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The Effects of Creatine Monohydrate and Conjugated Linoleic Acid on Growth Performance and Fiber Type, Fiber-related Genes and Metabolic Enzymes in the Tilapia Muscle

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Abstract

We analyzed the effects of creatine monohydrate (CMH) and conjugated linoleic acid (CLA) on muscle fiber type, related genes, metabolic enzymes, and growth performance in tilapia (*Oreochromis mossambicus*). The addition of CMH and CLA in the basal feed significantly reduced the daily intake of tilapia, indicating that CMH and CLA had positive effects on improving the tilapia feed conversion rate. CMH up-regulated the relative mRNA expressions of MyHC I, IIa/IIb in the tilapia muscle. The activities of oxidases (SDH, MDH), AMPK, and the mRNA expression levels of PGC-1α, MEF2C, and GLUT4 were increased but those of MyHC IIx and LDH activity were decreased. CLA increased the proportion of oxidative muscle fibers in tilapia muscle while enhancing the activities of MDH, SDH, and AMPK and increasing mRNA expressions of PGC-1α and GLUT4. However, it decreased the proportion of glycolytic muscle fiber types and LDH activity. CMH and CLA could activate the AMPK activity in the muscle, further up-regulating the gene expression in the AMPK pathway, enhancing oxidative metabolic ability, and transforming the muscle fiber type. The study provides a strategy for improving the quality of future tilapia culture by regulating muscle fiber type transformation through CMH and CLA supplementation.

Introduction

Muscle fiber growth is crucial in determining the yield and quality of aquatic products, and the heterogeneity of fiber types is an important characteristic of the muscle tissues of animals (Black and Olson, 1998). Based on polymorphisms of the myosin heavy chain (*MyHC*), muscle fibers can be divided into four types (Chen and Opara, 2013). Type *I* (slow oxidative, SO) muscle fiber has massive numbers of mitochondria, a high myoglobin content, and activity of aerobic metabolic enzymes but low ATPase activity, thus slowing down and making the contraction rate durable. Type *IIa* (fast oxidative, FO) muscle fibers contain myoglobin and remarkably high glycogen content, with two pathways of energy supply, including aerobic and glycolytic metabolism. Type *IIb* (fast glycolytic, FG) muscle fibers have fewer mitochondria, high glycogen, high ATPase activity, and high glycolytic enzyme activity, with fast and short contraction rates. The number of mitochondria, myoglobin content, cascade of enzyme activities, and metabolic and contractile characteristics

of *IIx* (fast oxido-glycolytic, FOG) muscle fibers lie between those of the *IIa* and *IIb* types (Chen and Opara, 2013). Therefore, if the proportion of oxidized muscle fibers is high, the flesh color, pH value, marbling score, and intramuscular fat content of the muscle are high. This results in tender muscle with high water-holding capacity and good quality (Li *et al.*, 2017).

AMP-activated protein kinase (AMPK) is a multi-subunit complex rich in serine/threonine. AMPK can regulate the metabolism of glucose, fat, and proteins in animal muscles by promoting mitochondrial biosynthesis and metabolic changes in the oxidized muscle phenotype (Hoppeler, 2016). Therefore, AMPK is known as the energy monitor of muscle cells. As an energy receptor in myocytes, AMPK activity is sensitive to changes in the concentration of high-energy phosphoric acid compounds owing to regulation by the ATP/AMP ratio. If energy consumption by muscle cells increases, AMPK can activate the metabolic rate of muscle cells to improve the re-synthesis of ATP. Simultaneously, it inhibits the consumption of ATP in muscle cell metabolism (Narkar *et al.*, 2008). AMPK activation increases glucose uptake by improving the expression of glucose transporter 4 (*GLUT4*) on the surface of the muscle membrane through activation. Moreover, AMPK can inhibit the activity of glycogen phosphorylase in muscle cells for glycogen synthesis during exercise, which is conducive to muscle development. AMPK can be activated through the following three pathways: exercise, activator, and special feed induction (Radhakrishnan *et al.*, 2021). Animal muscle development is dependent on nutrition. Improving the meat quality of economic animals through dietary nutrition is a research focus in the interest of the public.

Creatine monohydrate (CMH) in the animal liver or pancreas comprises glycine, arginine, and methionine. Approximately 95% of CMH is stored in the muscle. The effect of CMH's effect on muscle energy metabolism is related to the activation of AMPK (Liang *et al.*, 2013). Therefore, dietary supplementation of CMH is crucial for muscle growth and development. CMH is a monohydrate of creatine and the main form of exogenous creatine supplement. Following exogenous CMH supplementation, the phosphocreatine level in the muscle increases, which can enhance muscle production. Subsequently, glycogen decomposition and lactic acid generation in muscle can be delayed to slow down the decline in the post-mortem value. It can keep the humidity, color, and tenderness to improve the meat quality (Kamel *et al.*, 2023). CMH supplementation can reduce livestock and poultry fat stock to enhance muscle function and improve breeding quality (Sun *et al.*, 2022). Further systematical studies on whether CMH can effectively improve the quality of aquatic products, the scheme in aquatic production, and the effect of exogenous CMH supplementation on the metabolism of the organism are necessitated.

Conjugated linoleic acid (CLA) is a general term used for isomers with various conformations and positions of conjugated double-bond linoleic acid. Because CLA affects muscle formation and muscle fiber type during the early stages of growth and development of animals, dietary supplementation of CLA can increase the proportion of muscle fiber type *I* and the gene expression of *MyHC I*, *MyoG*, and *MyoD* (Qi *et al.*, 2015). CLA can affect meat color, tenderness, and pH value by regulating adipocyte differentiation, glucose and lipid metabolism, intramuscular fat deposition, muscle development, and muscle fiber types (Şahin *et al.*, 2022). CLA-enriched muscle can improve the structure of aliphatic acids in the muscle tissue to meat quality, playing an important role in providing economic meat of better quality for human consumption. Given the low CLA content in aquatic animals, adding CLA in aquaculture is of great value in improving the quality of products.

Tilapia (*Oreochromis mossambicus*) is a euryhaline and omnivorous fish, with the advantages of rapid growth, high yield, strong disease resistance, high meat yield, and delicious meat. Therefore, it is a fish with the highest production worldwide. It is also an excellent cultured fish promoted by the Food and Agriculture Organization of the United Nations (FAO). As a main future source of animal protein, tilapia is favored by consumers worldwide (Zhang et al., 2023). Improving the quality of tilapia farming is a critical scientific issue in the tilapia farming industry globally. In this study, tilapia were fed CMH and CLA. AMPK activity and relative mRNA levels of muscle fiber-related genes were quantitatively measured to assess the effects of dietary CMH and CLA on the muscle fiber type composition of tilapia along with their relationship with the AMPK metabolic pathway. This study provides a scientific basis for exploring the interaction between muscle fiber type conversion and glycolytic oxidase and the regulatory mechanism of the AMPK signaling pathway on muscle type conversion to propose strategies for improving the quality of tilapia culture.

Materials and Methods

Grouping of Experimental Animals

Two hundred healthy tilapia (*Oreochromis mossambicus*) (average length 6.10±0.57 cm, average weight 58.27±1.34 cm) were selected (provided by Guangxi Key Laboratory of Aquatic Genetic Breeding and Healthy Aquaculture, Nanning, Guangxi, China). Before the experiment, tilapia were placed in five circular tanks (5×3×1.8 m) for temporary adaptation to the breeding environment for one week. Tilapia were fed a basic diet not exceeding 5% of their weight twice a day (7:00 a.m. and 6:00 p.m.). Tanks were oxygenated for 1 hour each time, and water was changed by 10% in 2 weeks. The basic diet composition of the tilapia with a mass of 1 kg was as follows: corn (43.15%), wheat flour

(23.59%), soybean meal (18.21%), bran (11.78%), salt (0.28%), stone powder (0.83%), soybean oil (0.49%), yeast (0.22%), and premix (1.00%). Main nutrients included the crude protein mass fraction (9%), crude fat mass fraction (23%), and carbohydrate mass fraction (68%). The calculated metabolic energy was 14.21 MJ/kg. After one week of temporary adaptation, different proportions of CMH (Qingdao Auhai Biological Co., LTD., Qingdao, Shandong, China) and CLA (isomers c9, t11 and t10, c12 mass fractions were 28.5% and 30%, Qingdao Auhai Biological Co., LTD., Qingdao, Shandong, China) were added to the basic diet. Experiment fish were divided into the five following groups: Control, CMH (0.5%), CMH (1%), CLA (0.5%), and CLA (1%). After four weeks of feeding, the methods of oxygenation, feeding, and water change were consistent with those during temporary feeding. Tilapia were maintained in a fresh water tanks at 26±1°C at the Experimental Base of Guangxi Academy of Fishery Sciences, Qingshan Road No. 8, Nanning City, Guangxi Province, China. The experiment was conducted from July 2 to August 16, 2022.

Sample Collection

After feeding for four weeks, tilapia died due to ice water. Within 3 min, the skin was quickly peeled. The whole back muscle was cut into several parts and promptly put into a labeled DNase/RNase-Free cryogenic vial, and subsequently, in liquid nitrogen for quick freezing and stored at -80°C until further use.

Determination of Experimental Tilapia on Growth

The growth and daily intake of tilapia were recorded throughout the experimental period, and the daily mass gain of the tilapia was calculated.

Determination of AMPK Activity

The protein content of p-AMPK in samples was determined by biotin double antibody sandwich enzyme-linked immunosorbent assay, reflecting the phosphorylation level of AMPK. AMPK activity was expressed as the mass concentration of p-AMPK protein. The higher the mass concentration of p-AMPK protein, the higher the phosphorylation degree and the AMPK activity.

Determination of Relative mRNA expression Levels of Muscle Fiber-Related Genes

First, total RNA was extracted from the muscle tissue using extraction lysate (RNAiso Plus RNA, Takara Bio Co., LTD., Dalian, Liaoning, China). The Nucleic acid protein analyzer (NanoDrop2000, Thermo Fisher Scientific, USA) was used to detect the concentration and purity of the extracted total RNA ($A_{260\ nm}/A_{280\ nm}$). Integrity was tested based on the mass fraction using 1% agarose gel electrophoresis (Bio-Rad, USA). Extracted RNA was diluted to a concentration of 500 ng/μL. Using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time, Takara Bio, Dalian, China), cDNA was synthesized by reverse transcription and stored at -20°C. The primer sequence required for the experiment in tilapia was obtained from the literature (Livak and Schmittgen, 2001). Primers were designed and synthesized by ABOGEN Co., LTD. The specific sequence is shown in Table 1. Real-time fluorescence quantitative PCR (CFX96™, Bio-Rad, USA) was performed based on the chimeric fluorescence method. Using the synthetic cDNA as the template, amplification was performed using a real-time fluorescence quantitative kit (SYBR® Premix Ex Taq™ II, Takara Bio, Dalian, Liaoning, China). Three parallel and two negative controls were set for the steward gene and target gene, respectively. The

Table 1. Primer sequences used in the real-time polymerase chain reaction

Genes	Primer sequences (5'-3')	Fragment length/bp
18S rRNA	F: CCGTAACTTGACATTACC R: CCACCATATCGGAGCGTAGT	149
MyHC I	F: GCCGTCTACCAAGGAACTAC R: ACTTAACTCGTTGTCGCTC	79
MyHC IIα	F: CATTTCGATCAGTCTGGCTT R: CCATAGCATCAGGACACGA	219
MyHC IIb	F: ACAGACAACGTGAAAACG R: GCTGCAGTGTGACAAGGAGC	119
MyHC IIx	F: GGTCGAACAATCGTCCCATCG R: GGAGACTCCTTGGTGCTAACC	176
PGC-1α	F: AGGACGCACACAGTCG R: ACAAGTCGTGGTTAGAGGA	161
MEF2C	F: GACTGATGCAGAGCGA R: CAACGGACACGGATGACTGTA	172
GLUT4	F: CAGCTGTGAGCATGCTAGA R: GGGCGAGAGGGCTTTC	248

reaction mix, 25.0 µL in total, comprised SYBR® Premix Ex *Taq*™ II, 12.5 µL; upstream and downstream primers, 1.0 µL each; DNA template (10 ng/µL), 2.0 µL; dH_2O, 8.5 µL. Conditions for real-time quantitative PCR were as follows: the first step comprised pre-denaturation at 95°C for 30 s. A total of 35 cycles were performed with denaturation at 95°C for 5 s, 60°C annealing for 30 s, and extension at 72°C for 30 s. The final extension was at 72°C for 10 min. The amplified product was stored at 4°C. The $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative expression levels of target genes. The calculation formula (Livak and Schmittgen, 2001) was as follows:

$$\Delta\Delta Ct = (Ct_{\text{target gene}}-Ct_{\text{reference gene}})_{\text{treatment}}-(Ct_{\text{target gene}}-Ct_{\text{reference gene}})_{\text{untreated group}} \quad (1)$$

Determination of Related Metabolic Enzyme Activity

Detection kits for lactic dehydrogenase (LDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) enzymes were purchased from Nanjing Construction Institute of Bioengineering (Nanning, Jiangsu, China). The activities of LDH, SDH, and MDH were determined following the detailed instructions specified in the corresponding kit, and the results were calculated as the protein quantity; the unit was U/g.

Data Statistics and Analysis

Data normality and homogeneity was tested using T test and Levene's test in SPSS26 software. Data analysis was performed using the Origin 10.0 software. The results are expressed as mean ± standard deviation. Analysis of significant differences was performed by the one-way analysis of variance (ANOVA) method, and correlation analysis was performed by the Pearson test. Correlation coefficients were assessed using the *t*-test. $P<0.05$ suggested a significant difference.

Results

Growth Performance

After supplementation with CMH and CLA, the growth rate of tilapia was higher than that in the control group (Figure 1), and the growth rate in the high-dose group was higher compared to the low-dose group. After adding CMH and CLA, the daily intake of tilapia significantly decreased compared with that in the control group ($P<0.05$). However, no significant difference was detected between CHM and CLA groups ($P>0.05$) (Figure 2).

Muscle Fiber Type Composition

The relative mRNA expression of *MyHC IIa* in the high-dose CMH group was significantly higher than other groups ($P<0.05$). There was no obvious discrepancy in relative mRNA expression of *MyHC IIb* between low-dose and high-dose CMH groups ($P>0.05$), but their expressions were significant higher than those of the control group and CLA groups ($P<0.05$). Compared with the control group, the relative mRNA expression of *MyHC IIb* in high-dose CLA group and *MyHC IIx* in low-dose CLA group exhibited significant reduction ($P<0.05$). In addition, the relative mRNA expression of *MyHC I* in high-dose CLA group was significantly higher than the control group ($P<0.05$), but there was no significant change among other groups ($P>0.05$) (Figure 3).

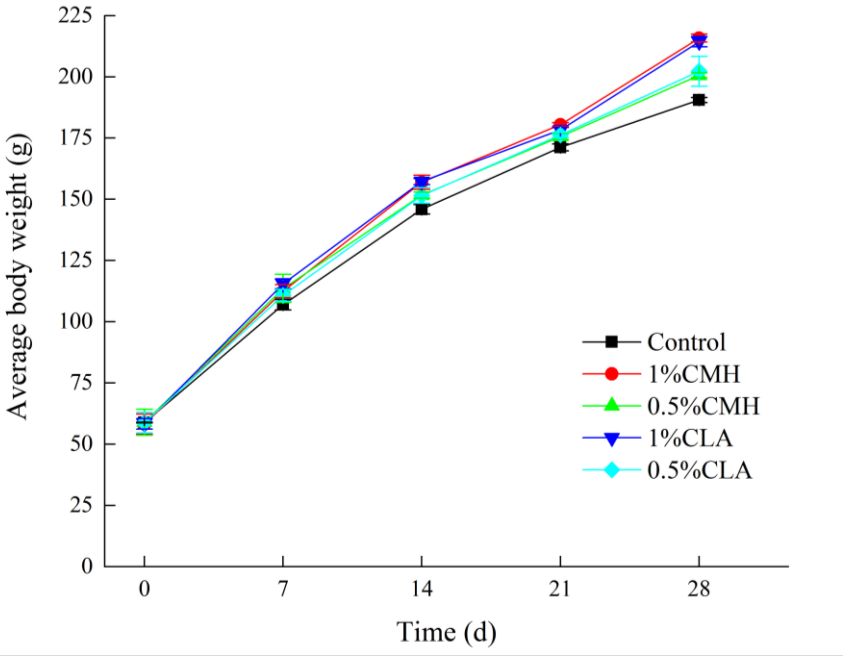

Figure 1. Effect of CMH and CLA on body mass of tilapia

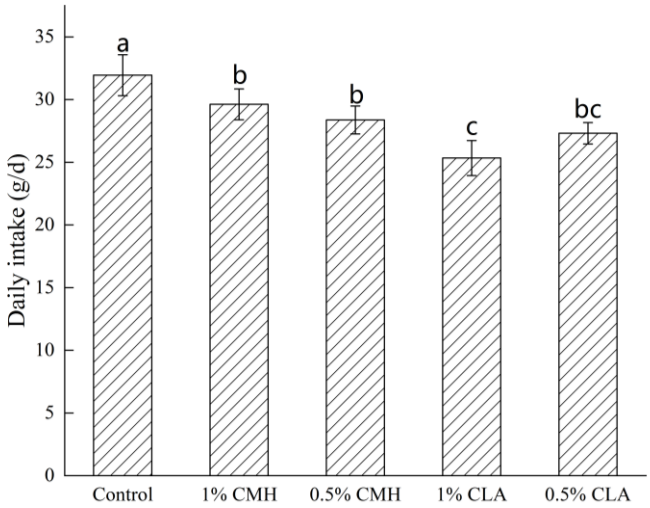

Figure 2. Effects of CMH and CLA on daily feed intake in tilapia. Different lower case letters indicated different significance.

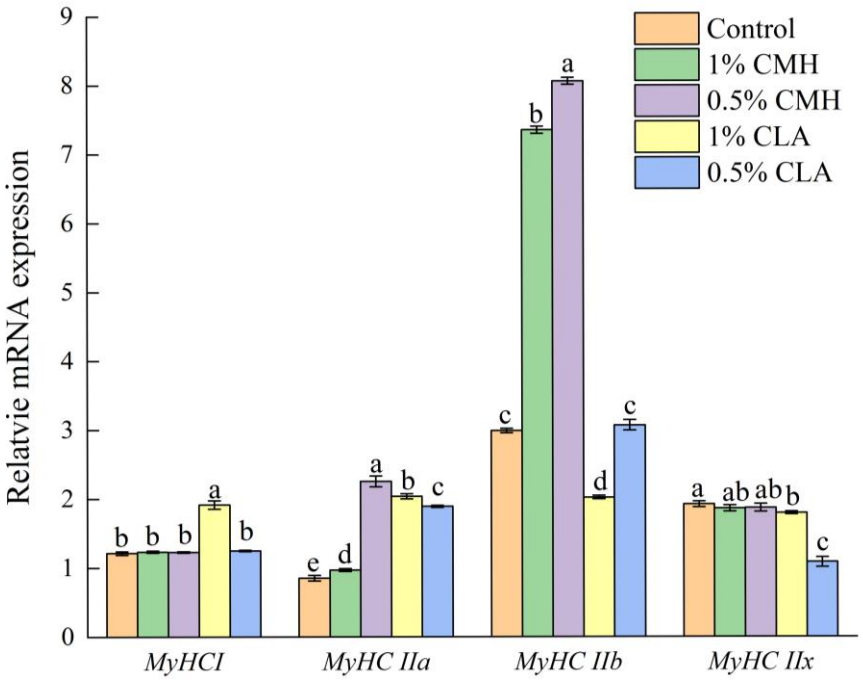

Figure 3. Effects of CMH and CLA on *MyHC* mRNA expression in tilapia gastrocnemius. Different lower case letters indicated different significance

Activities of LDH, SDH, and MDH

This experiment found that diets supplemented with CMH and CLA could significantly reduce the LDH activities in the tilapia muscle except that the low-dose CHM showed significant increase compared to the control group (P<0.05) (Figure 4a). Compared to the control group, the SDH activities in the low-dose CHM increased by 13.19%, and those in low-dose and high-dose CLA groups significantly raised by 39.10% and 26.09% (P<0.05), respectively (Figure 4b). In addition, the MDH activities in all groups were significantly higher than that of the control group (P<0.05), but the low-dose CMH group exhibited no significant alteration (P>0.05) (Figure 4c). The AMPK activities in low-dose of both CHM and CLA groups showed obvious increase compared to the control group *(*P<0.05). However, there was no obvious difference among control group, high-dose CHM group and high-dose CLA group (Figure 5).

Effect on the relative mRNA expressions of PGC-1α, MEF2C, and GLUT4

Compared with the control group, the relative mRNA expression of *PGC-1 α* in the muscle of tilapia in the low-dose CMH group was highest and increased by 168.54% (P<0.05). For the relative mRNA expression of *MEF2C*, groups with a low and high dose of CHM

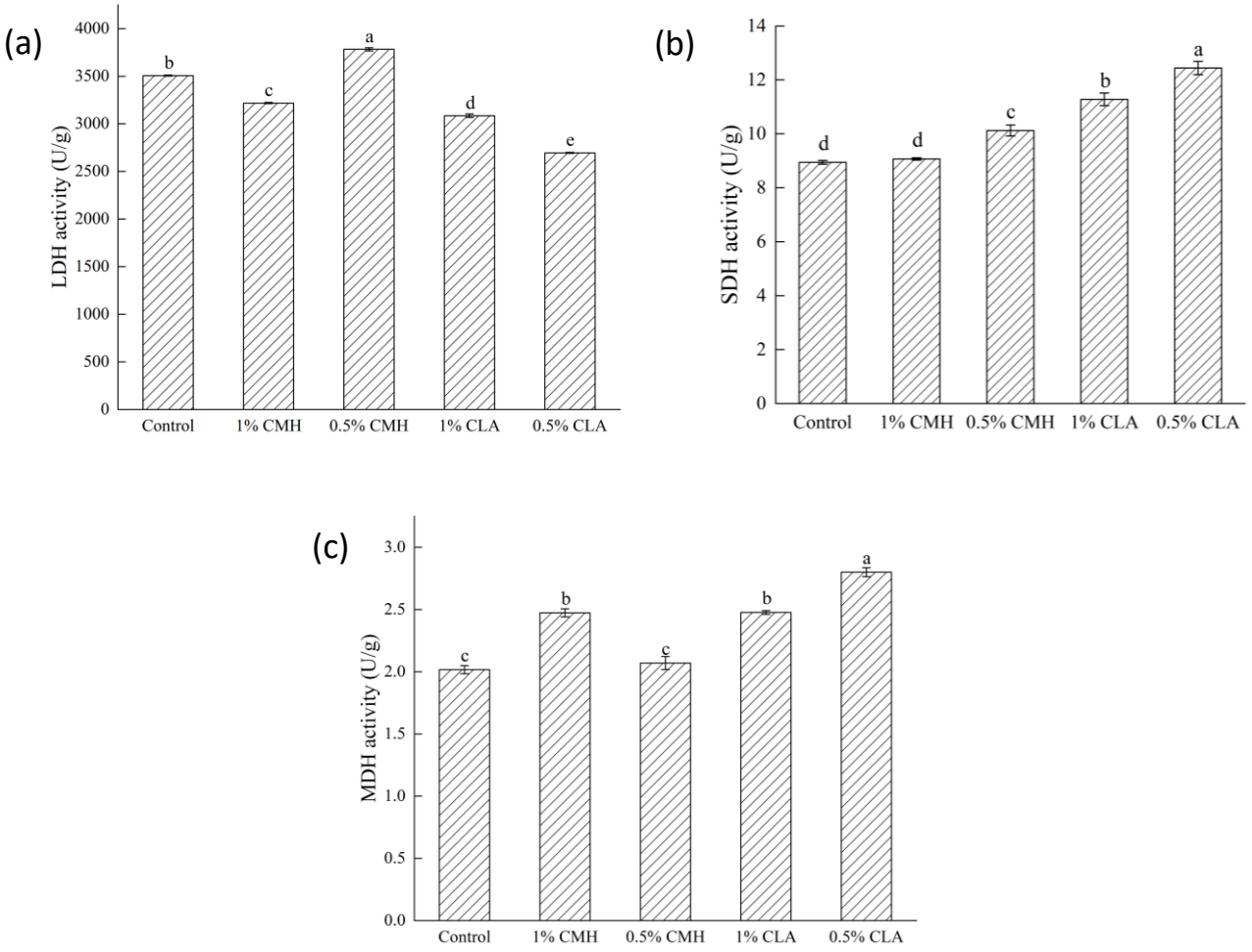

Figure 4. Effects of CMH and CLA on LDH activity (a), SDH activity (b) and MDH activity (c) in tilapia gastrocnemius. Different lower case letters indicated different significance.

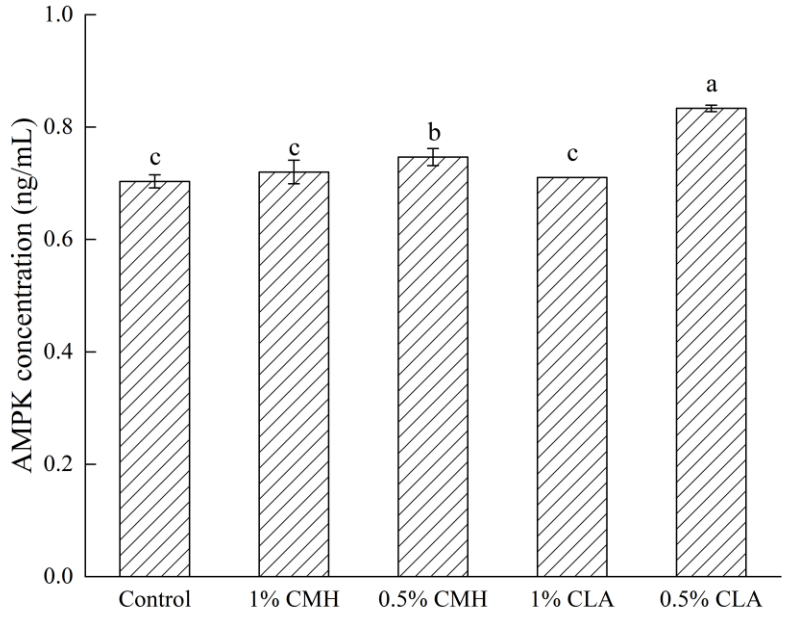

Figure 5. Effects of CMH and CLA on AMPK activity in tilapia gastrocnemius. Different lower case letters indicated different significance.

significantly increased by 42.97% and 35.14%, which was higher than those of CLA groups (P<0.05). Furthermore, the relative mRNA expressions of *GLUT4* in low and high dose of CMH groups obviously increased compared to the control group, which also significantly higher than those of CLA groups (P<0.05) (Figure 6).

Correlation Analysis between *Myhc* Expression and AMPK Activity, Muscle Fiber Transformation-related Gene Expression, and Enzyme Vitality

The activity of AMPK was positively correlated with the relative mRNA expression of *MyHC I*, *MyHC IIa*, and *MyHC IIx* but negatively correlated with that of *MyHC IIb*. LDH activity was positively correlated with the relative mRNA expression of *MyHC I*, *MyHC IIb*, and *MyHC IIx*, and significantly negatively correlated with the relative mRNA expression of *MyHC IIa* (P<0.05). The activities of SDH and MDH showed positive relation with the relative mRNA expressions of *MyHC I* and *MyHC IIa* but exhibited negative correlation with that of *MyHC IIb*. Finally, *PGC-1α*, *MEF2C*, and *GLUT4* all exerted positive regulatory effects on *MyHC I* and *MyHC IIa* muscle fibers, and negative regulatory effects on *MyHC IIb* and *MyHC IIx* muscle fibers (Table 2).

Discussion

Exogenous nutritional supplements significantly promote the muscle growth of fish, especially in promoting weight gain. The effect of exogenous nutritional supplements on mass is related to intake. The daily feed of individuals is limited when the total energy supply exceeds the biological requirement (Huang *et al.*, 2014). In this study, the addition of CMH and CLA to the basic diet had a significant positive effect on the growth rate of tilapia. However, it significantly reduced the daily intake of tilapia, indicating that CMH and CLA positively affected feed conversion. CLA can influence the growth of animals by regulating adipocyte differentiation, glucolipid metabolism, intramuscular fat deposition, muscle development, and muscle fiber types (Turkyilmaz and Esenbuga, 2022). CLA content in aquatic animals is small, and CLA is often added to improve growth in aquaculture. Therefore, studying the effect of CLA on the growth performance (such as weight) of aquaculture varieties is necessitated. Dietary CLA supplementation has no significant effect on the average daily gain mass of carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*), and the daily intake linearly decreases with the increase in CLA

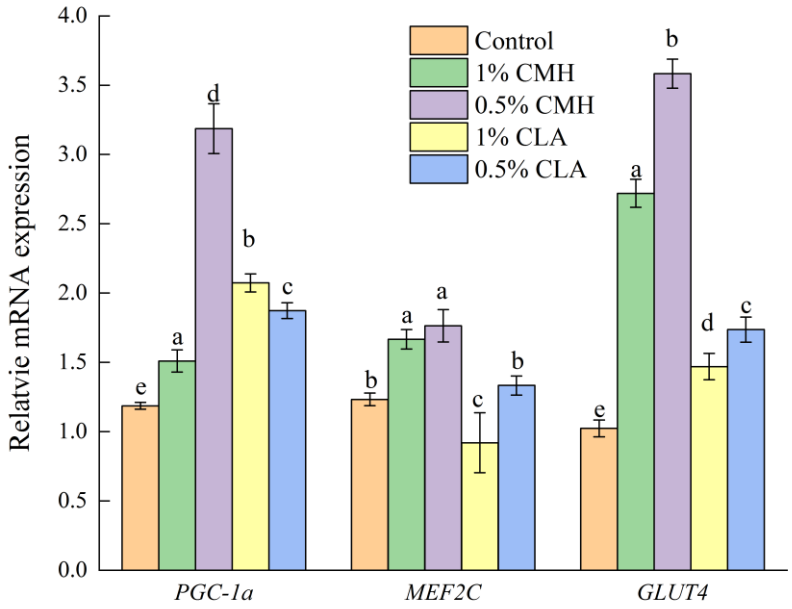

Figure 6. Effec.ts of CMH and CLA on muscle fiber type transition-related gene expression in tilapia gastrocnemius. Different lower case letters indicated different significance

Table 2. Analysis of correlation between *MyHC* expression and AMPK activity, muscle fiber transformation-related gene expression, and enzyme activities. * indicated significant correlation (P<0.05), ** expressed more significant correlation (P<0.01)

Muscle fiber types relative mRNA expression	Enzyme activities				Relative mRNA expression		
	AMPK	LDH	SDH	MDH	*PGC-1α*	*MEF2C*	*GLUT4*
MyHC I	0.265	0.068	0.333	0.863*	0.237	0.128	0.491
MyHC IIa	0.519	-0.658*	0.776	0.508	0.836*	0.219	0.433
MyHC IIb	-0.067	0.597	-0.228	-0.762**	-0.742	-0.637	-0.623
MyHC IIx	0.471	0.551	0.092	-0.459	-0.806*	-0.611	-0.678

supplementation (Bonafé *et al.*, 2017; Silva-Cavalcanti and Costa, 2009), consistent with the results of this study. It may be because the daily feeding energy increases with increasing CLA supplementation. When the dietary energy exceeds the requirement of tilapia, the feed intake is affected, thus reducing the daily intake.

Muscle fibers are divided into four types according to the specific *MyHC* types of bony fish muscle fibers, and the mRNA expressions of *MyHC I*, *MyHC IIa*, *MyHC IIb*, and *MyHC IIx* in perch muscle have been studied (Yang *et al.*, 2023). Molecular typing based on MyHC expression is more accurate and reliable compared to histochemical assessment (Willoughby and Nelson, 2002). The results of this study showed that dietary CMH increased the relative mRNA expression of *MyHC IIa* and *MyHC IIb* in tilapia muscle, and the effect of low-dose CMH on muscle fiber type composition was at a higher dose. Dietary addition of 0.5% CMH can significantly reduce the relative mRNA expression of *MyHC IIa* in the longissimus dorsi of salmon (Zybert *et al.*, 2019). Contrary to the results of this experiment, the reason for this difference may be attributed to the difference in animal species, supplement amount, muscle position, and other factors. This study found that dietary CLA supplementation can increase the expression in oxidized muscle fiber and decrease that in glycolic muscle fiber, suggesting that dietary CLA supplementation can promote the transformation of muscle fiber from the glycolic to oxidized type. Contrary to CMH addition, the effect of high-dose CLA on the composition of muscle fiber type is more obvious than low-dose CLA. The addition of 1.5% CLA to the diet can significantly improve the relative mRNA expression of *MyHC I* and *MyHC IIa.* It can significantly reduce the relative mRNA expression of *MyHC IIb* and *MyHC IIx* in catfish (Apple, 2022). Dietary CLA supplementation can significantly increase the relative mRNA expression of *MyHC I* and *MyHC IIa* and decrease that of *MyHC IIx* in the allogynogenetic crucian carp muscle. However, it has no significant effect on the relative mRNA expression of *MyHC IIb* (Huang *et al.*, 2014). The above results are consistent with the results of this study, suggesting that dietary CLA supplementation positively affects *MyHC I* and *MyHC IIa* muscle fibers.

The dietary addition of CMH can activate the AMPK signaling pathway and reduce glycolytic enzyme activity （Radhakrishnan *et al.*, 2021). Addition of unsaturated fatty acids (EPA/DHA) can improve the expression of AMPK in aquaculture fish meat and increase the proportion of oxidized muscle fibers (Geng *et al.*, 2010). AMPK plays an important role in muscle energy metabolism, which is closely related to muscle fiber type transformation. The transition from glycolic to oxidized muscle fiber types was hindered in the AMPK gene-deficient zebrafish (Geng *et al.*, 2010), indicating that AMPK had a positive feedback effect on oxidized muscle fibers. Different feeding environments lead to varying AMPK activities in the longissimus dorsal muscle of carp (*Cyprinus carpio*) (Cai *et al.*, 2023). The proportion of type *I*, *IIa*, *IIb*, and *IId/x* muscle fibers in different muscles is significantly different, and the activity of AMPK is also significantly different (Lee-young *et al.*, 2009). Therefore, after the addition of different doses of CMH and CLA to the basal diet of tilapia, changes in muscle AMPK activity of tilapia were measured. Dietary supplementation of polyunsaturated fatty acids can promote the expression of the AMPK gene (Yan *et al.*, 2011). After feeding grass carp (*Ctenopharyngodon idella*) with unsaturated fatty acids, the expression of the AMPK gene increased remarkably (Liu *et al.*, 2017). Adding CMH to the diet promotes the expression of AMPK mRNA (Overturf *et al.*, 2016), similar to the results of this experiment. Therefore, dietary supplementation of CMH and CLA can activate the AMPK signaling pathway, leading to changes in the tilapia tail muscle fiber types.

The peroxisome proliferator-activated receptor γ coactivator 1α (*PGC-1α*) is a major regulator of mitochondrial biogenesis. According to many studies, the expression of the *PGC-1α* gene can be induced through exercise and AMPK activation (Geng *et al.*, 2010). As a transcriptional coactivator (Scarpulla, 2002a), *PGC-1α* plays an important role in mitochondrial biogenesis (Scarpulla, 2011b). Its expression is correlated positively with the proportion of oxidized muscle fibers. Following the over-expression of *PGC-1α* in zebrafish, both the number and function of mitochondria in the zebrafish muscle are enhanced, while the proportion of type *I* muscle fibers is increased (Lin *et al.*, 2002). AMPK can directly or indirectly regulate the expression of *PGC-1α* mRNA in mouse myoblasts (Cantó *et al.*, 2009). Myocyte enhancer factor 2C (*MEF2C*) is a major coactivator of *PGC-1α*, which can improve oxidative metabolism by directly regulating the expression of *PGC-1α* mRNA (Cantó and Auwerx, 2010). The expression of the *MEF2C* gene in the muscle of grass carp (*Ctenopharyngodon idella*) fed unsaturated fatty acids increases significantly (Liu *et al.*, 2017). With glucose transporter 4 (*GLUT4*) being the upstream factor of *MEF2C*, *MEF2C* can promote glucose transport by binding *PGC-1α* to induce the expression of *GLUT4* (Wende *et al.*, 2005). In this study, except for high-dose CLA, the relative mRNA expression of *MEF2C* significantly decreased. The dietary addition of CMH and CLA could enhance the relative mRNA expression levels of *PGC-1α*, *MEF2C,* and *GLUT4*, consistent with the above results of increased AMPK activity. CMH exerted more significant effects on genes related to muscle fiber type transformation than CLA. The activation of the AMPK can significantly increase expressions of *GLUT4* and *PGC-1α*. An increase in the activity of oxidase and a decrease in that of the glycolytic enzyme (Suwa *et al.*, 2003; Chen *et al.*, 2018) are consistent with the results of this experiment.

LDH is a catalytic enzyme required for glycolysis for the production of lactic acid. It can reflect the activity of

anaerobic glycolysis in cells owing to regulation by the activity of the AMPK (Theret *et al.*, 2017). A significant negative correlation between LDH and the relative expression of *MyHC IIa* mRNA was identified because the decrease in the LDH activity elicited by increased AMPK activity improved the oxidation capacity of the energy substrate in tilapia muscle. Conversion of glycolytic muscle fibers to oxidized muscle fibers enhances the relative mRNA expression of *MyHC IIa*, a marker of rapid growth and protein deposition in fish (Vilchinskaya *et al.*, 2017). SDH and MDH are the rate-limiting enzymes of mitochondrial aerobic metabolism used to evaluate muscle oxidation activity, which is positively regulated by AMPK activity (Theret *et al.*, 2017). In this study, dietary supplementation of CMH and CLA significantly increased SDH and MDH activities in tilapia muscles. In addition, MDH showed a significant positive correlation with *MyHC I* and a highly significant negative correlation with *MyHC IIb*, showing that the muscle oxidase activity of tilapia increased. Simultaneously, dietary supplementation of CMH and CLA can significantly promote the mRNA expression of AMPK. This is followed by stimulation of increased expression of *MyHC I* and *MyHC IIa* and decreased expression of *MyHC IIb* in the tilapia muscle. CMH and CLA can increase the proportion of oxidized muscle fiber and decrease that of enzymatic muscle fiber.

Conclusion

This study found that CMH and CLA had positive effects on improving the tilapia feed conversion rate. At the same time, both CMH and CLA were able to activate the AMPK activity in muscle, up-regulating the AMPK pathway-related gene expression, enhancing the oxidative metabolic ability, and transforming the muscle fiber type. However, CMH up-regulated the relative expression of *MyHC I, IIa* and *IIb* mRNA in the tilapia muscle; CLA decreased the proportion of glycolytic muscle fiber types and LDH activity. This study contributed to studying the interaction of myofiber type transformation with glycolytic oxidase and the regulation mechanism of the AMPK signaling pathway on muscle type transformation. These research results also provided a strategy for improving the quality of future tilapia culture by supplementing CMH and CLA.

Ethical Statement

The study was approved by the insititutional review board (CWO) of Guangxi Academy of Fishery Science, Nanning, Guangxi, China. All trials were not need to provide an ethic documentation.

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Author Contribution

Huawei Ma and Xianda Bi: funding acquisition, conceptualization, methodology, formal analysis, investigation, writing-original draft.

Wei Ge and Zhide Ruan: conceptualization, methodology, formal analysis, writing-original draft, validation, investigation.

Zhichan He and Chuanyan Pan: writing-original draft, investigation.

Xu Luo and Min Lv: investigation, data curation, writing-review and editing.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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