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Article

The Impacts of Dietary Curcumin on Innate Immune Responses and Antioxidant Status in Greater Amberjack (*Seriola dumerili*) under Ammonia Stress

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Abstract: In this study, we investigated the effect of dietary curcumin on non-specific immune responses and antioxidative ability in *Seriola dumerili* under ammonia stress and post-recovery. Three diets were prepared to contain 0, 75, and 150 mg/kg of curcumin. A total of 225 greater amberjack (initial weight: 100.90 ± 0.03 g) were distributed into nine cylindrical tanks, constituting an experimental design with three treatments and three replicates. After 56 days of feeding, plasma, intestinal, and hepatic enzyme activities were evaluated. Then, an acute ammonia challenge experiment was conducted. Ten fish per tank were subjected to acute ammonia stress (total ammonia-N: 1000 mg/L) for eight minutes followed by six minutes of recovery. The results indicated that dietary curcumin significantly promoted intestinal and hepatic alkaline phosphatase (ALP) and acid phosphatase (ACP) levels as well as hepatic antioxidative enzymes such as superoxide dismutase (SOD), total antioxidant capacity (T-AOC), reduced glutathione (GSH), and glutathione peroxidase (GSH-Px) of greater amberjack. In addition, curcumin addition improved the activities of antioxidant enzymes, such as SOD, T-AOC, GSH, GSH-Px, and catalase (CAT), and reduced malondialdehyde (MDA) content in liver, spleen, head kidney, and brain tissues after post-recovery. The indexes related to immunity and antioxidant enzymes in the liver, gill, and spleen rose again to some extent, but they showed the worst recovery ability in the head kidney and brain tissue samples. These results indicate that dietary curcumin supplementation could increase non-specific immune responses, antioxidant ability, and enhance resistance to high ammonia stress in juvenile *S. dumerili*.

Keywords: curcumin; ammonia; enzyme activity; oxidative stress; *Seriola dumerili*

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1. Introduction

Whether in the wild or in an aquaculture environment, fish are often under stress (e.g., exposed to environmental contaminants, extreme conditions or water quality change, handling, transport, population density, or bacterial and viral invasion). These negative factors can lead to stress responses in fish species [1]. Between 60 and 80% of the nitrogenous waste excreted by fish species is ammonia. Ammonia is the final product of protein catabolism [2]. Ammonia includes ionized (NH_4^+) and unionized (NH_3) forms [3], and the toxic effects of ammonia is mainly attributed to NH_3, as it can diffuse freely along the concentration gradient to the gill membranes [2]. The accumulation of ammonia in water is a serious problem in today's high-density aquaculture environment. Ammonia concentrations in water can rise rapidly and lead to acute toxicity to aquatic organisms. Many

studies have demonstrated that excessively high concentrations of ammonia are detrimental to fish health, leading to immunosuppression and high mortality [4] and damage to many organs (such as the gill, liver, etc.) of fish species [5].

Curcumin (*Rhizoma curcumae* Longae) is the main active ingredient in turmeric [6,7]. It has extensively pivotal functions such as reducing inflammation as well as antioxidant, antitumor, and anti-stress properties in mammalian and aquatic animals [8,9]. Previous studies have shown many kinds of functions of curcumin in fish and other aquatic animals. Numerous studies have demonstrated that dietary curcumin could improve antioxidant capacity and immune function in tilapia (*Oreochromis niloticus*) [10], rainbow trout (*Oncorhynchus mykis*) [11], Japanese Sea bass (*Lateolabrax japonicus*) [12], and largemouth bass (*Micropterus salmoides*) [13]. In addition, dietary curcumin can play a protective role against environmental stress via promotion of antioxidant enzyme activities such as GSH-Px, SOD, and CAT activities [14,15]. In another study on tilapia, supplementation of curcumin also markedly enhanced anti-oxidative status during exposure to natural challenging cold temperatures [16].

Greater amberjack *Seriola dumerili* is a marine pelagic fish species with a circumglobal distribution throughout warm and tropical waters (such as in Australia, New Zealand, Japan, China, the United States, and Chile) [17]. This species has been targeted for commercial farming in Japan, Australia, and the Mediterranean region because of its high growth rate, superior flesh quality, and high commercial value [17,18]. It was reported that the aquaculture production of amberjack in 2021 reached more than 20,000 tons in China [19]. In the future, the artificial culture of *S. dumerili* has great potential in China. With the rise of high-density intensive farming, *S. dumerili* cultured in marine cages face more stressors, such as temperature, salinity, pH, bacteria, parasites, etc., which can seriously affect the healthy development of *S. dumerili*. However, to date, limited studies have been published examining the effects of dietary curcumin on resistance to ammonia stress, particularly changes in the activities of enzymes associated with various tissues in this fish. Therefore, in the present study, we tried to determine the effects of curcumin on the innate immune response and hepatic histology of *S. dumerili*, and we aimed to study the impacts of acute ammonia exposure on non-specific immune parameters and antioxidant abilities more comprehensively. We also sought to investigate the ability of recovery after ammonia exposure, which will provide some insights for disease prevention and/or stress attenuation.

2. Materials and Methods

All the experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of South China Sea Fisheries Institute. This study was recognized by the Ethics Committee of South China Sea Fisheries Institute (No. 20200815).

2.1. Experimental Diets

Fishmeal, corn gluten meal, and soybean meal were used as dietary protein sources. Fish oil and lecithin were used as lipid sources. Contents of crude protein and crude lipid were 49.7% and 12.7%, respectively, and this formulation (Table 1) has been shown to be nutritionally adequate for the growth of *S. dumerili* [20]. Three diets were formulated to supplement with levels of curcumin (0, 75 mg/kg, and 150 mg/kg) (Purity, ≥98%, Xi'an Bluegrass Biotechnology Co., Ltd., Shaanxi, China), which were named as control group, 75 mg/kg group, and 150 mg/kg group, respectively. The experimental diets were made by blending all the components well with oil and then adding distilled water until a stiff dough was produced. The pellets with 1 mm diameter were wet-extruded by a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) and then air-dried (26 °C, 3 days), put in plastic self-sealing bags, and stored at −20 °C until use.

Table 1. Ingredients and proximate composition of experimental diets.

Ingredient	(% of Dry Matter)
Fish meal	60
Corn gluten meal	8
Soybean meal	10
Corn starch	8
Microcrystalline cellulose	2
Fish oil	7
Lecithin	1
Vitamin mixture [1]	0.5
Mineral mixture [2]	0.5
Choline chloride	0.5
Betaine	0.5
Carboxyl-methyl cellulose	2
Total	100
Proximate composition	
Dry matter	87.9
Crude protein	49.7
Crude lipid	12.7
Crude ash	10.7
Gross energy (kJ/g)	18.5

[1,2] The specific components of vitamin premix and mineral premix refer to our previous literature [21].

2.2. Fish and Animal Husbandry

The feeding experiment was conducted in the Sanya Basement of the South China Sea Fisheries Research Institute, CAFS. Juvenile *S. dumerili* were bought in a commercial fish farm. Before the feeding experiment, fish were taken to 450 L cylindrical fiberglass tanks and fed with control diet to domesticate them for 14 days. The fish were fasted for 24 h before grouping. A total of 225 fish with uniform size (100.90 ± 0.03 g) were randomly divided into nine tanks and raised for 56 days. Fish were artificially fed twice a day (at 8:00 and 16:00) until apparent satiation. During the period, water quality parameters were as follows: temperature 29.0 ± 1.0 °C, dissolved oxygen >6.0 mg/L, pH 8.2–8.4, salinity 33.0 ± 0.04‰, and ammonia <0.01 mg/L, respectively. The photoperiod was the natural solar cycle throughout the whole process.

2.3. Acute Ammonia Challenge Experiment

After eight weeks of the feeding trial, the fish were starved for 24 h. Ten fish of similar specification were collected from each tank and subjected to an acute ammonia stress (total ammonia-N: 1000 mg/L) test in cylindrical tanks (100-L) according to the methods described in Zhang et al. [2,22]. The water temperature ranged from 28 °C to 30 °C, the flow rate was 2.2 L/min, and the DO was >6 mg/L. Ammonium chloride (NH_4Cl) was used as an ammonia source and added to required final ammonia contents. The fish were sampled at eight minutes, and the rest were taken to aerated water in a tank for six minutes of post-exposure recovery.

2.4. Sample Collection and Chemical Analyses

At the end of the rearing experiment, the fish were sampled from three fish per tank at 0, 8, and 14 min (8 + 6 min; they were taken after 6 min of post-exposure recovery). The fish were quickly netted and anesthetized with diluted eugenol (1:10,000; Shanghai Reagent Corp., China), and then three fish were randomly selected from each tank, and blood was collected through the caudal vein with 2 mL heparinized syringes. After collection, blood was centrifuged (3000× *g* at 4 °C for 10 min) to obtain plasma (stored at −80 °C for use). The liver, intestine, brain, gill, spleen, and head kidney samples were stored at −80 °C until enzyme activities were determined. The liver was taken and then placed in a fixed solution (4% paraformaldehyde) with 10 mL centrifuge tubes for morphological observation.

2.5. Blood Biochemical Parameter Measurements

Plasma alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were measured by ROCHE-P800 automatic biochemical analyzer (Roche, Basel, Switzerland) according to a standard kit method for each assay.

2.6. Tissues' Enzyme Activity Measurements

The liver, intestine, brain, gill, spleen, and head kidney samples were homogenized in cold phosphate buffer (diluted at 1:10) (phosphate buffer: 0.064 M at pH 6.4). Then the homogenate was centrifuged for 20 min (4 °C, 3000× *g*) and the supernatant was taken to determine alkaline phosphatase (ALP), acid phosphatase (ACP), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC), malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), amylase, lipase, and trypsase levels according to the protocols provided by commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China). ALP, ACP, SOD, CAT, T-AOC, MDA, GSH, GSH-Px, amylase, lipase, and trypsin activities were measured by a colorimetric method using wavelengths of 520 nm, 520 nm, 450 nm, 405 nm, 520 nm, 532 nm, 405 nm, and 412 nm, respectively. The Folin method was employed to measure the above six tissues' protein content (Lowry et al., 1951).

2.7. Statistical Analysis

The data in this study were tested for homogeneity of variances with the Levene test. All the data were subjected to one-way analysis of variance (ANOVA). When overall differences were significant, the Duncan's test was employed to compare means between treatments. The level of significant difference was $p < 0.05$. Statistical analysis was performed using the SPSS 25.0, and the results were expressed as mean ± SEM (standard error of the mean).

3. Results

3.1. Effect of Dietary Curcumin on Plasma ALP and ACP Activities of Greater Amberjack (Seriola dumerili)

The results of ALP were not significantly affected by dietary curcumin level ($p > 0.05$) (Table 2). Compared with the control group (0 mg/kg curcumin), the ACP was significantly improved in curcumin-addition groups ($p < 0.05$). There were no significant differences in ACP between curcumin treatment groups ($p > 0.05$).

Table 2. Plasma ALP and ACP of greater amberjack (*Seriola dumerili*) fed with diets containing different amounts of curcumin.

Dietary Curcumin (mg/kg)	0	75	150
ALP (king's unit/100 mL)	2.3 ± 0.15	2.17 ± 0.04	2.10 ± 0.10
ACP (king's unit/100 mL)	1.45 ± 0.02 ^{b}	2.67 ± 0.20 ^{a}	2.45 ± 0.09 ^{a}

The values are average ± standard error of three replications (n = 9). There was a significant difference in the average value of different superscript letters in the same row ($p < 0.05$).

3.2. Effect of Dietary Curcumin on Intestinal ALP and ACP Activities of Greater Amberjack (Seriola dumerili)

The results of intestinal ALP and ACP are shown in Table 3. The ALP and ACP levels in curcumin-addition groups were dramatically higher than that in the control treatment ($p < 0.05$). No statistical difference in ALP levels were observed between 75 mg/kg and 150 mg/kg of dietary curcumin groups ($p > 0.05$).

Table 3. Intestinal enzyme activities of greater amberjack (*S. dumerili*) fed with diets containing different amounts of curcumin.

Dietary Curcumin (mg/kg)	0	75	150
ALP (king's unit/gprot)	28,222 ± 2008 ^{b}	41,407 ± 2055 ^{a}	42,254 ± 3654 ^{a}
ACP (king's unit/gprot)	8710 ± 183 ^{b}	11,693 ± 419 ^{a}	11,330 ± 562 ^{a}

The values are average ± standard error of three replications (n = 9). There was a significant difference in the average value of different superscript letters in the same row ($p < 0.05$).

3.3. Effect of Dietary Curcumin on Hepatic Enzyme Activities of Greater Amberjack (Seriola dumerili)

Hepatic ALP levels in curcumin-addition groups were dramatically higher than those in the control treatment ($p < 0.05$) (Table 4). However, there were no significant differences in ALP between curcumin treatment groups. With the increase of dietary curcumin, the ACP levels were significantly promoted ($p < 0.05$). The ACP level in the 150 mg/kg group was significantly higher than those in control group and 75 mg/kg curcumin group ($p < 0.05$). Hepatic SOD and GPX activities in the group of 75 mg/kg dietary curcumin were dramatically higher than that in the control treatment ($p < 0.05$). However, there were no statistical differences in SOD and GPX between the control and 150 mg/kg dietary curcumin groups, respectively ($p > 0.05$). The T-AOC level in the 150 mg/kg dietary curcumin group was higher than that in the 75 mg/kg dietary curcumin group ($p < 0.05$), but it was not significantly different from that in the control group ($p > 0.05$). As for GSH, with the increase in dietary curcumin, it showed a trend of increasing and then decreasing. The GSH level in the 75 mg/kg dietary curcumin group was higher than that in the 150 mg/kg dietary curcumin group ($p < 0.05$), but it was not significantly different from that in control group ($p > 0.05$). However, with the increase of dietary curcumin, the CAT level showed a trend of increasing and then decreasing, but there was no statistical difference ($p > 0.05$). Inversely, the MDA level exhibited an opposite tendency, which was declining and then increasing.

Table 4. Hepatic enzyme activities of greater amberjack (*S. dumerili*) fed with diets containing different amounts of curcumin.

Dietary Curcumin (mg/kg)	0	75	150
ALP (king's unit/gprot)	16.62 ± 1.99 ^{b}	55.71 ± 4.07 ^{a}	52.25 ± 2.38 ^{a}
ACP (king's unit/gprot)	78.27 ± 4.48 ^{b}	90.44 ± 6.99 ^{b}	108.08 ± 1.91 ^{a}
SOD (U/mgprot)	6598 ± 772 ^{b}	8793 ± 768 ^{a}	5765 ± 204 ^{b}
CAT (U/mgprot)	172.89 ± 14.5	178.66 ± 4.88	160.87 ± 6.24
T-AOC (mM)	0.93 ± 0.02 ^{ab}	0.9 ± 0.01 ^{b}	0.98 ± 0.01 ^{a}
MDA (nmol/mgprot)	1.25 ± 0.11	1.16 ± 0.08	1.28 ± 0.05
GSH (μmol/gprot)	21.41 ± 1.37 ^{ab}	24.16 ± 1.05 ^{a}	18.12 ± 0.51 ^{b}
GPX (IU/mgprot)	9.19 ± 2.01 ^{b}	20.84 ± 1.06 ^{a}	11.29 ± 0.66 ^{b}

The values are average ± standard error of three replications (n = 9). There was a significant difference in the average value of different superscript letters in the same row ($p < 0.05$).

3.4. Intestinal Enzyme Activities in Greater Amberjack (S. dumerili) in Response to Acute Ammonia Exposure and Post-Recovery

The ALP level in the 75 mg/kg group after ammonia exposure was dramatically higher than that in the control treatment ($p < 0.05$) (Figure 1A). The ALP level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$). Intestinal ALP levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$) (Figure 1A). The intestinal ALP level in the control group after post-recovery was dramatically lower than that in the counterpart group after ammonia exposure ($p < 0.05$). Inversely, the ALP level in the 75 mg/kg group after post-recovery was significantly increased compared with the level after ammonia exposure ($p < 0.05$).

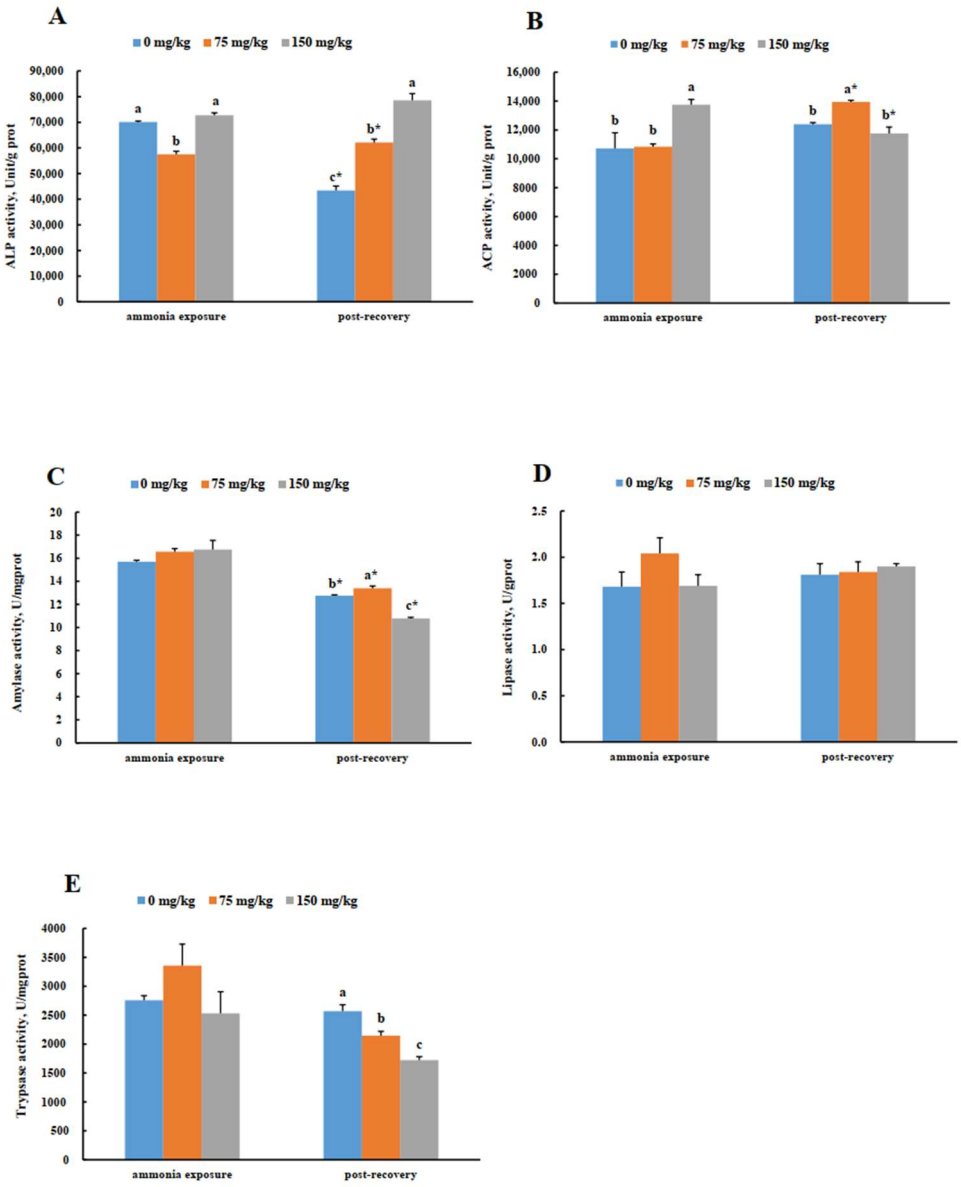

Figure 1. Intestinal ALP, ACP, amylase, lipase, and trypsase levels in greater amberjack (*S. dumerili*) fed dietary curcumin (**A**–**E**) and the response to acute ammonia exposure and post-recovery. Data are expressed as average ± standard error of the mean (SEM) (n = 9). The significant differences ($p < 0.05$) between values obtained in ammonia exposure and its post-recovery stage were determined using *t*-tests and are indicated by asterisks. Different letters indicate significant differences ($p < 0.05$) among different groups by Duncan's multi-range tests.

The ACP level in the 150 mg/kg group after ammonia exposure was dramatically higher than that in the control treatment ($p < 0.05$) (Figure 1B). The ACP level in the 75 mg/kg group was significantly higher than those in control and 150 mg/kg groups after post-recovery ($p < 0.05$). Compared with counterpart groups after ammonia exposure, the ACP level in the 75 mg/kg group after post-recovery was significantly increased ($p < 0.05$), while the ACP level in the 150 mg/kg group after post-recovery was significantly decreased ($p < 0.05$).

Compared to the control group after post-recovery, the amylase activity in the 75 mg/kg group after post-recovery was significantly increased ($p < 0.05$), while it was dramatically decreased in the 150 mg/kg group after post-recovery ($p < 0.05$) (Figure 1C). The amylase activities in the control, 75, and 150 mg/kg groups after post-recovery were signifi-

cantly decreased compared with the respective groups after ammonia exposure ($p < 0.05$).

Trypsase activity decreased significantly with increasing dietary curcumin addition, reaching a minimum in the 150 mg/kg group after post-recovery ($p < 0.05$) (Figure 1E). The trypsase activities in the 75 and 150 mg/kg groups after post-recovery were statistically lower than that in the control treatment ($p < 0.05$).

3.5. Hepatic Enzyme Activities in Greater Amberjack (S. dumerili) in Response to Acute Ammonia Exposure and Post-Recovery

The hepatic ALP level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 2A). Hepatic ALP levels in the 75 and 150 mg/kg groups after ammonia exposure were dramatically higher than that in the control treatment ($p < 0.05$). Hepatic ALP levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). The ALP level in the 150 mg/kg group after post-recovery was dramatically lower than that in the 75 mg/kg group after post-recovery ($p < 0.05$). Hepatic ALP levels in the control group and curcumin-addition groups after post-recovery were dramatically higher than those in the respective groups after ammonia exposure ($p < 0.05$).

Hepatic ACP levels were significantly decreased with increasing dietary curcumin addition, reaching a minimum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 2B). The ACP levels in the 75 and 150 mg/kg groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$). Similar results were also observed after post-recovery. The hepatic ACP levels in curcumin-addition groups after post-recovery were dramatically lower than that in the control treatment ($p < 0.05$). The hepatic ACP levels in the control, 75, and 150 mg/kg groups after post-recovery were dramatically higher than those in the respective groups after ammonia exposure ($p < 0.05$).

Hepatic SOD levels were significantly decreased with increasing dietary curcumin addition, reaching a minimum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 2C). The SOD levels in the 75 and 150 mg/kg groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$). The hepatic SOD levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). Compared with those three groups after ammonia exposure, the hepatic SOD levels in counterpart groups after post-recovery were statistically increased ($p < 0.05$).

CAT levels in the 75 and 150 mg/kg groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$) (Figure 2D). Compared with the control group after ammonia exposure, the hepatic CAT level in the control group after post-recovery was significantly increased ($p < 0.05$).

Hepatic T-AOC levels in curcumin-addition groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$) (Figure 2E). Compared with the control group after post-recovery, the hepatic T-AOC levels in curcumin-addition groups after post-recovery were significantly increased ($p < 0.05$). The hepatic T-AOC level in the 75 mg/kg group after post-recovery was dramatically raised compared with the higher curcumin-addition group (150 mg/kg) after post-recovery ($p < 0.05$). Compared with 75 and 150 mg/kg groups after ammonia exposure, the hepatic T-AOC level in 75 and 150 mg/kg groups after post-recovery was significantly increased ($p < 0.05$), respectively.

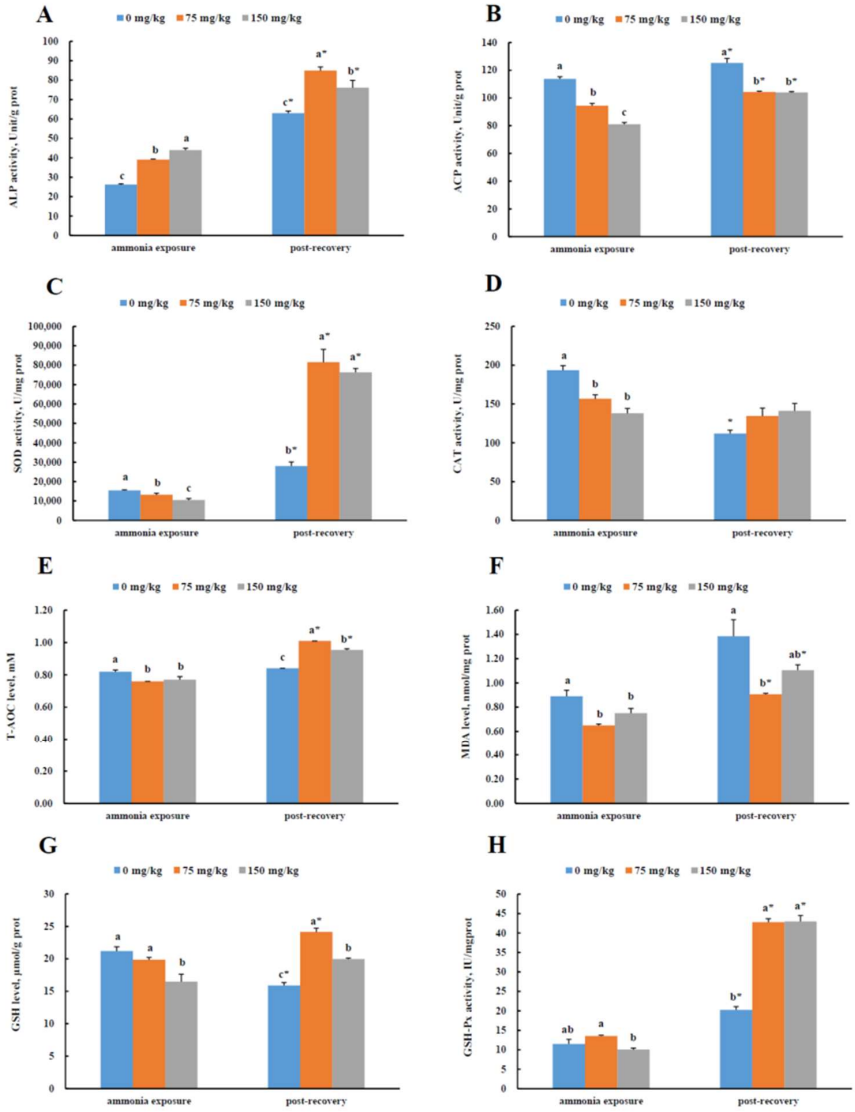

Figure 2. Hepatic ALP, ACP, SOD, CAT, T-AOC, MDA, GSH ， and GSH-PX levels in greater amberjack (*S. dumerili*) fed dietary curcumin (**A**–**H**) and the response to acute ammonia exposure and post-recovery. Data are expressed as average ± standard error of the mean (SEM) (n = 9). The significant differences ($p < 0.05$) between values obtained in ammonia exposure and its post-recovery stage were determined using *t*-tests and are indicated by asterisks. Different letters indicate significant differences ($p < 0.05$) among different groups as assessed by Duncan's multi-range tests.

Hepatic MDA levels in curcumin-addition groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$) (Figure 2F). The hepatic MDA level in the 75 mg/kg group after post-recovery was dramatically lower than that in the control treatment ($p < 0.05$). Compared with those three groups after ammonia exposure, the hepatic MDA levels in the control, 75, and 150 mg/kg groups after post-recovery were significantly increased ($p < 0.05$).

The hepatic GSH level in the 150 mg/kg group after ammonia exposure was dramatically lower than that in the control and 75 mg/kg treatments ($p < 0.05$) (Figure 2G). The hepatic GSH levels in the 75 and 150 mg/kg groups after post-recovery were dramatically higher than that in the control treatment after post-recovery ($p < 0.05$). The hepatic GSH level in the 75 mg/kg group after post-recovery was significantly higher than that in the 150 mg/kg groups after post-recovery ($p < 0.05$). The hepatic T-AOC levels in the control group after post-recovery was dramatically lower than that in the control treatment after ammonia exposure ($p < 0.05$). The hepatic T-AOC levels in the 75 mg/kg

group after post-recovery was dramatically higher than that in counterpart treatment after ammonia exposure.

The hepatic GSH-Px level in the 75 mg/kg group after ammonia exposure was higher than that in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 2H). No significant difference in hepatic GSH-Px level were observed between the control group and curcumin groups after ammonia exposure ($p < 0.05$). The hepatic GSH-Px levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). However, no statistical difference in hepatic GSH-Px levels was obtained between the 75 and 150 mg/kg groups after post-recovery ($p > 0.05$). Compared with those three groups after ammonia exposure, the hepatic GSH-Px levels in control, 75, and 150 mg/kg groups after post-recovery were statistically increased ($p < 0.05$).

3.6. Gill Enzyme Activities in Greater Amberjack (S. dumerili) in Response to Acute Ammonia Exposure and Post-Recovery

The gill ALP level in the 150 mg/kg group after ammonia exposure was dramatically lower than that in the control and 75 mg/kg treatments ($p < 0.05$) (Figure 3A). The gill ALP levels in the 75 and 150 mg/kg groups after post-recovery were dramatically lower than that in the control treatment ($p < 0.05$). Gill ALP levels in the control group and curcumin-addition groups after post-recovery were dramatically higher than those in the respective groups after ammonia exposure ($p < 0.05$).

Gill ACP levels were significantly decreased with increasing dietary curcumin addition, with a minimum in the 75 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 3B). The gill ACP levels in the 75 and 150 mg/kg groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$). Similarly, these results were also observed after post-recovery. The gill ACP levels in curcumin-addition groups after post-recovery were dramatically lower than that in the control treatment ($p < 0.05$). Compared with the control group after ammonia exposure, the gill ACP level in the control group after post-recovery was statistically increased ($p < 0.05$).

The gill SOD level was statistically increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$) (Figure 3C). Compared with the control group, gill SOD levels in the 75 and 150 mg/kg groups after post-recovery were statistically increased ($p < 0.05$). Gill SOD levels in the control and 75 mg/kg groups after post-recovery was dramatically lower than that in the control and 75 mg/kg treatments ($p < 0.05$), respectively.

The gill CAT level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 3D). The gill CAT level in the 75 mg/kg group was significantly higher than those in the control and 150 mg/kg groups after post-recovery ($p < 0.05$). The gill CAT level in the 75 mg/kg groups after post-recovery was dramatically higher than that in the counterpart group after ammonia exposure ($p < 0.05$).

The gill T-AOC level in the 150 mg/kg group after ammonia exposure was dramatically higher than that in the control and 75 mg/kg treatments ($p < 0.05$) (Figure 3E). Gill T-AOC levels in the curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). Compared with those two groups after ammonia exposure, the gill T-AOC level was significantly decreased in the control group after post-recovery, while it increased in the 75 mg/kg curcumin group after post-recovery ($p < 0.05$).

The gill MDA level in the 150 mg/kg group after ammonia exposure was dramatically lower than that in the control and 75 mg/kg treatments ($p < 0.05$) (Figure 3F). The gill MDA level in the 150 mg/kg groups after post-recovery was dramatically higher than that in the control and 75 mg/kg treatments ($p < 0.05$). Compared with the 150 mg/kg group after ammonia exposure, the gill MDA level was significantly increased in the 150 mg/kg group after post-recovery ($p < 0.05$).

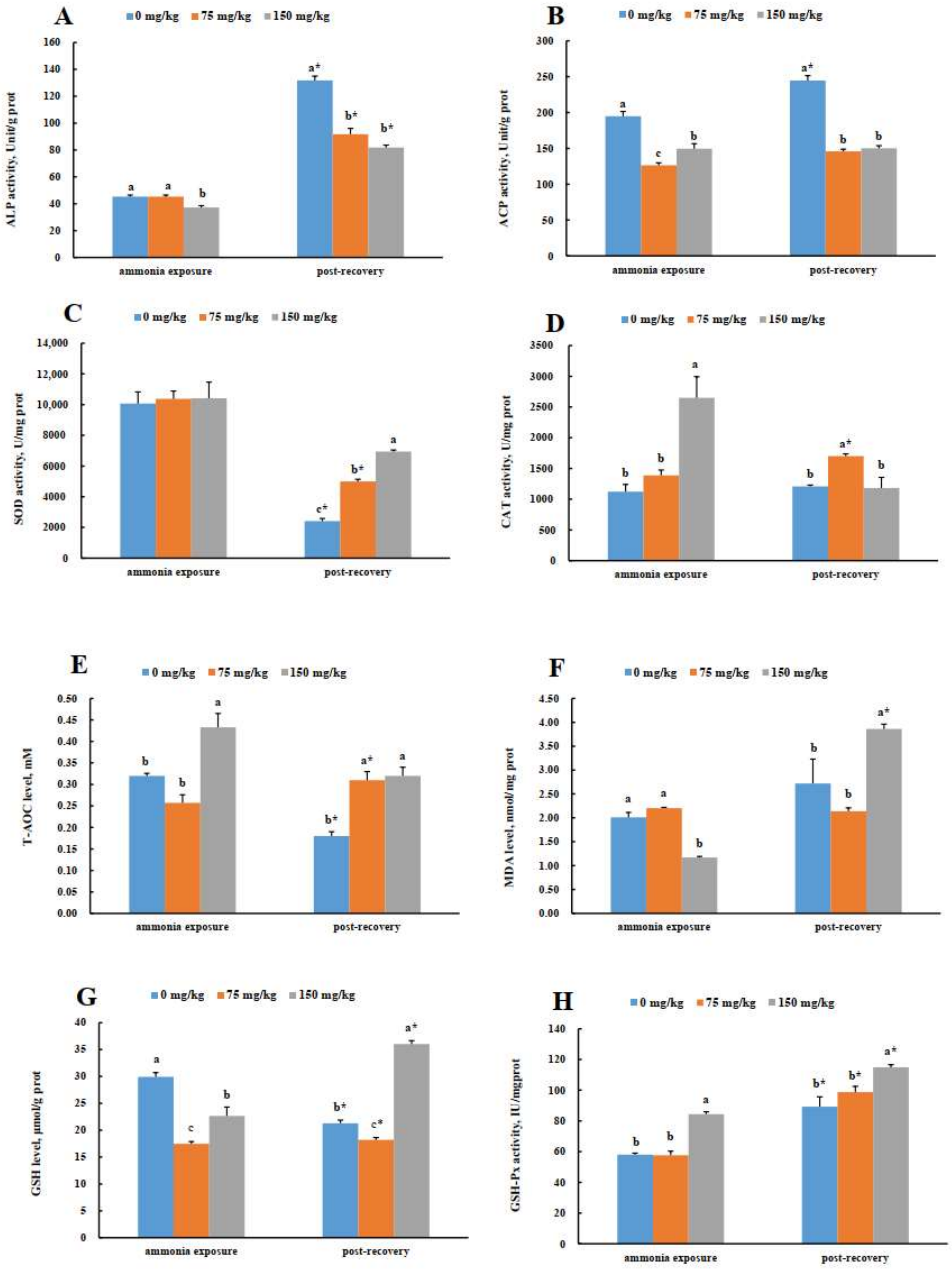

Figure 3. Gill ALP, ACP, SOD, CAT, T-AOC, MDA, GSH， and GSH-PX levels in greater amberjack (*S. dumerili*) fed dietary curcumin (**A–H**) and the response to acute ammonia exposure and post-recovery. Data are expressed as average ± standard error of the mean (SEM) (n = 9). The significant differences ($p < 0.05$) between values obtained in ammonia exposure and its post-recovery stage were determined using *t*-tests and are indicated by asterisks. Different letters indicate significant differences ($p < 0.05$) among different groups as assessed by Duncan's multi-range tests.

Gill GSH levels were significantly decreased with increasing dietary curcumin addition, with a minimum in the 75 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 3G). Gill GSH levels in the curcumin-addition groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$). Compared with the control group after post-recovery, gill GSH levels were statistically decreased in the 75 mg/kg group after post-recovery ($p < 0.05$), while they were significantly increased in the 150 mg/kg group after post-recovery ($p < 0.05$). Compared with those three groups after ammonia exposure, the gill GSH level was significantly decreased in the control group after post-recovery ($p < 0.05$), while it was increased in the 75 and 150 mg/kg group after post-recovery ($p < 0.05$).

The gill GSH-Px level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 3H). The gill GSH-Px level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$). Compared with those three groups after ammonia exposure, gill GSH-Px levels in the control, 75, and 150 mg/kg groups after post-recovery were noticeably increased ($p < 0.05$).

3.7. Spleen Enzyme Activities in Greater Amberjack (S. dumerili) in Response to Acute Ammonia Exposure and Post-Recovery

The spleen ALP level in the 150 mg/kg group after post-recovery were dramatically lower than those in the control and 75 mg/kg treatments ($p < 0.05$) (Figure 4A). Compared with the control and 75 mg/kg groups after ammonia exposure, spleen ALP levels in the control and 75 mg/kg groups after post-recovery were significantly increased ($p < 0.05$), respectively.

The spleen ACP level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 4B). ACP levels in the curcumin-addition groups after ammonia exposure were dramatically higher than that in the control treatment ($p < 0.05$). The spleen ACP level in the 75 mg/kg group after post-recovery was dramatically higher than that in the control treatment ($p < 0.05$), while it was dramatically lower in the 150 mg/kg group after post-recovery was obtained ($p < 0.05$). Compared with those three groups after ammonia exposure, spleen ACP levels were statistically increased in the control and 75 mg/kg groups after post-recovery ($p < 0.05$), while it was decreased in the 150 mg/kg group after post-recovery ($p < 0.05$).

Spleen SOD levels in the curcumin-addition group after ammonia exposure were dramatically higher than that in the control treatment ($p < 0.05$) (Figure 4C). Spleen SOD levels in the 75 mg/kg groups were significantly higher than that in the 150 mg/kg group after ammonia exposure ($p < 0.05$). Spleen SOD levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). Compared with those two groups after ammonia exposure, spleen SOD levels in the control group and 75 mg/kg groups after post-recovery were significantly decreased ($p < 0.05$).

The spleen CAT level in the 150 mg/kg group after ammonia exposure was dramatically higher than that in the control treatment ($p < 0.05$) (Figure 4D). The spleen CAT level in the 75 mg/kg group after post-recovery was dramatically higher than that in the control treatment ($p < 0.05$). The spleen CAT level in the 150 mg/kg group after post-recovery was dramatically lower than that in the counterpart group after ammonia exposure ($p < 0.05$).

The spleen T-AOC level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 4E). Compared with the control group, spleen T-AOC levels were significantly increased in the 75 and 150 mg/kg groups after ammonia exposure ($p < 0.05$). Similar results of spleen T-AOC were obtained after post-recovery. The spleen T-AOC levels were significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$). Spleen T-AOC levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). Compared with the 75 mg/kg group after ammonia exposure, the spleen CAT level was significantly decreased in the counterpart group after post-recovery ($p < 0.05$).

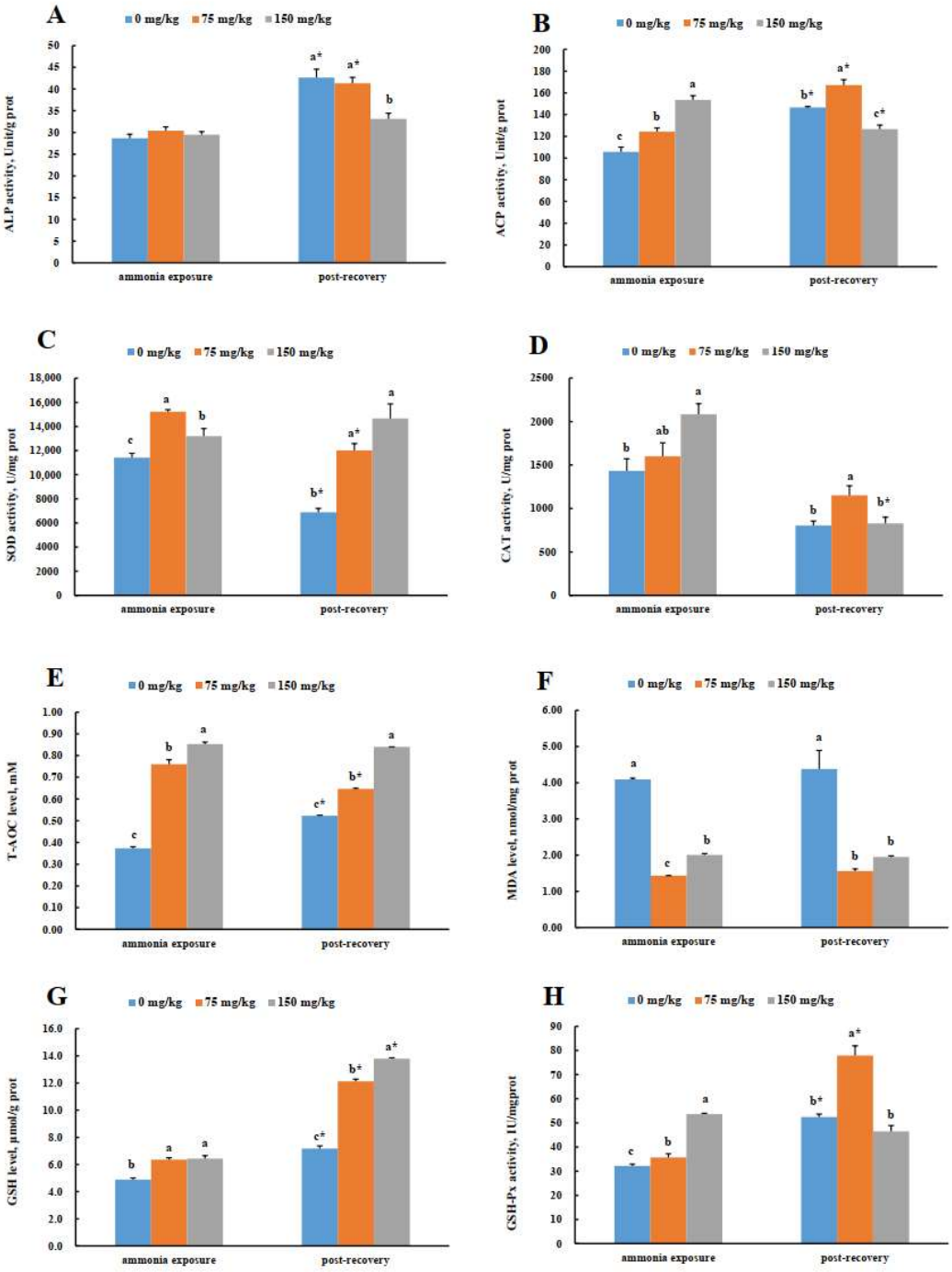

Figure 4. Spleen ALP, ACP, SOD, CAT, T-AOC, MDA, GSH ， and GSH-PX levels in greater amberjack (*S. dumerili*) fed dietary curcumin (**A**–**H**) and the response to acute ammonia exposure and post-recovery. Data are expressed as average ± standard error of the mean (SEM) (n = 9). The significant differences ($p < 0.05$) between values obtained in ammonia exposure and its post-recovery stage were determined using *t*-tests and are indicated by asterisks. Different letters indicate significant differences ($p < 0.05$) among different groups as assessed by Duncan's multi-range tests.

Spleen MDA levels in curcumin-addition groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$) (Figure 4F). The spleen MDA level in the 75 mg/kg group after ammonia exposure was statistically lower than that in the 150 mg/kg group after ammonia exposure ($p < 0.05$). Spleen MDA levels in curcumin-

addition groups after post-recovery were dramatically lower than that in the control treatment ($p < 0.05$).

Spleen GSH levels in curcumin-addition groups after ammonia exposure were dramatically higher than that in the control treatment ($p < 0.05$) (Figure 4G). Spleen GSH levels were dramatically increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$). Spleen GSH levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). Compared with those three groups after ammonia exposure, spleen GSH levels in the control, 75, and 150 mg/kg groups after post-recovery were significantly increased ($p < 0.05$).

Spleen GSH-Px levels were statistically increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 4H). Compared with the control group, spleen GSH-Px levels were significantly increased in the 75 and 150 mg/kg groups after ammonia exposure ($p < 0.05$). The spleen GSH-Px level in the 75 mg/kg group after post-recovery was dramatically higher than that in the control treatment ($p < 0.05$). Spleen GSH-Px levels in the control and 75 mg/kg groups after post-recovery were dramatically higher than those in the respective groups after ammonia exposure ($p < 0.05$).

3.8. Head Kidney Enzyme Activities in Greater Amberjack (S. dumerili) in Response to Acute Ammonia Exposure and Post-Recovery

Head kidney ALP levels were significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 5A). Compared with the control group, head kidney ALP levels were significantly increased in the 75 and 150 mg/kg groups after ammonia exposure ($p < 0.05$). Compared with the control group, head kidney ALP levels in the 75 and 150 mg/kg groups after post-recovery were statistically increased ($p < 0.05$). Head kidney ALP levels in the control and 150 mg/kg groups after post-recovery were dramatically higher than those in the respective treatments after ammonia exposure ($p < 0.05$).

The head kidney ACP level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$) (Figure 5B).

The head kidney SOD level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 5C). Head kidney SOD levels in the 75 and 150 mg/kg groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). The head kidney SOD level in the 75 mg/kg group after post-recovery was significantly higher than that in the 150 mg/kg groups after post-recovery ($p < 0.05$). Head kidney SOD levels in the control and 150 mg/kg groups after post-recovery were dramatically higher than those in the respective groups after ammonia exposure ($p < 0.05$).

The head kidney CAT level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 5D). Compared with the 150 mg/kg group after ammonia exposure, the head kidney CAT level in the 150 mg/kg group after post-recovery was dramatically lower than that in the counterpart group after ammonia exposure ($p < 0.05$).

The head kidney T-AOC level in the 75 mg/kg group after ammonia exposure was dramatically higher than that in the control treatment ($p < 0.05$) (Figure 5E). Head kidney T-AOC levels were significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$). Head kidney T-AOC levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). The head kidney T-AOC level in the 75 and 150 mg/kg groups after post-recovery was dramatically higher than those in the respective groups after ammonia exposure ($p < 0.05$).

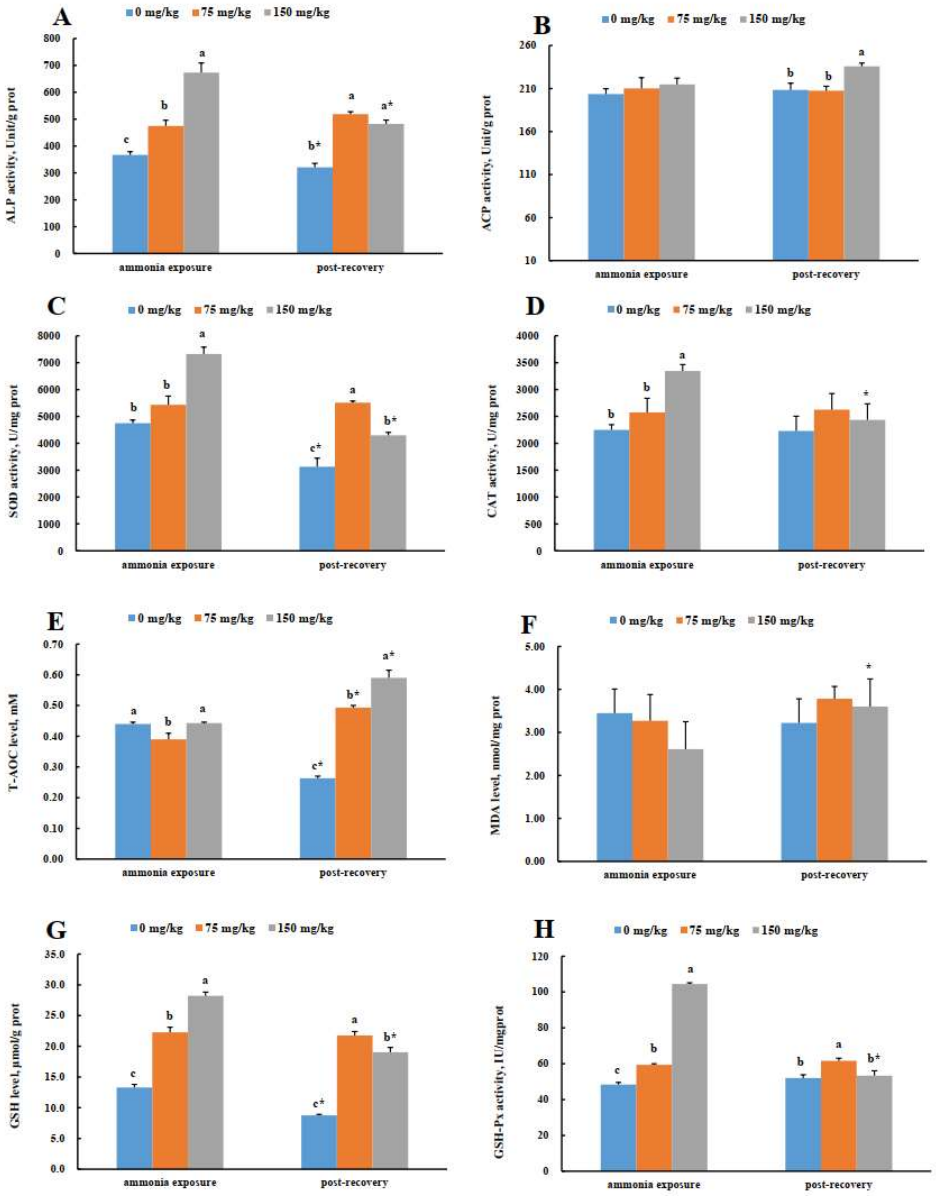

Figure 5. Head kidney ALP, ACP, SOD, CAT, T-AOC, MDA, GSH， and GSH-PX levels in greater amberjack (*S. dumerili*) fed dietary curcumin (**A–H**) and the response to acute ammonia exposure and post-recovery. Data are expressed as average ± standard error of the mean (SEM) (n = 9). The significant differences ($p < 0.05$) between values obtained in ammonia exposure and its post-recovery stage were determined using *t*-tests and are indicated by asterisks. Different letters indicate significant differences ($p < 0.05$) among different groups as assessed by Duncan's multi-range tests.

Compared with the 150 mg/kg group after ammonia exposure, the head kidney MDA level in the counterpart group after post-recovery was significantly increased ($p < 0.05$) (Figure 5F).

Head kidney GSH levels were significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 5G). Compared with the control group, head kidney GSH levels were significantly increased in the 75 and 150 mg/kg groups after ammonia exposure ($p < 0.05$). Head kidney GSH levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). The head kidney GSH level in the 75 mg/kg group after post-recovery was dramatically increased compared with the 150 mg/kg groups after post-recovery ($p < 0.05$). Head kidney GSH levels in control and

150 mg/kg groups after post-recovery were dramatically lower than those in the respective groups after ammonia exposure ($p < 0.05$).

Head kidney GSH-Px levels were significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 5H). Head kidney GSH levels in the 75 and 150 mg/kg groups after ammonia exposure were dramatically higher than that in the control treatment ($p < 0.05$). The head kidney GSH-Px level in the 75 mg/kg group after post-recovery was dramatically higher than that in the control treatment ($p < 0.05$). Compared with the control and 75 mg/kg groups after ammonia exposure, head kidney GSH-Px levels in the counterpart group after post-recovery were significantly increased ($p < 0.05$).

3.9. Brain Enzyme Activities in Greater Amberjack (S. dumerili) in Response to Acute Ammonia Exposure and Post-Recovery

The brain ALP level in the 150 mg/kg group after post-recovery was dramatically lower than that in the control treatment ($p < 0.05$) (Figure 6A). Compared with groups after ammonia exposure, brain ALP levels in the respective groups after post-recovery were noticeably declined ($p < 0.05$). Brain ACP levels were also observed among all groups after post-recovery ($p > 0.05$) (Figure 6B).

The brain SOD level was significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after ammonia exposure ($p < 0.05$) (Figure 6C). The brain SOD level in the 150 mg/kg group after ammonia exposure was dramatically higher than that in other treatments ($p < 0.05$). Compared with the control group after post-recovery, the brain SOD level was significantly increased in the 150 mg/kg group after post-recovery ($p < 0.05$). Compared with the 75 and 150 mg/kg groups after ammonia exposure, brain SOD levels in the respective groups after post-recovery were significantly increased ($p < 0.05$).

The brain T-AOC level in the 75 mg/kg group after post-recovery was dramatically higher than that in the control treatment ($p < 0.05$) (Figure 6E). Compared with counterpart groups after ammonia exposure, the brain T-AOC level in the control group after post-recovery was significantly decreased ($p < 0.05$), while the brain T-AOC level in the 75 mg/kg group after post-recovery was significantly increased ($p < 0.05$).

Brain MDA levels in curcumin-addition groups after ammonia exposure were dramatically lower than that in the control treatment ($p < 0.05$) (Figure 6F). The brain MDA level in the 75 mg/kg group after ammonia exposure was significantly lower than that in the 150 mg/kg group after ammonia exposure ($p < 0.05$). Brain MDA levels in curcumin-addition groups after post-recovery were dramatically lower than that in the control treatment ($p < 0.05$). The brain MDA level in the control and 150 mg/kg groups after post-recovery was dramatically lower ($p < 0.05$), while it was higher in the 75 mg/kg group after post-recovery than those in counterpart groups after ammonia exposure ($p < 0.05$).

Brain GSH levels were significantly increased with increasing dietary curcumin addition, reaching a maximum in the 150 mg/kg group after post-recovery ($p < 0.05$) (Figure 6G). Brain GSH levels in curcumin-addition groups after post-recovery were dramatically higher than that in the control treatment ($p < 0.05$). Compared with the 75 mg/kg group after ammonia exposure, the brain GSH level in the counterpart group after post-recovery was statistically decreased ($p < 0.05$).

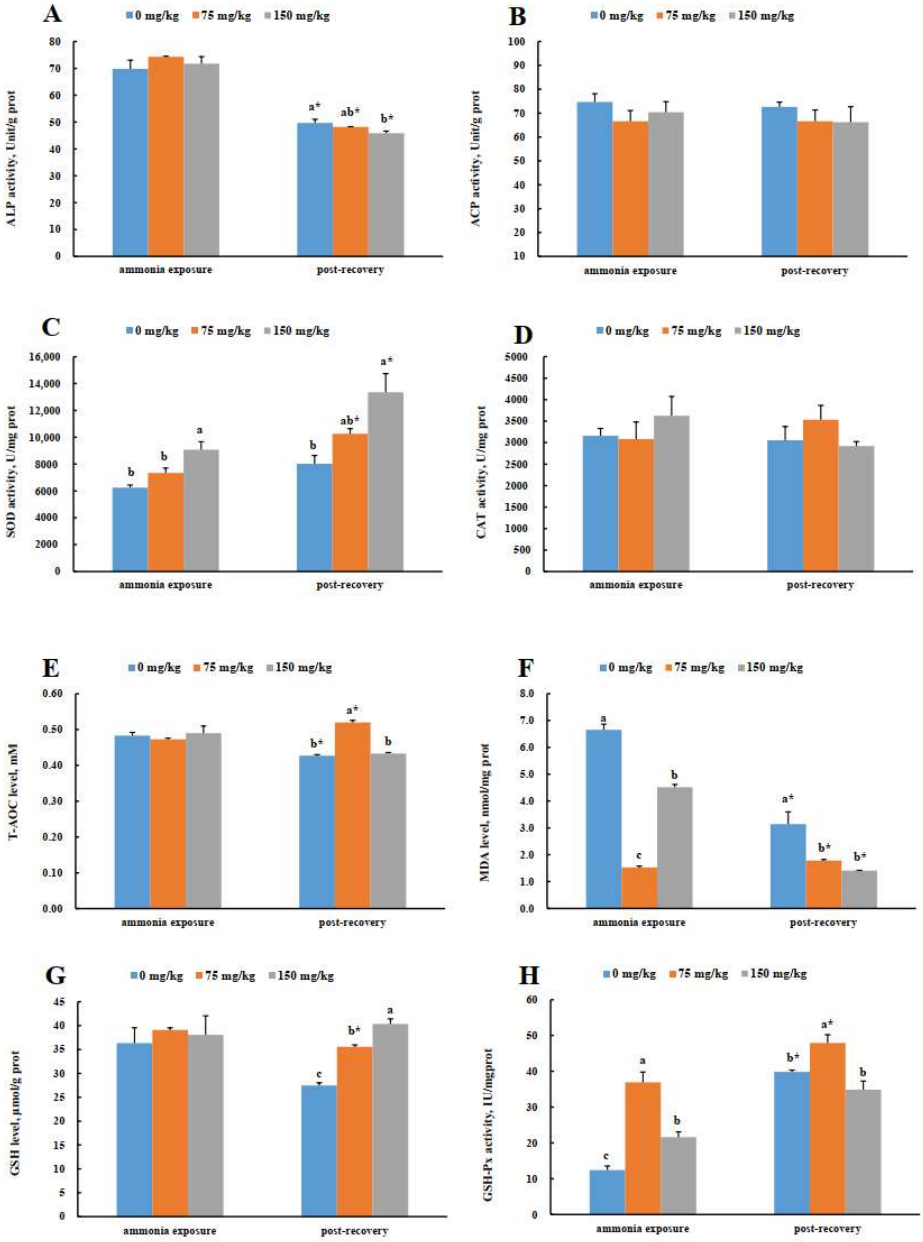

Figure 6. Brain ALP, ACP, SOD, CAT, T-AOC, MDA, GSH， and GSH-PX levels in greater amberjack (*S. dumerili*) fed dietary curcumin (**A**–**H**) and the response to acute ammonia exposure and post-recovery. Data are expressed as average ± standard error of the mean (SEM) (n = 9). The significant differences ($p < 0.05$) between values obtained in ammonia exposure and its post-recovery stage were indicated determined using *t*-tests and are indicated by asterisks. Different letters indicate significant differences ($p < 0.05$) among different groups as assessed by Duncan's multi-range tests.

Brain GSH-Px levels in curcumin-addition groups after ammonia exposure were dramatically higher than that in the control treatment ($p < 0.05$) (Figure 6H). The brain GSH-Px level in the 75 mg/kg group after ammonia exposure was significantly higher than that in the 150 mg/kg groups after ammonia exposure ($p < 0.05$). The brain GSH-Px level in the 75 mg/kg group after post-recovery was dramatically higher than that in the control treatment ($p < 0.05$). Compared with the control and 75 mg/kg groups after ammonia exposure, brain GSH-Px levels in counterpart groups after post-recovery were statistically increased ($p < 0.05$).

3.10. Effect of Dietary Curcumin on Hepatic Histological Changes of Greater Amberjack (Seriola dumerili)

Compared to the control group, liver tissues in the curcumin-addition groups had more intact morphology and showed no signs of cell swelling or increased vacuolation (Figure 7A–C).

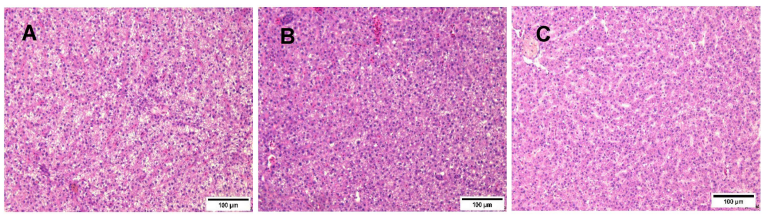

Figure 7. The microscopic hepatic structure of greater amberjack (*S. dumerili*) fed with diets containing different amounts of curcumin for eight weeks. (**A**) represents 0 mg/kg group; (**B**) represents 75 mg/kg group; and (**C**) represents 150 mg/kg group.

4. Discussion

Phosphatases, as part of the immune system, play a crucial role in protecting animals. Based on their catalytic optimum pH properties, phosphatases include two groups: alkaline phosphatases (ALPs) and acid phosphatases (ACPs) [23]. Phosphatases are pivotal lysosomal enzymes, and they are involved in humoral immune responses and degradation of exogenous nutrients, such as proteins, lipids, and carbohydrates [24]. ALPs are a superfamily of metalloenzymes. These enzymes exist in a wide range of organisms from bacteria to humans. ALPs can play an important catalytic role in the hydrolysis of phosphate monoesters to inorganic phosphate and the corresponding alcohols, phenols, and sugars [25]. ACP is a natural enzyme which exists widely in animals and plants. It plays an important role in many important immune defenses [26]. In acidic environments, ACP can play an immune role. It degrades or destroys pathogens by recognizing and catalyzing the hydrolysis of phosphate bonds of exogenous substances[27]. In the current study, ALP levels in the intestine and liver were significantly improved in fish fed with dietary curcumin compared with the control group. Similarly, the results of this study demonstrated that dietary curcumin promoted ACP levels in plasma, the intestine, and the liver of greater amberjack. All the results suggested that curcumin could enhance innate immune response in fish species, and curcumin has been considered to be an effective immunomodulator in both animals and humans [9].

The strength of the antioxidant capacity of the body defense system is closely related to health in fish [28,29]. Oxidative stress can lead to the generation of ROS and free radicals in fish [29,30]. In addition, a variety of antioxidant factors (e.g., SOD, T-AOC, GSH, and GSH-Px) are required to maintain immune system of fish [31]. Antioxidant capacity comprises both enzymatic and non-enzymatic antioxidant activities. Antioxidant enzymes include SOD, T-AOC, and GSH-Px, which form the first line of the organism's enzymatic defense mechanism against free radicals. T-AOC capacity is a comprehensive reflection of redox status in many aspects, such as the body, the antioxidant system of enzymes, and so on [28]. GSH is the primary antioxidant in the human body to counteract damage caused by free radicals, which is a contributing factor to aging and disease. In general, antioxidant defense in fish depends in some way on nutritional factors [32]. Curcumin is a natural ingredient that can also enhance the activities of antioxidative enzymes [33]. Curcumin exerts antioxidant effects mainly due to the presence of polyphenolic compounds as its main component [9,34]. Many plant extracts (also called herbal extracts) contain such substances, thus exerting antioxidant effects directly or indirectly [34,35]. These polyphenols can suppress oxidative stress and then prevent the catalytic mechanism of cytochrome P450CYP, and they can eventually neutralize ROS reagents [34]. The results of this study showed that dietary curcumin dramatically promoted the levels of hepatic SOD, T-AOC, GSH, and GSH-Px in greater amberjack. Similarly, previous studies showed that dietary

herbal extract can upregulate antioxidant-related gene expression in fish [34,36]. The activities of glutathione peroxidase and superoxide dismutase were increased in Siberian sturgeon (*Acipenser baerii*) treated with different levels of barberry fruit (BF) extract [37]. Wang et al. (2020) also found an increase in the activity of SOD in black sea bream (*Acanthopagrus schlegelii*) fed berberine [38]. However, a study on rainbow trout showed that the expression of antioxidant-related genes was downregulated by dandelion flower (DF) extract [34]. Similar results were also found in gilthead seabream (*Sparus aurata*) fed with palm fruit extracts [39]. The reason for this contradiction may be due to a time-lag effect between transcription and translation [40]. Further studies are needed to determine the reason for the changes in the expression of antioxidant genes and/or antioxidant enzyme activities in response to dietary levels of plant extract. Meanwhile, in the present study, the hepatic histomorphology in the curcumin group showed more intact morphology and no signs of cell swelling or increased vacuolation, indicating that curcumin addition plays a beneficial role in liver health. This result could also confirm that curcumin has hypolipidemic effects and may prevent the accumulation of fatty acids in liver cells that may result from metabolic imbalances and nonalcoholic steatohepatitis [41].

Previous studies have shown that the specific activities of immune-related enzymes in aquatic animals under ammonia stress were significantly reduced [42–44], and high concentrations of ammonia have a serious impact on the catalytic action of antioxidant enzymes and the stability of cell membranes in aquatic animals and thus disrupt the osmotic balance [45]. In this study, curcumin addition improved the activities of antioxidant enzymes and MDA content in the liver, spleen, head kidney, and brain tissues after post-recovery. This suggests that curcumin attenuated oxidative stress caused by ammonia stress via increasing antioxidant enzyme activities and decreasing the content of MDA. This result coincided with previous studies on Japanese Sea bass [12] and largemouth bass [13]. In this study, curcumin addition increased ALP levels in the liver and head kidney, but it decreased ALP levels in the gills, spleen, and brain. Meanwhile, curcumin addition increased ACP levels in the intestine and head kidney, but it decreased ACP levels in the liver and gills. This may be due to the fact that enzyme activity is influenced by a variety of factors. Environmental conditions such as pH, temperature, salt concentration, substrate concentration, activators, and inhibitors may alter the spatial structure of the enzyme, thus changing its rate of activity and/or ability to bind substrates [46]. This inconsistency requires further research to elucidate.

In aquatic environments, ammonia accumulation could result in acute toxicity to aquatic animals. In the process of resisting environmental toxic and harmful substances, the organs of aquatic animals (e.g., the gill, liver, spleen, etc.) play a pivotal role [5]. In this study, the fish showed the ability to regulate themselves. Specifically, the indexes associated with immunity and antioxidant enzymes in the liver, gill, and spleen rose again to some extent, but they showed the worst recovery ability in head kidney and brain tissue. Similar studies can be found in juvenile blunt snout bream [2]. In addition, compared with other groups, the 75 mg/kg curcumin group generally significantly increased the recovery level of related enzymes in these tissues. This suggested that adding 75 mg/kg of curcumin can enhance the antioxidant abilities and promote the overall health of the fish.

5. Conclusions

In conclusion, ammonia stress leads to metabolic changes and induces oxidative stress in juvenile *S. dumerili*. Reduced antioxidant enzyme activities revealed that exposure to high concentrations of ammonia may induce more severe oxidative stress. Recovery of antioxidant enzyme activities revealed that post-exposure recovery may attenuate oxidative stress to some extent. Dietary curcumin supplementation could increase non-specific immune responses, antioxidant ability, and enhance resistance to high ammonia stress in juvenile *S. dumerili*. The outcomes of this study will facilitate future research on the effects of ammonia toxicity in juvenile *S. dumerili* and provide technical support for prevention of disease and alleviation of stress.

Author Contributions: C.Z. contributed to the original idea. H.L. and Z.M. contributed with experimental design. Z.H., J.W., Y.W., W.Y., S.Z., J.H., R.Y. and C.Z. conducted the experiment and analyzed all the samples. All 10 authors contributed to the draft and final manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in strict accordance with the recommendation of the Ethics Committee of South China Sea Fisheries Institute. No protected species were used during the experiment.

Informed Consent Statement: Not applicable (Present study did not involve humans). Written informed consent has been obtained from all the patients to publish this paper.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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