY, Zhao YH, Wang WM. 1H NMR-based metabolomics approach reveals metabolic alterations in response to dietary imbalances in *Megalobrama amblycephala*. Metabolomics. 2017;13: 17.
6. Prisingkorn W, Prathomya P, Jakovliæ I, Liu H, Zhao YH, Wang WM. Transcriptomics, metabolomics and histology indicate that high carbohydrate diet negatively affects the liver health of blunt snout bream (*Megalobrama amblycephala*). BMC Genomics. 2017;18: 856.
7. Wolf M, Sauk J, Shah A, Smirnakis KV, Jimenez-Kimble R, Ecker JL, et al. Inflammation and glucose intolerance, a prospective study of gestational diabetes mellitus. Diabetes Care. 2004;27: 21–27.
8. Sun Q, Li J, Gao F. New insights into insulin, the anti-inflammatory effect and its clinical relevance. World J Diabetes. 2014;5: 89–96.
9. Mkhwanazi BN, Serumula MR, Myburg RB, Van Heerden FR, Musabayane CT. Antioxidant effects of maslinic acid in livers, hearts and kidneys of streptozotocin-induced diabetic rats, effects on kidney function. Ren Fail. 2014;36: 419–431.
10. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans, role of oxidative stress. Circulation. 2002;106: 2067–2072.

11. Laubertová L, Koňariková K, Gbelcová H, Ďuračková Z, Žitňanová I. Effect of walnut oil on hyperglycemia-induced oxidative stress and pro-inflammatory cytokines production. Eur J Nutr. 2015;54: 291–299.
12. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes, the atherosclerosis risk in communities study. Diabetes. 2003;52: 1799–1805.
13. González F. Inflammation in Polycystic Ovary Syndrome,underpinning of insulin resistance and ovarian dysfunction. Steroids. 2012;77: 300–305.
14. Francés DE, Ingaramo PI, Ronco MT, Carnovale CE. Diabetes, an inflammatory process, Oxidative stress and TNF-alpha involved in hepatic complication. J Biomedical Science and Engineering. 2013;6: 645–653.
15. Whiteman M, Chu SH, Jia LS, Rose P, Sabapathy K, Schantz JT, et al. The pro-inflammatory oxidant hypochlorous acid induces Bax-dependent mitochondrial permeabilisation and cell death through AIF-/EndoG-dependent pathways. Cell Signal. 2007;19: 705–714.
16. Keuper M, Blüher M, Schön MR, Möller P, Dzyakanchuk A, Amrein K, et al. An inflammatory micro-environment promotes human adipocyte apoptosis. Mol Cell Endocrinol. 2011;339: 105–113.
17. Polakof S, Panserat S, Soengas JL, Moon TW. Glucose metabolism in fish, a review. J Comp Physiol B. 2012;182: 1015–1045.
18. Chen SF, Zhao ZL, Ke LR, Li ZL, Li WX, Zhang ZL, et al. Resveratrol improves glucose uptake in insulin-resistant adipocytes via Sirt1. J Nutr Biochem. 2018;55: 209–218.
19. Liu S, Zheng ZD, Ji SH, Liu TT, Hou YH, Li SS, et al. Resveratrol reduces senescence-associated secretory phenotype by SIRT1/NF-κB pathway in gut of the annual fish *Nothobranchius guentheri*. Fish Shellfish Immun. 2018;80: 473-479.
20. Tan J, Fan L, Mao JJ, Chen B, Zheng LW, Zhang T, et al. Restoration of miR-34a in p53 deficient cells unexpectedly promotes the cell survival by increasing NF-κB activity. J Cell Biochem. 2012;113: 2903–2908.
21. Deng KY, Pan MZ, Liu JH, Yang MX, Gu ZX, Zhang Y, et al. Chronic stress of high dietary carbohydrate level causes inflammation and influences glucose transport through SOCS3 in Japanese flounder *Paralichthys olivaceus*. Sci Rep. 2018;8: 7415.
22. Yang Q, Liang HL, Mokrani A, Ji K, Yu H, Ge XP, et al. Dietary histidine affects intestinal antioxidant enzyme activities, antioxidant gene expressions and inflammatory factors in juvenile blunt snout bream (*Megalobrama amblycephala*). Aquacult Nutr. 2018; 25: 249–259.
23. Stirban A, Nandrean S, Kirana S, Götting C, Veresiu IA, Tschoepe D. Benfotiamine counteracts smoking-induced vascular dysfunction in healthy smokers. Int J Vasc Med. 2012;2012: 968761.
24. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, et al. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med. 2003;9: 294–299.
25. Beltramo E, Berrone E, Tarallo S, Porta M. Effects of thiamine and benfotiamine on intracellular glucose metabolism and relevance in the prevention of diabetic complications. Acta Diabetol. 2008;45: 131–141.
26. Tapias V, Jainuddin S, Ahuja M, Stack C, Elipenahli C, Vignisse J, Gerges M, et al. Benfotiamine treatment activates the Nrf2/ARE pathway and is neuroprotective in a transgenic mouse model of tauopathy. Hum Mol Genet. 2018;27: 2874-2892.
27. Serhiyenko V, Ponomarev AA, Segin VB, Azhmi S, Serhiyenko LM. Effects of benfotiamine on the insulin resistance state, some pro- and anti-inflammatory factors content in patients with type 2 diabetes mellitus and cardiac autonomic neuropathy. Russ J Cardiol. 2019;24: 78-82.
28. Xu C, Liu WB, Dai YJ, Jiang GZ, Wang BK, Li XF. Long-term administration of benfotiamine benefits the glucose homeostasis of juvenile blunt snout bream *Megalobrama amblycephala*, fed a high carbohydrate diet. Aquaculture. 2017;40, 74–83.
29. Xu C, Liu WB, Zhang DD, Shi HJ, Zhang L, Li XF. Benfotiamine, a lipid-soluble analog of vitamin B1, improves the mitochondrial biogenesis and function in blunt snout bream (*Megalobrama amblycephala*) fed high-carbohydrate diets

by promoting the AMPK/PGC-1β/NRF-1 axis. Front Physiol. 2018;9: 1079.

30. Xu C, Liu WB, Zhang DD, Cao XF, Shi HJ, Li XF. Interactions between dietary carbohydrate and metformin, implications on energy sensing, insulin signaling pathway, glycolipid metabolism and glucose tolerance in blunt snout bream *Megalobrama amblycephala*. Aquaculture. 2018;483: 183–195.
31. AOAC. (Association of Official Analytical Chemists). Official methods of analysis. AOAC, Arlington 1298, 1990.
32. Habte-Tsion HM, Ren MC, Liu B, Ge XP, Xie J, Chen R. Threonine modulates immune response, antioxidant status and gene expressions of antioxidant enzymes and antioxidant-immune-cytokine-related signaling molecules in juvenile blunt snout bream (*Megalobrama amblycephala*). Fish Shellfish Immunol. 2016;51: 189–199.
33. Zhou CP, Ge XP, Liu B, Xie J, Miao LH. Effect of high dietary carbohydrate on the growth performance and physiological responses of juvenile Wuchang bream, *Megalobrama amblycephala*. Asian-Australas J Anim Sci. 2013;26: 1598–1608.
34. Aebi H. Catalase "in vitro", Methods Enzymol. 1984;105: 121–127.
35. Zhao X, Ren X, Zhu R, Luo Z, Ren B. Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria-mediated apoptosis in zebrafish embryos. Aquat Toxicol. 2016;180: 56–70.
36. Xu C, Liu WB, Remø SC, Wang BK, Shi HJ, Zhang L, et al. Feeding restriction alleviates high carbohydrate diet-induced oxidative stress and inflammation of *Megalobrama amblycephala* by activating the AMPK-SIRT1 pathway. Fish Shellfish Immun. 2019;92: 637-648.
37. Shi HJ, Xu C, Liu MY, Wang BK, Liu WB, Chen DH, et al.Resveratrol improves the energy sensing and glycolipid metabolism of blunt snout bream *Megalobrama amblycephala* fed high-carbohydrate diets by activating the AMPK–SIRT1–PGC-1a network. Front Physiol. 2019;9: 1258.
38. Gao ZX, Luo W, Liu H, Zeng C, Liu XL, Yi SK, et al. Transcriptome analysis and SSR/SNP markers information of the blunt snout bream (*Megalobrama amblycephala*). PLoS One. 2012;7: e42637.
39. Ali MZ, Jauncey K. Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell 1822). Aquac Int. 2004;12: 169–180.
40. Huang HH, Feng L, Liu Y, Jiang J, Jiang WD, Hu K. Effects of dietary thiamin supplement on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac Nutr. 2011;17: e233–e240.
41. Yildirim NC, Danabas S. Assessment of immunomodulator biomarkers (Tnf-α, Il-1β and Il-6) in liver of Capoeta umbla for biomonitoring of pollution in uzuncayir dam lake (Tunceli, Turkey). Iran J Fish Sci. 2014;13: 653–666.
42. Wang B, Feng L, Chen GF, Jiang WD, Liu Y, Kuang SY, et al. Jian carp (*Cyprinus carpio* var. jian) intestinal immune responses, antioxidant status and tight junction protein mRNA expression are modulated via nrf2 and pkc in response to dietary arginine deficiency. Fish Shellfish Immunol. 2016;51: 116–124.
43. Zheng Z, Chen H, Zhao H, Liu K, Luo D, Chen Y et al. Inhibition of JAK2/STAT3-mediated VEGF upregulation under high glucose conditions by PEDF through a mitochondrial ROS pathway in vitro. Investig Ophthalmol Vis Sci. 2010;51: 64–71.
44. Takeuchi-Yorimoto A, Noto T, Yamada A, Miyamae Y, Oishi Y, Matsumoto M. Persistent fibrosis in the liver of choline-deficient and iron-supplemented lamino acid-defined diet-induced nonalcoholic steatohepatitis rat due to continuing oxidative stress after choline supplementation. Toxicol Appl Pharmacol. 2013;268: 264–277.
45. Cheng D, Kong H. The effect of Lycium barbarum polysaccharide on alcoholinduced oxidative stress in rats. Molecules. 2010;16: 2542–2550.
46. Oh SH, Witek RP, Bae SH, Darwiche H, Jung Y, Pi L, et al. Detection of transketolase in bone marrow-derived insulin-producing cells, benfotiamine enhances insulin synthesis and glucose metabolism. Stem Cells Dev. 2009;18: 37–46.
47. Rathanaswami P, Pourany A, Sundaresan R. Effects of thiamine deficiency on the secretion of insulin and the metabolism of glucose in isolated rat pancreatic islets. Biochem Int. 1991;25: 577–583.
48. Chen QQ, Liu WB, Zhou M, Dai YJ, Xu C, Tian HY, et al. Effects of berberine on the growth and immune performance in response to ammonia stress and high-fat dietary in blunt snout bream *Megalobrama amblycephala*. Fish Shellfish

Immunol. 2016;55: 165–172.

49. Liu B, Lei M, Hu T, Yu F, Xiao DM, Kang H. Inhibitory effects of SRT1720 on the apoptosis of rabbit chondrocytes by activating SIRT1 via p53/bax and NF-κB/PGC-1α pathways. J Huazhong Univ Sci Technol. 2016;36: 350–355.
50. Cantó C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol. 2009;20: 98–105.
51. Sauve AA. NAD^+ and vitamin B_3, From metabolism to therapies. J Pharmacol Exp Ther. 2007;324: 883–893.
52. Makarchikov AF. Vitamin B1, metabolism and functions. Biochem Suppl Series B Biomed Chem. 2009;3: 116–128.
53. Wu Y, Li X, Zhu JX, Xie W, Le W, Fan Z. Resveratrol-activated AMPK/SIRT1/autophagy in cellular models of Parkinson's disease. Neurosignals. 2011;19: 163–174.
54. Eo H, Jeon YJ, Lee M, Lim Y. Brown Alga *Ecklonia cava* polyphenol extract ameliorates hepatic lipogenesis, oxidative stress, and inflammation by activation of AMPK and SIRT1 in high-fat diet-induced obese mice. J Agric Food Chem. 2015;63: 349–359.
55. Li S, Zhao G, Chen L, Ding Y, Lian J, Hong G, et al. Resveratrol protects mice from paraquat-induced lung injury, the important role of SIRT1 and NRF2 antioxidant pathways. Mol Med Rep. 2016;13: 1833–1838.
56. Wang B, Sun J, Shi Y, Le G. Salvianolic acid B inhibits high-fat diet-induced inflammation by activating the Nrf2 pathway. J Food Sci. 2017;82: 1953–1960.
57. Jiang WD, Liu Y, Jiang J, Wu P, Feng L, Zhou XQ. Copper exposure induces toxicity to the antioxidant system via the destruction of nrf2/are signaling and caspase-3-regulated DNA damage in fish muscle, amelioration by myo-inositol. Aquat Toxicol. 2015;159: 245–255.
58. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev. 2013;27: 2179–2191.
59. Huang K, Chen C, Hao J, Huang J, Wang S, Liu P, et al. Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced upregulation of fibronetin and transforming growth factor-β1 in rat glomerular messangial cells. Mol Cell Endocrinol. 2015;399: 178–189.
60. Huang K, Gao X, Wei W. The crosstalk between Sirt1 and Keap1/Nrf2/ARE antioxidative pathway forms a positive feedback loop to inhibit FN and TGF-β1 expressions in rat glomerular mesangial cells. Exp Cell Res. 2017;361: 63–72.
61. Tong P, Peng QH, Gu LM, Xie WW, Li WJ. LncRNA-MEG3 alleviates high glucose induced inflammation and apoptosis of retina epithelial cells via regulating miR-34a/SIRT1 axis. Exp Mol Pathol. 2019;107: 102–109.
62. Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. Diabetes. 2003;52: 1256–1264.
63. Wachlin G, Augstein P, Schröder D, Kuttler B, Klöting I, Heinke P, et al. IL-1β, IFN-γ and TNF-α increase vulnerability of pancreatic beta cells to autoimmune destruction. J Autoimmun. 2003;20: 303–312.
64. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, et al. Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylation. EMBO J. 2004;23: 2369–2380.
65. Kume S, Haneda M, Kanasaki K, Sugimoto T, Araki S, Isshiki K, et al. SIRT1 inhibits transforming growth factor betainduced apoptosis in glomerular mesangial cells via Smad7 deacetylation. J Biol Chem. 2007;282:151–158.
66. Mellado-Gil JM, Aguilar-Diosdado M. High glucose potentiates cytokine- and streptozotocin-induced apoptosis of rat islet cells, effect on apoptosis-related genes. J Endocrinol. 2014;183: 155–162.
67. Mellado-Gil JM, Aguilar-Diosdado M. Assay for high glucose-mediated islet cell sensitization to apoptosis induced by streptozotocin and cytokines. Biol Proced Online. 2005;7: 162–171.
68. Hoffmann G, Breitenbücher F, Schuler M, Ehrenhofer-Murray AE. A novel sirtuin 2 (SIRT2) inhibitor with p53-dependent pro-apoptotic activity in non-small cell lung cancer. J Biol Chem. 2014;289: 5208–5216.
69. Kim EJ, Kho JH, Kang MR, Um SJ. Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. Mol Cell. 2007;28: 277–290.

8. Tables

Table 1. Formulation and proximate composition of the experimental diets

	C	HC	HCB1	HCB2	HCB3	HCB4
Formulation (%)						
Fish meal	8.00	8.00	8.00	8.00	8.00	8.00
Soybean meal	26.00	26.00	26.00	26.00	26.00	26.00
Rapeseed meal	17.00	17.00	17.00	17.00	17.00	17.00
Cottonseed meal	17.00	17.00	17.00	17.00	17.00	17.00
Fish oil	2.00	2.00	2.00	2.00	2.00	2.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Corn starch	12.00	25.00	25.00	25.00	25.00	25.00
Benfotiamine (mg/kg)	-	-	0.7125	1.425	2.85	5.7
Microcrystalline cellulose	13.00	-	-	-	-	-
Calcium biphosphate	1.80	1.80	1.80	1.80	1.80	1.80
Premix*	1.20	1.20	1.20	1.20	1.20	1.20
Proximate composition (*% air-dry basis*)						
Moisture	6.96	6.85	6.92	6.95	6.90	6.87
Crude lipid	5.93	5.71	5.78	5.66	5.77	5.87
Ash	8.46	8.28	8.12	8.23	8.34	8.20
Crude protein	29.82	30.12	30.31	30.03	30.02	30.11
Crude fiber	16.97	6.18	6.29	6.30	6.23	6.28
Nitrogen-free extract†	31.86	42.75	42.58	42.83	42.74	42.67
Energy (MJ/kg)	19.09	19.24	19.38	19.31	19.23	19.30

C, the control diet; HC, the high-carbohydrate diet; HCB1, the HC diet supplemented with 0.7125 mg/kg benfotiamine; HCB2, the HC diet supplemented with 1.425 mg/kg benfotiamine; HCB3, the HC diet supplemented with 2.85 mg/kg benfotiamine; HCB4, the HC diet supplemented with 5.7 mg/kg benfotiamine (the same below).

* Premix supplied the following minerals and/or vitamins (per kg): $CuSO_4 \cdot 5H_2O$, 2.0 g; $FeSO_4 \cdot 7H_2O$, 25 g; $ZnSO_4 \cdot 7H_2O$, 22 g; $MnSO_4 \cdot 4H_2O$, 7 g; Na_2SeO_3, 0.04g; KI, 0.026 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; Vitamin A, 900,000 IU; Vitamin D, 200,000 IU; Vitamin E, 4500 mg; Vitamin K_3, 220 mg; Vitamin B_1, 320 mg; Vitamin B_2, 1090 mg; Vitamin B_5, 2000 mg; Vitamin B_6, 500 mg; Vitamin B_{12}, 1.6 mg; Vitamin C, 5000 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60,000 mg.

† Calculated by difference (100 - moisture - crude protein - crude lipid - ash - crude fiber).

Table 2. Nucleotide sequences of the primers used to assay gene expressions by real-time PCR

Target gene	Forward (5'-3)	Reverse (5'-3)	Accession numbers or references
SIRT1	TCGGTTCATTCAGCAGCACA	ATGATGATCTGCCACAGCGT	[37]
Nrf2	GGGGAAGTCCTTGAACGGAG	AACCAGCGGGAATATCTCGG	[22]
Keap1	AATATCCGCCGGCTGTGTAG	TGAGTCCGAGGTGTTTCGTG	[22]
CAT	CAGTGCTCCTGATACCCAGC	TTCTGACACAGACGCTCTCG	[22]
Cu/Zn-SOD	AGTTGCCATGTGCACTTTTCT	AGGTGCTAGTCGAGTGTTAGG	KF479046.1
Mn-SOD	AGCTGCACCACAGCAAGCAC	TCCTCCACCATTCGGTGACA	KF195932.1
NF-κB	GAAGAAGGATGTGGGAGATG	TGTTGTCGTAGATGGGCTGAG	[38]
TNF α	TGGAGAGTGAACCAGGACCA	AGAGACCTGGCTGTAGACGA	KU976426.1
IL1β	ACGATAAGACCAGCACGACC	CTGTTTCCGTCTCTCAGCGT	[38]
IL6	CAGCAGAATGGGGGAGTTATC	CTCGCAGAGTCTTGACATCCTT	KJ755058.1
IL8	CAGAGAGTCGACGCATTGGT	ATTCACGGTGCTTTGTTGGC	[22]
IL10	GTGTTTTCGGGTGCAAGTGG	ATGAACGAGATCCTGCGCTT	[22]
P53	CAGCAGGAGCCAATCCATCA	ACGTACTCCCCAGACCTGAA	[38]
Bcl2	CCAACTCATCAGGAAACAA	GGGTGCTGCGGGTAAC	EU490408.1
Bax	ATCCAGCCAGCATCGT	CACTATCCCCAAGACCC	AF231015.1
Caspase 3	TCGTTCGTCTGTGTCCTGTTGAG	GCTGTGGAGAAGGCGTAGAGG	KY006115.1
Caspase 9	AATAAAGCACCGAGCG	GGGAGGAGGCCGATGAGCACTATCT	KM604705.1
EF1α	CTTCTCAGGCTGACTGTGC	CCGCTAGCATTACCCTCC	X77689.1

SIRT1, sirtuin 1; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, kelch-like ECH associating protein 1; CAT, catalase; Cu/Zn-SOD, copper/zinc superoxide dismutase; Mn-SOD, manganese superoxide dismutase; NF-κB, nuclear factor kappa B; TNF α, tumour necrosis factor α; IL1β, interleukin 1β; IL6, interleukin 6; IL8, interleukin 8; IL10, interleukin10; EF1α, Elongation factor 1α.

Table 3. Growth performance of blunt snout bream fed different experimental diets

Parameters	Diets					
	C	HC	HCB1	HCB2	HCB3	HCB4
Initial weight (g)	46.38±0.63	45.17±0.72	44.81±0.78	47.15±1.11	43.11±1.32	44.79±0.64
DGC	2.35±0.01^{ab}	1.81±0.01^{c}	2.01±0.02^{b}	2.63±0.01^{a}	2.01±0.02^{b}	1.83±0.02^{c}
GR_{MBW}	7.38±0.03^{b}	6.42±0.11^{c}	7.10±0.23^{b}	8.72±0.10^{a}	6.51±0.07^{c}	6.03±0.18^{c}
FI_{MBW}	13.99±0.52	11.31±0.11	11.42±0.04	12.98±0.50	10.41±0.21	9.49±0.03
FI	231.83±11.01^{a}	152.31±13.76^{bc}	163.89±9.13^{bc}	184.92±8.86^{c}	152.01±14.92^{bc}	131.29±6.31^{ab}
FER	0.57±0.01	0.56±0.02	0.63±0.01	0.71±0.03	0.64±0.01	0.60±0.01

Values are means ± S.E.M of four replications. Values in the same line with superscripts are significantly different (P<0.05).

Daily growth coefficient (DGC) (% day^{-1}) = 100 × ($W_f^{1/3}$ − $W_i^{1/3}$) / D.

Mean metabolic body weight (MBW) = [(W_i / 1000)$^{0.75}$ + (W_f / 1000)$^{0.75}$] / 2.

Growth rate per metabolic body weight (GR_{MBW}) (g kg^{-1} MBWday^{-1}) = (Body average weight gain / MBW) / D.

Feed intake per metabolic body weight (FI_{MBW}) (g kg^{-1} MBW day^{-1}) = DI / MBW / D.

Feed intake (FI) (g fish^{-1}) = total feed intake (g) / total fish number.

Feed efficiency ratio (FER) = total weight gain (g) / total feed intake (g).

where W_i and W_f are the initial and final fish average weights (g), repectively. D is the feeding days, DI is the dry feed intake per fish (g fish^{-1}) during the experimental period.

Table 4 Plasma and liver metabolites of blunt snout bream subjected to different treatments

Parameters	Diets					
	C	HC	HCB1	HCB2	HCB3	HCB4
Plasma metabolites						
AST (U/L)	20.31±0.91^{c}	37.11±1.01^{a}	29.86±0.76^{b}	20.72±1.49^{c}	21.22±1.39^{c}	36.20±0.33^{a}
ALT (U/L)	2.16±0.03^{b}	3.00±0.09^{a}	1.96±0.07^{b}	1.90±0.07^{b}	1.94±0.09^{b}	1.99±0.05^{b}
IL1β (ng/L)	3.13±0.12^{d}	12.03±0.17^{a}	9.32±0.04^{b}	4.39±0.03^{d}	7.81±0.21^{c}	7.29±0.18^{c}
IL6 (ng/L)	22.29±0.57^{d}	48.72±1.39^{a}	41.80±2.53^{a}	27.32±1.28^{c}	25.92±1.01^{c}	35.48±0.74^{b}
Liver metabolites						
T-AOC (U/mg protein)	1.11±0.03^{a}	0.45±0.01^{b}	0.80±0.02^{a}	0.88±0.04^{a}	0.93±0.21^{a}	0.81±0.02^{a}
SOD (U/mg protein)	17.90±0.14^{b}	9.28±0.45^{c}	11.39±0.72^{c}	21.48±0.31^{a}	15.16±0.92^{b}	17.10±0.29^{b}
CAT (U/mg protein)	25.29±0.23^{a}	8.12±0.70^{c}	10.21±0.39^{c}	23.12±0.83^{a}	20.14±1.38^{a}	17.15±0.19^{b}
MDA (nmol/mg protein)	4.73±0.02^{c}	11.50±0.41^{a}	7.10±0.15^{b}	5.09±0.03^{c}	5.44±0.52^{c}	7.19±0.05^{b}

Values are mean ± S.E.M of four replications. Means in the same line with different superscripts are significantly different ($P < 0.05$).

AST, alanine transaminase; ALT, aspartate aminotransferase; IL1β, interleukin 1β; IL6, interleukin 6; T-AOC, total anti-oxidation capacity; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde.

Figures

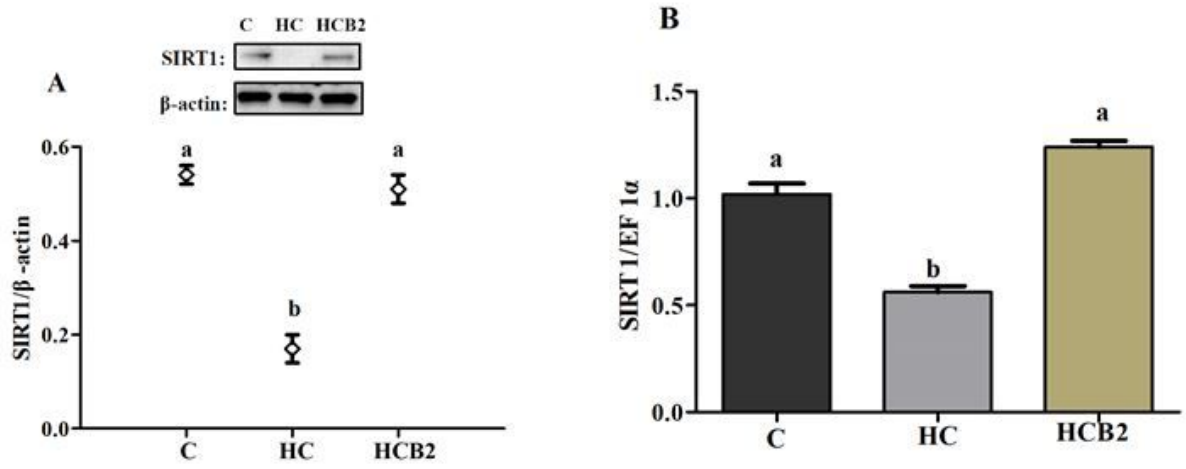

Figure 1

The expression of SIRT1 protein (A) and gene (B) in the liver of blunt snout bream fed different experimental diets. Gels were loaded with 20 mg total protein per lane. Each data represent the means ± SEM of four biological replicates. Bars assigned with different superscripts are significantly different (P < 0.05).

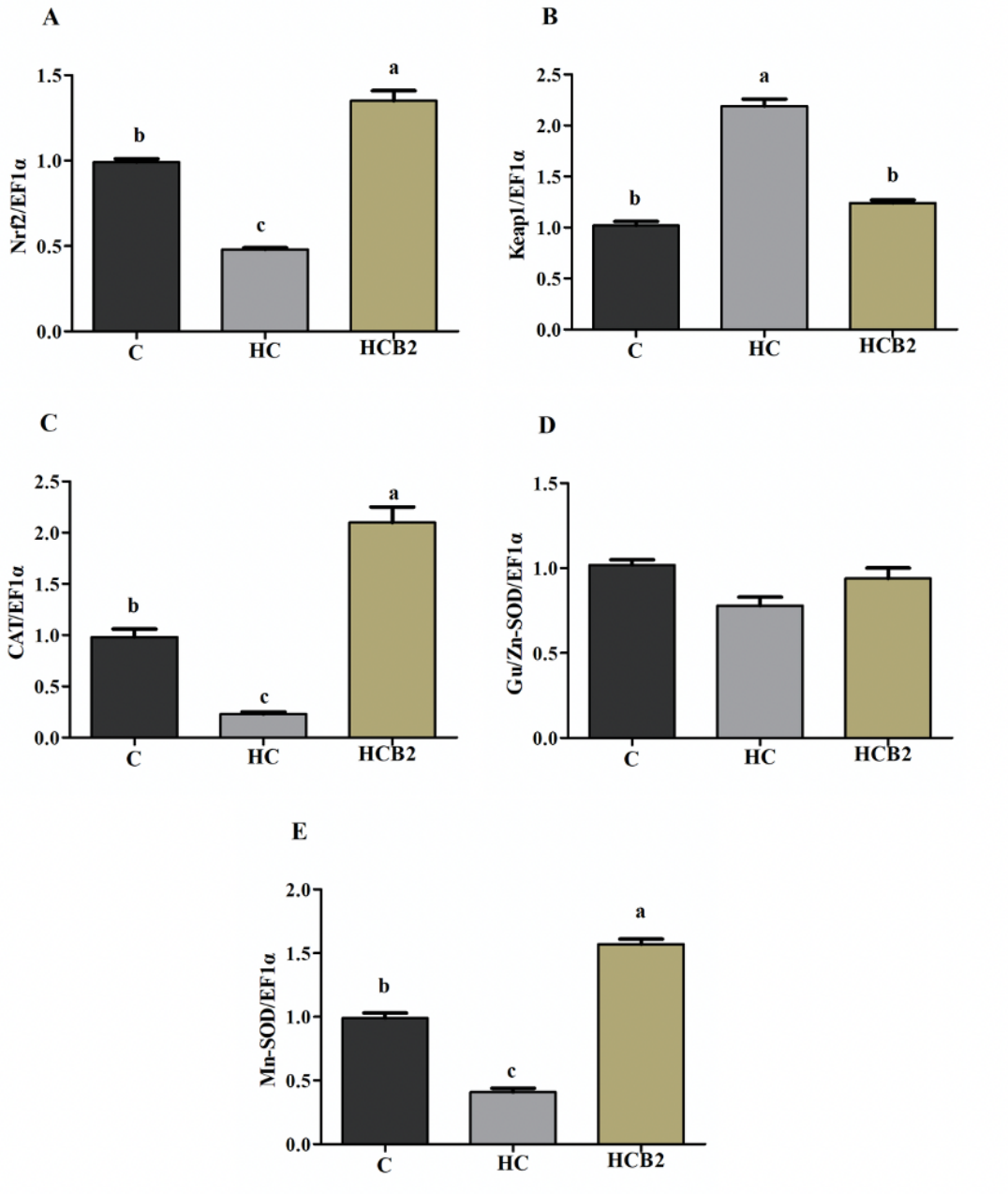

Figure 2

The expression levels of antioxidant-related genes in the liver of blunt snout bream subjected to different treatments. Values are mean ± S.E.M of four replications. Bars assigned with different superscripts are significantly different ($P < 0.05$).

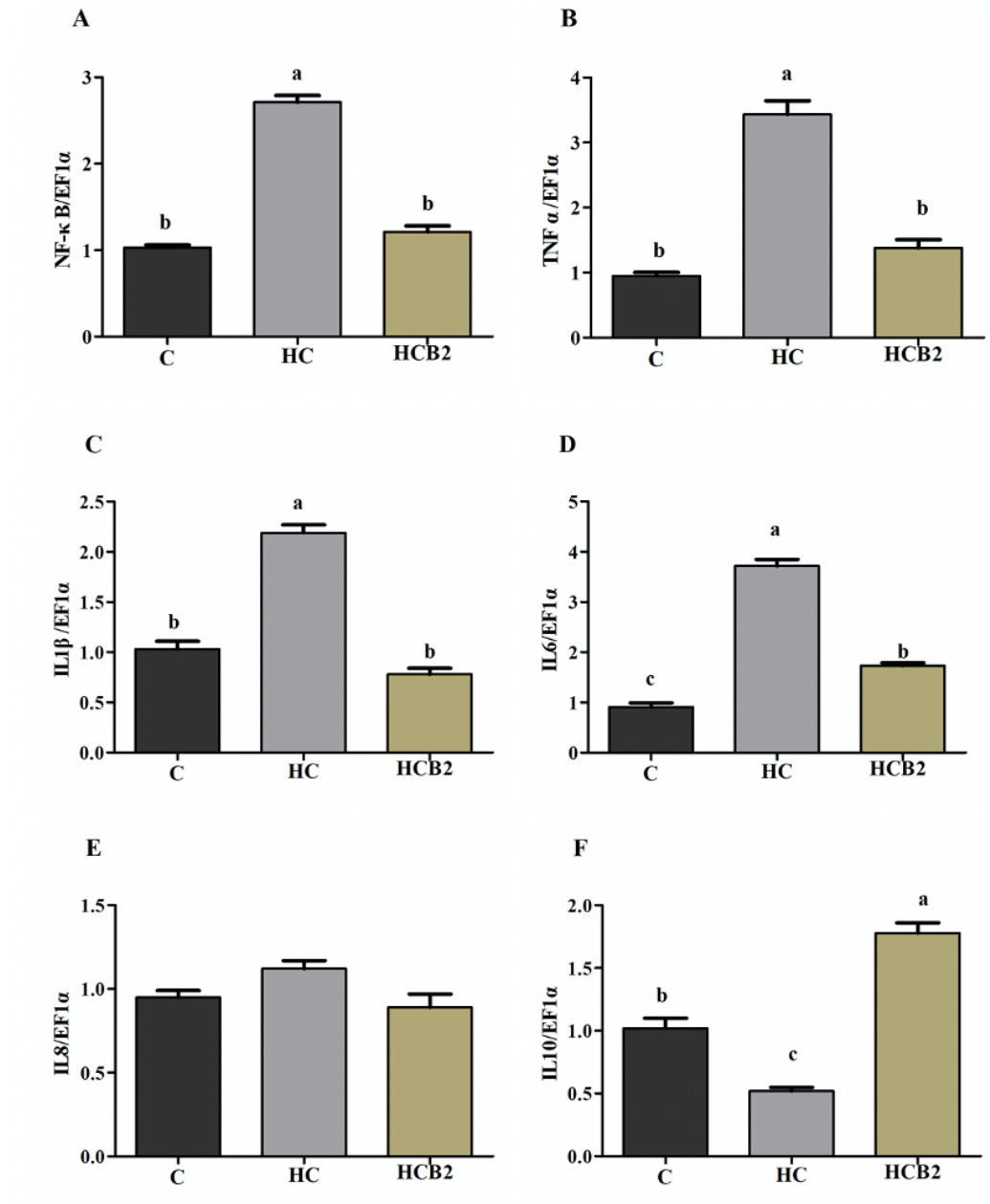

Figure 3

The expression levels of inflammation-related genes in the liver of blunt snout bream subjected to different treatments. Values are mean ± S.E.M of four replications. Bars assigned with different superscripts are significantly different ($P < 0.05$).

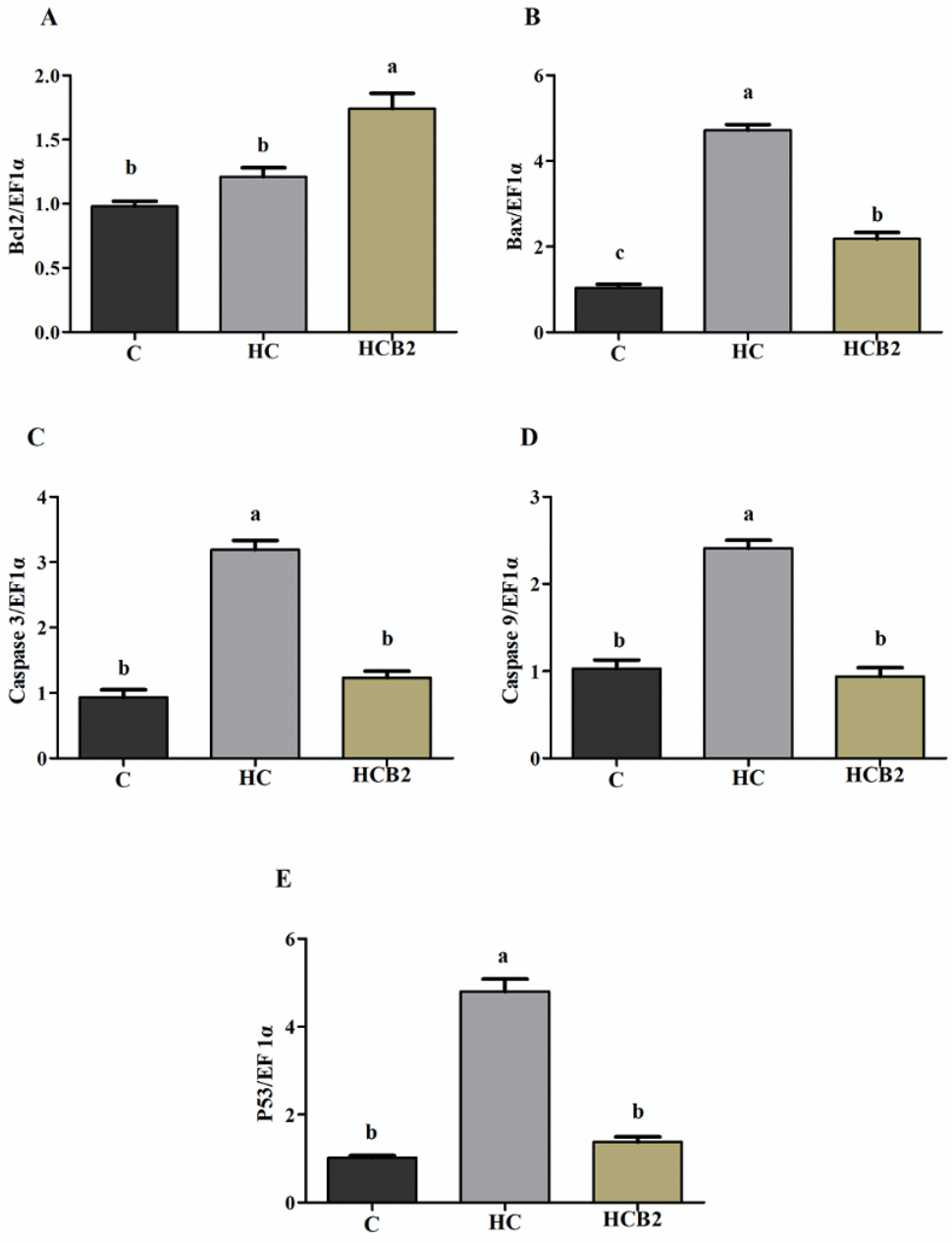

Figure 4

The expression levels of apoptosis-related genes in the liver of blunt snout bream subjected to different treatments. Values are mean ± S.E.M of four replications. Bars assigned with different superscripts are significantly different ($P < 0.05$).