Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 8371440, 11 pages
https://doi.org/10.1155/2022/8371440

Research Article

Effect of Different Dietary Zinc Sources on Growth, Element Deposition, Antioxidation, Lipid Metabolism, and Related Gene Expression in Hybrid Grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatus*)

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Received 24 May 2022; Revised 7 July 2022; Accepted 11 July 2022; Published 12 August 2022

Academic Editor: M Xue

This study investigated the effect of different supplements of Zn on the growth performance, antioxidant, lipid metabolism, and element deposition of hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatus*). A total of 180 juvenile groupers (bodyweight 10.02 ± 0.01 g) were divided into three groups of 20 fish each and fed with $ZnSO_4$, nanozinc oxide (Nano-ZnO), and dihydromyricetin-Zn (DMY-Zn) for 8 weeks(the content of Zn were 123,122.11, and 120.72 mg/kg). The findings revealed that DMY-Zn had a substantial impact on growth, feed conversion ratio, and protein efficiency ratio, whereas Nano-ZnO had a significant impact on feed conversion ratio and protein efficiency ratio. Furthermore, DMY-Zn had a positive effect on the high-density lipoprotein (HDL) content and significantly upregulated the lipid synthesis gene expression (FASN and ACACA) in the liver. The fish fed DMY-Zn had larger levels of catalase (CAT) in both the serum and liver, as well as Cu/Zn-superoxide dismutase (SOD) enzymes in muscle. In terms of antioxidant-related gene expression, the DMY-Zn treatment upregulated the expression of SOD1, Keap1b, and Mt2. Moreover, DMY-Zn also had a prominent effect on element deposition, increasing the concentration of Fe and Zn in the fish liver and muscle and Cu only in the fish muscle. The results revealed that a fish-fed diet enriched with DMY-Zn had significantly higher antioxidant and lipid metabolism activities than a $ZnSO_4$-fed diet. In conclusion, dietary DMY-Zn treatment appears to be effective in increasing hybrid grouper growth, element deposition, antioxidant activity, and lipid metabolism.

1. Introduction

Zinc (Zn) is an essential trace element that performs many functions in living cells, and their balanced level is a prerequisite for the completion of cellular activity [1]. It is necessarily required for the synthesis and repair of nucleic acids, as well as, structural integrity. Zn binds to more than 200 enzymes, including carbonic anhydrase, carboxypeptidase A, superoxide dismutase, alcohol dehydrogenase, alkaline phosphatase, and others, but it is primarily associated with proteins in cells. Zn homeostasis is extremely important for maintaining normal function [2] since it affects a variety of processes such as keratinization, epithelial tissue healing and hardening, immune system modulation, and insulin and adrenal glucocorticoid production [3–7]. On the other hand, Zn has antioxidant properties [8, 9] and their deficiency causes impaired cell-specific functions, oxidative damage, inhibition of iron absorption, inflammation, etc. [10–14]. Furthermore, its absence can lead to cognitive impairment, behavioral changes, and an effect on motor development [15]. Therefore, different Zn source supplements are used in this study to overcome the growth and health-related issues in hybrid grouper fish.

Zn oxide nanoparticles (ZnO-NPs) at a concentration of 20 mg/kg have been proven to boost growth performance and antioxidant status in catfish [16]. Chronic dietary exposure to ZnO-NPs (80 mg/kg) greatly improved antioxidant defenses in medaka fish without having a deleterious impact on it [17]. However, ZnO-NPs produce reactive oxygen species (ROS) and may interact with biological macromolecules (proteins or DNA) to cause dysfunction by altering antioxidant defense mechanisms, histomorphology, and oxidative stress genes [18]. High soluble $ZnSO_4$ sources may cause olfactory organs to damage and affect testicular function, glucose metabolism, etc. [19–21]. Also, Zn complexes are generally less toxic and have fewer side effects [22]; besides, they have higher bioavailability to fish than inorganic Zn [23]. Zn^{2+} can support variable coordination geometry and facilitates rapid ligand exchange. Meanwhile, dihydromyricetin (DMY) has the effects of scavenging free radicals, antioxidation, anti-inflammatory, antitumor, and preventing fatty liver [24–27]. Moreover, DMY can be stable complexes with metal ions due to the higher degree of delocalization and large conjugation bond (Π) system. Furthermore, it has synergistic or antagonistic effects with some trace metal elements for certain physiological activities. Metal chelation is important for the binding of coenzymes, cofactors, and enzymes. In addition, the bioactivated polysaccharide metal complex has strong biocompatibility and biodegradability, making it a safe and nontoxic feed additive. Moreover, a bioconditioned polydihydromyricetin-fused Zn nanoparticle (PDMY-Zn NPs) presented impressive microbial inhibition and catalytic degradation [28]. There still has not been any research that has examined the impact of DMY-Zn as a food supplement on hybrid groupers.

The hybrid grouper is a cross between a female tiger grouper (*Epinephelus fuscoguttatus*) and a male giant grouper (*Epinephelus lanceolatus*). The meat of the grouper is fresh and tender; meanwhile, it grows rapidly and is disease resistant [29–31]. It can be eaten or used as an ornamental fish. Especially in the Asia-Pacific region, due to its dietary requirements, growth and production efficiency, environmental adaptability, and other qualities are keeping advantages of aquaculture [32]. China is one of the major grouper producer countries and has a huge breeding potential on a commercial scale [33]. However, due to changes in body size and nutritional requirements at different times, the implementation of formula feed is limited [34]. The feed made from Chinese herbs has been shown to be advantageous to aquaculture animals [35, 36]; however, there are only a few studies on the effects of Chinese herb extract on hybrid groupers. Therefore, it was decided to conduct this study to assess how different Zn source base diets affected grouper growth, serum biochemical indices, antioxidant capability, lipid metabolism, and associated gene expression.

2. Materials and Methods

2.1. Experimental Diets. $ZnSO_4$, Nano-ZnO, and DMY-Zn were used to make three different experimental diets. Each Zn-sources (30 mg/kg) was precisely weighed and then dissolved in nitric acid. The contents of Zn level in diet were determined by an inductively coupled plasma atomic emission spectrometer (ICAP-6000 Series). Feed formula and nutritional composition are shown in (Table 1).

Table 1: Composition of ingredients and nutrients levels of the test diets (%).

Ingredients	$ZnSO_4$	Nano-ZnO	DMY-Zn
Fish meal	30	30	30
Casein	30	30	30
Fish oil	6	6	6
Wheat flour	24.45	24.45	24.45
Soya bean lecithin	1	1	1
Choline chloride	0.5	0.5	0.5
$Ca(H_2PO_4)_2$	0.5	0.5	0.5
Vitamin premix [1]	1	1	1
Phagostimulant	0.5	0.5	0.5
Antioxidant	0.05	0.05	0.05
Mineral premix [2]	1	1	1
Microcrystalline cellulose	5	5	5
Nutritional ingredient			
Crude ash (%)	9.26	9.38	9.09
Crude protein (%)	53.03	52.67	52.33
Crude lipid (%)	9.17	9.28	9.34
Moisture (%)	2.34	2.28	3.64
Dietary Zn level ($mg \cdot kg^{-1}$)	123	122.11	120.72

(1) Vitamin premix provides per kilogram of feed: vitamin A 30 mg, vitamin D3 3 mg, vitamin E 12.5 mg, vitamin K3 28 mg, vitamin B1 30 mg, vitamin B2 6 mg, vitamin B6 4 mg, vitamin B12 0.02 mg, niacinamide 20.0 g, D-calcium pantothenate 12.0 g, folic acid 1.5 mg, biotin B7 0.4 mg, inositol 200 mg, and vitamin C 200 mg. (2) Mineral premixes are provided per kg of feed: copper 50.0 mg, manganese 80.0 mg, iron 500.0 mg, selenium 2.0 mg, iodine 4.0 mg, cobalt 2.5 mg, potassium 100.0 mg, sodium 100.0 mg, and magnesium 150.0 mg. (3) Crude ash, crude protein, crude fat, and Zn contents were measured.

2.2. Experimental Plan. In this study, different Zn source supplements were used as a diet to investigate the impact on grouper fish (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatus*). The experimental study was carried out at Hengxing Aquaculture, Zhanjiang, Guangdong, China. Initially, the grouper was fed with basal supplements for 14 days to acclimatization. After that, 180 juvenile hybrid grouper with a bodyweight of 10.02 ± 0.01 g were randomly distributed into 3 groups based on Zn source ($ZnSO_4$, Nano-ZnO, and DMY-Zn). Each group consist of three replications of 20 fish that were regularly fed in the morning (9:00 am) and evening time (4:00 pm). The experiment was carried out in a 400-liter fiberglass tank with a sea water-like flowing rate (4 L/min velocity) and salinity (30‰). The diet of the fish was adjusted according to their body weight, and it continued for 8 weeks (feed intake is about 6% of body weight). The following parameters: growth characteristics, feed intake, daily gain, and mortality rate of grouper fish were measured during the feeding trial.

2.3. Sample Collection. The fish feed was halted one day after the experiment was completed, and all of the fish from each

replication were weighed. Nine fish were chosen at random from each group (3 per replication) for further analysis. To obtain serum, the blood sample was first taken and allowed to cool at 4°C for 12 hours before being centrifuged (4000 rpm, 15 minutes). Separated fish livers were weighed and immediately dipped in liquid nitrogen until stored in a refrigerator at -80°C. Furthermore, grouper fish muscle samples were obtained and kept in ziplock bags for mineral analysis.

2.4. Growth Parameters Analysis. The growth parameters such as survival rate (SR), weight gain rate (WGR), feed conversion ratio (FCR), special growth rate (SGR), condition factor (CF), protein efficiency ratio (PR), liver body ratio, and viscera body ratio of grouper fish were calculated using the following formulas:

$$\begin{aligned}
\text{Survival rate (SR, \%)} &= \frac{Nt}{N0} \times 100,\\
\text{Weight gain rate (WGR, \%)} &= \frac{Wt - W0}{W0} \times 100,\\
\text{Feed conversion ratio, FCR} &= \text{F}/(\text{Wt} - \text{W0})\\
\text{Special growth rate (SGR, \%/d)} &= \frac{\ln Wt - \ln W0}{t} \times 100,\\
\text{Condition factor (CF, g/cm}^3) &= \frac{W}{L^3},\\
\text{Protein efficiency ratio (PER, \%)} &= \frac{Wt - W0}{P},\\
\text{Liver body ratio} &= \left(\frac{Wl}{Wt}\right),\\
\text{Viscera body ratio} &= \left(\frac{Wv}{Wt}\right).
\end{aligned} \tag{1}$$

Nt and $N0$ are the number of experimental fish at the end and beginning of the experiment, respectively; Wt and $W0$ are the weight of experimental fish at the end of the experiment and the beginning of the experiment (g); t is the experiment time (d); F is a dry weight of ingested feed (g); L is the body length (cm) of grouper; W is the body weight (g); Wl is the liver weight (g); Wv is the visceral weight (g); and P is protein intake (g).

2.5. Analysis of Enzymes Activities. The activity of Cu-Zn superoxide dismutase (CuZn-SOD), total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) in the serum, liver, and muscle was measured by the kit of Nanjing, Jiancheng, Institute of Biological Engineering (full-wavelength microplate analyzer -1510). The contents of lipoprotein lipase (LPL), hepatic lipase (HL), and total esterase in the liver were determined by a kit from Nanjing Jiancheng, Institute of Biological Engineering.

2.6. Analysis of Serum Biochemical Index. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AKP), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (T-CHO), and triglyceride (TG) contents were measured using kits from Nanjing, Jiancheng, Bioengineering Research Institute.

2.7. Analysis of Element Deposition. The samples of 20.00 mg were accurately weighed and dissolved in nitric acid. The contents of Fe, Cu, Mn, and Zn in the liver and muscle were determined by an inductively coupled plasma atomic emission spectrometer (ICAP-6000 Series).

2.8. Real-Time Quantitative Analysis. The expression levels of genes related to antioxidant (SOD, CAT, Keap1b, and Mt2) and lipid metabolism (PPARa, CPT-1,LPL, and FASAN) were measured. Total RNA was extracted from the liver using the TRIzol reagent. The expression of lipid metabolism and antioxidation-related genes were detected by RT-PCR. Follow the TB Green PrimeScript RT-PCR kit's instructions. In Table 2, the primers were mentioned. The $2^{-\Delta\Delta Ct}$ technique was used to calculate the relative mRNA expression levels of the target genes [37].

2.9. Statistical Analysis. SPSS 26.0 was used to conduct a statistical analysis of the data. The mean and standard error (mean ± SEM) were used to express all of the results. In the statistical analysis, a one-way ANOVA was used, as well as, Duncan's multiple range test. The significance level of data was analyzed at a p value of 0.05.

3. Results

3.1. Growth Parameter. The effects of different zinc sources on the growth performance of grouper (Table 3). Overall, DMY-Zn had the greatest impact on grouper body weight, with a weight gain rate of 831 percent. In addition, DMY-Zn significantly increased the final weight, weight gain rate, and specific growth rate ($p < 0.05$) as compared to Nano-ZnO and $ZnSO_4$. On the other hand, DMY-Zn and Nano-ZnO significantly boosted the protein efficiency ratio with the value of 2.22 and 2.19, respectively, as compared to $ZnSO_4$ (2.04). The survival rate, hepatosomatic ratio, viscerosomatic ratio, and fatness were all unaffected by different Zn sources.

3.2. Biochemical Analysis. The activities of GOT, GPT, and T-CHO were considerably reduced by DMY-Zn and Nano-ZnO. The HDL content was substantially greater in DMY-Zn (0.22 ± 0.06 mmol/L) than in $ZnSO_4$ (0.16 ± 0.05 mmol/L), and Nano-ZnO (0.12 ± 0.02 mmol/L; Table 4). The fish feed by Nano-ZnO had a significantly lower TG content than $ZnSO_4$ and DMY-Zn ($p < 0.05$). Furthermore, there were no significant differences in AKP activity between different Zn sources.

3.3. Antioxidant Activity. The impact of different Zn sources was evaluated at a level of significance ($p < 0.05$) against the antioxidant capacity of grouper fish in the serum, liver, and muscle (Table 5). In the serum, the CAT and T-SOD and T-AOC activity were considerably enhanced by DMY-Zn and

Table 2: The gene's primer sequences.

Primer names	Forward and reverse primers sequence (5′ to 3′)	Accession no.
GAPDH[1]-F/R	TTGTGGCGATCAATGACCCT/CACCCCATTTGATGTTGGCG	>XM_033641056.1
PPARa[2]-F/R	AAGTTTGTTCGCAGAGCCGA/AAGGCGAATCTGGGAGGAAC	>XM_033614774.1
CPT-1[3]-F/R	GAGAGCACAGGAGACGACAG/CAGGCTGAGGAAAGCTGTGA	>XM_033635682.1
LPL-1[4]-F/R	TACTCCCAAACCACAACTCCT/TTTCTGGATGTCGGGAGACC	>XM_033622741.1
FASN[5]-F/R	ATGGTCAACTGCTTACGGCA/GCTTGGTGCTTCTGAGGACT	>XM_033612145.1
ACACA[6]-F/R	ATCGATCTGCCGGACACAAA/GGCTCTTCTTAGCAGCACCA	>XM_033611873.1
SOD1[7]-F/R	AGCATGGTTTCCACGTCCAT/CTGTCCGCATCAGTAGGACC	>XM_033633905.1
CAT[8]-F/R	CAGAAGCGCAATCCCCAAAC/CTTGCAGTAGAACCGCTTGC	>XM_033635388.1
Keap1b[9]-F/R	TGTGATGGGCGGATACGATG/AATGCAGTGACTCCCAGAGC	>XM_033623805.1
Mt2[10]-F/R	ATAAAAAGAGCCGCCCATGC/GCAATCGCAAGGGTCCATTT	>XM_033634179.1

[1]GAPDH: glyceraldehyde-3-phosphate dehydrogenase; [2]PPARa: peroxisome proliferator-activated receptor α; [3]CPT-1: carnitine palmityl transferase -1; [4]LPL: lipoprotein lipase; [5]FASN: fatty acid synthetase; [6]ACACA: acetyl-coa carboxylase α; [7]SOD1: superoxide dismutase 1; [8]CAT: catalase; [9]Keap1b: Kelch-like ECH-associated protein 1b; [10]Mt2: metallothionein 2.

Table 3: Effects of different zinc sources on the growth performance of grouper fish.

Item	$ZnSO_4$	Nano-Zn	DMY-Zn	p value
$W0$ (g)	10.03 ± 0.01	10.02 ± 0.01	10.02 ± 0.01	0.178
Wt (g)	87.64 ± 0.96^b	89.39 ± 1.97^b	93.29 ± 1.40^a	0.020
WGR (%)	774.14 ± 8.90^b	792.38 ± 20.12^b	831.03 ± 14.49^a	0.023
SGR (%·d^{-1})	3.87 ± 0.02^b	3.91 ± 0.04^b	3.98 ± 0.03^a	0.023
PER	2.04 ± 0.03^b	2.19 ± 0.05^a	2.22 ± 0.03^a	0.003
FCR	0.92 ± 0.02^a	0.87 ± 0.02^b	0.86 ± 0.01^b	0.009
SR (%)	98.89 ± 1.92	100.00	98.89 ± 1.92	0.630
Liver body ratio (%)	2.56 ± 0.09	2.49 ± 0.23	2.72 ± 0.30	0.492
Viscera body ratio (%)	9.38 ± 0.25	9.26 ± 0.28	9.64 ± 0.42	0.397
CF (%)	3.46 ± 0.07	3.42 ± 0.11	3.40 ± 0.09	0.695

The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

Table 4: Effects of different zinc sources on serum biochemical indices.

Item	$ZnSO_4$	Nano-Zn	DMY-Zn	p value
GOT/AST (U/L)	238.24 ± 4.4^a	194.31 ± 7.09^b	199.49 ± 21.92^b	0.014
GPT/ALT (U/L)	354.58 ± 8.29^a	289.18 ± 7.97^b	288.09 ± 23.68^b	0.003
AKP (mg/mL)	8.22 ± 1.21	7.44 ± 0.96	8.86 ± 1.09	0.345
HDL (mmol/L)	0.16 ± 0.05^{ab}	0.12 ± 0.02^b	0.22 ± 0.06^a	0.070
LDL (mmol/L)	0.71 ± 0.05^a	0.3 ± 0.05^c	0.42 ± 0.05^b	<0.001
T-CHO (mmol/L)	1.73 ± 0.2^a	0.91 ± 0.28^b	1.11 ± 0.06^b	0.006
TG (mmol/L)	0.87 ± 0.05^a	0.46 ± 0.1^b	0.86 ± 0.06^a	0.001

The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

Nano-Zn, respectively. In terms of liver activity, DMY-Zn was more significant (5.04 U/mL) against CAT activity than Nano-Zn and $ZnSO_4$ (2.98 U/mL and 4.18 U/mL, respectively). Furthermore, the T-AOC activity was statistically at par among DMY-Zn and $ZnSO_4$ but substantially higher than that of Nano-ZnO.

The activity of Cu/Zn-SOD in the muscle was considerably raised by DMY-Zn and Nano-ZnO ($p < 0.05$) but the DMY-Zn supplement had a significantly greater effect than Nano-ZnO and $ZnSO_4$. Also, Nano-ZnO and DMY-Zn both significantly boosted T-AOC activity in the muscle cell.

3.4. Enzyme Activities of Lipid Metabolism. The lipid metabolism of grouper fish was evaluated by calculating the activity of LPL, HL, and total esterase. The effect of different Zn-based dietary supplements on lipid metabolism was shown in Table 6. Among different Zn sources, DMY-Zn was responsible to enhance the LPL activity with the highest

Table 5: Effects of different zinc sources on the antioxidant capacity.

Item	$ZnSO_4$	Nano-Zn	DMY-Zn	p value
Serum				
CAT (U/mL)	5.88 ± 0.53^{b}	5.54 ± 1.09^{b}	7.49 ± 0.55^{a}	0.043
T-SOD (U/mL)	63.95 ± 1.63^{c}	78.9 ± 2.06^{a}	71.42 ± 1.41^{b}	<0.001
Cu/Zn-SOD (U/mL)	41.19 ± 7.82	51.2 ± 0.77	49.13 ± 4.39	0.119
T-AOC (mM)	1.34 ± 0.05^{b}	1.85 ± 0.02^{a}	1.44 ± 0.09^{b}	<0.001
Liver				
CAT (U/mL)	4.18 ± 0.13^{b}	2.98 ± 0.27^{c}	5.04 ± 0.12^{a}	<0.001
T-SOD (U/mL)	23.88 ± 0.45	25.98 ± 2.66	20.66 ± 2.87	0.073
Cu/Zn-SOD (U/mL)	18.94 ± 2.29	20 ± 1.79	19.07 ± 3.67	0.874
T-AOC (mM)	0.02 ± 0^{a}	0.01 ± 0^{b}	0.02 ± 0.01^{a}	0.041
Muscle				
CAT (U/mL)	0.29 ± 0.04	0.29 ± 0.05	0.33 ± 0.02	0.348
T-SOD (U/mL)	4.32 ± 0.8	3.92 ± 0.48	4.87 ± 1.67	0.599
Cu/Zn-SOD (U/mL)	2.5 ± 0.49^{c}	4.16 ± 0.25^{b}	5.71 ± 0.69^{a}	0.001
T-AOC (mM)	0.6 ± 0^{b}	0.63 ± 0.01^{a}	0.61 ± 0.01^{ab}	0.011

The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

Table 6: Effects of different zinc sources on enzyme activities of lipid metabolism in the liver.

Item	$ZnSO_4$	Nano-Zn	DMY-Zn	p value
LPL (U/mL)	0.08 ± 0.04^{b}	0.14 ± 0.03^{ab}	0.17 ± 0.02^{a}	0.026
HL (U/mL)	0.07 ± 0.04	0.17 ± 0.1	0.13 ± 0.05	0.269
Total esterase(U/mL)	0.16 ± 0.08	0.31 ± 0.12	0.3 ± 0.07	0.161

The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

value of 0.17 U/mL. Also, Zn dietary supplements (DMY-Zn, Nano-Zn, and $ZnSO_4$) had a similar effect on HL and total esterase activity.

3.5. Element Deposition. In muscle, DMY-Zn-based diets were responsible for significantly increased Cu deposition as compared to Nano-Zn and $ZnSO_4$ (Table 7). In comparison to $ZnSO_4$ and Nano-ZnO, DMY-Zn treatment resulted to considerably higher for Fe deposition. Furthermore, both DMY-Zn and Nano-ZnO treatments were having a similar effect on Zn deposition but were significantly higher than $ZnSO_4$.

Regarding the liver, DMY-Zn treatment significantly increased Fe and Zn deposition, followed by Nano-Zn and $ZnSO_4$. There was no significant difference were seen in Mn and Cu deposition between different treatment groups (Table 7).

3.6. Relative Gene Expression. DMY-Zn had a considerable effect on SOD1 (Figure 1(a)) and Keap1b (Figure 1(c)) in antioxidation-related genes. In the case of MT2 (Figure 1(d)) relative expression, both DMY-Zn and Nano-ZnO were statistically at par but significantly upregulated than ZnSO4.

Regarding lipid metabolism genes, the genes involved in lipid oxidation, CPT-1, were significantly upregulated by Nano-ZnO. The rest of the expression of lipid metabolism genes such as PPARa, ACACA, and FASN were significantly improved by DMY-Zn. (Figure 2).

Table 7: Effects of different zinc sources on tissue element deposition.

Item	$ZnSO_4$	Nano-Zn	DMY-Zn	p value
Muscle				
Cu	4.53 ± 0.43^{b}	2.77 ± 1.02^{c}	6.59 ± 0.34^{a}	0.001
Fe	23.64 ± 1.18^{b}	24.67 ± 2.28^{b}	29.32 ± 1.50^{a}	0.022
Mn	3.42 ± 1.13	2.6 ± 0.27	3.45 ± 1.56	0.601
Zn	16.93 ± 2.71^{b}	33.83 ± 4.20^{a}	29.02 ± 1.32^{a}	0.001
Liver				
Cu	29.87 ± 2.44	33.83 ± 4.20	29.02 ± 1.32	0.177
Fe	$147.74 \pm 10.80_{c}$	$171.01 \pm 12.89_{b}$	$200.21 \pm 7.12_{a}$	0.003
Mn	3.18 ± 0.54	4.03 ± 0.58	3.35 ± 0.26	0.153
Zn	83.44 ± 4.30^{b}	86.39 ± 3.88^{ab}	90.89 ± 1.30^{a}	0.094

The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

4. Discussion

Zn is a trace element with regulatory functions and a role as a cofactor in over 3,000 Zn metalloproteins that affect almost

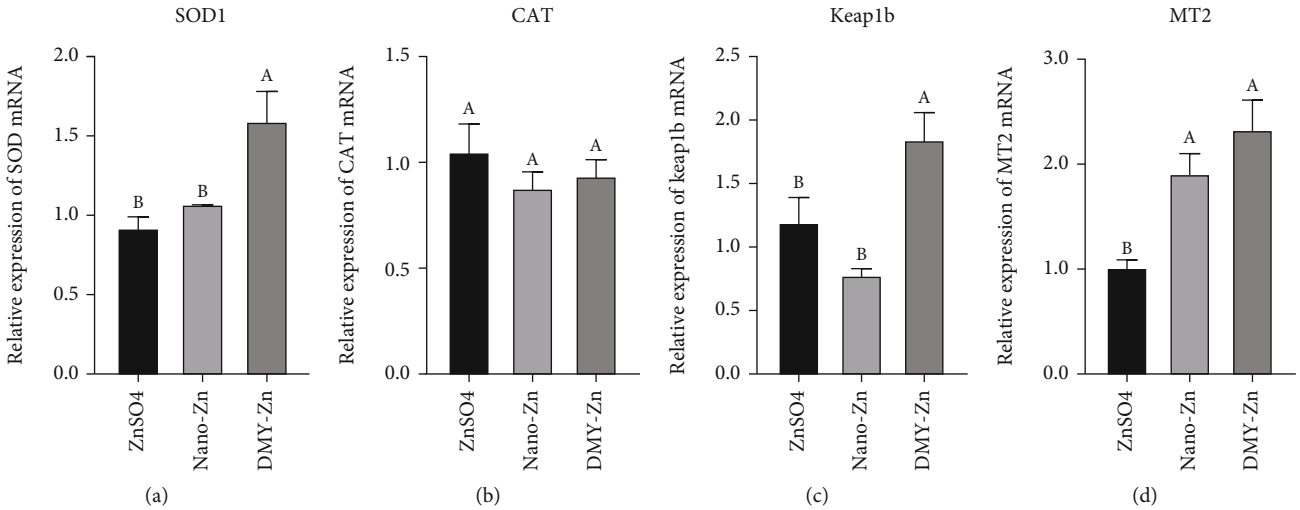

Figure 1: Relative mRNA expression of antioxidant-related genes. (a) Relative mRNA expression of SOD1. (b) Relative mRNA expression of CAT. (c) Relative mRNA expression of Keap1b. (d) Relative mRNA expression of Mt2. The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

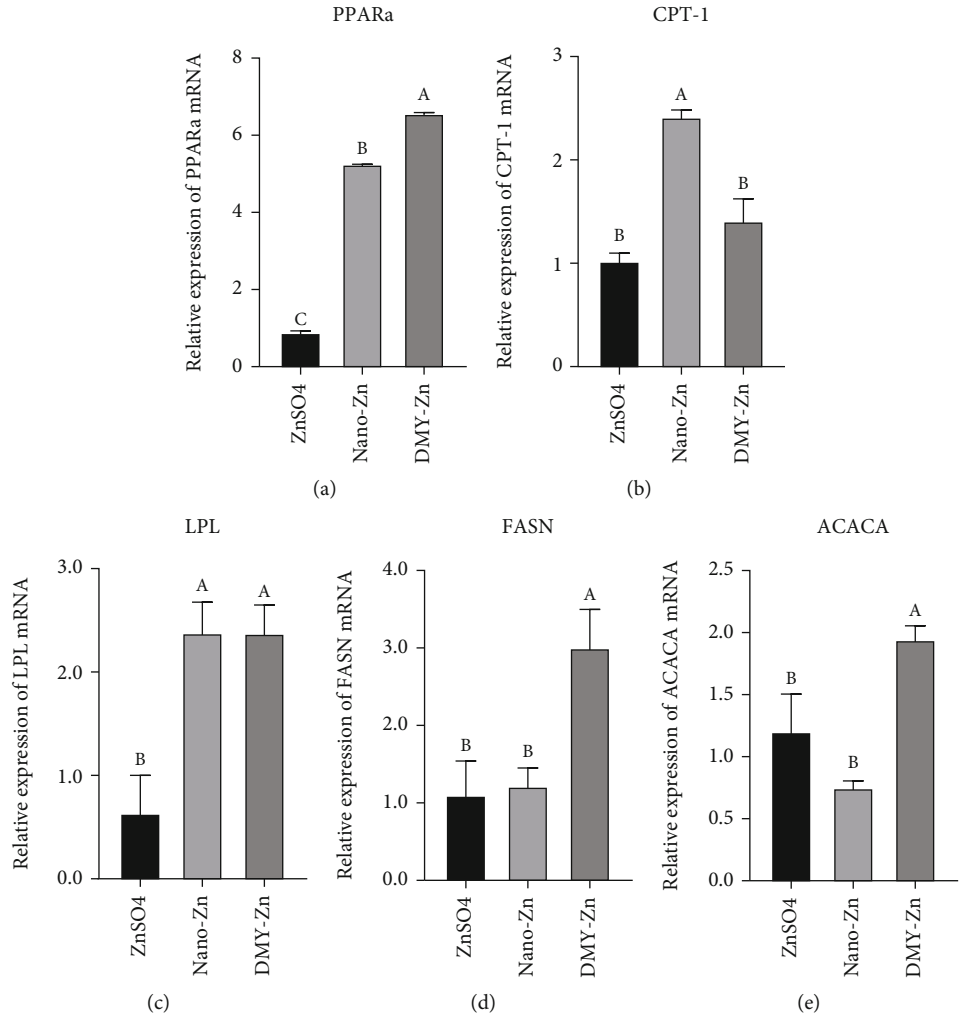

Figure 2: Relative mRNA expression of lipid metabolism genes. (a) Relative mRNA expression of PPARa. (b) Relative mRNA expression of CPT-1. (c) Relative mRNA expression of LPL. (d) Relative mRNA expression of FASN. (e) Relative mRNA expression of ACACA. The significant difference is exhibited in the same column with different superscript letters ($p < 0.05$).

every aspect of cell biology [38]. Dietary supplementation based on different Zn sources has been shown to promote grouper growth [39]. In our study, we found that DMY-Zn had the greatest impact on grouper body weight, with an 831 percent weight gain rate and a high PER. In previous studies, the Zn amino acid chelate has been shown to improve FCR and WG [40, 41]. Furthermore, Zn methionine and Nano-Zn were found to increase muscle tissue mitosis and cell differentiation by increasing growth hormone (GH) and insulin-like growth factor 1 (IGF-1) [42]. Zn content and their bioavailability are directly linked to growth and development. Several studies have found that organic Zn has a higher Zn bioavailability than inorganic Zn because it promotes intestinal release and facilitates Zn absorption [43–45]. Furthermore, polyphenols have also been found to cross-link with intestinal mucins, modulating barrier properties and thus nutrient absorption. This could affect bioavailability because mucus binding is important for increasing Zn absorption [46].

Serum biochemical indexes are key indicators of liver function, with serum ALT and AST concentrations being the most widely used to assess liver disease [47, 48]. Moreover, elevated ALT levels are associated with insulin resistance, which reduces glucose absorption and utilization efficiency [49]. Insulin resistance does not prevent the liver from producing glucose, making it easier to accumulate lipids in the liver, resulting in elevated blood glucose and triglycerides, which are linked to the development of fatty liver [50, 51]. Simultaneously, Zn, insulin, and proinsulin can form soluble polymers, which help insulin's physiological and biochemical effects. On the other hand, the toxicity of Zn sources may be one of the factors affecting the elevation of alanine aminotransferase [52]. HDL is a type of lipoprotein found in the blood that delivers cholesterol to the liver and helps it to be metabolized. Also, it has antioxidant and antiapoptotic properties which help to fight against atherosclerosis in the human body [53]. Elevated levels of LDL can contribute to the buildup of cholesterol in arterial walls, which leads to arteriosclerosis. At the same time, elevated HDL levels inhibit LDL oxidation and prevent cholesterol from being moved outside, promoting lipid metabolism in the liver [54, 55]. Nano-ZnO has a lower HDL than $ZnSO_4$ and DMY-Zn, which could be related to more reactive oxygen species being produced and accumulated [56]. Although Nano-ZnO and DMY-Zn treatments were shown to reduce cholesterol production, also, several investigations found that dietary Zn supplementation had minimal effect on cholesterol levels [57]. DMY-Zn has a good protective impact on the liver when compared to other Zn-based diets, as determined by serological indicators. As a result, it has the potential to be an effective Zn additive.

Antioxidant enzymes play a critical function in protecting the body from oxidative damage. Once peroxide has been generated in the body, REDOX action can be used to convert peroxide into less hazardous or harmless molecules, as well slowing the rate of oxidation. In normal circumstances, O_2 is oxidized to H_2O; however, in an 'escape' situation, the body creates the superoxide anion radical O^{2-}. In this situation, SOD turns it to the less harmful H_2O_2, which is then converted to H_2O and O_2 by CAT. At the same time, glutathione catalase (GSH-PX) also promotes the decomposition of H_2O_2 [58]. Zn's antioxidant action can be linked to its role as a cofactor of SOD in the cytoplasm and extracellular, as well as its involvement in metallothionein synthesis and the ability to keep free radical scavenger enzymes active [59]. In this study, DMY-Zn increased the enzyme activities of CAT, SOD in the serum, and CAT in the liver. Moreover, the activity of Cu/Zn-SOD was higher in DMY-Zn (organic) than that of $ZnSO_4$ (inorganic), which was similar to the finding in rainbow trout [60]. According to a previous study, organic Zn has a stronger antioxidant activity than inorganic Zn such as Zn glycine (Zn-Gly) enhanced serum T-AOC, AKP, and Cu/Zn SOD while lowering nitric oxide levels when compared to $ZnSO_4$ [61]. According to another study, Zn methionine (Zn-Met) and Nano-ZnO reduced the amount of malondialdehyde (MDA) and boosted the activities of CAT, SOD, and GSH-Px than ZnO [62]. In addition to the role of Zn ions, plant antioxidants may prevent cell damage induced by inflammatory cytokines [63]. In the case of plant-derived Zn, the addition of *E. prolifera* polysaccharide Zn chelate (EP-Zn) to a diet significantly improves the antioxidant activity in muscles and reduces the protein breakdown caused by oxidative stress, resulting in improved growth performance [64]. In vitro, antioxidant evaluation of fritillaria polysaccharide Zn revealed that fritillaria polysaccharide Zn (FUP-Zn) has a stronger ·OH scavenging activity than fritillaria polysaccharide (FUP) [65]. Zn is a cofactor of the antioxidant enzyme and may have an indirect effect on its activity. The finding revealed that DMY-Zn stimulated the synthesis of antioxidant enzymes and gene expression of antioxidant enzymes more effectively than other Zn sources. The effect of Zn on transcription factors plays a role in the regulation of the Zn-dependent antioxidant system [66, 67]. Also, it protects against oxidative stress by increasing the transcription factor Nrf2-ARE, upregulating antioxidant gene expression, stimulating the synthesis of metallothionein, etc. [68]. The induction of antioxidant enzyme genes is regulated by a variety of cellular signaling pathways and transcription factors. The gene expression level of SOD1 is linked to the Keap-Nrf2 pathway, in which Nrf2 is activated in the cytoplasm and transported into the nucleus to bind with the antioxidant response element (ARE), which could explain the increased expression of the antioxidant enzyme [69]. However, the continuous accumulation of Nrf2 in the nucleus might have negative consequences such as free radical damage, apoptosis, and tumorigenesis. The overexpression of Keap1 stimulates Nrf2 degradation, hence regulating Nrf2 abundance via response regulation [70]. Besides, metallothionein has a high affinity for Zn and plays an important role in maintaining Zn homeostasis [71], as well as being a powerful free radical scavenger. Organic Zn has been proven to improve MT mRNA expression in the liver and intestinal system of laying hens [72]. However, after intravenous Zn infusion, there was no change between organic and inorganic Zn in the pancreas [73]. In conclusion, DMY-Zn has stronger antioxidant enzyme activity than $ZnSO_4$ and Nano-ZnO, and it might be responsible to regulate the activation of Nrf2 to boost the antioxidant capacity.

In vivo, the enzymes HL and LPL are both involved in fat metabolism. LPL decomposes TG into fatty acids and monotriglycerides for tissue oxidation, energy supply, and storage. HL can prevent excessive cholesterol accumulation in extrahepatic tissues [74]. Zn can control metabolism in the body and, as a supplement, can promote glucose absorption and reduce lipid deposition [75]. Studies have shown that a higher concentration of Zn in the dietary supplement was responsible for significantly reducing lipid content in the liver and muscle and improving LPL activity in both of them. Dietary Zn addition also affected several lipogenic enzymatic activities and the expression of genes [76]. Zn oxide nanoparticles have been found to reduce lipid content in egg yolks, possibly due to a decrease in lipid formation and an increase in lipid digestion [77]. The results of our study showed that both Nano-ZnO and DMY-Zn had positive effects on lipid oxidation. PPARa is a nuclear receptor protein that regulates transcription factor expression. It plays a vital role in cell differentiation, development, and metabolism, as well as, its upregulation can accelerate lipid oxidation. DMY-Zn showed a higher expression level of PPARa might be due to more bioavailability of Zn in dietary supplements is thought to benefit the DNA binding activity of PPARa. Free Zn^{2+} boosts the binding of metal response element-binding transcription factor (MTF-1) in the PPARa promoter region, resulting in the activation of genes related to autophagy and lipidolysis [78]. LPL promotes tissue oxidation by breaking down triglycerides into glycerol and fatty acids. In addition, CPT-1 is the rate-limiting enzyme in fatty acid oxidation, with its primary function being to catalyze the fatty acids through oxidation into mitochondria when the body or tissues are low on energy. The higher dose of dietary Zn supplementation enhances lipid metabolism and inhibits fat accumulation in the liver by upregulating CPT-1 and PPARa levels while downregulating FASN and ACACA levels [79]. Surprisingly, ACACA and FASN, which are involved in the fatty acid synthesis, were strongly expressed in DMY-Zn supplemented to grouper. Lipid accumulation is caused by the imbalance between lipid synthesis and decomposition. Despite lipid accumulation, the load can be reduced by influencing other activities that promote lipid efflux from the liver [80]. This might be related to the reduction of ACC activity through phosphorylation of AMP-activated protein kinase (AMPK), which leads to a decrease in malonyl-CoA levels and hence the removal of inhibition of carnityl-transferase [81]. Different additives had a different outcomes; therefore, it still needs further research work to understand the mechanism.

Fe, Cu, Mn, and Zn are vital trace elements that play a critical role in growth, development, metabolism, and other processes. Mineral additives are commonly utilized as feed supplements due to their essential requirements and feed content. Furthermore, changes in the level of one mineral element will impact the content of other mineral elements, such as the antagonism of copper and Zn, as well, as iron overload affecting manganese homeostasis, etc. [82–84]. In addition, the affinity of different zinc sources to target organs was different, and the affinity of ZnO to all organs was higher than $ZnSO_4$ and protein zinc. At the same time, high doses of Zn lead to an increase in elemental deposition [85]. A study found that there were no significant differences in Cu, Fe, and Mn contents in tissues except the liver and pancreas after long-term exposure to 50, 500, and 5000 mg/kg of Nano-ZnO [86]. Furthermore, as the amount of Zn supplementation increased, the levels of Cu and Fe in the liver and muscle of Pelodiscus sinensis decreased [87]. It is worth mentioning that in our experiment, compared with $ZnSO_4$, DMY-Zn supplementation increased the 24% and 36% iron content in the liver and muscle, respectively, while Nano-ZnO-based diet increased the 15% iron content in muscle. Moreover, the DMY-Zn treatment also improved the Cu content.

5. Conclusions

The purpose of our study is to determine a better Zn-based nutritional diet for grouper (Epinephelus fuscoguttatus and E. lanceolatus) growth and development at enzymatic and molecular levels. In comparison to Nano-Zn and $ZnSO_4$, the results of this study show that DMY-Zn increased growth performance and CAT activity in the liver and serum. Furthermore, it enhances the deposition of Fe and Zn in the muscle and serum of fish. DMY-Zn is also involved in the upregulation of antioxidant gene expression (SOD1 and Keap1b) as well as lipid metabolism gene expression (PPARa, FASN, and ACACA). As a result, it can be concluded that a dietary supplement based on DMY-Zn has outstanding performance in the production of grouper fish. Further research will be carried out in order to optimize the dose of DMY-Zn in aquaculture.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors wish to express their gratitude for the financial support from the Guangdong and Hong Kong Joint Innovation Field (2017A050506055), National Innovation and Entrepreneurship Program for College Students (202011347008), Guangzhou science and technology plan projects (20190701003320206003), Graduate Innovation Program (KA210318537), and Scientific and Technological Innovation Strategy Special Fund Project of Guangdong Province (Pdjh2021a0247).

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