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Effects of ammonia on growth, digestion and antioxidant capacity in juvenile yellow catfish *Pelteobagrus fulvidraco* (Richardson, 1846)

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Summary

Effects of ammonia stress on food ingestion, growth, digestion and antioxidant capacity were investigated in juvenile yellow catfish *Pelteobagrus fulvidraco* (Richardson) with initial body weights of 20.24 ± 0.18 g. The fish were reared in triplicate in 15 experimental tanks at a rate of 30 fish per tank for 56 days. Water was maintained at a dissolved oxygen (DO) level of over 6.2 mg L^{-1}, pH 7.2–7.6, and temperature of 29.0 ± 1.5°C under a natural 12L: 12D photoperiod. Survival, food ingestion (FI), specific growth rate (SGR), food conversion efficiency (FCE), apparent digestibility coefficient (ADC), total antioxidant capacity (T-AOC), levels of glutathione (GSH) and malonaldehyde (MDA), and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) of the juveniles were determined in total ammonia nitrogen (NH_3-N+NH_4-N) levels of 0 (control group), 3.36, 6.72, 13.44 and 26.88 mg L^{-1}. The results show that the survival, FI, SGR, FCE, and ADC decreased significantly with an increase in total ammonia nitrogen ($p < .05$), and a significantly negative correlation between SGR and total ammonia nitrogen levels ($p < .05$). T-AOC, SOD, CAT, GSH-PX and GSH in the blood, liver and gills were found to decline significantly with an increase in the total ammonia nitrogen level ($p < .05$), while the MDA in the blood, liver and gills was elevated significantly with the increase in total ammonia nitrogen ($p < .05$). The results indicate a threshold in the induction of the T-AOC and activities of antioxidant enzymes in yellow catfish tissues with a total ammonia nitrogen increase. In the present study the total ammonia nitrogen threshold thus changed from 6.72 mg L^{-1} in the juvenile yellow catfish.

1 | INTRODUCTION

Ammonia nitrogen is one of the major toxicants in aquaculture environment, which beyond a certain level leads to physiological dysfunction and becomes fatal or lethal to animals when in higher concentrations (Hegazi, Attia, & Ashour, 2010; Medeiros, Lopez, Sampaio, Romano, & Rodrigue, 2016; Sun, Wang, Li, & Yang, 2014). Poor growth, lower immunity, higher susceptibility to bacterial pathogens, and thus high mortality were observed in animals exposed to long-term high concentrations of ammonia nitrogen (Kasturi, Lutz, & Peter, 2006; Seongdeok, Pyong Kih, & Joong-Kyun, 2014; Sun et al., 2012). The food conversion ratio in aquaculture was found to increase in animals with long-term exposure to ammonia nitrogen (Foss, Vollen, & Øiestad, 2003). Adverse physiological and hispathological reactions, including congestion and hemorrhaging in tissues, organs, blood composition and destruction of erythrocytes, were found in fish exposed to sub-lethal concentration of unionized ammonia (Pinto et al., 2016; Seongdeok et al., 2014; Thurston et al., 1984). The total antioxidant capacity (T-AOC) is a comprehensive evaluation index including an antioxidant enzyme system and non-enzymatic

system for oxidative stress as a whole (Tan, He, Yan, & Liang, 2005). Components of the antioxidant defense system could be changed in fish exposed to ammonia pollutants; as a result, antioxidant enzymes could induce redox cycling, suggesting oxidative stress and lipid peroxidation and oxydative damage by the pollutants (Sun et al., 2014; Zhao, 2006).

Yellow catfish (*Pelteobagrus fulvidraco*) is an economically important candidate for commercial aquaculture. However, knowledge on rearing technology seems inadequate; although the fish is cultivated traditionally at high densities and feeding rates in China, the problems of high FCR and low yield persist (Hagopian & Riley, 1998). It is surmised that the low yield is probably due to high concentrations of ammonia, hydrogen sulfide and nitrite in ponds. It is known that ammonia derived from feces and remaining feed is transformed into inorganic ammonia nitrogen due to interactions with microorganisms in ponds (Hargreaves, 1998). To improve on this knowledge, the present study investigated the effects of four total ammonia-nitrogen concentrations on the performance of juvenile *Peltobagrus fulvidraco* using the parameters of food ingestion, growth performance, digestion and antioxidant defense as assessment criteria.

2 | MATERIALS AND METHODS

2.1 | Materials and facility

Healthy, disease-free and with no apparent injuries, 5000 yellow catfish *P. fulvidraco* juveniles were purchased from the Seedling Breeding Base, State Freshwater Fisheries Research Center, Bihai Demonstration Garden of Agricultural Science and Technology in Hubei Province. The juveniles were held for 2 weeks in 12 indoor concrete tanks each 2.1 m length × 1.6 m width × 1.1 m height and 3.0 m^3 capacity in a natural photoperiod rhythm of 12L: 12D. Water in all tanks was aerated for 24 h, except when feeding. Dissolved oxygen (6.8 ± 0.6 mg L^{-1}) and water temperature (29.0 ± 1.5°C) inside all tanks were measured daily for 2 weeks. Juveniles were fed to satiation ad libitum twice a day at 07.00 hr and 18.00 hr.

As an inertia indicator, 1% of Cr_2O_3 was added to the feed used in present experiment. Ingredients and approximate diet compositions are given in Table 1. The 2 mm Ø pellet feed was first dried in a 60°C oven and maintained at 4°C prior to feeding.

The experimental facility consisted of 15 separate circular fiberglass tanks each 80 cm Ø × 70 cm in height and a 180-L volume. The *P. fulvidraco* juveniles with body weights of (20.24 ± 0.18) g (n = 450) were fasted for 1 day and then moved from the holding tanks into 15 experimental tanks at a rate of 30 fish per tank and reared for 56 days in triplicate. Chemical NH_4Cl (analytical reagent, AR) used in the experiment was dried to a constant weight and prepared for stock solution at a concentration of 10 g L^{-1}, then diluted to the applied concentrations. Except when fish were feeding, water in the circular fiberglass tanks was aerated for 24 hr under a natural photoperiod of 12L:12D. Water quality parameters during the feeding trial are given in Table 2. Total ammonia nitrogen (NH_3-N+NH_4-N) concentrations were measured by Nessler's reagent colorimetric method with a precision of 0.01 mg L^{-1} daily in each tank (Chen, 2006). The total ammonia nitrogen level was maintained at a concentration of below 0.20 mg L^{-1} in the control group. The solutions of apparent concentrations were regulated by the stock solution in time, and two-thirds of the experimental solution was exchanged in a 24-hr interval.

TABLE 1 Ingredients and approximate experimental diet composition for yellow catfish *Pelteobagrus fulvidraco*

Ingredients	Contents (%)
White fishmeal[a]	35.00
Fish oil	1.00
α-starch	5.00
Soybean meal[b]	36.00
Wheat flour	17.00
Soybean oil	1.00
Immune polysaccharide	0.10
Mineral premix[c]	3.40
Vitamin premix[d]	0.30
Vitamin C	0.10
Choline chloride	0.10
Chromic oxide	1.00
Approximate composition (% dry matter)	
Dry matter	94.23
Crude protein	40.54
Crude fat	3.87
Ash	9.28
Gross energy (kJ g^{-1})	17.28

[a]Imported from Peru.
[b]By-product of soaked-oil soybean without squeezing.
[c]Mineral premix (mg kg^{-1} diet): NaCl, 500; $MgSO_4{\cdot}7H_2O$, 7500; $NaH_2PO_4{\cdot}2H_2O$, 12500; KH_2PO_4, 16000; $Ca(H_2PO_4)_2{\cdot}H_2O$,100 000; $FeSO_4$, 1250; $C_6H_{10}CaO_6{\cdot}5H_2O$, 1750; $ZnSO_4{\cdot}7H_2O$, 176.5; $MnSO_4{\cdot}4H_2O$, 81; $CuSO_4{\cdot}5H_2O$, 15.5; $CoSO_4{\cdot}6H_2O$, 0.5; KI, 1.5; starch, 22.5.
[d]Vitamin premix (mg kg^{-1} diet): thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 2; folic acid, 5; calcium pantothenate, 50; inositol, 100; niacin, 100; biotin, 5; starch, 3226; ascorbic acid, 111; vitamin A, 110; vitamin D_3, 20; vitamin E (DL-α-tocopherol acetate), 100; vitamin K_3 (menadione sodium bisulphite), 10.

The concentration of un-ionized ammonia was determined from $[NH_3]$ (mg L^{-1}) = f $[NH_4^+ + NH_3]$ (mg L^{-1}), where f is defined as the percentage of un-ionized ammonia at a specific pH. The value of f can be calculated using $f = 1/[10^{(pKa - pH)} + 1]$, with pKa the dissociate constant for ammonia calculated using pKa = 0.09018 + 2729.92/T, where T is the temperature in Kelvin (K) (Emerson, Russo, Lund, & Thurston, 1975).

2.2 | Experiment design

The experiment design was carried out according to the acute toxicity by Zhang et al. (2012), and composed five groups with total ammonia nitrogen concentrations: 0 mg L^{-1} (control group), 3.36 mg L^{-1}, 6.72 mg L^{-1}, 13.44 mg L^{-1} and 26.88 mg L^{-1}. In the experiment, the

TABLE 2 Mean (±SD) water quality parameters during a 56 days feeding trial at various total ammonia nitrogen concentrations (TAN, mg L^{-1}).

TAN (mg L^{-1})	0	3.36	6.72	13.44	26.88
Actual TAN (mg L^{-1})	0.08 ± 0.01	3.15 ± 0.17	6.46 ± 0.20	13.15 ± 0.26	25.41 ± 0.73
NH_3-N (mg L^{-1})	0.00 ± 0.00	0.07 ± 0.00	0.13 ± 0.00	0.28 ± 0.01	0.56 ± 0.02
Water temperature (°C)	29.0 ± 1.5	29.0 ± 1.5	29.0 ± 1.5	29.0 ± 1.5	29.0 ± 1.5
Dissolved oxygen (mg L^{-1})	6.95 ± 0.45	6.98 ± 0.53	7.04 ± 0.42	6.93 ± 0.48	6.80 ± 0.51
pH	7.42 ± 0.09	7.43 ± 0.09	7.45 ± 0.10	7.46 ± 0.08	7.48 ± 0.07

yellow catfish juveniles were fed to satiation ad libitum at 7.00 hr and 18.00 hr, and the feed amount was recorded daily. Feed remnants were siphoned 40 min after feeding, dried to constant weight and then weighed. Quantitative feed was soaked in water for 1 hr, then siphoned, dried, and weighed in order to estimate the remnant recovery and to adjust the food ingestion. Feces were siphoned 2 hr after feeding, dried to constant weight and then weighed for use according to Zhang et al. (2011).

2.3 | Preparation and storage of samples

Fish were fasted for 1 day at the end of the experiment, 15 individuals were then sampled from each experimental tank, and anesthetized immediately by MS-222 at a dose of 200 mg L^{-1}. The blood sample was taken from the caudal vein and put into vials numbered according to the tank, placed at 4°C for 4 hr, then centrifuged at 2500 r min^{-1} for 15 min. The supernatant or serum was put into a 1.5 ml centrifugal tube, sealed and refrigerated at −20°C until used.

Fish samples were placed in an ice tray to collect gill filaments and open the abdominal cavity to remove the liver for analysis. Both the liver and the gill filaments were rinsed with 0.85% saline, and dried on the surface with filter paper. The liver and gill filaments in the same tank were weighed collectively, recorded, marked and maintained at low temperatures for detection of antioxidant capacity.

2.4 | Determination of antioxidant capacity

Fluid of crude enzyme extract was prepared from the frozen liver and gill filaments of yellow catfish. The samples were put in a 50 ml beaker, minced and homogenized on the ice plate, with 0.85% normal saline added at 4°C as the tissue was being homogenized. The 20% crude fluids of enzyme extract were prepared from the tissue homogenates via frozen centrifugation at 12000 r min^{-1} for 30 min at 4°C. According to Sun et al. (2012), the total antioxidant capacity (T-AOC), activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), glutathione (GSH) and malonaldehyde (MDA) in the tissues of yellow catfish were determined using the kit developed by the Nanjing Jiancheng Biological Engineering Institute (China).

2.5 | Determination and calculation of indices

Food ingestion (FR), specific growth rate in wet weight (SGRw), specific growth rate in dry weight (SGRd), specific growth rate in protein (SGRp), specific growth rate in energy (SGRe), feed conversion efficiency in wet weight (FCEw), feed conversion efficiency in dry weight (FCEd), feed conversion efficiency in protein (FCEp), and feed conversion efficiency in energy (FCEe) were estimated as:

$$\mathrm{FR},\%\,\mathrm{day}^{-1} = \mathrm{FI}\times 100/[(W_t + W_0)/2]\times t \quad (1)$$

$$\mathrm{SGRw},\%\,\mathrm{day}^{-1} = 100\times(\ln W_t - \ln W_0)/t \quad (2)$$

$$\mathrm{SGRd},\%\,\mathrm{day}^{-1} = 100\times[\ln (W_t\times \mathrm{CD}_t) - \ln (W_0\times \mathrm{CD}_0)]/t \quad (3)$$

$$\mathrm{SGRp},\%\,\mathrm{day}^{-1} = 100\times[\ln (W_t\times \mathrm{CP}_t) - \ln (W_0\times \mathrm{CP}_0)]/t \quad (4)$$

$$\mathrm{SGRe},\%\,\mathrm{day}^{-1} = 100\times[\ln (W_t\times \mathrm{CE}_t) - \ln (W_0\times \mathrm{CE}_0)]/t \quad (5)$$

$$\mathrm{FCEw},\% = 100\times(W_t - W_0)/\mathrm{FI} \quad (6)$$

$$\mathrm{FCEd},\% = 100\times(W_t\times \mathrm{CD}_t - W_0\times \mathrm{CD}_0)/(\mathrm{FI}\times \mathrm{CD}) \quad (7)$$

$$\mathrm{FCEp},\% = 100\times(W_t\times \mathrm{CP}_t - W_0\times \mathrm{CP}_0)/(\mathrm{FI}\times \mathrm{CP}) \quad (8)$$

$$\mathrm{FCEe},\% = 100\times(W_t\times \mathrm{CE}_t - W_0\times \mathrm{CE}_0)/(\mathrm{FI}\times \mathrm{CE}) \quad (9)$$

where: FI, total food ingestion per fish (g); W_t, final wet body weight (g); W_0, initial wet body weight (g); CD_t, final dry body weight (%); CD_0, initial dry body weight (%); CP_t, protein percent of final wet body weight (%); CP_0, protein percent of initial wet body weight (%); Ce_t, energy value of final body weight (kJ g^{-1}); Ce_0, energy value of initial body weight (kJ g^{-1}); CD, dry material in diet (%); CP, dietary protein (%); CE, dietary energy (kJ g^{-1}); t, experimental period (day).

Apparent digestibility coefficient in dry matter (ADCd), apparent digestibility coefficient in crude protein (ADCp), and apparent digestibility coefficient in gross energy (ADCe) were estimated as:

$$\mathrm{ADCd},\% = 100\times(1 - C_1/C_2) \quad (10)$$

$$\mathrm{ADCp},\% = 100\times(1 - C_1P_2/C_2P_1) \quad (11)$$

$$\mathrm{ADCe},\% = 100\times(1 - C_1E_2/C_2E_1) \quad (12)$$

where: C_1, Cr_2O_3 content in feed (%); C_2, Cr_2O_3 content in feces (%); P_1, protein content in feed (%); P_2, protein content in feces (%); E_1, energy content in feed (%); E_2, energy content in feces (kJ g^{-1}).

2.6 | Statistical analysis

Statistical analysis was completed using Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA). Data were analyzed by one-way ANOVA after a homogeneity of variance test, and data were expressed as mean ± SE. When significant differences were found, Duncan's multiple range tests were used to identify differences among experimental groups. Differences were considered significant at the level $p < .05$.

3 | RESULTS

3.1 | Feeding and growth performance

Yellow catfish survival decreased significantly with the increase in ammonia levels ($p < .05$) (Fig. 1). In the control, *P. fulvidraco* had a 98.88% average survival at the end of the experiment. Yellow catfish exposed to total ammonia nitrogen for 56 days had a 91.11% survival rate at 3.36 mg L^{-1}, 86.67% at 6.72 mg L^{-1}, 73.33% at 13.44 mg L^{-1}, and 58.89% at 26.88 mg L^{-1}.

Feeding, growth and feed conversion efficiency of yellow catfish at various total ammonia nitrogen concentrations are given in Table 3, showing the significant effects on the fish ($p < .05$). The FR and SGR including SGRw, SGRd, SGRp, and SGRe, and FCE including FCEw, FCEd, FCEp and FCEe decreased significantly with an increase in the total ammonia nitrogen concentration ($p < .05$). There was a significantly negative correlation between SGR and the total ammonia nitrogen level in the experiment ($p < .05$) (Fig. 2).

Apparent digestibility including ABCd, ADCp, and ADC significantly declined with elevated total ammonia nitrogen concentrations (Fig. 3) ($p < .05$). There was a significantly lower apparent digestibility coefficient in crude protein (ADCp) at a total ammonia nitrogen level of 26.88 mg L^{-1} than in the other total ammonia nitrogen concentrations ($p < .05$).

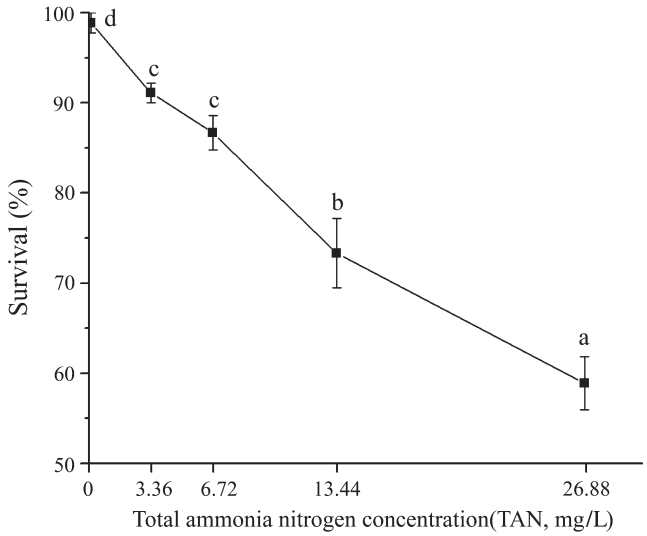

FIGURE 1 Relationship between survival rate of yellow catfish *Pelteobagrus fulvidraco* and total ammonia nitrogen concentration (TAN, mg L^{-1}) after 56 days feeding trial at 29.0 ± 1.5°C and pH 7.45 ± 0.18. Values (mean ± SE, $n = 3$) with different letters in same line = significantly different ($p < .05$). There were 90 individuals (30 fish per replicate) in each test condition.

3.2 | Antioxidant capacity

The T-AOC and activities of various antioxidant enzymes and GSH levels in blood, liver and gills were significantly reduced with elevated total ammonia nitrogen concentrations (Table 4) ($p < .05$). However, the MDA levels in blood, liver and gills were significantly increased with elevated total ammonia nitrogen concentrations ($p < .05$). A threshold was determined over which the T-AOC and activities of antioxidant enzymes in juvenile yellow catfish would respond to the total ammonia nitrogen levels. There was no significant difference in T-AOC or in activities of antioxidant enzymes in yellow catfish at a total ammonia nitrogen concentration of 3.36 mg L^{-1} compared to the control group; significant inhibition of T-AOC, and activities of antioxidant enzymes in yellow catfish at total ammonia nitrogen concentrations of 6.72, 13.44 and 26.88 mg L^{-1} compared to the control group indicated that the threshold changed at 6.72 mg L^{-1} in the present experiment (Table 4).

There was significantly lower T-AOC in blood and liver in the juveniles exposed to total ammonia nitrogen concentration of 6.72 mg L^{-1} for 56 days than those in the control group ($p < .05$). In the gills, however, there was no significant difference in T-AOC in the fish exposed to total ammonia nitrogen concentration of 6.72 mg L^{-1} for 56 days from those in the control group ($p > .05$), indicating that the T-AOC in blood and liver was more sensitive to ammonia than the T-AOC in gills under long-term ammonia stress. Similarly, the change in SOD activity was found to be more sensitive to ammonia concentrations in blood and gill filaments than in the liver; the CAT and GSH were more sensitive to ammonia concentrations in liver and gill filaments than those in the blood; the change in GSH-PX activity was found to be more sensitive to the ammonia concentration in the blood than in the liver and gill filament; the change in MDA content was found to be relatively sensitive to ammonia concentrations in the blood, liver and gill filament.

4 | DISCUSSION

In the present experiment, yellow catfish survival decreased with the elevated total ammonia nitrogen concentrations (Fig. 1), roughly comparable to the survival of toothed-tongue seabass (*Dicentrarchus labrax*) exposed to high total ammonia nitrogen concentrations (TAN 21.7–22.3 mg L^{-1}) for 10 days (Lemarié et al., 2004). Similar results were also obtained in *Macrobrachium rosenbergii* (Naqvi, Adhikari, Pillai, & Sarangi, 2007) and *Oplegnathus fasciatus* (Seongdeok et al., 2014). Ammonia can severely damage the antioxidant enzyme system in animals, intensify lipid peroxidation, to some extent reduce their immunity and disease resistance, and thereby leading to high mortalities (Wang, 2007; Zhao, 2006).

TABLE 3 Effects of total ammonia nitrogen concentration (TAN, mg L^{-1}) on food intake (FR, % day^{-1}), survival (%), wet body weight (g), specific growth rate (SGR) and feed conversion efficiency (FCR) in yellow catfish during the 56 days feeding trial.

TAN (mg L^{-1})	0	3.36	6.72	13.44	26.88
IBW	20.20 ± 0.56	20.07 ± 0.21	20.59 ± 0.25	20.38 ± 0.13	20.07 ± 0.32
FBW	32.77 ± 0.55d	30.05 ± 0.38c	29.79 ± 0.60c	24.60 ± 0.29b	23.25 ± 0.17a
FR	1.27 ± 0.02c	1.25 ± 0.02bc	1.24 ± 0.01bc	1.21 ± 0.02b	1.12 ± 0.02a
SGRw	0.86 ± 0.03c	0.72 ± 0.03b	0.66 ± 0.03b	0.34 ± 0.02a	0.26 ± 0.02a
SGRd	0.83 ± 0.02c	0.69 ± 0.01b	0.62 ± 0.01b	0.30 ± 0.01a	0.26 ± 0.01a
SGRp	0.92 ± 0.04d	0.74 ± 0.02c	0.70 ± 0.01c	0.40 ± 0.02b	0.27 ± 0.04a
SGRe	0.88 ± 0.03d	0.73 ± 0.02c	0.62 ± 0.05b	0.27 ± 0.02a	0.22 ± 0.02a
FCEw	66.72 ± 1.33c	54.22 ± 2.11b	51.06 ± 1.99b	27.79 ± 2.10a	23.15 ± 0.76a
FCEd	16.68 ± 0.29d	13.52 ± 0.39c	12.53 ± 0.11b	6.39 ± 0.12a	6.05 ± 0.42a
FCEp	27.37 ± 0.71d	21.32 ± 0.92c	20.69 ± 0.44c	12.87 ± 0.77b	9.15 ± 1.03a
FCEe	20.65 ± 0.69d	16.43 ± 0.27c	14.21 ± 0.91b	6.70 ± 0.47a	5.87 ± 0.75a

Values (mean ± SE, n = 3) with different letters in same line are significantly different (p < .05). IBW, initial body weight; FBW, final body weight; SGRw, specific growth rate in wet weight (% day^{-1}); SGRd, specific growth rate in dry weight (% day^{-1}); SGRp, specific growth rate in protein (% day^{-1}); SGRe, specific growth rate in energy (% day^{-1}); FCEw, feed conversion efficiency in wet weight (%); FCEd, feed conversion efficiency in dry weight (%); FCEp, feed conversion efficiency in protein (%); FCEe, feed conversion efficiency in energy (%).

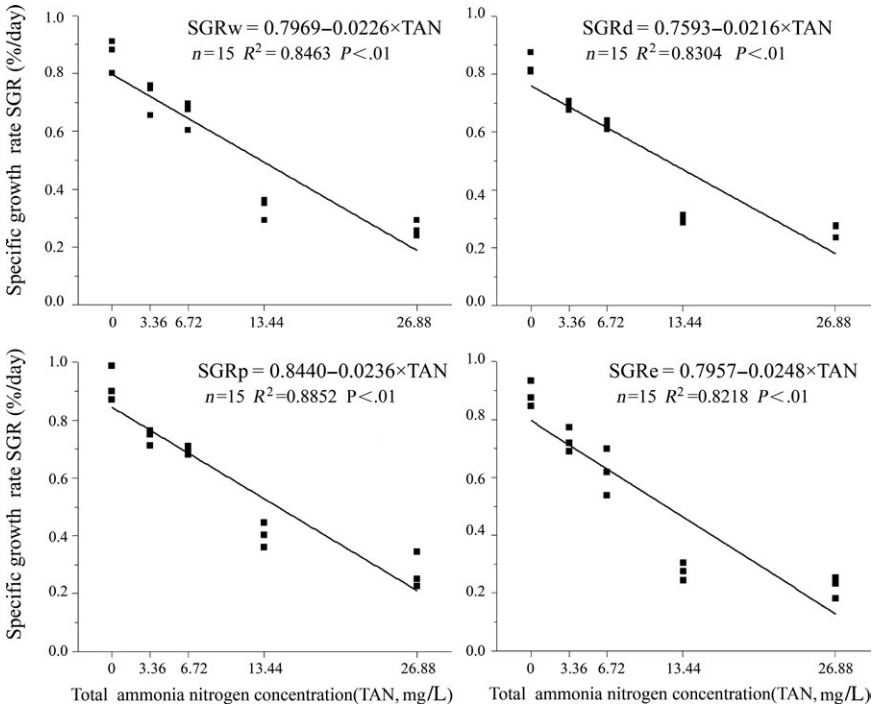

FIGURE 2 Linear regression curve between specific growth rate (SGR, % day^{-1}) and total ammonia nitrogen concentration (TAN, mg L^{-1}) after 56 days feeding trial at 29.0 ± 1.5°C and pH 7.45 ± 0.18. There were 90 *Pelteobagrus fulvidraco* (30 fish per replicate) in each test condition. "*n*" value = all test replicates (three replicates per test condition).

Many investigations noted that fish under long-term ammonia exposure showed poor growth (Arillo, Margiocco, Melodia, Mensi, & Schemone, 1981; Frances, Nowak, & Allan, 2000; Lemarié et al., 2004; Rasmussen & Korsgaard, 1996; Wajsbrot, Gasith, Diamant, & Popper, 1993). In the present study, the specific growth rate including SGRw, SGRd, SGRp and SGRe in *P. fulvidraco* was found to decrease with an increase in the total ammonia nitrogen concentration, and was significantly lower (p < .05) at 3.36 mg L^{-1} of total ammonia nitrogen concentration equal to 0.07 mg L^{-1} of unionized ammonia-nitrogen than that of the control group, which is consistent with the ammonia concentration under inhibition of growth in rainbow trout (*Oncorhynchus mykiss*) exposed to 0.02 mg L^{-1} of unionized ammonia nitrogen (Arillo et al., 1981). It was reported that Senegal sole (*Solea senegalensis*) exposed to 23.2 mg L^{-1} TAN for 52 days had a specific growth rate 1/50th of that in the control group (Wilson et al., 2007). However, Madison, Dhillon, Tufts, and Wang (2009) reported that a 52-day low ammonia exposure level led to improved growth in pikeperch (*Sander vitreus*).

Animals under stress showed poor growth due to decreased food intake whereby most of the energy was used to combat stress instead of being invested in metabolic growth, resulting in decrease in a feed conversion efficiency (Foss, Siikavuopio, Sæther, & Evensen, 2004). In

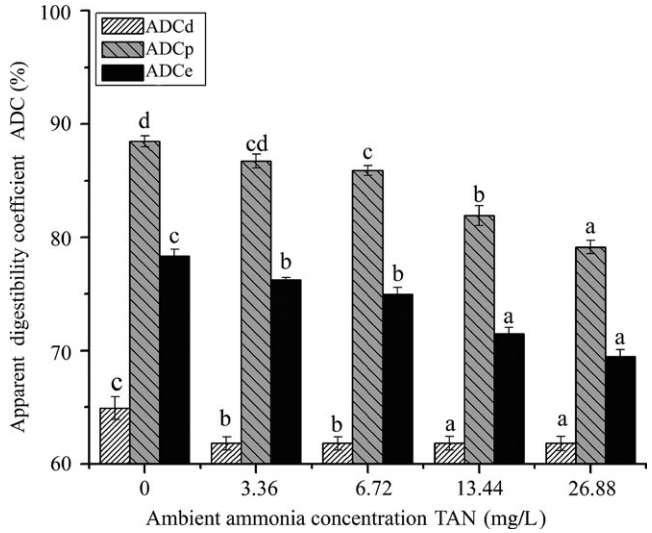

FIGURE 3 Effects of total ammonia nitrogen concentration (TAN, mg L^{-1}) on apparent digestibility coefficients in yellow catfish *Pelteobagrus fulvidraco* after 56 days feeding trial at 29.0 ± 1.5°C and pH 7.45 ± 0.18. There were 90 individuals (30 fish per replicate) in each test condition.

(Table 3; Fig. 3). The conclusion is that poor growth in animals involves inhibition of food intake and digestion, owing to the ammonia stress.

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) as the main antioxidant enzymes in an antioxidant immune system have a protective effect on oxidative damage induced by free radicals (Vijayavel, Gomathi, Durgabhavani, & Balasubramanian, 2004; Wang et al., 2004). GSH is a main non-protein sulfhydryl compound in tissues and the substrate of GSH-PX and glutathione S-transferase (GST), which require GSH for decomposition of hydrogen peroxide (Zhang, 1978). Thus, SOD, CAT, GSH-PX, GSH and GSSG constitute a simple antioxidant immune system, and protect organisms from oxidative damage (Lopes, Pinheiro, & Santos, 2001; Qujeqa, Aliakbarpour, & Kalavi, 2004; Sakai, Murata, Yamauchi, Sekiya, & Ukawa, 1992; Wang et al., 2004). Malonaldehyde (MDA) as the final decomposition product of lipid peroxidation indirectly reflects the level of lipid peroxidation, and can be used to evaluate free radical activity (Liu, 2006; Tan et al., 2005; Wang, 2007).

In our study, T-AOC in blood, liver and gills were shown to be in decline with the increase in total ammonia nitrogen levels (Table 4), indicating that ammonia exposure results in metabolism disorder,

TABLE 4 Antioxidant index in blood, liver and gills of yellow catfish exposed to various total ammonia nitrogen concentrations (TAN, mg L^{-1}) for 56 days.

		Ambient ammonia level (TAN) (mg L^{-1})				
Antioxidant index	**Tissue**	**0**	**3.36**	**6.72**	**13.44**	**26.88**
T-AOC (U ml^{-1}) (U mg $protein^{-1}$)	Blood	17.95 ± 0.96d	15.92 ± 1.07 cd	14.84 ± 0.77bc	12.83 ± 0.51ab	11.30 ± 1.00a
	Liver	1.66 ± 0.04d	1.58 ± 0.05 cd	1.47 ± 0.03c	1.26 ± 0.06b	1.11 ± 0.03a
	Gills	1.31 ± 0.03c	1.26 ± 0.05c	1.23 ± 0.06bc	1.06 ± 0.07b	0.87 ± 0.07a
SOD (U ml^{-1}) (U mg $protein^{-1}$)	Blood	65.29 ± 2.39c	61.40 ± 2.31c	53.69 ± 1.89b	49.71 ± 1.90b	42.31 ± 2.43a
	Liver	15.44 ± 1.69c	15.46 ± 1.16c	12.37 ± 0.91bc	10.67 ± 0.86ab	7.93 ± 0.65a
	Gills	14.12 ± 1.50c	11.63 ± 0.73bc	10.66 ± 1.04b	7.22 ± 0.61a	5.79 ± 0.58a
CAT (U ml^{-1}) (U mg $protein^{-1}$)	Blood	0.38 ± 0.03c	0.37 ± 0.01c	0.32 ± 0.02bc	0.30 ± 0.01b	0.23 ± 0.01a
	Liver	0.64 ± 0.02d	0.60 ± 0.03 cd	0.55 ± 0.02bc	0.48 ± 0.02b	0.39 ± 0.03a
	Gills	0.35 ± 0.01c	0.33 ± 0.02bc	0.30 ± 0.02b	0.29 ± 0.01b	0.24 ± 0.01a
GSH-PX (U ml^{-1}) (U mg $protein^{-1}$)	Blood	744.34 ± 14.62c	715.18 ± 11.67c	656.47 ± 18.43b	622.24 ± 17.44b	532.89 ± 14.24a
	Liver	42.97 ± 2.28b	43.33 ± 2.62b	41.31 ± 2.56b	36.58 ± 1.98ab	31.39 ± 1.27a
	Gills	60.22 ± 4.00c	60.95 ± 1.95c	55.97 ± 2.35c	48.07 ± 1.46b	31.92 ± 1.57a
GSH (mg L^{-1}) (mg g $protein^{-1}$)	Blood	6.46 ± 0.11b	6.21 ± 0.21ab	6.21 ± 0.22ab	5.89 ± 0.07a	5.74 ± 0.09a
	Liver	4.21 ± 0.13d	3.80 ± 0.05d	3.26 ± 0.08c	2.61 ± 0.15b	2.21 ± 0.09a
	Gills	0.52 ± 0.02c	0.49 ± 0.01bc	0.46 ± 0.01b	0.40 ± 0.01a	0.40 ± 0.87a
MDA (nmol ml^{-1}) (nmol mg $protein^{-1}$)	Blood	20.87 ± 0.88a	22.16 ± 1.09a	28.08 ± 0.87b	32.86 ± 0.59c	40.17 ± 1.37d
	Liver	0.68 ± 0.03a	0.86 ± 0.03a	1.21 ± 0.13b	1.72 ± 0.10c	2.51 ± 0.11d
	Gills	0.52 ± 0.03a	0.60 ± 0.04a	0.81 ± 0.05b	0.91 ± 0.08bc	1.05 ± 0.03c

Values (mean ± SE, n = 3) followed by different letters in the same line are significantly different (p < .05).

the present study, besides feed intake, specific growth rate as a stress index of total ammonia nitrogen level, feed conversion efficiency and apparent digestibility, as well the specific growth rate were found to be in decline with an increase in total ammonia nitrogen levels including total descending antioxidant capacity, and resistance to disease in fish. Low SOD activity was observed in the yellow catfish exposed to high ammonia nitrogen levels, especially SOD activity in gill filaments (Table 4) being only one-third of that in the control

group fish, implying that long term ambient ammonia exposure leads to induction of large amounts of O_2^- and exhaustion of SOD, which is consistent with the findings in southern catfish (*Silurus meridionalis*) (Zhao, 2006), and common carp (*Cyprinus carpio*) (Wang, 2007). There was much lower SOD activity in gill filaments than in the blood and liver under ammonia exposure, attributed to the fact that gill filaments are in direct contact with the total ammonia nitrogen in water and function as a barrier for ammonia to enter the fish body, thus the decline in SOD activity in gill filaments. Wang (2007) assumed that accumulation of free radicals in cells due to inhibition of removal of free radicals derived from low SOD activity and ATP or energy level during removal of free radicals caused microcirculation change. On the other hand, the accumulation of free radicals in cells resulted in the ischemia and hypoxia in cells, which further aggravated the metabolic disorders and tissue damage, resulting in peroxidation stress.

The findings in the present study revealed that activities of CAT, GSH-PX and GSH were in decline with elevated total ammonia nitrogen concentrations in the blood, liver and gills (Table 4). Liu et al. (2007) reported that activities of CAT and GSH in the blood, hepatopancreas and gills and GSH-PX activity in the hepatopancreas and gills were declining in the Pacific white leg shrimp (*Litopenaeus vannamei*) exposed to total ammonia nitrogen concentrations of 20 mg L^{-1} for 72 hr, which are consistent with our findings. However, this fact is attributed to a decrease in H_2O_2 reduction caused by the serum GSH-PX specific binding of reduced glutathione (GSH) and initial metabolism of peroxides under high H_2O_2 levels only during 72 hr ambient ammonia concentration exposure (Wang et al., 2004).

There were significantly higher MDA contents in blood and gills: two times higher in blood and gills and nearly three times higher in the liver of yellow catfish than those in the control group, and showing high levels of lipid peroxidation and an increase in activity of free radicals in the body. The liver as the largest organ of metabolism and detoxification might be a candidate for a higher level of lipid peroxidation and activity of free radicals than those of blood and gills. The oxidative stress in blood, liver and gills is ammonia concentration dependent, which is consistent with the findings by Wang (2007). However, the increase in MDA content and decrease in activity of antioxidant enzymes are not always consistent (Abele, Tesch, Wencke, & Pörtner, 2001; Martinez-Alvarez et al., 2002), as observed in the present study.

We found that there was a threshold of total ammonia nitrogen concentration, which induced a significant increase in antioxidant enzyme activity in yellow catfish. We suggest that yellow catfish show less stress or have adapted to an ammonia stress level at a low total ammonia nitrogen concentration. However, the activities of antioxidant enzymes were induced to rise and then decline in fish in a low total ammonia nitrogen concentration. It is uncertain whether non-ammonia stress even occurred in the activities of antioxidant enzymes, which recovered to the level of the fish in the control group (Sakai et al., 1992).

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