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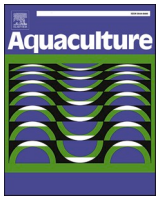

Effects of dietary citric acid on growth performance, mineral status, body and muscle composition, muscle growth and mTOR signaling in yellow catfish *Pelteobagrus fulvidraco* fed with low-manganese diets

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ABSTRACT

The positive impacts of dietary citric acid (CA) on aquaculture species have been studied. However, the wide application of CA in aquatic-feed requires a comprehensive understanding of its nutritional functions. Here, an 8-week feeding trial was performed to determine whether dietary CA supplementation could improve manganese (Mn) utilization and thus reduce Mn excretion by investigating the effect of dietary CA on growth performance, mineral statues, body and muscle composition, muscle growth and mTOR signaling of yellow catfish *Pelteobagrus fulvidraco* fed with low-manganese (L-Mn) diets. Four isonitrogenous and isolipidic diets were formulated and fed to triplicated groups of fish. A suitable Mn diet containing 5.7 mg kg^{-1} Mn was set as the control, while the diets without extra Mn addition was regarded as L-Mn group. The other two diets were supplemented with 30 g kg^{-1} CA into the control and L-Mn diet, respectively. The results showed that the weight gain, specific growth rate, feed efficiency, protein content in muscle, iron concentration in serum, and Mn content in whole body and muscle were significantly decreased after L-Mn diet and resumed by dietary CA addition ($P < 0.05$). Dietary low-Mn markedly decreased the diameters of muscle fibers, which was alleviated by CA supplementation. Dietary Mn and CA interacted to affect the density of the muscle fiber density, the mRNA expression of muscle development-, myocyte myogenic and myocyte enhancer factors-related genes (*P*-interaction <0.05). Besides, expression of genes in muscle involved in mTOR signaling including *mtor*, *s6* and *eif4e* were significantly down-regulated in fish fed L-Mn diet compared to the control. CA supplementation reversed the L-Mn diet induced decrease of their mRNA levels. Furthermore, the protein expression of p-mTOR, S6 and p-S6 were significantly decreased after L-Mn diet, and were upregulated by CA supplementation in the muscle ($P < 0.05$). Taken together, this study revealed that CA as a feed additive could improve growth performance, mineral bioavailability, muscle growth and protein synthesis in yellow catfish under the condition of Mn deficiency. These positive effects may be partially attributed to dietary CA supplementation improving Mn absorption and bioavailability. This study also has a significance for the development of environmentally friendly feeds for the aquatic species.

1. Introduction

The success of aquaculture relies on the presence of commercially available feed that is nutritionally balanced, cost-effective, and eco-friendly. The minerals are the important components of aquatic feed essential for the normal development and growth of fish (Hardy and Kaushik, 2022). Although many researchers recognized the critical role of minerals in the various life processes, such as normal growth, metabolism and muscle development, there remains a scarcity of research on mineral nutrition in fish (Antony Jesu Prabhu et al., 2016). Among the indispensable trace minerals, manganese (Mn) is one of the most vital trace minerals necessary for normal growth, vertebral development and antioxidant in fish (Tan et al., 2012). It plays a key role in modulating several biological processes including protein, lipid and carbohydrate metabolisms (Aschner and Erikson, 2017; Martins et al., 2020). Diet is the primary source of Mn supply for fish. Fish fed a Mn-

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deficient diet showed an adverse impact, such as growth retardation, skeletal deformities, reduced Mn-SOD activity, poor reproductive performance, and decreased tissue Mn content (Tan et al., 2012; Liu et al., 2018; Musharraf and Khan, 2021). Therefore, an appropriate amount of Mn is necessary to maintain normal growth and physiological metabolism of fish. The dietary Mn requirements for major aquaculture fish species have been exhaustively reviewed by Antony Jesu Prabhu et al. (2016) and major fish species such as fingerling channel catfish, *Ictalurus punctatus* (Gatlin and Wilson, 1984); gibel carp, *Carassius auratus gibelio* (Pan et al., 2008); yellow catfish, *Pelteobagrus fulvidraco* (Tan et al., 2012; Xu et al., 2023a, 2023b); grass carp, *Ctenopharyngodon idella* (Liang et al., 2015); cobia, *Rachycentron canadum* (Nie et al., 2016); large yellow croaker, *Larimichthys crocea* (Zhang et al., 2016a), hybrid grouper, *Epinephelus lanceolatus* × *E. fuscoguttatus* (Liu et al., 2018) and fingerling *Labeo rohita* (Hamilton) (Musharraf and Khan, 2021); largemouth bass, *Micropterus salmoides* (Song et al., 2023) have been estimated to range between 2.4 and 24.9 mg kg^{-1} of diet. However, Mn supplementation in aquatic feeds is usually done with inorganic salts and the most used salt being Mn sulphate ($MnSO_4$), which have a lower bioavailability. Undigested Mn from fish diet is discharged into the water through feces, leading to environmental Mn pollution, further threatening the safety of aquatic animals and human (Wasserman et al., 2006; Fordahl et al., 2012). Diets supplemented with organic acids have been widely proven to have beneficial efficacies for fish, such as improved growth (Goosen et al., 2011; Zhu et al., 2015; Zhang et al., 2016b; Dai et al., 2018), enhanced nutrition availability, raised activities of digestive enzymes and improved the intestinal health (Zhang et al., 2016b; Dai et al., 2018; Zhao et al., 2019). Therefore, dietary organic acids may be beneficial in improving some negative effects induced by Mn deficient diet, and may be an effective way to decrease environmental Mn pollution in aquaculture.

Citric acid (CA), a type of nutritional organic acid additives, has been documented to improve growth, enhance the bioavailability of minerals in diet, improve intestinal health in various aquaculture species (Zhu et al., 2015; Zhang et al., 2016b; Zhao et al., 2019; Xu et al., 2020). It is generally believed that the decrease in gastric pH caused by CA will increase the activity of digestive enzymes, thereby enhancing nutrient utilization (Zhang et al., 2016b; Dai et al., 2018). A large number of studies have shown that dietary CA can increase growth performance, improve feed utilization and intestinal digestion and absorption function in piglets (Suiryanrayna and Ramana, 2015; Nguyen et al., 2020; Deng et al., 2021). Several studies focus on the impacts of citric acid on growth, utilization of mineral, immune responses and metabolism of aquatic animals (Zhu et al., 2015; Zhang et al., 2016b; Ng and Koh, 2017; Dai et al., 2018; Xu et al., 2020). Moreover, studies have shown that dietary citric acid has protective effects on intestinal function in turbot (Chen et al., 2018; Zhao et al., 2019). However, so far, the interaction between CA and mineral elements was rarely studied in fish.

The growth performance of the whole body is closely associated with muscle growth, and fish skeletal muscle acts as both the major reservoir of proteins and amino acids and a highly adaptable tissue with exuberant metabolism, exhibiting strong plasticity to accommodate metabolic variations (Wolfe, 2006). Importantly, muscle growth mainly includes the proliferation and hypertrophy of myofibers (Luo et al., 2019). The muscle growth is primarily the hypertrophy of myofibers in most vertebrates, because of myofibers don't grow quickly after birth (Zanou and Gailly, 2013). However, during most of the fish life cycle, both proliferation and hypertrophy of myofibers exist (Wang et al., 2021a). The proliferation and hypertrophy of myofibers are modulated by several transcription factors, including myostatin (Mstn) and myogenic regulatory factors (Mrfs) (Song et al., 2022). The Mrfs include myogenic factor 5 (Myf5), and myogenic regulatory factor 4 (Mrf4), myogenin (Myog) and myoblast determination protein (Myod). These transcription factors participate in the complicated process of generation and differentiation of muscle (Zammit, 2017). It was documented those diets supplemented with optimal Se improved the hypertrophic growth of the muscle in zebrafish and rainbow trout (Wang et al., 2021a; Wang et al., 2021b). However, the adaptive changes in muscle development and their regulatory mechanisms have not been well understood so far, especially when mineral elements are limited. Therefore, it is necessary to explore whether the addition of citric acid to a low-Mn diet has a positive effect on muscle growth for fish.

The mammalian target of rapamycin (mTOR) is widely recognized as the prominent controller of growth and protein translation (Laplante and Sabatini, 2012; Liu and Sabatini, 2020). The mTOR modulates translation of protein by modulating its target genes, including the p70 ribosomal S6 protein kinase 1 (S6K1/p70S6k) and the 4E-binding protein-1 (4E-BP1) (Laplante and Sabatini, 2012). Studies have pointed out that mTOR pathway can be activated by different stimuli, such as biochemical and mechanical intervention, thereby promoting protein synthesis in muscle (Chen et al., 2019; You et al., 2019). Nevertheless, the mechanisms of protein synthesis involved in mTOR pathway in muscle following nutritional intervention remain to be fully elucidated.

Yellow catfish (*Pelteobagrus fulvidraco*), an economic omnivorous freshwater fish, is considered an excellent candidate for freshwater farming and highly favored by Asian consumers for its delicious meat (Luo et al., 2011). As far as we know, there has not yet studies assessing the impact of citric acid supplementation in L-Mn diet on growth performance, mineral status, muscle growth and protein synthesis for yellow catfish. Thus, this study aimed to assess the suitability of CA as a beneficial feed additive to increase growth performance, Mn utilization and muscle growth in yellow catfish fed low Mn diet. The study would contribute to develop the environmentally friendly feeds, too.

2. Materials and methods

2.1. Ethical standards

All the experimental protocols involving the management and use of fish were conducted on the basis of the ethical guidelines of Huazhong Agricultural University (HZAU) and were allowed by the Ethical Committee of HZAU.

2.2. Diet preparation

A 2 × 2 factorial design with three duplications was utilized in present study. A total of four isonitrogenous and isolipidic semi-purified diets were prepared to contain two Mn levels (0 and 8 mg kg^{-1}) and two citric acid supplementation levels (0 and 30 g kg^{-1}). The dose of citric acid was used after previous reports (Zhang et al., 2016b; Dai et al., 2018). $MnSO_4 \cdot H_2O$ was used as Mn source. Fish meal, casein, gelatin, were utilized as protein sources. Soybean oil and fish oil were utilized as lipid sources. The diets were formulated to meet the optimum dietary Mn requirements of juvenile yellow catfish (Tan et al., 2012). The submerged diets were prepared with a Mn-free mineral premix in this study. Citric acid (99.5%, Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China) was supplemented in the experimental diets, named the control, CA group, Low-Mn group, and Low-Mn + CA group, respectively. In brief, all dry feed ingredients were ground and screened through a 80-meshsieve. First, vitamin premix was mixed with cellulose, and the mineral premix was mixed with fish meal. Subsequently, these two mixtures were mixed together. After that, additional dry ingredients were added to these pre-mixed mixtures and mixed thoroughly until achieving a homogeneous mixture. Then, the fish oil and soybean oil were mixed together, and added to the dry ingredients for further mixture. Afterwards, all of these ingredients were mixed thoroughly with cold-distilled water using a Groove-type mixer (CH-50, Changzhou Golden Ball Drying Equipment Co., Ltd., China). The mixture was then extruded through a meat grinder with a 2-mm diameter die (TY-432, Shanghai Taiyi Machinery, China). After natural drying, all the diets were kept at −20 °C until use. Formulation and proximate composition

of all diets are listed in Table 1. Actual Mn contents in diets were measured using ICP-OES (Optima™ 8000, Waltham, MA, USA), and the contents are 5.7, 5.7, 2.6 and 2.7 mg kg^{-1} for the control, CA group, low-Mn group, and low-Mn + CA group, respectively.

2.3. Animals rearing and sampling

The protocols of fish management were consistent with our recent publications (Zhao et al., 2020a; Xu et al., 2023a, 2023b). For the feeding trial, fish were acquired from a local fish farm in Wuhan (Hubei, China). First, the fish were fed a mixed experimental diet for two weeks to adapt to the experimental environments. After the acclimatization, 360 healthy yellow catfish (2.00 ± 0.01 g, mean ± SEM) were distributed into 12 circular fiberglass tanks (250 L), each tank with 30 fish. The experiment was performed in triplicate for each dietary treatment, and fish were given the diets to apparent satiety twice a day (8:00 am and 4:00 pm) for 8 wks. During the feeding experiment, water temperature ranged from 27.9 to 29.5 °C; NH_4-N, dissolved oxygen and NO_2-N were 0.08 ± 0.02 mg L^{-1} and 6.44 ± 0.15 mg L^{-1}, 0.007 ± 0.001 mg L^{-1}, respectively. Mn concentration in rearing water was also determined twice a week, and was below the minimum detection limit of ICP-OES (< 1 μg/L).

Following the 8-week feeding experiment, all fish were fasted for 24 h prior to sampling. Fish from each tank were anaesthetized with MS-222 (100 mg L^{-1} water), then weighed and counted to calculate survival rate, specific growth rate (SGR) and weight gain (WG). Next, 15 fish each tank were randomly selected, and measured for body length and weighed individually, and dissected quickly on ice to collect the liver tissues, viscera and mesenteric fat for evaluation of hepatosomatic index (HSI), viscerosomatic index (VSI) and visceral adipose index (VAI). 3 fish each tank were randomly chosen to assay the composition and mineral content of whole-body. 6 fish were randomly chosen each tank, and blood samples from caudal vein and the dorsal muscle from the same position of each fish were rapidly collected. Blood was placed at 4 °C for 6 h, and then centrifuged with 3500 *g* min^{-1} for 10 min at 4 °C (Centrifuge CT15RE; Hitachi, Japan). Then serum was obtained, immediately kept at −80 °C to determine the mineral content. The muscle samples were frozen quickly in liquid nitrogen and kept at −80 °C refrigerator for genes and proteins analysis. 3 fish each tank was collected and the muscle was sampled for analysis of composition and mineral content. Another 3 fish from each tank were randomly picked out and the muscle were obtained, and then fixed quickly in 4% paraformaldehyde for H&E staining. The calculation formulas for parameters are as follows:

$$\text{HSI}\ (\%) = 100\% \times \text{liver weight}\ (g)/\text{body weight}\ (g).$$

$$\text{VSI}\ (\%) = 100\% \times \text{viscera weight}\ (g)/\text{body weight}\ (g).$$

$$\text{VAI}\ (\%) = 100\% \times \text{visceral adipose tissue weight}\ (g)/\text{body weight}\ (g).$$

Table 1
Feed formulation and proximate analysis of experimental diets.

Ingredients (g kg^{-1})	Control	CA	Low-Mn	Low-Mn + CA
Casein	360	360	360	360
Gelatin	25	25	25	25
White fish meal	100	100	100	100
Fish oil	25	25	25	25
Soybean oil	25	25	25	25
Wheat starch	250	250	250	250
Ascorbyl-2-polyphosphate	10	10	10	10
$Ca(H_2PO_4)_2{\cdot}H_2O$	10	10	10	10
NaCl	10	10	10	10
Betaine	10	10	10	10
Vitamin premix [1]	5	5	5	5
Mineral premix (Mn-Free) [2]	5	5	5	5
Cellulose	164.992	134.992	165	135
Citric acid (CA)	0	30	0	30
$MnSO_4{\cdot}H_2O$	0.008	0.008	0	0
Proximate analysis (% dry matter basis)				
Moisture	5.59	5.50	5.39	5.35
Ash	4.09	4.21	4.12	4.19
Crude protein	43.41	43.59	43.02	43.28
Lipid	7.53	7.69	7.88	7.65
Mn content (mg kg^{-1} diet)	5.7	5.7	2.6	2.7

[1] Vitamin premix (mg or IU per kg diet): retinylacetate, 10,000 IU; cholecalciferol, 1000 IU; all-rac-a- tocopheryl acetate, 30 IU; menadione nicotinamide bisulfite, 7; thiamine hydrochloride, 6; riboflavin, 3; pyridoxine hydrochloride, 12; D-calcium pantothenate, 30; niacin, 50; biotin, 1; folic acid, 6; cyanocobalamine, 0.03.

[2] Mineral mixture (mg kg^{-1} diet): $Ca(H_2PO_3)_2{\cdot}H_2O$, 1000; $FeSO_4{\cdot}_7H_2O$, 40; $ZnSO_4{\cdot}_7H_2O$, 40; $CuSO_4{\cdot}5H_2O$, 2; $CaIO_3{\cdot}6H_2O$, 3; Na_2SeO_3, 0.05; $CoSO_4$, 0.05.

2.4. Chemical composition determination of experimental diets, fish and serum

Moisture, ash, crude protein, and crude lipid contents in the diets and fish (including whole fish and dorsal muscle) were determined in triplicates according to Tan et al. (2012). Mn content in the diet, and Mn, Cu, Zn, Mg, Fe and Ca in serum, whole-body and muscle were detected using the inductively coupled plasma-optical emission spectrophotometer (ICP-OES) (Optima™ 8000) according to the protocols as described in our recent study (Xu et al., 2023a, 2023b).

2.5. Histological analysis

The histological analysis of the muscle was similar to previous publication (Zhao et al., 2018). Briefly, the muscle samples fixed in 4% paraformaldehyde were dehydrated using gradient ethanol, cleared with xylol, and subsequently embedded within paraffin. Then, the paraffin-embedded samples were sliced into 7 μm thickness using the Leica Rm2016 microtome (Berlin, Germany), Next, the slices were stained with hematoxylin and eosin, and sealed with neutral resin. The slices were photographed using an optical microscope with a digital camera (Olympus BX53, Tokyo, Japan). The diameter and density of myofibers were determined using Image-Pro Plus software (Media Cybernetics, USA).

2.6. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted with RNAiso Plus reagent (9109, Takara Biomedical Technology, Beijing, China) and transcribed into the first strand cDNA using a cDNA Synthesis Kit (CW2582, Cwbio IT Group, Beijing, China). qPCR assays were performed according to the methods as described in the recent publication (Xu et al., 2023a, 2023b). The specific primers sequences of genes are shown in Table S1. 10 housekeeping genes (*b2m, tuba, rpl7,* β*-actin, ubce, hprt, tbp, gapdh, elfa* and *18S rRNA*) were chose to evaluate their transcription stability. Analysis of the best two housekeeping genes using geNorm(*https://genorm.cmgg.be/*) (Pfaffl, 2001), and the $2^{-\Delta\Delta Ct}$ method was utilized to analyze the relative mRNA expression of genes.

2.7. Western blotting assay

We used the western blotting to test the expression levels of several proteins in muscle, such as mTOR, p-mTOR, S6 and p-S6. The methods for western blotting were described in our recent publications (Zhao et al., 2020b; He et al., 2023). Briefly, muscle tissues were lysed with RIPA buffer (BL504A, Biosharp, Hefei, China) on ice for 30 min. The proteins were then separated by SDS-PAGE, and then transferred to PVDF membranes. Next, the membranes were blocked with 8% (*w/v*) non-fat dry milk in TBST buffer at room temperature for 2 h. They were washed 3 times with TBST buffer for 5 min each time, and were then incubated with specific primary antibodies, such as rabbit anti-mTOR (1:1000, A2445; Abclonal, Wuhan, China), anti-p-$mTOR^{S2448}$ (1:2000,

AP0094; Abclonal), anti-S6 (1:1000, A6058; Abclonal) and anti- p-S6$^{S235/236}$ (1:1000, AP0538; Abclonal) for overnight at 4 °C. Subsequently, they were incubated with HRP-conjugated anti-rabbit IgG antibody (1:10000, BL003A; Biosharp). Finally, the protein bands were recorded by a Fusion FX6 Spectra imaging system (Vilber, Paris, France), and the densities of protein were quantified by Image-Pro Plus software.

2.8. Statistical analysis

Before statistical analysis, the Kolmogorov–Smirnov test was applied to assess the normality of all the data, and Bartlett's test was carried out to analyze the homogeneity of the variances among the treatments. The two-way ANOVA was applied to analyze all the data. When a 2-way ANOVA showed a significant P interaction ($P < 0.05$), the significant differences among all treatments were tested by Duncan's multiple-range test using SPSS 19 software. The values are presented as the means ± SEM (standard error of the mean). The difference was considered significant at $P < 0.05$.

3. Results

3.1. Growth performance, feed utilization, and morphological parameters

Dietary Mn and CA interacted to affect the FBW, WG, SGR, feed conversion ratio (FCR) and VAI (P-interaction <0.05) (Table 2). Low-Mn diet decreased the FBW, WG, SGR and FCR, but increased VAI, compared with the control. However, CA addition alleviated the low-Mn-induced decrease in FBW, WG, SGR and FCR. In addition, dietary CA alleviated the low-Mn-induced increase of VAI. FCR, feed intake (FI), HSI, VSI, CF, and survival were not remarkably affected by the interaction between dietary CA and Mn addition (P-interaction ≥0.05).

3.2. Whole body and muscle proximate composition

Dietary Mn and CA interacted to influence the lipid content of whole body and the protein content of muscle (P-interaction <0.05) (Table 3). CA supplementation alleviated dietary low-Mn-induced increase of crude lipid content in whole body, and attenuated dietary low-Mn-induced decrease of crude protein content in the muscle. However, dietary Mn and CA did not affect the moisture, ash and crude protein contents in whole body, and the moisture, crude lipid and ash contents in muscle (P-interaction ≥0.05).

3.3. Serum, whole body and dorsal muscle mineral composition

As shown in Table 4, dietary Mn and CA interacted to influence the Cu, Zn and Fe contents in the serum of yellow catfish (P-interaction <0.05). CA diet relieved the low-Mn-induced increase of serum Cu content. Dietary low-Mn decreased the Zn and Fe contents in the serum, compared with the control. Dietary low-Mn-induced change of Fe content was reversed by dietary CA. However, Mn, Mg and Ca contents in the serum were not notably influenced by the interaction of dietary Mn and CA (P-interaction ≥0.05).

Dietary Mn and CA interacted to affect the Mn and Fe content in the muscle and whole body of yellow catfish (P-interaction <0.05), but not Cu, Zn, Mg and Ca contents in muscle and whole body (P-interaction ≥0.05) (Table 5). Mn contents in the whole body and muscle were decreased by dietary low-Mn, but elevated by dietary CA addition. As well as CA diet alleviated the low-Mn-induced increase of Fe content in the muscle and whole body.

3.4. The mRNA expression of muscle Mn metabolism

Next, to explore the impacts of dietary CA on Mn metabolism, we determined the mRNA expression of Mn transporter-related genes. Dietary Mn and CA interacted to influence the mRNA level of *zip8* and *dmt1* in the muscle of yellow catfish (P-interaction <0.05), but not *zip14* and *znt10* (Fig. 1). Low Mn diet upregulated the mRNA expression of *zip8* and *dmt1*, and CA addition alleviated the L-Mn induced upregulation of their expression.

3.5. The transversal morphology of muscle tissue

Dietary low-Mn obviously decreased the diameters of muscle fibers, but alleviated by CA addition (Fig. 2A). Compared with the control, low-Mn diet increased the ratio of muscle fibers under 20 μm, but reduced the ratio of myofibers greater than or equal to 50 μm. However, CA addition significantly increased the ratio of myofibers greater than or equal to 50 μm, compared to L-Mn group (Fig. 2B). Dietary Mn and CA interacted to affect the density of the muscle fiber density (P-interaction

Table 2
Combined effects of dietary Mn and CA on growth performance, feed utilization, morphological parameters and survival of juvenile yellow catfish after 8 week[1].

Diets	Control	CA	L-Mn	L-Mn + CA	Two-way ANOVA P-value		
					Mn	CA	Mn × CA
IBW(g/fish)	1.987 ± 0.009	2.000 ± 0.025	1.997 ± 0.018	2.013 ± 0.022	NS	NS	NS
FBW (g/fish)	14.190 ± 0.474^{c}	14.427 ± 0.381^{c}	11.233 ± 0.228^{a}	13.067 ± 0.102^{b}	0.000	0.014	0.041
WG[2]	614.445 ± 27.126^{c}	621.062 ± 10.379^{c}	462.943 ± 9.347^{a}	549.623 ± 12.043^{b}	0.000	0.022	0.041
SGR[3]	3.509 ± 0.067^{c}	3.527 ± 0.026^{c}	3.085 ± 0.030^{a}	3.341 ± 0.033^{b}	0.000	0.012	0.023
FI (g/fish)	14.641 ± 0.162	15.438 ± 0.091	12.174 ± 0.242	14.537 ± 0.208	0.000	0.000	NS
FCR[4]	1.151 ± 0.032^{a}	1.216 ± 0.030^{ab}	1.435 ± 0.036^{c}	1.314 ± 0.024^{b}	0.000	NS	0.017
HSI[5]	1.737 ± 0.132	1.497 ± 0.034	1.473 ± 0.048	1.433 ± 0.037	NS	NS	NS
VSI[6]	7.123 ± 0.410	7.277 ± 0.066	6.407 ± 0.078	6.763 ± 0.198	0.030	NS	NS
VAI[7]	0.760 ± 0.029^{a}	0.750 ± 0.060^{a}	0.960 ± 0.050^{b}	0.690 ± 0.045^{a}	NS	0.018	0.025
CF[8]	1.713 ± 0.029	1.613 ± 0.013	1.627 ± 0.023	1.623 ± 0.014	NS	0.040	NS
Survival[9]	75.556 ± 1.111	74.444 ± 2.940	78.889 ± 1.111	73.333 ± 1.924	NS	NS	NS

[1] Values are means ± SEM; $n = 3$ tanks. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). CA, citric acid; CF, condition factor; FBW, final mean body weight; FCR, feed conversion ratio; FI, feed intake; HSI, hepatosomatic index; IBW, initial mean body weight; L-Mn, low manganese; NS, no significance; SGR, specific growth rate; VAI, visceral adipose index; VSI, viscerosomatic index; WG, weight gain.
[2] WG (%) = 100% × (FBW − IBW)/IBW.
[3] SGR (% d^{-1}) = 100% × [ln (FBW) − ln (IBW)]/d.
[4] FCR = dry feed fed (g) / wet weight gain (g).
[5] HSI (%) = 100% × liver weight (g) / body weight (g).
[6] VSI (%) = 100% × viscera weight (g) / body weight (g).
[7] VAI (%) = 100% × visceral adipose tissue weight (g) / body weight (g).
[8] CF (%) = 100% × (live weight, g) / (body length, cm)^{3}.
[9] Survival (%) = 100% × final fish number / initial fish number.

Table 3
Combined effects of dietary Mn and CA on whole body and muscle composition of yellow catfish (percentage of live weight basis).

Diet	Control	CA	L-Mn	L-Mn + CA	Two-way ANOVA *P*-value		
					Mn	CA	Mn × CA
Whole body							
Moisture	77.855 ± 0.865	77.212 ± 0.472	75.092 ± 0.452	76.808 ± 0.142	0.020	NS	NS
Crude protein	13.006 ± 0.673	12.772 ± 1.207	14.753 ± 0.265	13.703 ± 0.234	NS	NS	NS
Crude lipid	7.540 ± 0.719^{a}	7.922 ± 0.119^{a}	9.841 ± 0.360^{b}	7.918 ± 0.225^{a}	0.026	NS	0.026
Ash	3.427 ± 0.138	3.773 ± 0.208	3.804 ± 0.222	3.688 ± 0.254	NS	NS	NS
Muscle							
Moisture	80.975 ± 0.341	80.662 ± 0.193	82.517 ± 0.622	80.867 ± 0.254	NS	0.036	NS
Crude protein	16.311 ± 0.318^{b}	16.958 ± 0.022^{b}	15.088 ± 0.269^{a}	16.735 ± 0.037^{b}	0.009	0.001	0.044
Crude lipid	3.190 ± 0.592	2.912 ± 0.191	2.367 ± 0.238	2.864 ± 0.489	NS	NS	NS
Ash	1.241 ± 0.036	1.271 ± 0.032	1.080 ± 0.044	1.216 ± 0.018	0.013	0.040	NS

Values are means ± SEM; n = 3 tanks. "NS" indicates no significance. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test).

Table 4
Combined effects of dietary Mn and CA on mineral composition of serum in yellow catfish.

Diets	Control	CA	L-Mn	L-Mn + CA	Two-way ANOVA *P*-value		
					Mn	CA	Mn × CA
Mn (mg L^{-1})	0.184 ± 0.020	0.183 ± 0.047	0.221 ± 0.024	0.193 ± 0.031	NS	NS	NS
Cu (mg L^{-1})	0.998 ± 0.094^{a}	0.596 ± 0.073^{a}	2.507 ± 0.236^{b}	0.654 ± 0.114^{a}	0.001	0.000	0.001
Zn (mg L^{-1})	13.826 ± 0.863^{b}	9.622 ± 1.508^{a}	8.101 ± 0.296^{a}	8.247 ± 0.269^{a}	0.004	NS	0.041
Fe (mg L^{-1})	4.122 ± 0.589^{b}	4.226 ± 0.201^{b}	1.804 ± 0.261^{a}	5.144 ± 1.150^{b}	NS	0.032	0.041
Mg (mg L^{-1})	36.720 ± 0.302	36.711 ± 1.187	33.220 ± 1.102	34.063 ± 1.438	0.023	NS	NS
Ca (mg L^{-1})	140.652 ± 6.304	136.191 ± 4.816	140.514 ± 6.694	129.676 ± 13.069	NS	NS	NS

Values are means ± SEM; n = 3 tanks. "NS" indicates no significance. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test).

Table 5
Combined effects of dietary Mn and CA on mineral composition of whole body and muscle in yellow catfish (on a live weight basis).

Diets	Control	CA	L-Mn	L-Mn + CA	Two-way ANOVA *P*-value		
					Mn	CA	Mn × CA
Whole body							
Mn	1.988 ± 0.025^{c}	1.95 ± 0.10^{c}	1.141 ± 0.070^{a}	1.570 ± 0.044^{b}	0.000	0.020	0.008
Cu	0.319 ± 0.056	0.248 ± 0.075	0.359 ± 0.049	0.322 ± 0.091	NS	NS	NS
Zn	13.420 ± 0.446	11.864 ± 0.635	13.422 ± 0.937	12.537 ± 1.072	NS	NS	NS
Fe	20.661 ± 1.362^{a}	22.462 ± 0.670^{ab}	23.822 ± 0.460^{b}	21.642 ± 0.589^{ab}	NS	NS	0.047
Mg	0.220 ± 0.003	0.250 ± 0.006	0.247 ± 0.019	0.235 ± 0.012	NS	NS	NS
Ca	10.442 ± 0.350	12.469 ± 0.564	11.749 ± 1.399	11.868 ± 0.929	NS	NS	NS
Muscle							
Mn	0.447 ± 0.024^{c}	0.464 ± 0.005^{c}	0.254 ± 0.024^{a}	0.360 ± 0.012^{b}	0.000	0.010	0.041
Cu	0.626 ± 0.105	0.464 ± 0.064	0.478 ± 0.078	0.532 ± 0.094	NS	NS	NS
Zn	4.743 ± 0.060	5.089 ± 0.371	4.616 ± 0.555	5.489 ± 0.394	NS	NS	NS
Fe	16.388 ± 0.569^{a}	14.822 ± 1.307^{a}	21.429 ± 1.334^{b}	12.941 ± 1.687^{a}	NS	0.005	0.028
Mg	0.185 ± 0.011	0.209 ± 0.002	0.179 ± 0.008	0.202 ± 0.007	NS	0.013	NS
Ca	0.376 ± 0.053	0.378 ± 0.072	0.246 ± 0.034	0.220 ± 0.052	0.029	NS	NS

Values are means ± SEM; n = 3 tanks. "NS" indicates no significance. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). Unit for Mn, Zn, Cu and Fe: mg kg^{-1}, for Ca and Mg: g kg^{-1}.

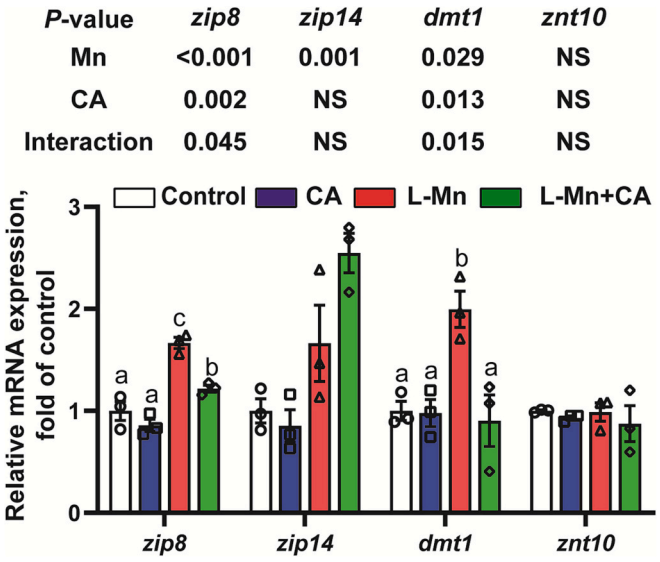

Fig. 1. Effects of dietary Mn and CA on the mRNA expression of Mn absorption and transport-related genes in muscle of yellow catfish. Relative mRNA expression values were normalized to housekeeping genes (*rpl7* and *ubce*) expressed as a ratio of the control. Values are means ± S.E.M. $n = 3$ replicate tanks, which were used as three biological replicates. 6 fish were sampled for each tank and used as technical replicates. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). *dmt1*, divalent metal transporter 1; NS, no significance; *zip8*, solute carrier family 39 (metal ion transporter), member 8; *zip14*, solute carrier family 39 (metal ion transporter), member 14; *znt10*, solute carrier family 30 member 10. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

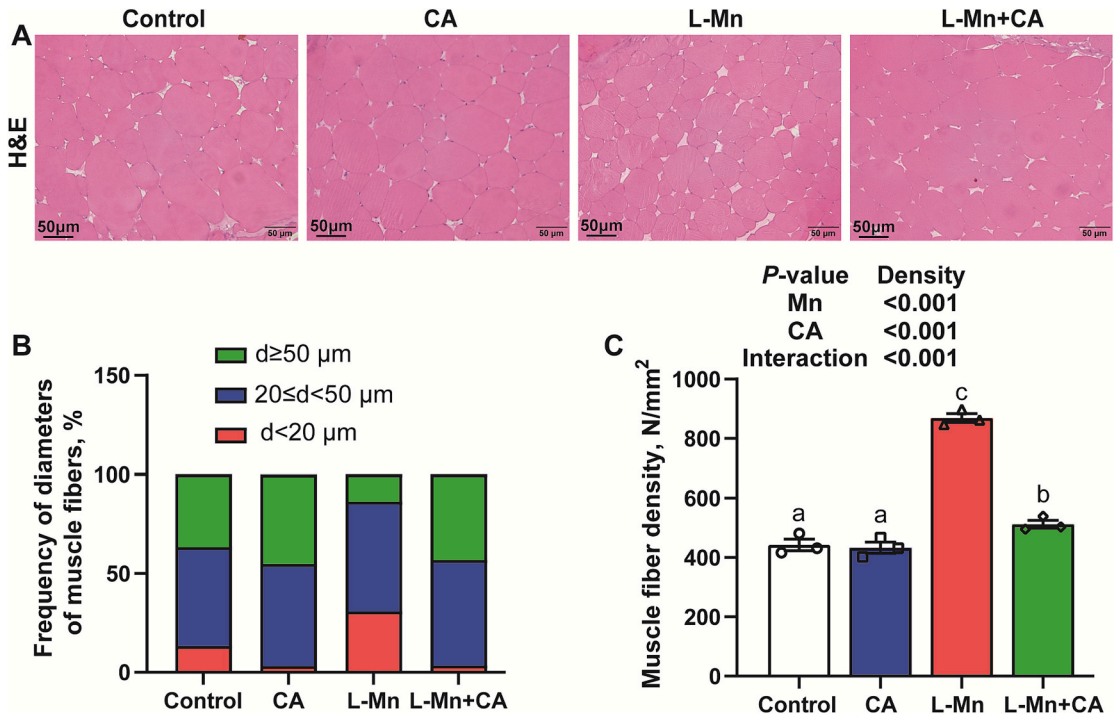

Fig. 2. Effects of dietary Mn and CA on transverse section microstructure of muscle (A), frequency of diameters of muscle fibers (B), and the density of muscle fibers (C) in yellow catfish after 8 weeks. Photomicrographs (×400) and scale bar (50 μm). Values are means ± S.E.M. (n = 3). Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

<0.05) (Fig. 2C). Compared with the L-Mn group, dietary CA and L-Mn co-treatment significantly reduced the muscle fiber density.

3.6. The mRNA level of muscle growth-related genes

The mRNA expressions of muscle growth-related genes are shown in Fig. 3. Dietary Mn and CA interacted to affect the mRNA expression of genes relevant with muscle development, myocyte myogenesis and myocyte enhancer factors in yellow catfish (*P*-interaction <0.05). Dietary L-Mn increased the mRNA levels of *myhc*, *mef2c* and *mrf4*, and the CA addition alleviated the L-Mn induced increase of their mRNA expression. The expression of *myod* was significantly lower in L-Mn

P-value	*myhc*	*pax7*	*mef2a*	*mef2c*	*mef2d*	*myod*	*myog*	*mrf4*	*myf5*	*mstn*
Mn	0.004	NS	NS	0.049	NS	NS	<0.001	NS	NS	NS
CA	0.002	NS	NS	0.011	NS	0.002	NS	<0.001	0.007	NS
Interaction	0.006	NS	NS	0.010	NS	0.047	NS	0.009	NS	NS

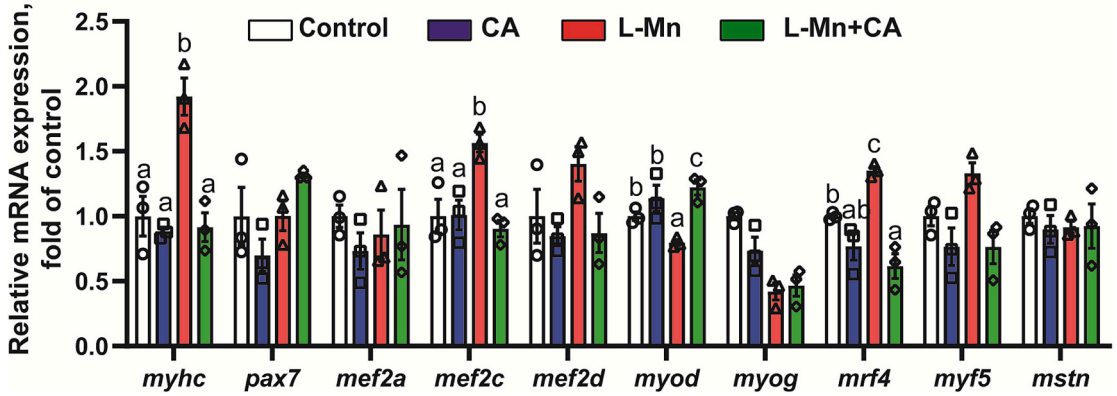

Fig. 3. Effects of dietary Mn and CA on the mRNA expression of muscle growth, development, myocyte myogenic regulatory factors and myocyte enhancer factors in muscle of yellow catfish. Relative mRNA expression values were normalized to housekeeping genes (*rpl7* and *ubce*) expressed as a ratio of the control. Values are means ± S.E.M. n = 3 replicate tanks, which were used as three biological replicates. 6 fish were sampled for each tank and used as technical replicates. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). *mef2a*, myocyte enhancer factor 2a; *mef2c*, myocyte enhancer factor 2c; *mef2d*, myocyte enhancer factor 2d; *mrf4*, myogenic regulatory factor 4; *myf5*, myogenic factor 5; *myhc*, myosin heavy chain; *myod*, myoblast determination protein; *myog*, myogenin; *mstn*, myostatin; NS, no significance; *pax7*, paired-homeobox transcription factor 7. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

group than in the control ($P < 0.05$), and CA supplementation reversed the L-Mn-induced downregulation of *myod* expression. However, there was no significant difference in *pax7*, *mef2a*, *mef2d*, *myog*, *myf5* and *mstn* mRNA expression among the groups (*P*-interaction ≥0.05). Overall, these data indicated that dietary CA abolished the negative effects of L-Mn diet on muscle growth and development.

3.7. The mRNA and protein expressions of mTOR signaling pathway

The mTOR is a renowned regulator of growth and protein synthesis (Laplante and Sabatini, 2012; Liu and Sabatini, 2020). Here, we examined the influence of dietary Mn and CA on mTOR signaling. Dietary Mn and CA interacted to influence the mRNA expression of *mtor*, *s6*, *4ebp1* and *eif4e* in the muscle of yellow catfish (*P*-interaction <0.05) (Fig. 4). Compared to the control, L-Mn diet significantly reduced the expression of genes involved in mTOR signaling including *mtor*, *s6* and *eif4e*, and CA supplementation reversed the L-Mn-induced decrease of their mRNA levels. Meanwhile, CA addition attenuated the L-Mn-induced upregulation of *4ebp1* mRNA expression.

The interaction of the L-Mn and CA also affected the protein expression of p-mTOR, p-S6, S6 and the ratio of p-mTOR/mTOR, but not the ratio of mTOR-β-actin, and p-S6/β-actin (Fig. 5). Dietary CA addition alleviated the L-Mn-induced reduction of the p-mTOR/mTOR ratio and p-mTOR, p-S6 and S6 protein expression in the muscle (Fig. 5). Taken together, the L-Mn diet caused abnormal expressions of mTOR signaling genes in muscle, and CA addition could avert these abnormal changes.

4. Discussion

This study showed that the dietary L-Mn significantly decreased the WG and SGR of yellow catfish, suggesting that dietary Mn was an indispensable element for the fish growth. Similarly, growth reduction in fish response to Mn deficiency in diets has been documented in many previous investigations (Ye et al., 2009; Tan et al., 2012; Liang et al., 2015; Zhang et al., 2016a; Liu et al., 2018; Musharraf and Khan, 2021). Furthermore, we revealed that dietary CA addition improved the WG and SGR of yellow catfish, which is similar to other findings in beluga (Khajepour and Hosseini, 2012), yellow catfish (Zhu et al., 2015) and large yellow croaker (Zhang et al., 2016b). In contrast, studies pointed out that dietary organic acid didn't impact growth of tilapia (Ng et al., 2009), rainbow trout (Gao et al., 2011) and turbot (Dai et al., 2018). The discrepancy in growth could be due to the type of fish species, the type of organic acid and dosage used (Ng and Koh, 2017). The CA-induced growth improvement might be attributed to enhanced mineral availability, recovery of intestinal digestive function and reduction of intestinal oxidation (Zhang et al., 2016b; Dai et al., 2018).

In nutritional researches, the compositions of whole body and muscle, especially the lipid and protein contents, are of significant interest owing to their direct association with meat quality (Zehra and Khan, 2016). In current study, muscle protein content remarkably decreased in the L-Mn group, compared with control group. Similar reduction in muscle protein content was also observed in prawn fed a low Mn diet (Asaikkutti et al., 2016). Moreover, the addition of CA increased the content of muscle protein, indicating that CA might promote protein digestibility. Other studies reported that the addition of citric acid significantly improved protein digestibility (Zhu et al., 2015; Afsharmanesh and Pourreza, 2005). However, the lipid content in whole-body was higher in the L-Mn group than the control, in agreement with previous investigations in grass carp (Liang et al., 2015) and yellow catfish (Tan et al., 2012). CA addition in a L-Mn diet decreased the L-Mn-induced increase of body lipid content, suggesting that CA improved the utilization of fat.

Mineral interactions play a vital role in their metabolism (Tan et al., 2012). In present study, dietary Mn and CA have no interaction effect on the concentration of Mn, Mg and Ca in serum, but CA addition rescued the reduction of serum Zn and Fe in the L-Mn diet; CA addition decreased the L-Mn-induced increase of Cu concentration in serum. Similarly, Antony Jesu Prabhu et al. (2019) pointed out that a L-Mn diet has the lowest Zn and Mn concentrations in plasma, but the concentration of Cu in plasma was not sensitive to dietary Mn levels. Meanwhile, Cu, Zn, Mg and Ca contents were not influenced by dietary Mn and CA, but CA addition significantly increased the L-Mn diet-induced

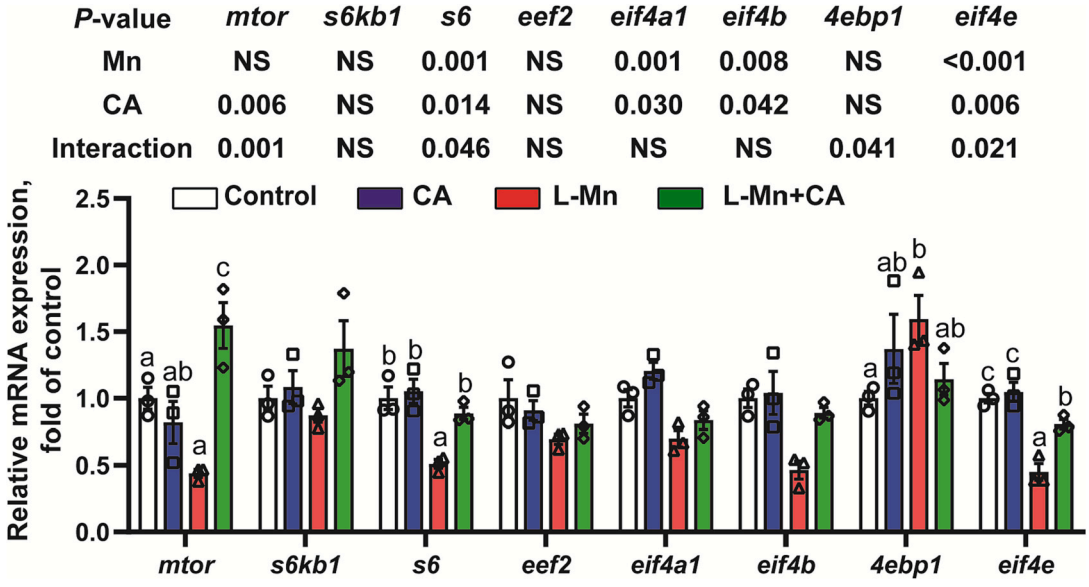

Fig. 4. Effects of dietary Mn and CA on the mRNA expression of mTOR signaling pathway related genes in muscle of yellow catfish. Relative mRNA expression values were normalized to housekeeping genes (*rpl7* and *ubce*) expressed as a ratio of the control. Values are means ± S.E.M. n = 3 replicate tanks, which were used as three biological replicates. 6 fish were sampled for each tank and used as technical replicates. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). *4ebp1*, eukaryotic translation initiation factor 4E binding protein 1; *eef2*, eukaryotic translation elongation factor 2; *eif4a1*, eukaryotic translation initiation factor 4a1; *eif4b*, eukaryotic translation initiation factor 4B; *eif4e*, eukaryotic translation initiation factor 4E; *mtor*, mammalian/mechanistic target of rapamycin; NS, no significance; *s6*, ribosomal protein S6; *s6kb1*, ribosomal protein S6 kinase beta 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

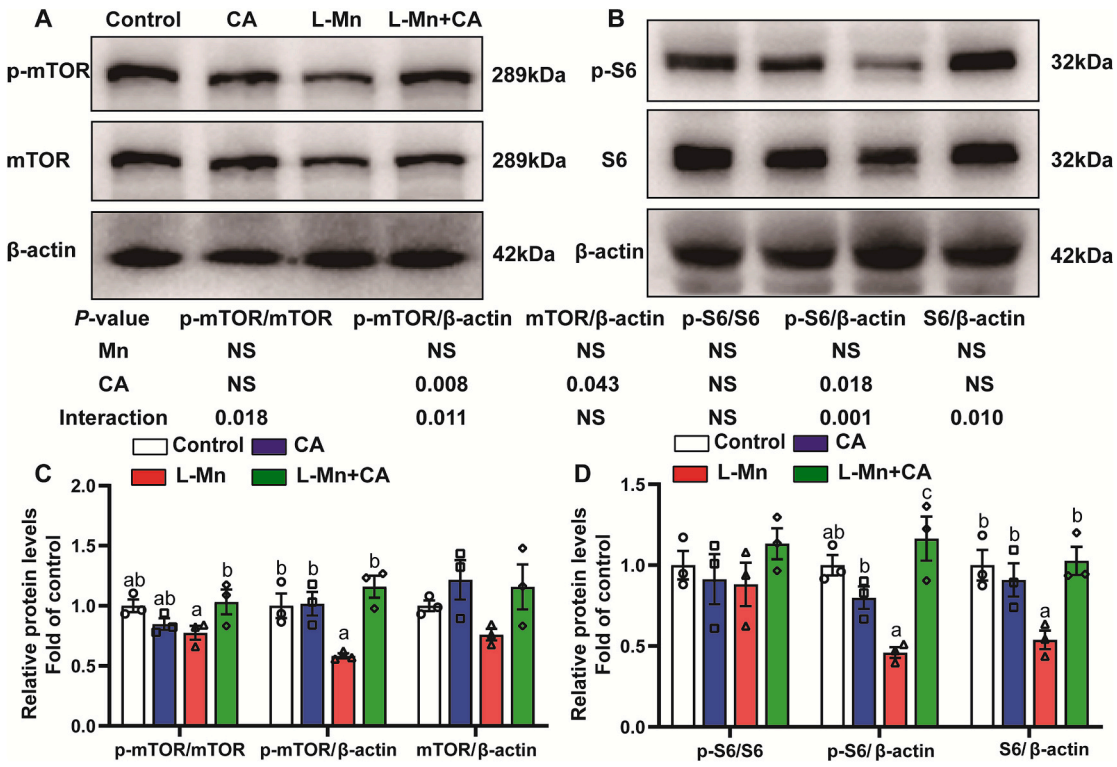

Fig. 5. Effects of dietary Mn and CA on the key protein expression of mTOR signaling pathway in muscle of yellow catfish. (A, C) Western blot analysis and quantification of p-mTOR and mTOR; (B, D) Western blot analysis and quantification of p-S6 and S6. Values are means ± S.E.M. n = 3 replicate tanks. Labeled means without a common letter differ, $P < 0.05$ (2-factor ANOVA, Duncan post hoc test). NS, no significance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

decrease of Mn content in muscle and whole body, and significantly decreased the L-Mn-induced increase of Fe content in muscle and whole body. Similarly, the decrease of the Mn content in whole body fed with L-Mn diet was also reported by other researchers in Atlantic salmon (Maage et al., 2000), cobia (Liu et al., 2013), gibel carp (Pan et al., 2008), yellow catfish (Tan et al., 2012), and grass carp (Liang et al., 2015). In our study, an increase in whole body and muscle Fe concentration was observed in L-Mn diet, compared to the control. Similarly, several studies also showed the increased Fe concentration at lower Mn levels than those in the optimal Mn levels (Liu et al., 2018; Nie et al., 2016; Tan et al., 2012), while another study showed no significant difference in whole body Fe by increasing Mn levels (Ye et al., 2009). Study pointed out that competition between Mn and Fe for the same uptake binding sites might be the probable reason for increased Fe concentration at lower dietary (Luo et al., 2017). In this study, CA addition elevated the L-Mn-induced decrease of the Mn content in the muscle and whole body, suggesting that CA could promote the availability of Mn. Studies also found that dietary CA could regulate the availability of minerals. For example, CA addition enhanced Zn content in rainbow trout (Sugiura et al., 1998) and beluga (Khajepour and Hosseini, 2012). However, supplementation of 2 g kg^{-1} CA in diet didn't affect the utilization of mineral in yellow catfish (Zhu et al., 2015), in contrast with our study. It is noted that the dose of CA adopted in our research was 30 g kg^{-1} diet. Thus, the different results may be owing to the dose of supplemental CA.

Studies have suggested that divalent metal transporter 1 (DMT1), ZIP8 (SLC39A8), ZIP14 (SLC39A14) and ZNT10 (SLC30A10) play important roles in maintaining Mn homeostasis in the organisms and cells (Fujishiro et al., 2012; Winslow et al., 2020). The *zip8*, *zip14* and *znt10* were expressed in the muscle of yellow catfish (Chen et al., 2020; Song et al., 2020). To date, there are no studies on mechanisms associated with the Mn metabolism in muscle. In this study, we revealed that, compared with the control, L-Mn diet increased the mRNA levels of *zip8* and *dmt1* in muscle of yellow catfish. Increased *zip8* and *dmt*1 mRNA expression caused the enhanced Mn absorption in response to L-Mn, which might ultimately maintain the Mn homeostasis.

Dietary nutritional addition also influenced the fillet quality. Muscle fiber, as the fundamental unit of muscle, is recognized as one of the most crucial factors influencing the fish quality (Huang et al., 2022). Muscle growth in fish occurs through proliferation and hypertrophy of myofibers (Wang et al., 2021a). The proliferation of myofibers is an increase in the amount of new muscle fibers. Hypertrophic growth will continue until the myofiber attains the maximum diameter (Huang et al., 2022). Studies suggested that fish growth is the result of muscle fiber proliferation and hypertrophy, which are influenced by both genotype and external factors (such as temperature, feed, ecological environment) (Nathanailides et al., 2011; Zhao et al., 2018; Jiang et al., 2021). The features of myofiber comprise its type, diameter, density, and cross-sectional area (Huang et al., 2022). Here, the fish fed L-Mn diet exhibited higher myofiber density than the control group. In our study, the ratio of muscle fibers measuring 50 μm or more was significantly decreased, while the ratio of myofiber that measuring under 20 μm was notably increased in the L-Mn group, compared with control. Meanwhile, CA addition increased the L-Mn-induced decrease of myofiber diameter and increase of muscle density, meaning that CA facilitated an increase in the diameters of myofibers. To our knowledge, this is the first investigation to report the influence of dietary Mn and CA on growth of fish muscle. Myosin heavy chain (Myhc) is the major component of thick filaments and affects the thick filaments formation and muscle development (Hettige et al., 2020). Paired box 7 (Pax7) plays an important role in maintaining the proliferation and differentiation of muscle satellite cells, and the loss of Pax7 could lead to poor differentiation of muscle (Koganti et al., 2020). Myogenic regulatory factors (Mrfs) comprise myogenic factor 5 (Myf5), myogenin (Myog) and myogenic differentiation (Myod) (Zammit, 2017). Myf5 is highly conserved in fish and promotes the myoblasts formation (Zhao et al., 2018). Myod is

involved in the increase of myoblasts during the muscle development and facilitates the transformation of muscular cell into myocytes (Megeney and Rudnicki, 2011). In contrast, the loss of Myod didn't influence muscle differentiation, suggesting that both overlapped in function (Zammit, 2017). Myog promotes differentiation of myogenic cell and accelerates myoblasts exit from the cell cycle and takes shape multinucleated muscle fibers (Mastroyiannopoulos et al., 2013). Mrf4 is a myogenic determinant that functions in both determination and differentiation (Zammit, 2017). Myocyte enhancer factor 2 (Mef2) family genes consist of four subtypes (*mef2a*, *mef2b*, *mef2c* and *mef2d*) and modulate development and growth of muscle by binding to muscular enhancers or promoters (Lu et al., 2021). Here, we showed that L-Mn diet obviously up-regulated the relative mRNA expression of *myhc*, *mef2c* and *mrf4*, whereas down-regulated the *myod* mRNA expression in the muscle, compared with control. The elevated expression of these genes in the L-Mn diet group reflected a higher possibility of muscle regeneration in this group. Azm et al. (2021) reported the decreased MRFs gene expression in the DDGS diet group. Xu et al. (2019) suggested that in a 14-day fasting and 14-days refeeding treatment, the mRNA expression of MRFs achieved a maximum value on day 21. Both of these studies showed that muscle regeneration occurred in growth-restricted fish, similar to our study.

The mTOR signaling pathway plays a significant role in cellular growth and proliferation by modulating protein translation and energy homeostasis (Laplante and Sabatini, 2012; Liu and Sabatini, 2020). The mTOR is an evolutionarily conserved protein kinase and initiates translation and stimulates protein synthesis via S6 and 4E-BPs (Laplante and Sabatini, 2012). Studies have shown that the overexpression of mTOR is closely related to activated anabolism, such as protein synthesis, lipogenesis, and lipid deposition (Kennedy and Lamming, 2016; Liu and Sabatini, 2020). In present study, dietary CA addition alleviated L-Mn-induced decrease in mRNA level of *mtor* and *s6* and the protein levels of p-mTOR, mTOR, p-S6. Meantime, dietary supplementation of CA increased L-Mn-induced decrease of protein content in muscle levels. Therefore, the activated mTOR signaling pathway could be a molecular characteristic in the CA-fed fish. However, the potential mechanism by which CA activates mTOR signaling pathway needs to be further evaluated.

5. Conclusion

In summary, for the first time, this study clearly revealed that there were interactive effects between Mn and CA on growth performance, mineral utilization, muscle growth and mTOR signaling pathway of yellow catfish. Dietary CA addition increased the Mn absorption and contents in the body and muscle of fish under the condition of Mn deficiency. Meanwhile, CA facilitated muscle growth largely by increasing the diameters of myofibers, therefore enhancing the growth performance under Mn deficiency diet. In addition, CA addition in the L-Mn diet enhanced the protein synthesis in muscle mainly by activating mTOR signaling. These positive effects may be partially attributed to dietary CA supplementation improving Mn absorption and bioavailability. Thus, CA appears to be a hopeful feed additive to develop more eco-friendly feed for yellow catfish through reducing dietary Mn addition.

CRediT authorship contribution statement

Tao Zhao: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Jie-Jie Xu:** Investigation, Writing – review & editing. **Yannis P. Kotzamanis:** Data curation, Writing – review & editing. **Dian-Guang Zhang:** Methodology, Writing – review & editing. **Yi-Chuang Xu:** Formal analysis, Writing – review & editing. **Hua Zheng:** Formal analysis, Writing – review & editing. **Ya-Kang Han:** Formal analysis, Writing – review & editing. **Zhi Luo:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing 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.

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.2024.740569.

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