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Dietary sodium acetate improves high-fat diet utilization through promoting differential nutrients metabolism between liver and muscle in Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

High-fat diet (HFD) often causes many negative effects and impairs fish growth. Short-chain fatty acids (SCFAs) such as acetates modify metabolic disorders and regulate body homeostasis. However, the effects of sodium acetate on alleviating HFD in fish is currently unknown. This study investigated the role of using dietary sodium acetate to alleviate adverse effects caused by HFD in fish. Three replicates (thirty fish each) of 4.8 ± 0.30 g Nile tilapia (*Oreochromis niloticus*) were fed with control diet (Con), high-fat diet (HFD) or HFD containing sodium acetate diet (HFD + NaAc) for eight weeks. After the feeding trial, Nile tilapia fed with HFD increased significantly tissue lipid deposition, reduced insulin sensitivity and suppressed glucose and lipid metabolism in both liver and muscle, accompanied with signs of metabolic disorders and liver damage. Moreover, HFD feeding inhibited muscle protein synthesis and impaired fish growth performance. However, the fish fed on HFD + NaAc improved significantly oxidative stress, inflammation, apoptosis and injury in liver compared to those fed on HFD. More importantly, dietary sodium acetate supplementation enhanced insulin sensitivity, promoted glucose catabolic utilization and protein synthesis in muscle through activation of insulin and mTOR signaling pathways, respectively, and markedly improved the growth performance. In contrast, dietary sodium acetate promoted hepatic pentose phosphate pathway, hepatic glycogen accumulation, and activated lipid catabolism to alleviate hepatic lipid deposition. Our study illustrates that sodium acetate alleviates differentially the adverse effects induced by feeding Nile tilapia with HFD in muscle and liver. SCFAs such as acetate may be used for improving HFD utilization in fish nutrition.

1. Introduction

High-fat diet (HFD) has been widely used in modern aquaculture because of their low cost, reduced nitrogen emissions and protein-saving effects (Beamish and Medland, 1986; Li et al., 2012; Kong et al., 2020). Dietary lipids are an important energy source and also supply essential fatty acids and phospholipids, which play a significant role in fish nutrition (Watanabe, 1982). However, feeding HFD for long-term usually causes many negative effects on fish such as metabolic disorders (Du et al., 2006; Ning et al., 2017), metabolic diseases and impair immunity (Zhou et al., 2019a), which in turn lead to reduced fish resistance to pathogens, increased mortality and inhibited growth performance (Qian et al., 2021). Extensive studies have shown that feeding HFD caused abnormal lipid accumulation (Lu et al., 2013), leading to impaired energy supply for glucose and lipid metabolism (Figueiredo-Silva et al., 2012), inducing oxidative stress and organ injury (Li et al., 2020b), accompanied by inflammatory reactions in fish (Jia et al., 2020). To ameliorate the negative effects caused by HFD, various feed additives have been used in aquaculture production. For example, dietary curcumin supplementation improved HFD utilization in large yellow croaker (*Pseudosciaena crocea*) by reducing hepatic lipid deposition, increasing antioxidant activity and polyunsaturated fatty acid (PUFA)

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content (Ji et al., 2021). Studies in blunt snout bream (*Megalobrama amblycephala*) found that berberine alleviated HFD-induced hepatic steatosis and dysfunction by promoting fatty acid oxidation (Zhou et al., 2019a). Similarly, taurine in European sea bass (*Dicentrarchus labrax*) (Coutinho et al., 2017) and tea polyphenols in tilapia (*Oreochromis niloticus*) (Qian et al., 2021) have been reported to alleviate HFD-induced lipid accumulation and oxidative stress through enhancing antioxidant capacity and lipid catabolic capacity. Therefore, the negative effects caused by HFD feeding could be alleviated by using appropriate feed additives.

Organic acids are preservatives and food additives with a long history, which are used to prevent food spoilage and extend the shelf life of perishable food ingredients (Ricke, 2003). Short-chain fatty acids (SCFAs, primarily acetate, propionate and butyrate) are specific organic acids mainly produced by gut microbes through anaerobic fermentation (He et al., 2020). Previous studies have found that SCFAs have multiple biological effects, such as regulating energy metabolism (Den Besten et al., 2013), alleviating oxidative stress, enhancing immunity and promoting growth (Canfora et al., 2015; Wendy and Erika, 2021). Acetate, as an important SCFA, has received increasing attention (Hamer, 2007; Kim et al., 2021). In mammals, acetate plays a significant regulatory role in insulin sensitivity and body weight control by enhancing energy expenditure and fat oxidation (González Hernández et al., 2019). Moreover, acetate inhibited lipogenesis in liver, activated lipolysis to reduce fat accumulation in adipose tissue, and promoted glucose uptake in muscle tissue, thereby worked against obesity and associated type 2 diabetes in rats (Yamashita et al., 2009). In humans, intestinal acetate infusion promoted fat oxidation and improved systemic substrate metabolism, suggesting that intestinal acetate may treat or prevent metabolic disorders as a nutritional supplement (Van der Beek et al., 2016). In addition, acetate had also beneficial effects in improving intestinal inflammation and apoptosis, and maintaining T cells function in mice (Piekarska et al., 2011; Balmer et al., 2016). Some of the adverse effects ameliorated with acetate are also caused by HFD. Therefore, acetate has potential for alleviating negative effects induced by feeding fish with HFD.

Indeed, acetate has been reported in studies related to metabolism, growth and internal environment in cultured fish. Accordingly, dietary sodium acetate supplementation improved glucose homeostasis, increased energy retention and promoted weight gain in zebrafish (*Danio rerio*) (Zhang et al., 2020; Wang et al., 2021). Moreover, acetate-producing bacteria or sodium acetate supplementation alleviated metabolic disturbances, intestinal inflammation and improved growth caused by HCD in Nile tilapia (Li et al., 2020c; Xu et al., 2022). These studies indicate that sodium acetate plays a significant role in metabolic homeostasis and physiological regulation. To this end, sodium acetate has been recognized as a promising feed additive for aquafeed industry. However, most studies on sodium acetate have been focused on normal and high carbohydrate nutritional contexts, and relatively little studies have been done on high fat nutritional condition in fish. Interesting, a study conducted by Yu et al. (2022) reported that sodium acetate alleviated dietary sodium palmitate-caused lipid deposition, oxidative stress and apoptosis in blunt snout bream. However, the mechanism used to alleviate these adverse effects has not been explored. Thus, it is essential to further explore the biological effects and mechanisms of dietary sodium acetate in high-fat nutritional contexts in order to understand deeply the application of acetate in aquafeeds formulation.

Nile tilapia is increasingly used as a model fish for nutritional research because of its fast growth rate, highly resistant to disease and wide distribution (Zhou et al., 2022). Thus, the current study explored the role of dietary sodium acetate to alleviate the negative effects induced by feeding HFD in Nile tilapia. To achieve the purpose, Nile tilapia fingerlings were fed with a control diet (Con), HFD or HFD containing sodium acetate (HFD + NaAc) for eight weeks. Afterwards, the biochemical and molecular indexes related to growth, liver and muscle nutrient metabolism, and liver health were systemically assayed. The knowledge gained point to a potential use of acetate in improving HFD utilization in fish nutrition.

2. Materials and methods

2.1. Ethical statement

We conducted all experiments strictly in accordance with the Chinese Guide for the Care and Use of Laboratory Animals. Our study was authorized by the Animal Experimentation Ethics Committee of East China Normal University (F20190101).

2.2. Feed ingredients and formulation

To explore the effect of sodium acetate in HFD, three isonitrogenous (400 g/kg protein) purified diets were formulated. The diets were Con (60 g/kg fat), HFD (120 g/kg fat) and HFD + NaAc (120 g/kg fat; 1.8 g/kg sodium acetate). The dose of NaAc (1.8 g/kg) was referenced from our previous studies in which the dose of 1.5 to 1.8 g/kg NaAc efficiently promoted growth in fish (Li et al., 2020c; Xu et al., 2022). The basal diet (Con) included appropriate fat, protein and carbohydrate levels to satisfy the normal physiological needs of Nile tilapia. Soybean oil was used as the lipid source and casein and gelatin were used as the protein sources in the experimental diets (Table 1). The diets were prepared according to our previous study (Zhou et al., 2022). The prepared diets were naturally dried in a ventilated room and stored at −30 °C for later use.

2.3. Experimental fish

Sixty hundred Nile tilapia (about 2.8 g) were obtained from Menghu Aquaculture Farm (Zhanjiang, China) and acclimatized in four 500 L tanks under suitable culture conditions. During this time, fish were fed a

Table 1
Formulation and proximate composition (dry matter basis) of the experimental diets.

Ingredients (%)	Con	HFD	HFD + NaAc
Casein	320	320	320
Gelatin	80	80	80
Soybean oil	60	120	120
Corn starch	300	300	300
Sodium acetate[1]	0	0	1.8
Vitamin premix[2]	12	12	12
Mineral premix[3]	12	12	12
Ca(H2PO4)2	10	10	10
CMC	30	30	30
Cellulose	169.75	109.75	107.95
DMPT	1	1	1
Choline chloride	5	5	5
BHT	0.25	0.25	0.25
Total	1000	1000	1000
Proximate composition (g / kg dry matter)			
Dry matter	902	898	903
Crude protein	385	382	379
Crude lipid	58	117	119
Ash	51	54	55

[1] Sodium acetate (Purity: ≥ 99%) purchased from Sigma-Aldrich company (CAS number: 127–09-3, Merck, Germany).

[2] Vitamin premix (mg or IU/kg): 500,000 IU/kg Vitamin A; 50,000 IU/kg Vitamin D_3; 2500 mg/kg Vitamin E; 1000 mg/kg Vitamin K_3; 5000 mg/kg Vitamin B_1; 5000 mg/kg Vitamin B_2; 5000 mg/kg Vitamin B_6; 5000 mg/kg Vitamin B_{12}; 25,000 mg/kg Inositol; 10,000 mg/kg Pantothenic acid; 100,000 mg/kg Cholin; 25,000 mg/kg Niacin; 1000 mg/kg Folic acid; 250 mg/kg Biotin; 10,000 mg/kg Vitamin C.

[3] Mineral premix (g/kg diet): 314.0 g/kg $CaCO_3$; 469.3 g/kg KH_2PO_4; 147.4 g/kg $MgSO_4{\cdot}7H_2O$; 49.8 g/kg NaCl; 10.9 g/kg Fe (II) gluconate; 3.12 g/kg $MnSO_4{\cdot}H_2O$; 4.67 g/kg $ZnSO_4{\cdot}7H_2O$; 0.62 g/kg $CuSO_4{\cdot}5H_2O$; 0.16 g/kg KJ; 0.08 g/kg $CoCl_2{\cdot}6H_2O$; 0.06 g/kg NH_4 molybdate; 0.02 g/kg $NaSeO_3$.

commercial diet containing 33% protein and 5% fat (Tongwei, China). After acclimatization, 270 Nile tilapia (4.8 ± 0.30 g) were randomly grouped into three treatments, each with three replicates (90 fish each treatment) and fed with the experimental diets. The fish in each tank were fed three times a day at 4% of their body weight for eight weeks in a 200 L water tank containing clean dechlorinated freshwater (water temperature: 28 ± 1.5 °C; pH: 7.7 to 8.3; total ammonia nitrogen: 0.05 to 0.08 mg/L). The fish in all experimental diets were reared under 12 h: 12 h light: dark photoperiod condition. Besides, fish in each tank were weighed every two weeks to adjust the amount of feed. The amount of diet used for each treatment was recorded for determination of feed efficiency.

2.4. Determination of growth and feed performance

During the trial, all fish from each tank were weighed every two weeks using a Precision Electronics Balance (Jiewote, JTS-30LC, China) to calculate the weight gain (WG) until the eighth week. After eight weeks, all fish were fasted for 24 h, and anesthetized by using 7 g/L MS-222 (Sigma, USA) for counting and subsequent sampling. The total amount of dry feed consumed was used to calculate the feed intake (FI) and feed efficiency (FE). We counted the final fish number from each treatment to determine the survival rate (SR). The SR, WG and FE were determined by the following formulae:

$$\mathrm{SR}\ (\%) = \frac{\text{Initial fish number} - \text{Final fish number}}{\text{Initial fish number}} \times 100$$

$$\mathrm{WG}\ (\%) = \frac{[\text{Final fish weight} - \text{Initial fish weight}](\mathrm{g})}{\text{Initial fish weight}\ (\mathrm{g})} \times 100$$

$$\mathrm{FE}\ (\%) = \frac{[(\text{Final tank biomass} + \text{dead fish}) - \text{Initial tank biomass}](\mathrm{g})}{\text{Total feed intake in each tank}\ (\mathrm{g})} \times 100$$

$$\mathrm{FI}\ (\mathrm{g/fish}) = \frac{\text{Feed consumption}\ (\mathrm{g})}{\text{Fish number}} \times 100$$

2.5. Blood collection and organ indices calculation

Twelve fish were randomly selected from each treatment, anesthetized as described above, and individual body weight and length were measured to determine condition factor (CF). Thereafter, blood was drawn from the tail vein using 2 mL syringes. The obtained blood was immediately centrifuged at 3000 rpm for 12 min at 4 °C to obtain the serum. The collected serum was immediately kept at −20 °C for later analysis. Next, the fish were dissected to obtain the carcass, viscera, liver and intraperitoneal fat. They were weighed to calculate carcase ratio (CR), viscera somatic index (VSI), intraperitoneal fat ratio (IPF) and hepatosomatic index (HSI). Part of the muscle and liver tissues were frozen in liquid nitrogen and then kept at −80 °C for subsequent analysis. The CR, VSI, IPF, HSI and CF, were determined based on the following equations:

$$\mathrm{CR}\ (\%) = \frac{\text{Carcase weight}\ (\mathrm{g})}{\text{Fish weight}\ (\mathrm{g})} \times 100$$

$$\mathrm{VSI}\ (\%) = \frac{\text{Viscera weight}\ (\mathrm{g})}{\text{Fish weight}\ (\mathrm{g})} \times 100$$

$$\mathrm{IPF}\ (\%) = \frac{\text{Intraperitoneal fat weight}\ (\mathrm{g})}{\text{Fish weight}\ (\mathrm{g})} \times 100$$

$$\mathrm{HSI}\ (\%) = \frac{\text{Liver weight}\ (\mathrm{g})}{\text{Fish weight}\ (\mathrm{g})} \times 100$$

$$\mathrm{CF}\ (\%) = \frac{\text{Fish weight}\ (\mathrm{g})}{\text{Fish length}\ (cm^3)} \times 100$$

2.6. Biochemical measurement

Nine fish were randomly collected from each treatment to evaluate the protein, lipid and biochemical indexes. The glucose, pyruvate, lactate (LD), non-esterified fatty acids (NFFA), triglycerides (TG), malondialdehyde (MDA), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in serum, liver or muscle were determined by using specific commercial assay kits (Jiancheng, China). Muscle and liver glycogen content were analyzed by using specific commercial kits (Jiancheng, China), according to the manufacturer's protocol. The serum insulin level was determined by using fish enzyme-linked immunosorbent assay (ELISA) kit (No. HB90131–QT, Shanghai Hengyuan Biological, China) based on the instructions of the manufacturer. Briefly, the methods of chloroform-methanol (1:2, v:v) extraction was used to analyze crude lipid of whole fish (Bligh and Dyer, 1959). Whole body and muscle crude protein content was measured by Kjeltec™ 8200 (FOSS, Sweden).

2.7. Glucose tolerance test (GTT)

Glucose tolerance test was administered as previously described (Liu et al., 2018). Blood was obtained from the tail vein of Nile tilapia for the measurement of glucose and insulin levels, after 0, 0.5, 1.0, and 3.0 h of intraperitoneal glucose injection. The glucose tolerance of Nile tilapia was determined based on the fluctuations in blood insulin and glucose levels during the set time as indicated above. For detailed experimental procedures see description in a previous study (Li et al., 2020a).

2.8. Histological analysis

Six fresh liver tissues from each treatment were collected randomly for hematoxylin and eosin (H & E) and Oil Red O staining, respectively, as described previously (Zhou et al., 2022). To analyze liver tissue damage, liver sections from six fish per treatment were subjected to the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit (red fluorescence, Sevier), based on the instructions from the manufacturer. Paraffin-embedded liver tissues were pre-treated, and sections were dewaxed and rehydrated following the kit instructions. The liver sections were incubated with working solution and proteinase K, followed by fixation in paraformaldehyde solution, then permeabilized in sodium citrate solution, and finally samples were tagged by using TUNEL reaction mixture. After staining, the liver sections were photographed and analyzed by a fluorescent microscope (Nikon Corporation, Tokyo, Japan).

2.9. Quantitative real time polymerase reaction (qPCR)

Fresh liver and muscle tissue (eight fish/per treatment) were randomly collected and immediately frozen in liquid nitrogen for RNA extraction. Total RNA was isolated using Trizol reagent (Takara, Japan) and reverse-transcribed using a PrimeScript™ RT reagent Kit (No. R323–01, Vazyme Biotech Co., Nanjing, China). The obtained cDNA was unified in concentration before qPCR. The *β-actin* and elongation factor 1α (*ef1α*) were used as housekeeping genes to normalize target gene expression levels. All qPCR primers used in this study are shown in Table 2. qPCR reaction system was performed as described before (Zhou et al., 2022). The expression levels of genes were determined according to the comparative ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen, 2001).

2.10. Protein expression analysis by Western blot (WB)

Muscle tissue lysates (six fish/per treatment) were isolated by using RIPA buffer with complete Protease Inhibitor Cocktail (NCM Biotech Co., Suzhou, China) on ice. The protein lysate was separated by 8 to 12% SDS-PAGE (NCM Biotech Co., Suzhou, China), and then transferred to

Table 2
Primer sequences accession numbers for real-time PCR analysis.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	GenBank NO	Amplification efficiency (%)
gk	GACATGAGGACATTGACAAGGGAA	CTTGATGGCGTCTCTGAGTAAACC	XM_003451020.2	90
pfk	AACCTGTGTGTGATTGGAGGTGAT	CGTGATCTTACCGGCTTTAACAAG	XM_003441476.2	98
pk	CAGCATAATCTGCACCATCGGT	ATGAGAGAAGTTAAGACGGGCGA	XM_005472621.3	99
ldha	CACGCAGGAGGCTGTCTTTA	CATCGCCTCACCCTTCAACT	XM_003440392.5	94
ldhb	TTCATCCTTCACCAGCCACC	GTCACCTTGTTCCTGGGAGG	XM_003456028.5	91
pepck	TGGAAGAACAAACCTTGGCG	TGGGTCAATAATGGGACACTGTCT	XM_003448375	97
g6pdh	ACGGACGAAGAACAGATGGG	CCGTCTCTGAACAACCACCA	XM_003448158.5	99
glut1	TCTTCATCCCCGCTGTGATACA	TTCTTCAGCACTGATTTGGCCTT	NM_001279727.1	102
glut2	CATTGGCATTCTAATCAGCCAGGT	TTGTAATATTGCTGGCGCTCCA	XM_003442884.5	96
glut4	GCAGGAGGAAAGCCATGCTTATA	ATCATTTCAAAGGAGCGGCAGA	XM_003458705.4	97
ir	CTGCCCCAAAACGGAAAAGG	ACCTCGATCTGGTAGCTGGT	XM_005476595.4	103
akt	AAGAAAGGCAAGAGAAACGGA	TTCTCGATGGTGGTTACCTGG	XM_005453313.4	93
pdh	ATGAGCGATCCTGGCGTTAG	AAGGGGTTGGGTGGGTTATG	XM_003446838.5	104
idh	ACGCATCGCTGAGTACGCCTT	AGACCGTCTGACATCCGCATGA	XM_003437590.5	95
cs	AGCACCACAGTTTACCAG	AGTGTTGACAAACCCAGA	XM_003438897	98
pparα	GTTCCTCAAGAGTCTCCGCC	AAAGAGCTAGGTCGCTGTCG	KF871430	96
cpt1a	TTTCCAGGCCTCCTTACCCA	TTGTACTGCTCATTGTCCAGCAGA	XM_003440552	103
aco	AGTCCCACTGTGAGCTCCATCAA	CAGACCATGGCAGTTTCCAAGA	KF918710	92
acc	TAGCTGAAGAGGAGGGTGCAAGA	AACCTCTGGATTGGCTTGAACA	XM_005471970	95
fas	TCATCCAGCAGTTCACTGGCATT	TGATTAGGTCCACGGCCACA	GU433188	98
acly	AAAAGCTTTGATGAGCTTGGGG	TACAGTGGGAGGAGGCAACTCTT	XM_003442027	96
acss2	GCGTGATGCGGACCATTTAC	TGTCGTCTATCCTCCCCGTT	XM_005477776.4	100
dgat2	GCTTGAATTCTGTCACCCTGAAGA	ACCTGCTTGTAGGCGTCGTTCT	XM_003458972	105
hsl	AGTTCACTCCAGCCATTCGG	TGGCTGCTACCCCTATTCCT	XM_005463937.4	99
atgl	GACACATGCTGCAAAGCACT	ACCAGGACGTTTTCTCCGTC	XM_003440346.5	91
ansn	CAGAAGACTGACAGCGTGGTGA	GGTGTTGGAGCCTTGTGGAAGT	XM_019355582.1	98
glns	AAGCTGGTGCTGTGTGAAGT	TCGACTACGTCCCTGCCATA	NM_001279668.1	97
bckdh	ACGCCATCTCTACCCCTACA	ACACGGATGGACAGCATACC	XM_003459112.5	105
gdh1	GGTTCCAGGACAAGACCTTCGT	TCTCCGACTCCGACGCACTT	XM_003457465.3	96
tnf-α	CAGAAGCACTAAAGGCGAAGAACA	TTCTAGATGGATGGCTGCCTTG	NM_001279533	97
il-1β	GAGCACAGAATTCCAGGATGAAAG	TGAACTGAGGTGGTCCAGCTGT	XM_019365841.1	101
il-10	CAGCAGCAGGAGCATCAGCATT	CACAGGAGGACGGTCTGAGAAGT	KP645180.1	98
tgf-1β	AAGAGGAGGAGGAATACTTTGCCA	GAAGCTCATTGAGATGACTTTGGG	NM_001311325.1	107
caspase-3	GGAGTGGACGATACAGACGCAAA	TGAAGCTGTGTGACTGGGGCTT	NM_001282894.1	95
gst	TAATGGGAGAGGGAAGATGG	CTCTGCGATGTAATTCAGG	NM_001279635.1	96
cat	TTCAGCAGCACTTGCAGTTT	TTGGTCATATCGCACAGCCC	XM_003447521.5	94
β-actin	AGCCTTCCTTCCTTGGTATGGAAT	TGTTGGCGTACAGGTCCTTACG	KJ126772.1	99
ef1a	ATCAAGAAGATCGGCTACAACCCT	ATCCCTTGAACCAGCTCATCTTGT	KJ123689.1	101

Glycolysis: Glucokinase (*gk*); Phosphofructokinase (*pfk*); Pyruvate kinase (*pk*).
Gluconeogenesis: Phosphoenolpyruvate carboxykinase (*pepck*).
Pentose phosphate pathway: glucose 6-phosphate dehydrogenase (*g6pd*).
Lactic acid metabolism: Lactic dehydrogenase (*ldha/b*).
Insulin signal pathway: Insulin receptor (*ir*); Glucose transporter 1 (*glut1*); Glucose transporter 2 (*glut2*); Glucose transport 4 (*glut4*); serine/threonine kinase (*akt*).
Fatty acid oxidation: Carnitine palmitoyltransferase 1a (*cpt1a*); Acyl-coenzyme A oxidase (*aco*).
Lipolysis: Hormone-sensitive lipase (*hsl*); Adipose triglyceride lipase (*atgl*).
Lipogenesis: ATP citrate lyase (*acly*); Fatty acid synthase (*fas*); Acetyl-CoA carboxylase (*acc*); Acyl-CoA synthetase 2 (*acss2*).
Tricarboxylic acid cycle (*tca*): Citrate synthase (*cs*); Isocitrate dehydrogenase (*idh*); pyruvate dehydrogenase (*pdh*).
Amino metabolism: Asparagine synthetase (*asns*); Glutamine synthetase (*glns*); Branched amino acid dehydrogenase (*bckdh*); Glutamate dehydrogenase1 (*gdh1*).
Inflammation and apoptosis: Tumor necrosis factor alpha (*tnf*-α); Interleukin-1 beta (*il-1*β); Interleukin-10 (*il-10*); Tumor growth factor-1 beta (*tgf-1*β).
Antioxidant capacity: Glutathione S-transferase (*gst*); Catalase (*cat*).
Transcriptional regulation factor: Peroxisome proliferator activated receptor alpha (*ppar*α).
Reference genes: elongation factor 1-alpha (*ef1α*); *β-actin*

PVDF membranes. Blots were visualized by using LI-COR Biosciences (Odyssey Clx). The antibodies Tubulin was used as internal reference protein. Specific information on all antibodies used in this study is shown in Table 3. All antibodies were used at a dilution of 1:1000.

Table 3
Antibodies for Western Blot analysis experiments.

Antibodies	Source	Identifier
Mouse monoclonal anti- Tubulin	HUABIO	M1501–180
Rabbit polyclonal anti- mTOR	CST	#2972
Rabbit polyclonal anti- P-mTOR (Ser2448)	CST	#2971
Rabbit monoclonal anti- S6	CST	#2217
Rabbit monoclonal anti- P—S6 (Ser235/236)	CST	#4856

2.11. Statistical analyses

The data were tested for normality by using the Shapiro-Wilk test and homogeneity of variance by using Levene's test. All the data for measured parameters were tested by using a two-tailed Student's *t*-test in GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). Specific comparisons were made for the mean values between Con and HFD; Con and HFD + NaAc; and HFD and HFD + NaAc. Results with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of HFD and HFD + NaAc on growth, feed performance and organ indexes

The results indicated that the Nile tilapia fed the HFD reduced

markedly SR than those fed the Con (Fig. 1A; $P < 0.05$). The fish fed the HFD had lower weight gain than those fed the Con after six weeks (Fig. 1B; $P < 0.05$). After eight weeks, the Nile tilapia fed the HFD had lower final weight than those fed the HFD + NaAc (Fig. 1B; $P < 0.05$). Similarly, fish fed the HFD also had lower body length (Fig. 1C) and FE (Fig. 1E) compared to the other two treatments ($P < 0.05$). FI of fish in the HFD treatment was significantly lower than that of fish in the Con treatment (Fig. 1D; $P < 0.05$), while there was no statistical difference between fish in the HFD and HFD + NaAc treatments ($P > 0.05$). In addition, the fish fed the HFD increased markedly CF (Fig. 1F), VSI (Fig. 1G), HSI (Fig. 1H) and IPF (Fig. 1I) compared to those fish fed the Con ($P < 0.05$). However, these parameters did not differ significantly between HFD and HFD + NaAc treatments ($P > 0.05$). Together, dietary sodium acetate supplementation increased FE and improved growth of Nile tilapia, but it had no significant effect on HFD-induced organ indices.

3.2. Effects of HFD and HFD + NaAc on serum biochemical indicators related to glucose and lipid metabolism

The Nile tilapia fed the HFD had markedly higher serum glucose (Fig. 2A), insulin (Fig. 2B) and TG (Fig. 2E) levels than those fed the Con and HFD + NaAc ($P < 0.05$). The fish fed the HFD increased significantly serum NEFA levels (Fig. 2F; $P < 0.05$) compared to those fed the Con. However, serum NEFA had comparable levels between the HFD and HFD + NaAc treatments (Fig. 2F; $P > 0.05$). The fish fed the HFD reduced significantly serum pyruvate levels (Fig. 2C) compared to those fed the Con, but did not alter significantly serum lactate levels (Fig. 2D). Interestingly, the fish fed the HFD + NaAc reduced significantly serum pyruvate (Fig. 2C) and lactate levels than those fed the HFD (Fig. 2D). After intraperitoneal glucose injection, the fish fed the HFD had consistently higher serum glucose (Fig. 3A) and insulin (Fig. 3B) levels than the other two treatments ($P < 0.05$). The Nile tilapia fed the HFD + NaAc had a similar serum glucose profile as the fish fed the Con ($P > 0.05$), but had lower serum insulin levels (Fig. 3A and B; $P < 0.05$). These results suggest that HFD caused high serum glucose, insulin and lipids levels, indicating the occurrence of disturbances in glucose and lipid metabolism and insulin resistance in Nile tilapia. However, dietary sodium acetate supplementation increased insulin sensitivity and alleviated these adverse changes in Nile tilapia.

3.3. Effects of HFD and HFD + NaAc on liver glucose metabolism

The HFD treatment fish had significantly lower glycogen level

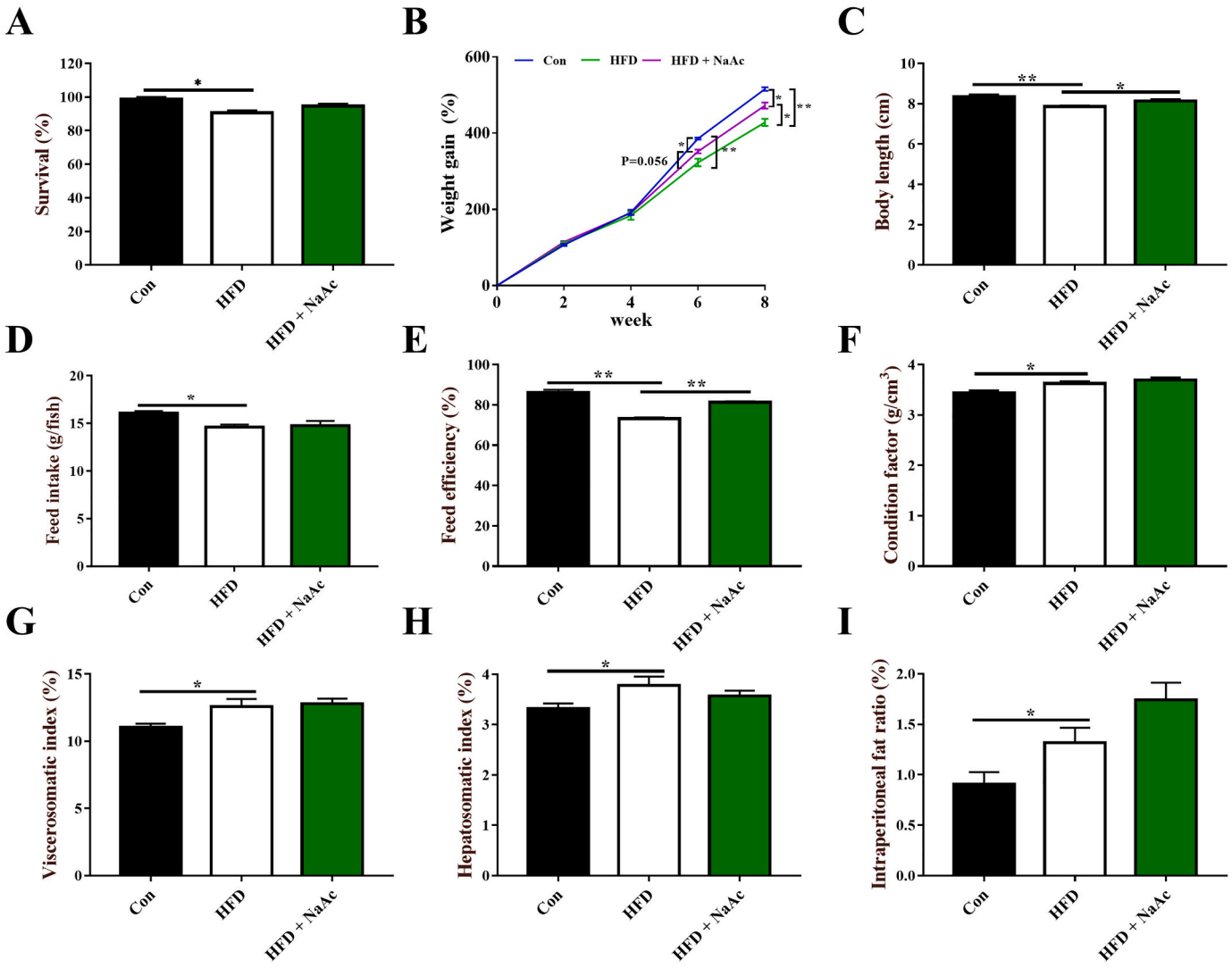


Fig. 1. The survival rate, growth performance, feed efficiency and organ indexes parameters of Nile tilapia. (A) survival, (B) weight gain (WG), (C) body length, (D) feed intake (FI), (E) feed efficiency (FE), (F) condition factor (CF), (G) viscerosomatic index (VSI), (H) hepatosomatic index (HSI) and (I) Intraperitoneal fat ratio (IPF). Values are mean ± SEM for three replicates. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively.

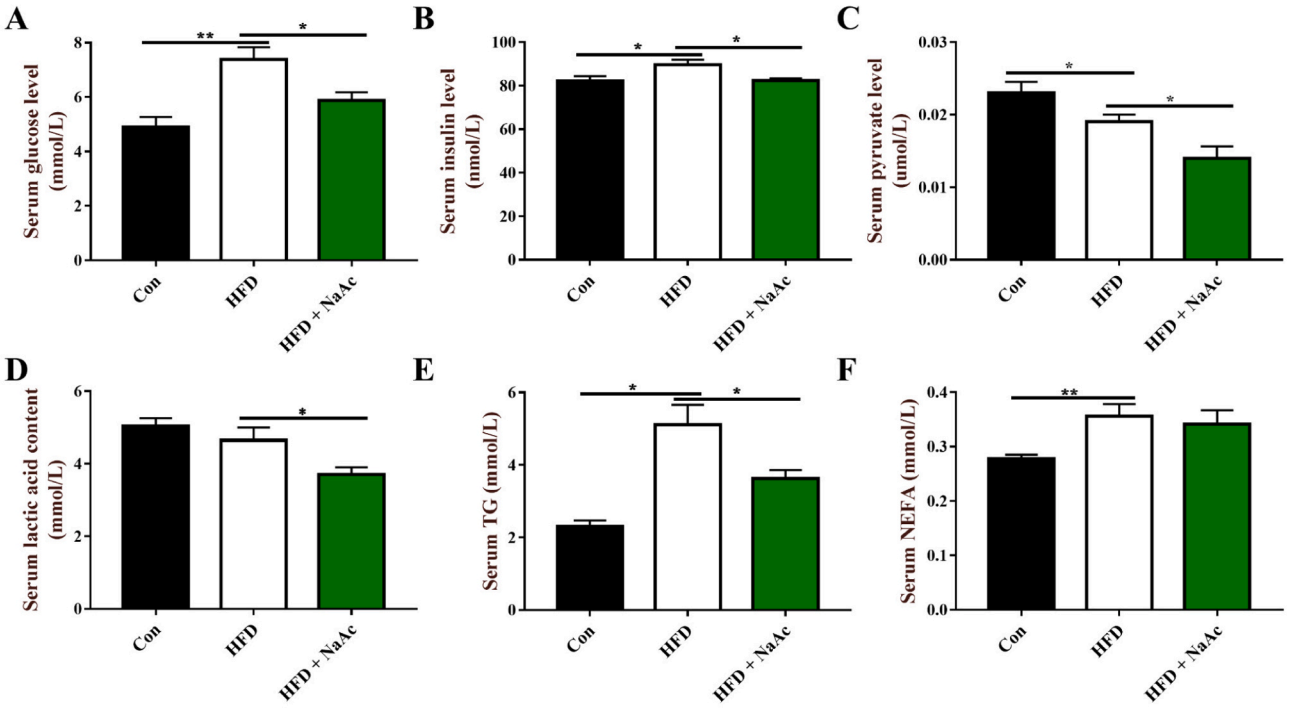


Fig. 2. Serum glucose and lipid-related parameters of Nile tilapia during the study. (A) serum glucose, (B) serum insulin, (C) serum pyruvate, (D) serum lactic acid, (E) serum TG and (F) serum NEFA. Values are mean ± SEM for nine fish. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively.

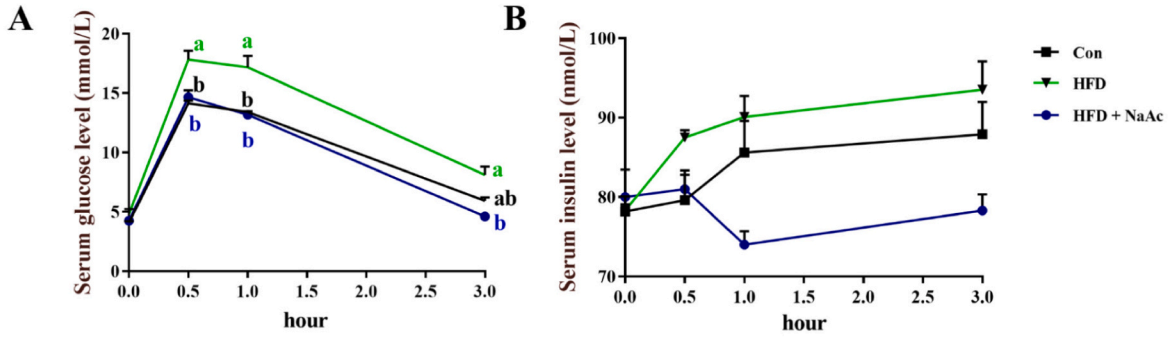


Fig. 3. The glucose tolerance test (GTT) of Nile tilapia during the study. Serum glucose (A) and insulin (B) levels at different time points after intraperitoneal glucose injection. Values are mean ± SEM for six fish. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. Different letters indicate a significant difference ($P < 0.05$).

(Fig. 4A), and higher pyruvate (Fig. 4B) and lactate (Fig. 4C) levels in the liver than the other two treatments ($P < 0.05$). Next, we measured mRNA expression levels of key genes in the hepatic glycolysis (*gk* and *pk*), tricarboxylic acid (TCA, *pdh* and *cs*) cycle, lactate metabolism (*ldha* and *ldhb*), gluconeogenesis (*pepck*) and pentose phosphate pathways (PPP, *g6pdh*). Results showed that the measured key genes for hepatic glycolysis and TCA cycle were significantly down-regulated in the fish fed HFD and HFD + NaAc compared to those fed the Con. Interestingly, the expression of these genes was not significant different between fish fed the HFD and HFD + NaAc (Fig. 4D; $P > 0.05$). The fish fed the HFD had significantly higher mRNA expression level of hepatic lactate metabolism gene *ldhb* than the other two treatments ($P < 0.05$). No marked difference in fish was detected in the expression of *ldha* among the three treatments (Fig. 4D; $P > 0.05$). The fish fed the HFD downregulated *6pdh* and *pepck* genes compared to those fed the Con and HFD + NaAc (Fig. 4D; $P < 0.05$). Moreover, feeding the fish with HFD and HFD + NaAc significantly down-regulated the insulin pathway-related genes, including *ir*, *akt* and *glut2*, compared to the Con (Fig. 4E; $P < 0.05$). The results indicated that dietary sodium acetate addition did not improve insulin sensitivity, hepatic glycolysis and TCA processes inhibited by feeding fish with HFD, but increased glycogen accumulation and promoted gluconeogenesis and PPP in liver.

3.4. Effects of HFD and HFD + NaAc on muscle glucose metabolism

The Nile tilapia fed the HFD + NaAc reduced muscle glycogen level compared to those fed the Con and HFD (Fig. 5A; $P < 0.05$). The fish fed the HFD had lower muscle pyruvate level (Fig. 5B) than Con and HFD +

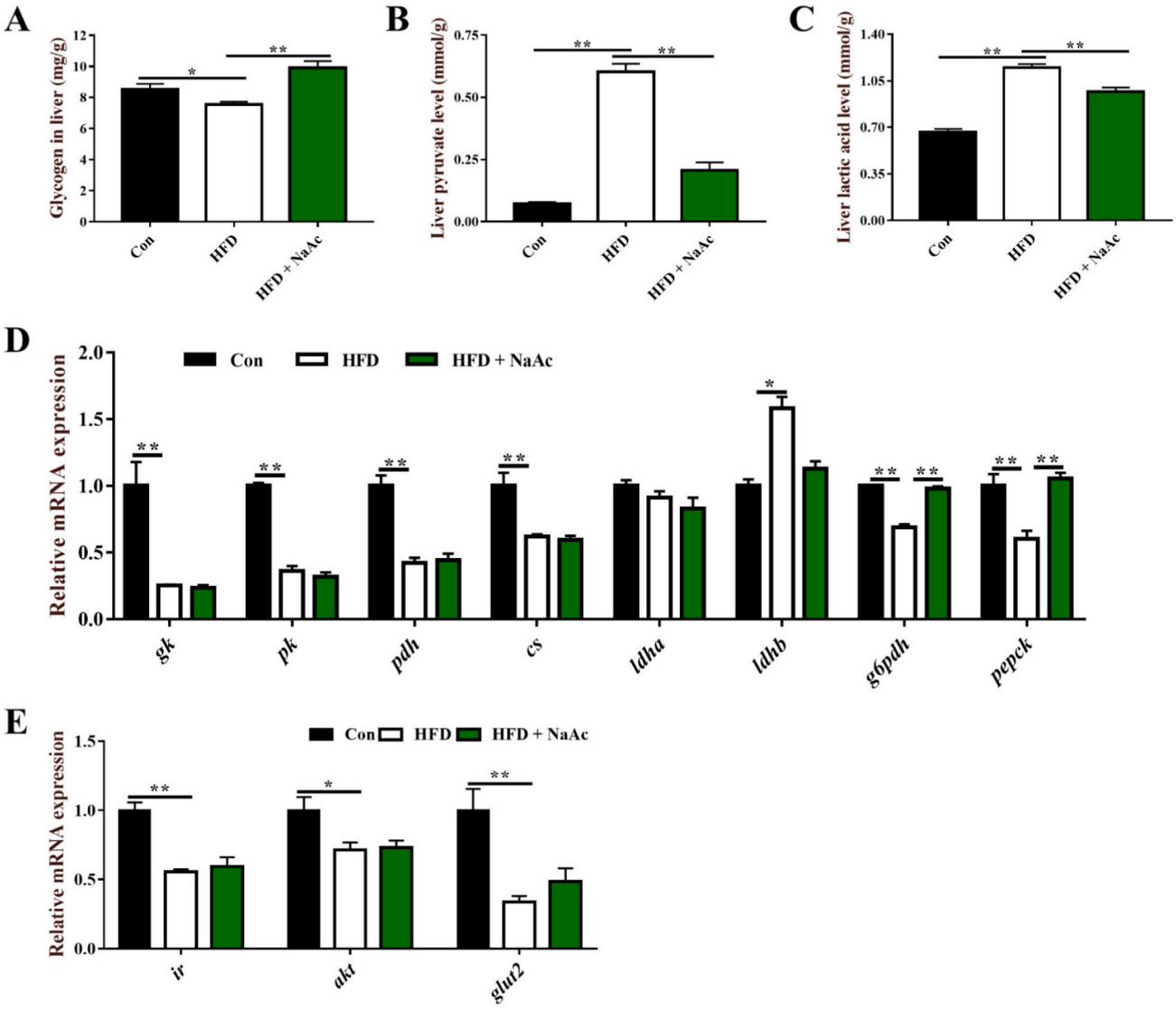


Fig. 4. The liver glucose metabolism in Nile tilapia during the study. (A) liver glycogen content, (B) liver pyruvate level, (C) liver lactic acid level, (D) the mRNA expression levels of glucose metabolism key genes and (E) the mRNA expression levels of insulin signaling pathway related genes. Values are mean ± SEM for eight fish. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively.

NaAc ($P < 0.05$). The fish fed the HFD had higher muscle lactate level than those fed the Con (Fig. 5C; $P < 0.05$), but not between the HFD and HFD + NaAc treated fish ($P > 0.05$). Also, we examined glucose metabolism related genes in muscle and found that the expression of key genes in the glycolysis (*gk, pfk,* and *pk*), TCA cycle (*pdh*5) and PPP (*g6pdh*) were down-regulated in HFD-fed fish compared to the Con-fed fish ($P < 0.05$), but no significant changes in lactate metabolism genes (*ldha* and *ldhb*) between the two treatments (Fig. 5D; $P > 0.05$). In contrast, the fish fed the HFD + NaAc significantly up-regulated the expression of *gk, pfk, pk* and *pdh* compared to those fed the HFD (Fig. 5D; $P < 0.05$). However, the expression of *ldha*, *ldhb, cs* and *g6pdh* genes did not change significantly between the two treatments (Fig. 5D; $P > 0.05$). Further, we examined insulin pathway-related genes. The results revealed that fish fed the HFD down-regulated significantly *akt* and *glut4* genes compared to those fed the Con (Fig. 5E; $P < 0.05$), while the expression of *ir* and *glut1* genes did not change significantly (Fig. 5E; $P > 0.05$). However, feeding the fish with HFD + NaAc significantly upregulated most insulin pathway-related genes such as *ir*, *ak*t and *glut1*, but not *glut4*, compared to feeding the fish with HFD (Fig. 5E; $P < 0.05$). In general, feeding the Nile tilapia with the HFD reduced insulin sensitivity and impaired glycogen synthesis, while dietary sodium acetate improved insulin functions and stimulated glucose catabolism in fish muscle.

3.5. Effects of HFD and HFD + NaAc on liver and muscle lipid metabolism

As expected, the fish fed HFD increased markedly TG (Fig. 6A) and NEFA (Fig. 6B) levels in liver compared the fish fed Con, whereas dietary supplementation with sodium acetate decreased liver TG and NEFA levels ($P < 0.05$). Correspondingly, the fish fed HFD increased markedly lipid droplets in liver tissue sections compared to those fed Con or HFD + NaAc (Fig. 6C; $P < 0.05$). In addition, we measured the genes associated with lipid synthesis and catabolism in liver. The results showed that the fish fed HFD down-regulated markedly the expression of *fas*, *acc*, *acly*, *cpt1a* and *atgl* genes compared to those fed the Con ($P < 0.05$). Nevertheless, the expression of these genes did not change significantly between the fish fed the HFD and HFD + NaAc (Fig. 6D; $P > 0.05$). The

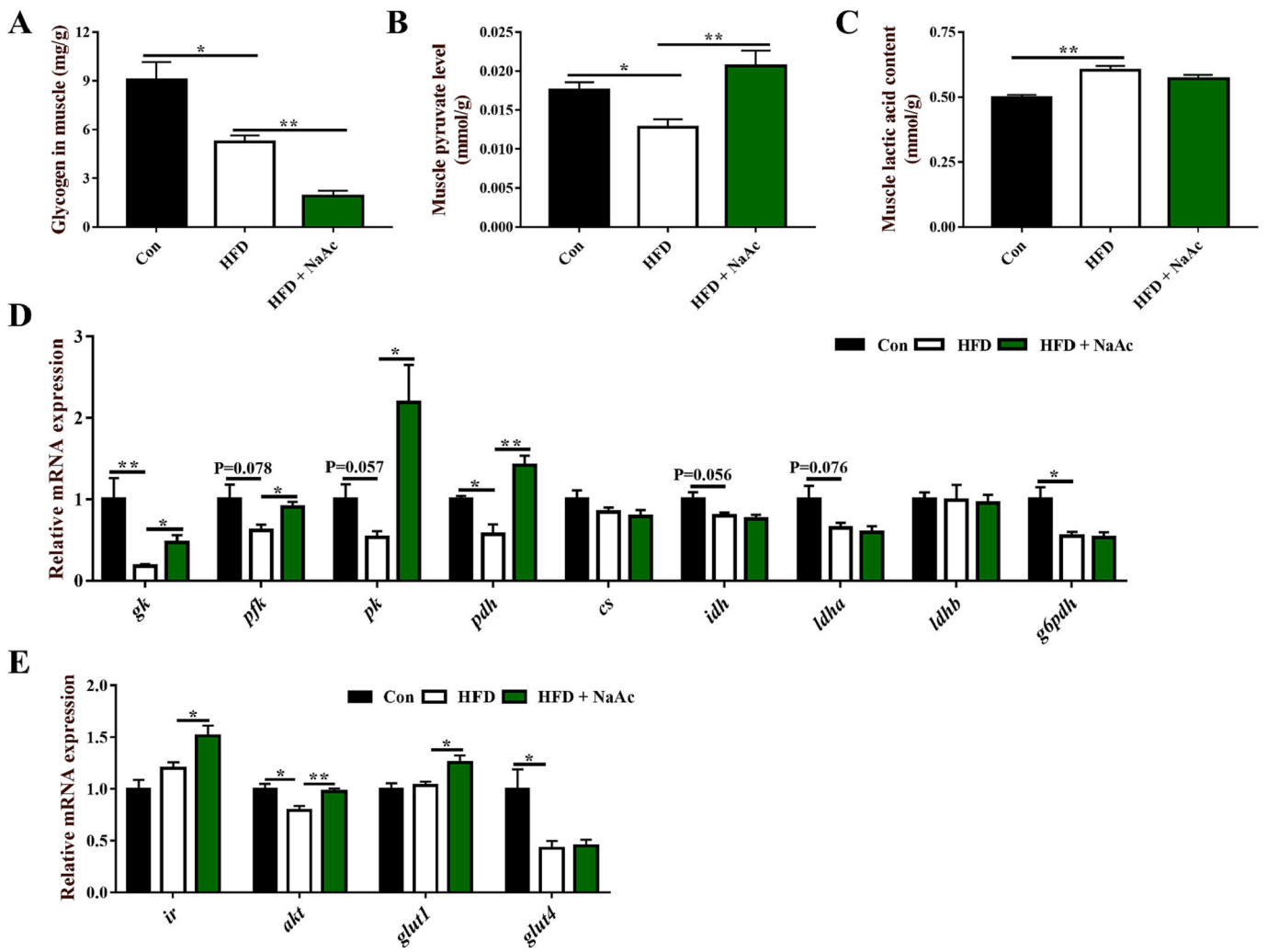


Fig. 5. The muscle glucose metabolism in Nile tilapia during the study. (A) muscle glycogen content, (B) muscle pyruvate level, (C) muscle lactic acid level, (D) the mRNA expression levels of glucose metabolism key genes and (E) the mRNA expression levels of insulin signaling pathway related genes. Values are mean ± SEM for eight fish. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively.

fish fed the HFD + NaAc had higher expression of *ppara* and *aco* genes than the fish fed the HFD (Fig. 6D; $P < 0.05$). However, the expression of *ppara* and *aco* genes did not differ between the fish fed the Con and HFD ($P > 0.05$). The fish fed HFD had higher mRNA expression level of *hsl* gene than those fed the Con ($P < 0.05$). However, no significant alteration in *hsl* gene between the HFD and HFD + NaAc (Fig. 6D; $P > 0.05$). In fact, we also assessed lipid metabolism in muscle and whole body. The results indicated that dietary sodium acetate supplementation had no significant effect on the HFD-induced changes in lipid metabolism (Fig. S1). Accordingly, the main target organ for dietary sodium acetate to regulate lipid metabolism is the liver rather than muscle, where it mainly activates lipid catabolism in the peroxisome.

3.6. Effects of HFD and HFD + NaAc on muscle amino acid and protein metabolism

Protein and amino acid metabolism are closely linked to protein synthesis and growth in fish. The fish fed the HFD had lower carcass ratio (Fig. 7A) and muscle protein content (Fig. 7B), than those fed the Con and HFD + NaAc ($P < 0.05$). The whole body protein content did not vary markedly in fish fed the three diets (Fig. 7C; $P > 0.05$). The fish fed the HFD did not affect significantly the expression of amino acid catabolic genes (*bckdh* and *gdh1*), but significantly down-regulated amino acid anabolic-related genes (*gln*s and *asns*) expression compared to those fed the Con (Fig. 7D; $P < 0.05$). However, fish fed the HFD + NaAc down-regulated significantly *bckdh* and *gdh1* genes expression, but up-regulated *gln*s gene expression compared to those fed the HFD (Fig. 7D; $P < 0.05$). Besides, the fish fed the HFD down-regulated significantly P-mTOR and P—S6 proteins expression levels compared to those fed the Con (Fig. 7E; $P < 0.05$). Contrastingly, the fish fed the HFD + NaAc had significantly higher expression levels of P-mTOR and P—S6 proteins compared to those fed the HFD, suggesting that the mTOR activity was increased in the NaAc treatment (Fig. 7E; $P < 0.05$). Therefore, HFD supplementation with sodium acetate increase muscle protein deposition by activating the mTOR pathway to promote amino acid and protein anabolism and inhibit amino acid catabolism in muscle.

3.7. Effects of HFD and HFD + NaAc on liver injury, inflammation and apoptosis

To determine the effect of dietary sodium acetate on the liver health, we evaluated the status of liver injury, inflammation and apoptosis. The fish fed the HFD had higher serum ALT (Fig. 8A), serum AST (Fig. 8B) and hepatic MDA (Fig. 8C) levels compared to those fish fed the Con and HFD + NaAc ($P < 0.05$). In contrast, the fish fed the HFD had significantly lower SOD activity in the liver than those fed the Con and HFD + NaAc (Fig. 8D; $P < 0.05$). Meanwhile, the fish fed the HFD + NaAc increased the mRNA expression level of *gst* gene than those fed the Con

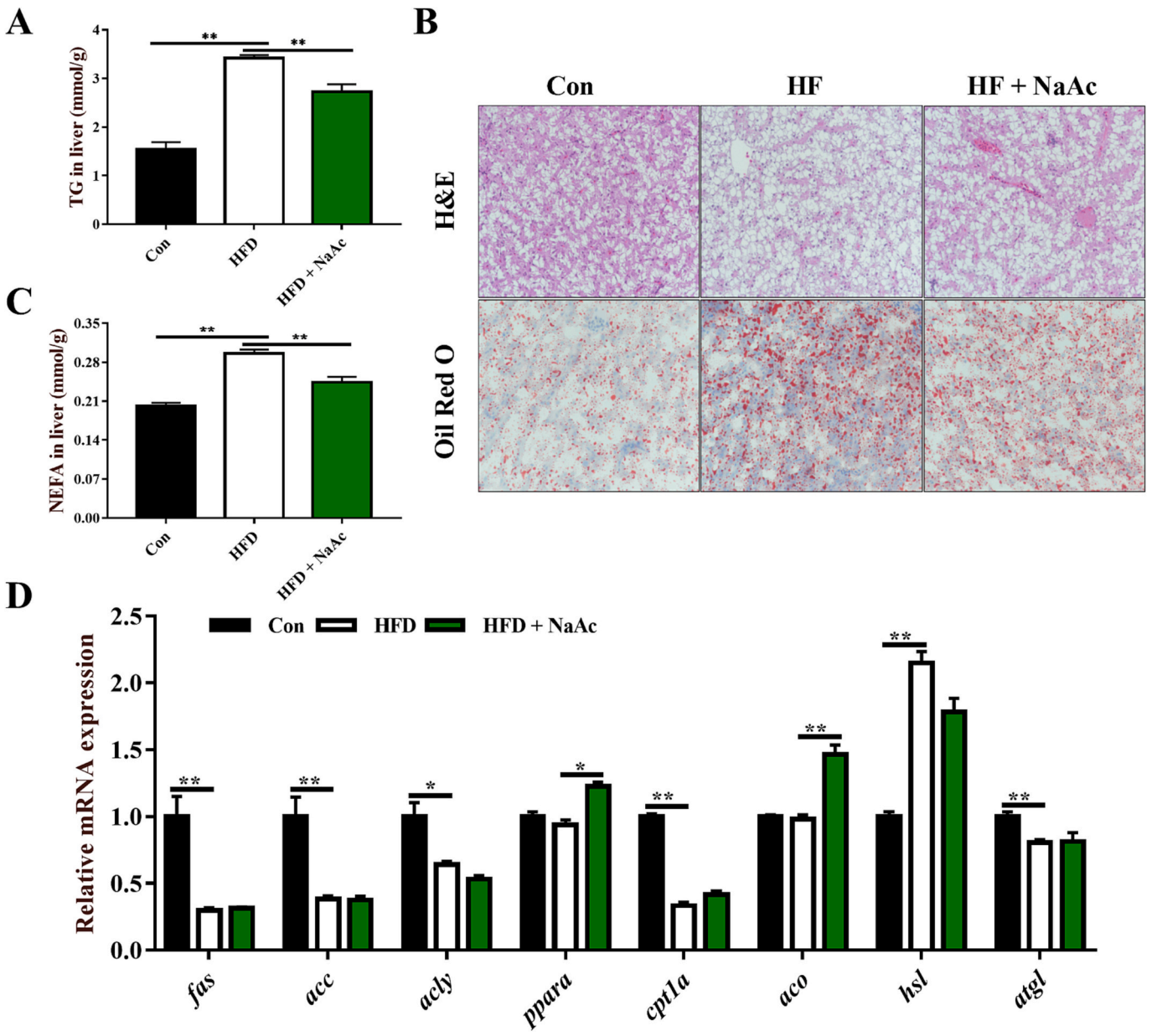


Fig. 6. The liver lipid metabolism in Nile tilapia the liver during the study. (A) liver TG content, (B) the liver tissue H & E and oil red O sections of the liver, (C) liver NEFA content and (D) the mRNA expression levels of lipid metabolism key genes. Values are mean ± SEM for eight fish. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and HFD (Fig. 8E; $P < 0.05$). In addition, Nile tilapia fed the HFD up-regulated the expression of the pro-inflammatory genes *tnf-a* and *il-1b* compared to those fed the Con and HFD + NaAc (Fig. 8E; $P < 0.05$). The fish fed the HFD down-regulated the anti-inflammatory gene (*il-10*) expression compared to those fed the Con ($P < 0.05$), while no marked difference was detected of *il-10* gene expression between fish fed the HFD and HFD + NaAc (Fig. 8E; $P > 0.05$). The expression of *cat* and *tgf-b* genes did not change significantly in fish among all three dietary treatments (Fig. 8E; $P > 0.05$). Besides, the fish fed the HFD had significantly higher expression of the cysteine-aspartic acid protease 3 (*caspase 3*) gene compared to those fed the Con and HFD + NaAc (Fig. 8F; $P < 0.05$). Similarly, the fish fed the HFD exhibited the stronger apoptotic signal in liver sections than those fed the other two diets (Fig. 8G). Collectively, dietary sodium acetate supplementation alleviated HFD-induced liver oxidative stress, inflammation and apoptosis, thereby improved liver health in Nile tilapia.

4. Discussion

Currently, the application of HFD in aquaculture are difficult to avoid because of economic efficiency. However, HFD has multiple negative effects that can lead to poor growth of farmed fish (Du, 2014; Yan et al., 2015). Therefore, improving the utilization efficiency of HFD by using cost-effective additives is a viable response strategy. SCFAs have been shown to possess multiple biological effects and are considered as a promising feed additive (Den Besten et al., 2013; Zhang et al., 2020). In the present study, we found that dietary sodium acetate supplementation alleviated the inhibited growth, metabolic disorders and liver damage caused by feeding HFD in Nile tilapia. However, the metabolic regulation of dietary sodium acetate appeared to be obviously organ-dependent as the detailed metabolic processes summarized in Fig. 9.

HFD-induced glucose metabolism disorders have been extensively

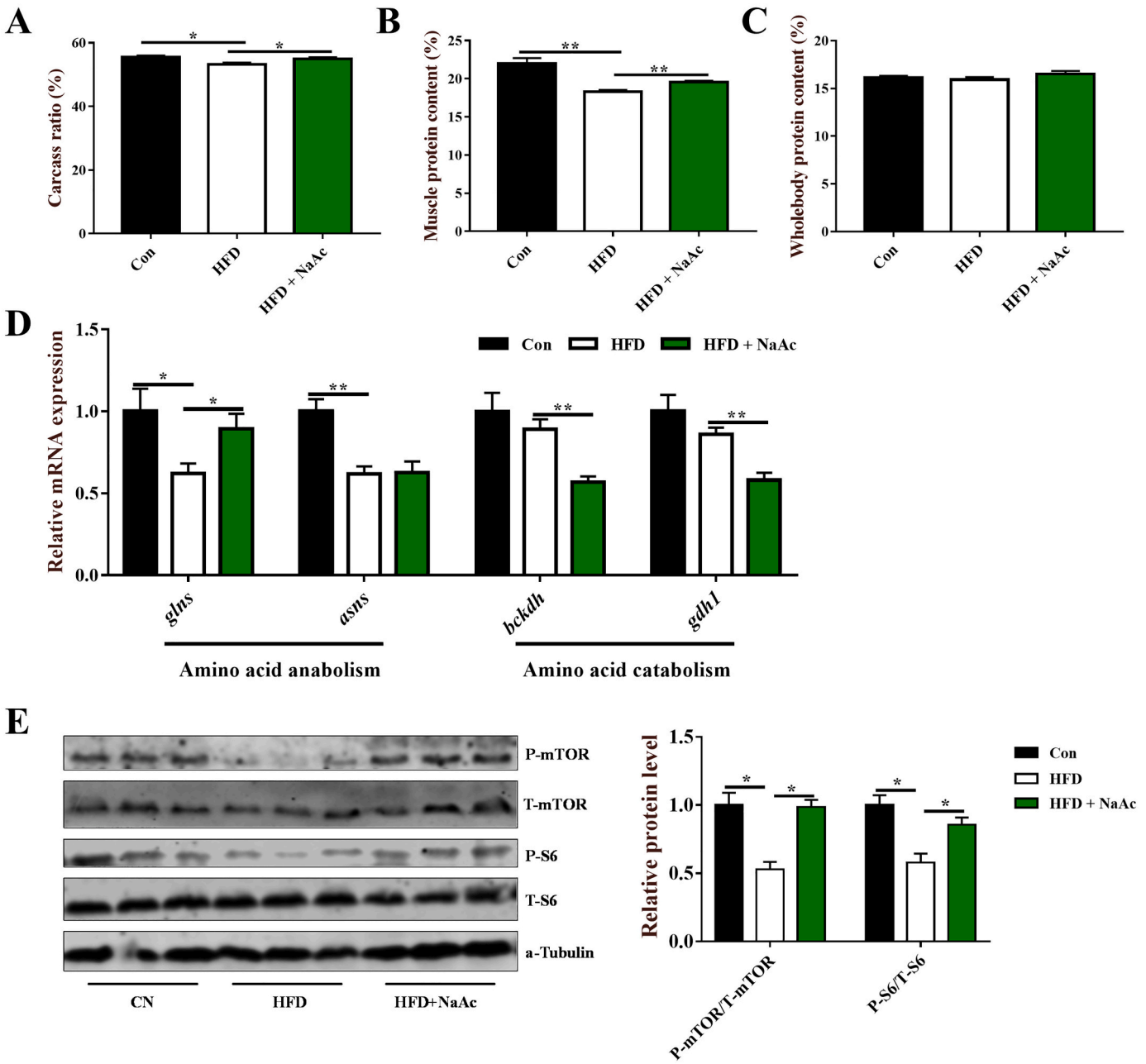


Fig. 7. The muscle protein and amino acid metabolism in Nile tilapia during the study. (A) carcass ratio (CR), (B) muscle protein content, (C) wholebody protein content, (D) the mRNA expression levels of amino acid metabolism genes, (E) the protein expression levels of protein synthesis regulator (P-mTOR: Phosphorylated mTOR; P-S6: Phosphorylated S6). Values are mean ± SEM for eight fish. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively.

studied and closely associated with reduced insulin sensitivity (Lichtenstein and Schwab, 2000; Figueiredo-Silva et al., 2012). However, relatively limited studies have been conducted on the role of sodium acetate in regulating this process. A study in mammals reported that sodium acetate improved glucose homeostasis in the body by enhancing insulin sensitivity (Olaniyi and Amusa, 2020). Similarly, studies in fish have also shown that dietary sodium acetate supplementation reduced markedly serum glucose levels and improved the metabolic status of zebrafish (Wang et al., 2021) and Golden Pompano (*Trachinotus ovatus*) (Xun et al., 2022). In the present study, the GTT test showed that the dietary sodium acetate addition alleviated the HFD-induced glucose intolerance but decreased serum insulin levels in Nile tilapia. A similar phenotype was observed in the dichloroacetate- or mildronate-treated Nile tilapia, and these studies indicated that the improved glucose metabolism by dichloroacetate or mildronate was associated with the increased insulin efficiency rather than insulin secretion (Li et al., 2020a; Luo et al., 2022). However, dietary sodium acetate supplementation did not restore the HFD-induced inhibition of hepatic glycolysis and TCA cycle processes, but instead promoted the expression of *pepck*, a key gene for gluconeogenesis, suggesting that the hepatic insulin pathway did not appear to be activated. We also noted that dietary sodium acetate supplementation reduced hepatic pyruvate and lactate levels, further suggesting an increase in hepatic gluconeogenesis. Only the expression of *g6pdh*, a key gene in hepatic PPP, and hepatic glycogen deposition were increased after dietary sodium acetate supplementation in the present study. The study conducted on GIFT tilapia also showed that *g6pdh* plays a significant role in maintaining endocrine status and liver glucose metabolism (Qiang et al., 2014, 2016). In addition, the PPP

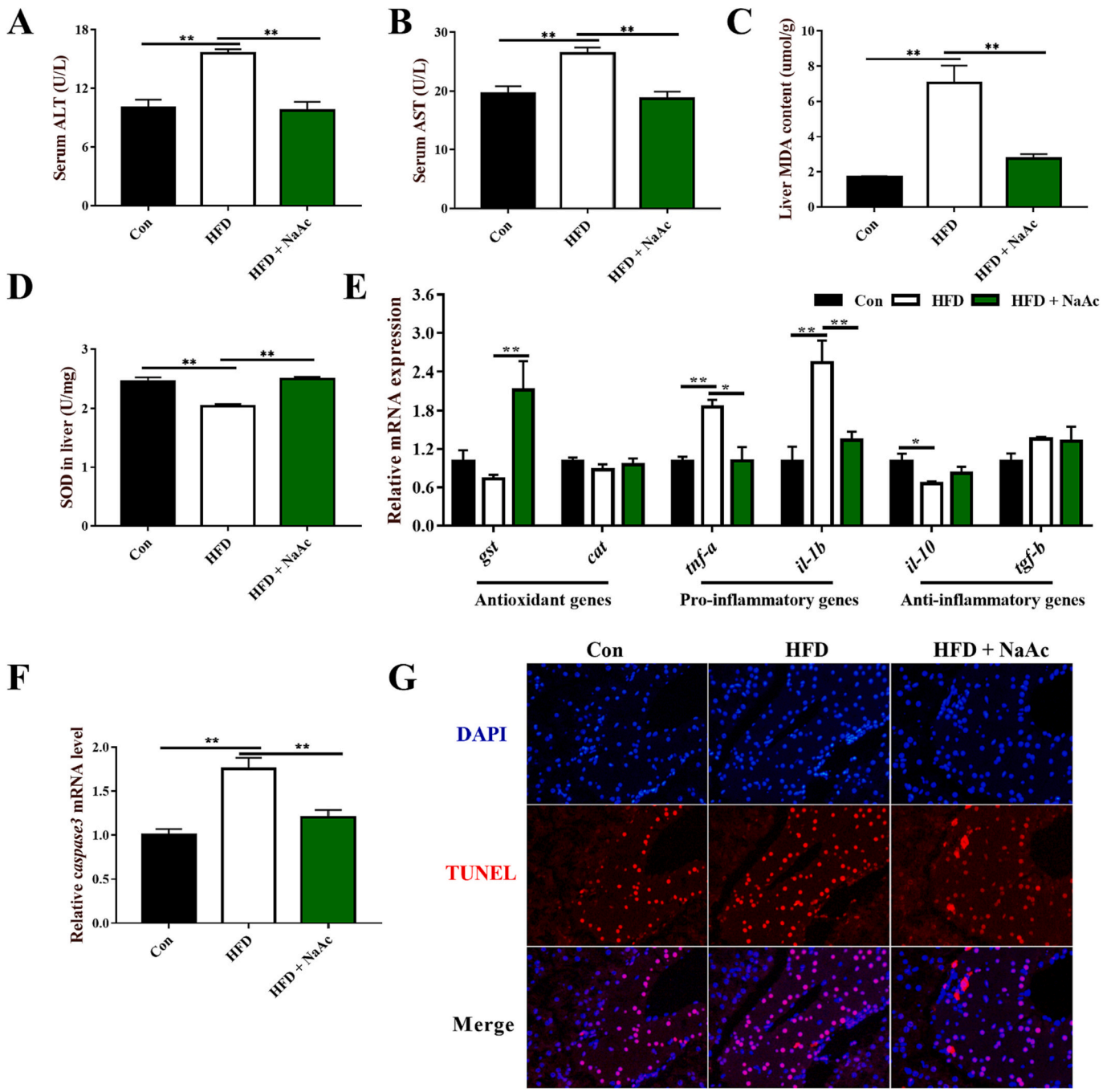


Fig. 8. The liver injury, inflammation and apoptosis in Nile tilapia during the study. (A) serum ALT level, (B) serum AST level, (C) liver MDA content, (D) liver SOD activity, (E) the mRNA expression levels of antioxidant and inflammatory genes, (F) the casepase3 gene expression levels, and (G) the liver TUNEL staining (DAPI: blue-fluorescent DNA stain; TUNEL: apoptosis fluorescence staining). Values are mean ± SEM for eight fish. The * and ** represent significant difference ($P < 0.05$) and highly significant difference ($P < 0.01$), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is involved in the *de novo* synthesis of nucleotides, which is closely related to cell growth (Ali et al., 2022). Therefore, enhanced PPP also contributes to improved hepatic glucose metabolism and hepatocyte growth. Taken together, dietary sodium acetate supplementation increase hepatic glucose consumption mainly by promoting the PPP and glycogen accumulation, but does not improve hepatic glucose catabolism utilization in Nile tilapia fed with HFD.

Contrastingly, muscle tissue had better insulin sensitivity than liver tissue upon dietary sodium acetate supplementation in the present study. Similar to liver tissue, HFD also inhibited the insulin signaling pathway and glycolysis in muscle tissue, but dietary supplementation with sodium acetate activated the insulin signaling pathway and promoted glycolysis and TCA cycle processes. Correspondingly, dietary sodium acetate supplementation reduced muscle glycogen levels and increased muscle pyruvate levels, indicating an increase in glycolytic flux. A previous study in Nile tilapia also found that muscle tissue appeared to be more sensitive to insulin signaling responses (Zhou et al., 2022). A recent review in mammals suggested multiple roles for insulin in skeletal muscle, which is considered to be the most important tissue for determining blood glucose (Sylow et al., 2021). Thus, enhanced

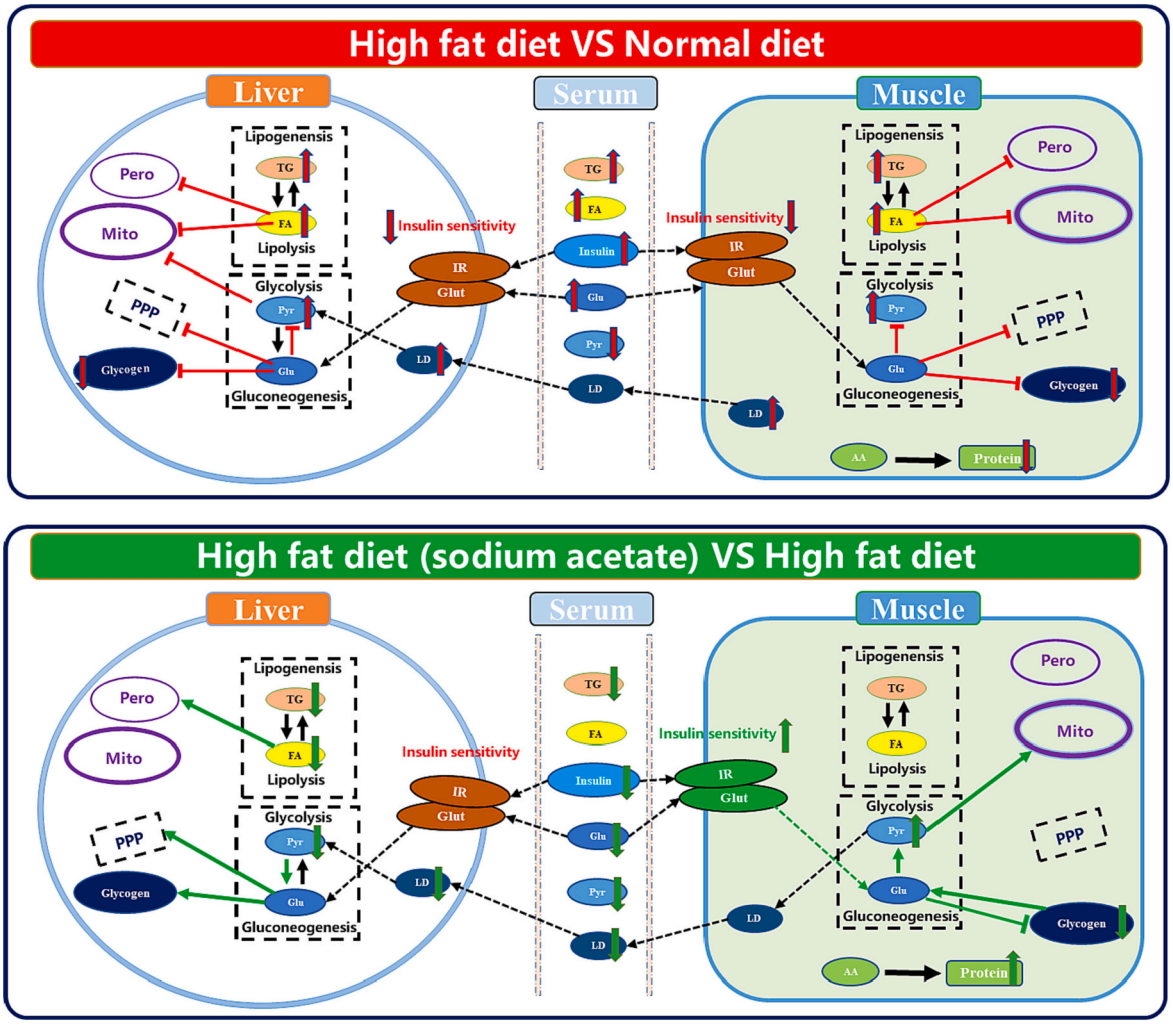


Fig. 9. Altered liver and muscle glucose and lipid metabolism and muscle protein metabolism in Nile tilapia fed with high-fat diet or high-fat containing sodium acetate diet. Note: The red and green arrows represent the effects (promotion/inhibition; increase/decrease) of high-fat diet and high-fat containing sodium acetate diet on tissue metabolic processes or metabolites, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

muscle glucose catabolism may contribute more in alleviating HFD-induced hyperglycaemia. Here, we also noted that muscle glycogen levels were markedly reduced despite the fact that dietary sodium acetate supplementation activated the insulin signaling pathway. In addition, we also observed that the genes related to glycolysis, including *gk*, *pfk*, and *pk*, were also up-regulated in the fish fed sodium acetate. These results suggest that, dietary sodium acetate supplementation in HFD enhance glucose catabolism in muscle tissue to provide more glucose-sourced energy supply rather than glycogen synthesis. However, dietary sodium acetate supplementation did not promote *g6pdh* gene expression in muscle as it did in the liver tissue, suggesting that the PPP may have different regulatory pathways in liver and muscle tissues. Interestingly, HFD-fed Nile tilapia dramatically increased pyruvate levels despite the inhibition of glycolysis in liver, which may be associated with enhanced Cori cycling (Cori, 1981). This is because lactate content was elevated in both muscle and liver in Nile tilapia fed with HFD, while the expression of *ldhb* gene, a key enzyme responsible for catalyzing the conversion of lactate to pyruvate, was also upregulated in the liver, suggesting an increased lactate flow from the muscle to the liver. This also reflects that liver and muscle can work together through the lactic acid cycle to coordinate glucose metabolism during glucose metabolism disorders triggered by feeding Nile tilapia with HFD. In general, dietary sodium acetate increases insulin sensitivity in Nile tilapia muscle tissue, but not liver tissue, thereby promotes glucose catabolism.

Unlike glucose metabolism, dietary sodium acetate supplementation mainly improved liver lipid metabolism, while it had no positive effect on muscle lipid metabolism. Similar to other studies, HFD caused increased lipid deposition in most Nile tilapia tissues, including serum, liver, muscle, and abdominal adipose (Yan et al., 2015; Li et al., 2020b). However, the present study revealed that dietary sodium acetate supplementation mainly increased peroxisomal lipid catabolism through activation of hepatic *pparα/Aco* pathway, thereby reduced HFD-induced hepatic lipid deposition in Nile tilapia. Studies in black seabream (*Spondyliosoma cantharus*) and tilapia also found that the activation of the *pparα/Aco* pathway alleviated HFD-induced hepatic lipid deposition (Zhang et al., 2019; Jin et al., 2020). Besides, dietary sodium acetate addition also can activate AMP-activated protein kinase (AMPK) and reduce liver lipid deposition in Nile tilapia (Xu et al., 2022). Actually, AMPK is considered to be a key target in the regulation of lipid catabolism and its activators have been heavily used as lipid-lowering agents (Zhou et al., 2019a; Zhou et al., 2019b). In addition, PPAR is a downstream target of AMPK, and AMPK/PPAR signaling pathway is a major regulatory pathway of lipid catabolism (Diniz et al., 2021). Therefore, the AMPK/ppar signaling pathway may be an important target for lipid lowering promoted by dietary sodium acetate in liver of Nile tilapia.

Also, peroxisomal beta-oxidation has been reported to be stronger in liver than in muscle and the former tissue is the main target for pparα (Ning et al., 2016), which may be one of the reasons for dietary sodium acetate to induce a more pronounced lipid-lowering effect in the liver than in muscle. In mammals, dietary sodium acetate supplementation promoted beta-oxidation in adipose tissue and induced their browning to increase thermogenesis, thereby reduced lipid deposition induced by HFD (Canfora et al., 2015; Sahuri-Arisoylu et al., 2016). Nevertheless, in the current study dietary sodium acetate supplementation did not alleviate HFD-induced intraperitoneal fat deposition in Nile tilapia. This may be related to the fact that the function of adipose tissue in fish is different from that of mammals, as fish are poikilothermic animals that do not require adipose tissue thermogenesis to maintain body temperature (Haesemeyer, 2020). In short, dietary sodium acetate improves lipid metabolism in HFD-fed Nile tilapia mainly by promoting liver lipid catabolism.

We also observed that sodium acetate supplementation in HFD increased carcass ratio and muscle protein levels and improved HFD-induced growth depression in Nile tilapia. This may be linked to the activation of the muscle insulin signaling pathway induced by dietary sodium acetate in Nile tilapia. Insulin can regulate cell metabolism and promote cell proliferation via the PI3K/AKT/mTOR pathway (Haar et al., 2007). In the current study, sodium acetate addition in HFD remarkably activated the muscle mTOR/S6 pathway, which in turn upregulated protein synthesis in the ribosome (Mok et al., 2013). However, the amino acid catabolism was not remarkably depressed by the HFD, which could be related to inhibited glucose and fatty acid catabolism in the muscle. The lower muscle protein content in the HFD-fed fish is because the Nile tilapia body needed to maintain basic energy production through protein catabolism when the energy supply from glucose and fat was blocked. Contrastingly, sodium acetate supplementation in HFD inhibited muscle amino acid catabolism and promoted amino acid anabolism, which contributed to muscle amino acid accumulation. The increased amino acid levels can also activate the mTOR pathway as protein synthesis substrates and promote protein synthesis (Jewell et al., 2013). These results suggest that sodium acetate promotes protein anabolism in muscle tissue through the mTOR pathway.

The liver plays an irreplaceable role in maintaining the internal environment and homeostasis as the central metabolic organ (Berghe, 1991). Numerous studies have demonstrated that high energy diets (including high fat/high carbohydrate) often cause liver oxidative stress, inflammation and damage that can seriously affect health and growth in both fish and mammals (Kesh et al., 2016; Han et al., 2020; Qian et al., 2021). In the current study, feeding the Nile tilapia with HFD triggered marked oxidative stress, inflammation, apoptosis and damage in liver, which were largely relieved by dietary supplementation with sodium acetate. MDA is the main product of lipid peroxidation and is considered a marker of oxidative stress in the body (Xun et al., 2022). In the present study, dietary sodium acetate was able to depress the HFD-induced MDA content increase in Nile tilapia liver, indicating that oxidative stress was mitigated. This may be associated with increased antioxidant capacity of fish, as dietary sodium acetate increased liver SOD activity and *gst* gene expression in the present study. Similarly, studies on blunt snout bream showed that dietary sodium acetate alleviated hepatic oxidative stress and apoptosis induced by HFD (sodium palmitate), which was closely related to the increased antioxidant capacity of sodium acetate to reduce reactive oxygen species (ROS) and MDA levels in hepatocytes (Yu et al., 2022). Moreover, the present study showed that dietary sodium acetate inhibited the expression of pro-inflammatory genes *tnf*-α and *il-1*β (downstream of MAPK and NF-κB signaling pathways) induced by HFD in liver. Other studies have also shown that dietary sodium acetate suppressed inflammatory response via MAPK and NF-κB signaling pathways in Nile tilapia (Li et al., 2020c) and golden pompano (Xun et al., 2022). These indicate that MAPK and NF-κB signaling pathways may be important targets of sodium acetate in regulating inflammatory responses of fish. Therefore, one of the important reasons for dietary sodium acetate to attenuate liver injury may be its combined effect of alleviating oxidative stress, inflammatory response and apoptosis. Another reason for the alleviation of liver injury may be related to the lipid-lowering effect of dietary sodium acetate. Many studies in fish have shown that reducing hepatic lipid deposition with lipid catabolic activators ameliorated HFD-induced liver injury, such as metformin (Zhou et al., 2019b) and berberine (Zhou et al., 2019a). These results indicate that sodium acetate alleviates inflammation, oxidative stress, cell apoptosis and damage in the liver of Nile tilapia.

In conclusion, dietary sodium acetate addition alleviated the adverse effects induced by feeding Nile tilapia with HFD, but with a clear difference in the two major metabolic tissues (liver and muscle). Dietary sodium acetate mainly improved insulin sensitivity in Nile tilapia muscle, increased catabolic utilization of muscle glucose, and promoted muscle protein synthesis. However, dietary sodium acetate only promoted hepatic PPP and hepatic glycogen accumulation. Dietary sodium acetate supplementation alleviated HFD-induced hepatic lipid deposition to some extent by activating lipid catabolism. Moreover, sodium acetate improved liver health status by reducing oxidative stress, apoptosis and inflammatory responses. Therefore, sodium acetate may be a promising feed additive to alleviate HFD-induced metabolic dysfunction and liver damage in fish. In future, SCFAs and their derivative salts should be considered in the formulation of aquafeeds to improve the utilization of HFD.

Author contributions

Zhenyu Du and **Wenhao Zhou** designed the experiments; **Wenhao Zhou** carried out the experimental work. **Wenhao Zhou** wrote the manuscript under the direction of **Zhenyu Du**. **Ruixin Li, Yuan Luo**, **Jiong Ren** and **Fang Qiao** assisted with the experimental work. **Samwel Mchele Limbu** and **Meiling Zhang** contributed to critical revision of the manuscript.

Declaration of Competing Interest

We declare that the authors have no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.739142.

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