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Effect of CO_2 on acid-base regulation and growth performance of basa catfish *(Pangasius bocourti)*

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ABSTRACT

This study is aimed to evaluate the effects of different carbon dioxide (CO_2) levels on acid-base regulation and growth performance of basa catfish (Pangasius bocourti). The study included two experiments, (1) effect of different CO_2 levels (1%, 2%, 3%) on fish blood physiological parameters, and (2) effect of CO_2 levels on fish growth performance. In the first experiment, the experimental setup consisted of a big tank (1 m^3) that recirculated water to 4 smaller tanks (200 L) with 45 fish in each. The water partial pressure of carbon dioxide (pCO_2) was controlled with an Oxyguard Pacific box coupled with a G10 ps CO_2 probe and a K01svpld pH probe (Oxyguard International A/S, Farum, Denmark). The blood samples were collected at 0, 1, 6, 24, 48, 72, 96 and 168 hrs. after equilibration to investigate pH_e, pCO_2, $[HCO_3^-]$ and $[Cl^-]$ in plasma. The grow-out experiment was set up for 60 days and fish was weighed at the day 0, 30 and 60. The results showed that, after 1 hr. of CO_2 exposure, pH_e was significantly decreased (7.51±0.01 in control fish and 7.28±0.02 in the fish exposed to CO_2 3%, this parameter was recovered after 6 hrs. pCO_2 and $[HCO_3^-]$ increased at all CO_2 exposed groups. After 168 hrs., pCO_2 and $[HCO_3^-]$ in plasma of 3% CO_2 exposed fish were significantly increased and reached the values of 20.7±1.35 mmHg and 22.2±1.16 mM, respectively; those pCO_2 and $[HCO_3^-]$ values were 2.7 and 3.2-fold as high as the values of control fish. $[Cl^-]$ concentration in plasma of fish in 2% and 3% CO_2 treatments were significantly decreased after 48 hrs. of CO_2 exposure in comparison with control treatment ($p<0.05$). Besides, weight gain, daily weight gain and specific growth rate were significantly decreased, while feed conversion ratio increased with the increase of CO_2 concentrations ($p<0.05$). In conclusion, carbon dioxide was found to have significantly effects on acid-base regulation and growth performance of fish. Increasing of carbon dioxide in aquaculture systems should be regulated.

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1 INTRODUCTION

CO_2 content in the atmosphere has been increased with the economic development or with the industrialization process. CO_2 was 278 ppm in 1750s and increased to 390.5 ppm in 2011 (Flato *et al.,* 2013). The value of CO_2 is estimated to reach 421-936 ppm in the year of 2100. The surface water absorbs about one third of total CO_2 content that results in the decrease of pH. It is estimated that the pH value will decrease 0.3-0.4 unit in the end of 21st century if compared to current value (Hartmann *et al.*, 2013). CO_2 is a toxic element to aquatic animals. CO_2 in aquatic system may come from the atmosphere, the respiratory of cultured species as well as microbio activities. The CO_2 concentration mostly increases in the period of no photosynthesis of phytoplankton and reduces the pH of cultured ponds (Wurts and Durborow, 1992). The present of CO_2 in aquatic environment may influence growth performance as well as the change in physiology of aquatic animals. The water-breathing fish is more sensitive to CO_2 than air-breathing one, because of the lower CO_2 partial pressures of their body fluids; therefore, high concentration of CO_2 in water influences the diffusion of CO_2 from blood to environment (Ultsch and Jackson,1996). CO_2 is a small non-polar molecule, which can easy pass the cell membrane and acidify the blood of animal due to the activity of carbonic anhydrase that converts CO_2 to carbonic acid. The biological activities of cell is strongly depended on pH, so the increase of CO_2 in cellular fluid will cause disorder of normal metabolism of a cell (Ishimatsu *et al.*, 2005). Besides, the increase of CO_2 concentration in water influences the acid-base equilibrium process in fish body fluid (Brauner *et al.*, 2004), increases respiratory rhythm (Gilmour, 2001), increases HCO_3^- concentration (Damsgaard *et al.*, 2015) and decreases plasma Cl^- (Cameron and Iwama, 1987). In fresh water aquatic system, CO_2 concentration is high due to high nutrition which results in low oxygen and high CO_2 concentration (Wilmer, 1934; Ulsch, 1987; Furch and Junk, 1997 cited in Regan *et al.,* 2016). In striped catfish (*Pangasianodon hypophthalmus*) intensive culture pond, the CO_2 partial pressure was 4.5 kPa (33.75 mm Hg) (Damsgaard *et al.*, 2015), so the animal in such environment suffers with increased CO_2 concentration in water.

The increasing of CO_2 caused from climate change may influence the natural fish in Mekong Delta including basa (or river) catfish (*Pangasius bocourti*) which is one of important cultured species. As basa catfish is a non-air breathing fish, its capable of standing up to environmental changes is lower than the air-breathing fish. High concentration of CO_2 in cultured pond may affect its health and growth performance. Besides, studies on this fish were mainly on seed production, nutritional requirement, culture technique, and etc. but the effect of toxic gases (such as CO_2) on the fish is not yet documented in both national and international studies. Generally, this study was conducted to investigate the effect of high water CO_2 concentration on blood acid-base regulation, growth performance, as well as adaptability of the fish.

2 METHODOLOGY

2.1 Experimental fish

Fish was purchased from hatchery in Dong Thap province and acclimated in 2 m^3 tanks for at least 2 weeks prior to experimentation. Fish were maintained in well-aerated water in outdoor tanks on a natural photoperiod; fed commercial pellets (30% of crude protein, 3-5% crude lipid) twice a day; withheld feeding one day prior to experimentation. The size of fish for physiological and growth performance studies were 19.5±1.15 g and 13.8±0.042 g, respectively.

2.2 Experimental design

Experiment 1: Effect of CO_2 on acid-base regulation of *P. bocourti*

The experiment was designed with four treatments and four replicates, this experiment included control treatment without CO_2 addition, 1%, 2% and 3% of CO_2 addition corresponding to 3.4±0.01, 15.5±0.08, 27.9±0.05 and 44.7±0.04 mg CO_2 per liter, respectively. Fish were stocked into experimental tanks for 2 days prior to CO_2 addition with a density of 45 ind. per 200 L of water. The CO_2 concentration was controlled with oxy guard system, Denmark. The designed CO_2 concentration of each treatment was maintained in a big tank (1 m^3) and pumped to experimental tanks in a circulation system. Fish samples were collected at 0, 1, 6, 24, 48, 72, 96 and 168 hrs. after designed CO_2 concentration was reached. For each sampling point, blood of three fish was collected and divided into two parts; the first one was used to analyze partial pressure of carbon dioxide (pCO_2), extracellular pH (pH_e) using iSTAT analyzer (Abbott Laboratories, Abbott Park, Illinois, USA) with test Cartridge CG3$^+$; and the second was centrifuged at 6000 rpm to collect plasma for analyzing [Cl^-] with MKII Chloride Analyzer 926s (Sherwood, UK). Water quality parameters (pH, temperature and oxygen) were measured twice a day with Mettler toledo (USA).

The true value for pH_e and pCO_2 was temperature compensated from the fish tank temperature after

measured by iSTAT analyzer, using the equations from iSTAT manual. Plasma [HCO_3^-] was calculated from Henderson and Hasselbach equation and appropriate pK and CO_2 values (Boutilier *et al*., 1985).

$[HCO_3^-] = \alpha CO_2 . pCO_2 . 10^{pHe-pK}$

Experiment 2: Effect of CO_2 on growth performance and survival of *P. bocourti*

Experiment included three treatments and three replicates in which control treatment with no CO_2 addition, and 1%, 2% and 3% of CO_2 addition corresponding to 4.31±0.94, 15.5±0.69, 28.5±0.58 and 43.7±1.14 mg CO_2 per liter, respectively. The duration of experiment was 60 days, and stocking density was 30 ind. per 200 L of water. CO_2 was added and controlled similarly to experiment 1. Fish was fed twice a day with the amount of 3% of body weight using commercial pellet (30% crude protein and 3-5% crude lipid); and uneaten feed was recorded after 30 minutes of feeding. Water was exchanged weekly with ratio of 30% of total volume. Water quality parameters (pH, temperature and oxygen) were measured twice a day using YSI professional plus (USA). Fish weight was measured at day 0, 30 and 60 after commencing the experiment to evaluate growth parameters such as weight gain (WG), daily weight gain (DWG), specific grow rate (SGR) as well as feed conversion ratio (FCR). Fish survival rate was also calculated in the end of experiment.

Investigation parameters

$$\text{Survival rate (\%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} x\ 100$$

Weight gain (g): WG=Wt-Wo

Daily weight gain (g/day): $DWG = \frac{Wt-Wo}{t}$

Specific growth rate (%/day): $SGR = \frac{Ln(Wt)-Ln(Wo)}{t} x100$

Where: W_0: initial weight (g); Wt: final weight (g); and t: time (day)

Feed conversion ratio (FCR)= $\frac{\text{Feed Intake}}{\text{Final weight + Weight of mortality - Initial weight}}$

2.3 Statistics

Mean and standard error was calculated using Microsoft Excel version 2016. The difference among treatments was determined according to one-way ANOVA by Duncan test with SPSS 16.0. A probability (P) value at the 0.05 level was considered as significant. Graphs were made from SigmaPlot 12.5 and Microsoft Excel version 2016.

3 RESULTS

3.1 Effect of CO_2 on acid-base regulation of *P. bocourti*

Water temperature and dissolved oxygen during the experimental period varied from 29.3 to 29.6°C and 6.99 to 7.06 mg/L, respectively. pH of treatments decreased with the increase of CO_2 concentrations and actual CO_2 concentrations were almost similar to the designed treatment levels (Table 1).

Table 1: Water quality parameters of acid-base regulation experiment

Treatments		Control	1% CO_2	2% CO_2	3% CO_2
Tem. (°C)		29.6±0.07	29.5±0.15	29.6±0.08	29.3±0.07
Oxygen (mg/L)		7.00±0.03	7.06±0.01	6.99±0.04	7.06±0.02
pH		7.26±0.03	6.98±0.01	6.62±0.03	6.39±0.05
CO_2	(mg/L)	3.42±0.01	15.5±0.08	27.9±0.05	44.7±0.04
	%	0.24±0.00	1.00±0.00	1.99±0.00	3.01±0.01

3.1.1 Effect of CO_2 on pH_e of P. bocourti

The results showed that the pH_e of all treatments decreased after 1 hour of CO_2 exposure (Fig. 1 and Fig. 3A). The pH_e of control treatment was 7.51±0.01, but the pH_e was 7.26±0.08 and 7.28±0.02 in 2% and 3% CO_2 treatments, respectively. The pH_e of high CO_2 treatments was significantly lower than that of the control and 1% CO_2 treatments ($p<0.05$). After 6 hrs. of CO_2 exposure, the pH_e of CO_2 exposure treatments was recovered and approached to the pH_e of control treatment at 24 hrs. At 48 and 168 hrs., the pH_e of CO_2 exposure treatments was higher than that of control treatment, but there was no significant difference among the treatments ($p>0.05$).

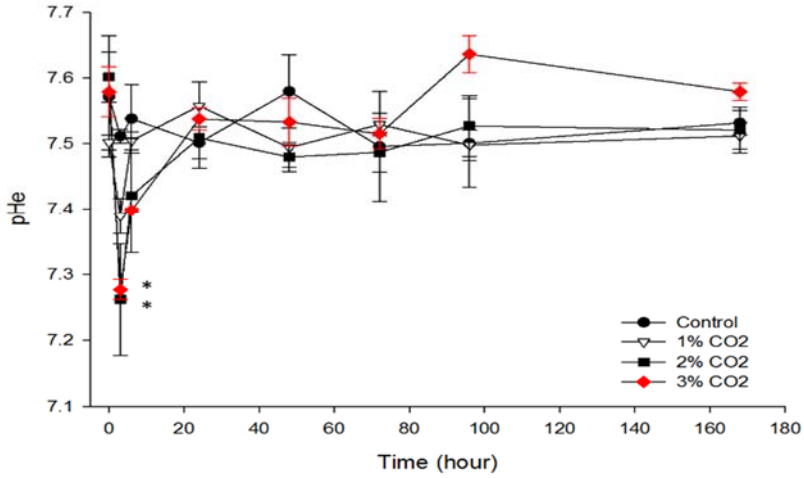

Fig. 1: Change of pH_e of *P. bocourti* exposed to different CO_2 concentrations

Asterisk (*) shows the significant difference ($p<0.05$) among control and CO_2 treatments in a sampling period

3.1.2 Effect of CO_2 on pCO_2 in blood of P. bocourti

The analyzed result showed that pCO_2 in fish blood of exposure treatments significantly increased if compared to that of control treatment (Fig. 2). At the sampling point of 1 and 6 hrs., the pCO_2 values of 3% CO_2 exposure fish were significantly increased faster than other treatments ($p<0.05$). During the experimental period from 24 to 168 hrs., the pCO_2 values of fish in exposure treatments were high and significantly different to control treatment ($p<0.05$). At sampling point of 168 hrs., the pCO_2 value of 3% CO_2 treatment was 2.7 times as high as the control, and the pCO_2 of 1%, 2% and 3% treatments was 10.4±0.91; 11.3±0.25 and 20.7±1.35 mmHg, respectively. The pCO_2 values of 1% and 2% CO_2 exposure treatments changed lightly, but no significant difference between these was found ($p>0.05$).

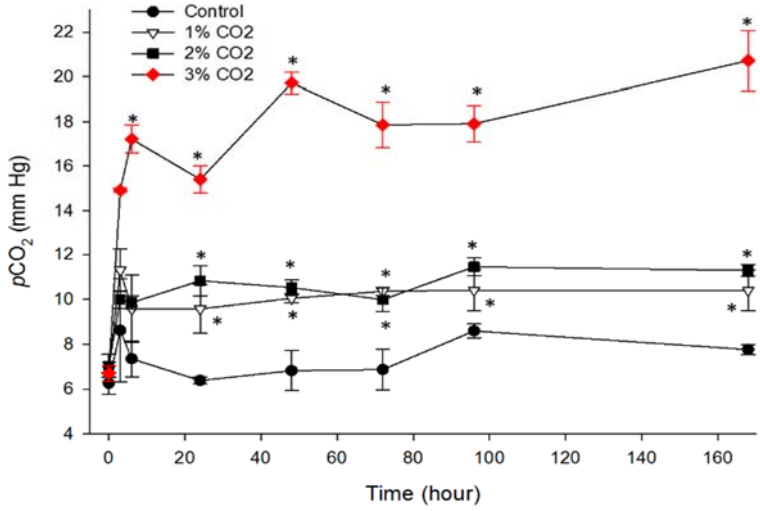

Fig. 2: Change of pCO_2 of *P. bocourti* exposed to different CO_2 concentrations

Asterisk (*) shows the significant difference ($p<0.05$) between control and CO_2 exposed fish in a sampling period

3.1.3 Effect of CO_2 exposure on plasma [HCO_3^-] of P. bocourti

The varies of [HCO_3^-] of CO_2 exposure treatments were similar to that of pCO_2. In the period of 24 and 168 hrs., [HCO_3^-] of all CO_2 exposure treatments was significantly higher than that of the control treatment ($p<0.05$). The concentration of HCO_3^- plasma of control and 3% CO_2 treatments at 0 hr. was 6.76±0.68 mM and 7.00±0.35 mM, respectively. At the sampling of 168 hrs., [HCO_3^-] of 3% CO_2 treatment (22.2±1.16 mM) was significantly higher than that of the control treatment (6.96±0.14 mM). There was no significant difference between the [HCO_3^-] in plasma of 1% and 2% CO_2 treatments (Fig. 3B). Therefore, the acid-base regulation of *P. bocourti* was significantly affected by CO_2 exposure (Fig. 3A).

3.1.4 Effect of CO_2 exposure on plasma [Cl^-] of P. bocourti

The change of [Cl^-] was different from [HCO_3^-]; [Cl^-] of CO_2 exposure treatments significantly decreased, compared to that of the control treatment in the period of 48 and 168 hrs. (Fig. 4). The concentrations of ion Cl^- of 2% and 3% CO_2 treatments were 92.6±1.83 and 92.0±3.7 mM, respectively, while that value of the control treatment was 101±1.21 mM at 48 hrs. At 168 hrs. after exposure, [Cl^-] of high CO_2 concentration treatments (2 and 3%) was 92.9±0.66 and 88.6±1.63 mM, respectively; the values were significantly different from that of the control treatment ($p<0.05$). No significant difference was found in other sampling periods ($p<0.05$).

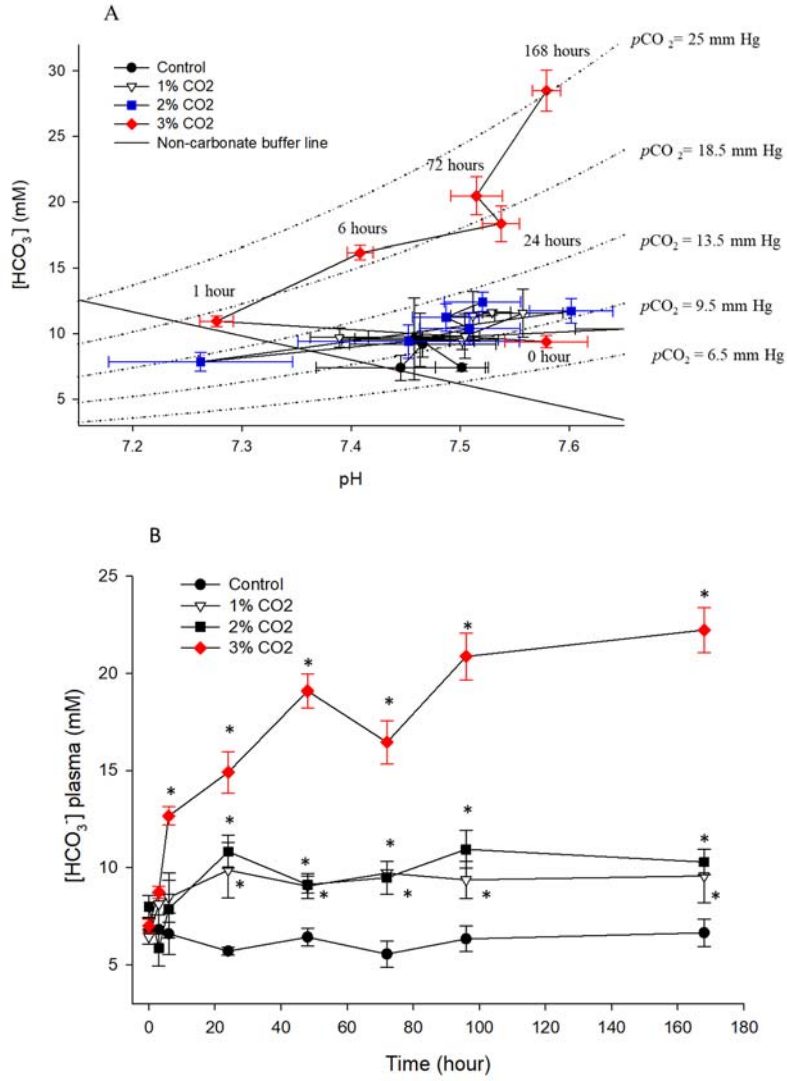

Fig. 3: Davenport diagram (A), plasma [HCO_3^-] (B) of *P. bocourti* exposed to different CO_2 concentration

Asterisk (*) shows the significant difference ($p<0.05$) among control and exposure treatments in a sampling period

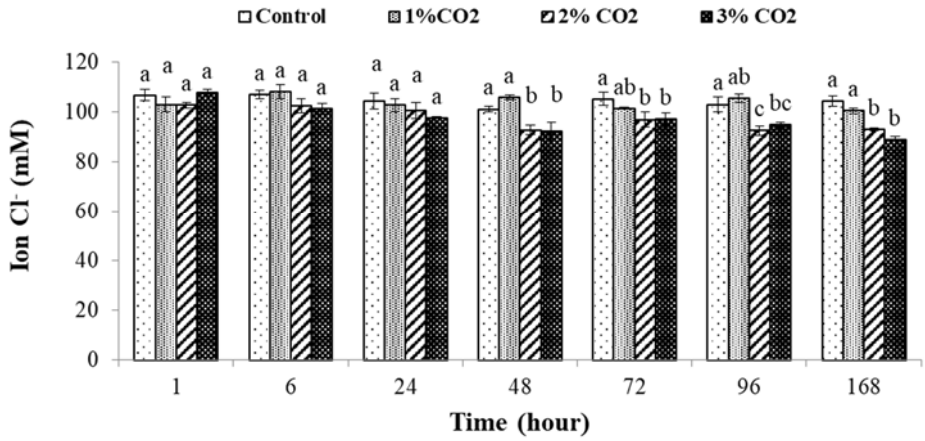

Fig. 4: Change of blood [Cl^-] of *P. bocourti* exposed to different CO_2 concentrations

Different letters (a, b, c) in a sampling period showed a significant difference ($p<0.05$)

3.2 Effect of CO_2 exposure on *P. bocourti* growth performance

Environmental parameters of this experiment are shown in Table 2. Water temperature and dissolved oxygen varied between 27.4-27.7°C and 7.05-7.23 mg/L, respectively. Water pH of treatments decreased with the increase of CO_2 concentrations. The pH values and the actual CO_2 concentrations of experiments are shown in Table 2.

Table 2: Environmental parameters of growth performance experiment

Treatment	Control	1% CO_2	2% CO_2	3% CO_2
Temperature (°C)	27.7±0.56	27.4±0.55	27.6±0.50	27.7±0.38
Oxygen (mg/L)	7.23±0.32	7.09±0.32	7.13±0.22	7.05±0.28
pH	7.18±0.29	6.87±0.28	6.65±0.33	6.41±0.47
CO_2 (mg/L)	4.31±0.94	15.5±0.69	28.3±0.58	43.7±1.14
CO_2 %	0.28±0.06	1.00±0.00	2.00±0.00	3.00±0.00

3.2.1 Growth performance

The results showed that after 30 days of rearing, the WG of control treatment (25.2 g) was significantly higher than that of 1%, 2% and 3% CO_2 treatments ($p<0.05$). No difference was found among exposure treatments ($p>0.05$) (Table 3). At the 60th day, the WG values of *P. bocourti* at CO_2 exposure treatments were significantly lower than those of control treatment, and the differences were found among all treatments ($p<0.05$). The WG values of control and 3% CO_2 treatment were highly different (Table 3).

After 30 days and 60 days of expriment, SGR and DWG of fish at CO_2 exposed treatments decreased with the increase of CO_2 concentrations, and were significantly different to those of the control treatment (Table 3). After 60 days, SGR and DWG of control treatment were 2.92±0.03 %/day and 1.10±0.01 g/day while those values of 3% CO_2 treatment were 2.60±0.02 %/day and 0.87±0.01 g/day, respectively.

Table 3: WG, SGR and DWG of *P. bocourti* exposed to different CO_2 concentrations

Treatment	WG30 (g)	DWG30 (g/day)	SGR30 (%/day)	WG60 (g)	DWG60 (g/day)	SGR60 (%/day)
Control	25.2±0.77^a	0.84±0.03^a	3.46±0.08^a	65.8±0.74^a	1.10±0.01^a	2.92±0.03^a
1% CO_2	20.2±0.41^b	0.67±0.01^b	3.00±0.04^b	61.1±1.41^b	1.02±0.02^b	2.81±0.03^b
2% CO_2	20.1±0.33^b	0.67±0.01^b	3.00±0.03^b	56.6±0.43^c	0.94±0.01^c	2.72±0.01^c
3% CO_2	19.3±0.08^b	0.64±0.00^b	2.91±0.01^b	52.3±0.73^d	0.87±0.01^d	2.60±0.02^d

Values are expressed as mean±SE. Different letters (a, b, c) in the columns signify a significant difference ($p<0.05$)

3.2.2 FCR and feed intake (FI)

FCR and FI increased with the increase of CO_2 concentration. After 30 days, the FCR of control treatment (0.92±0.03) was significantly lower than that of 1% CO_2 (1.13±0.03), 2% CO_2 (1.16±0.02), and 3% CO_2 (1.24±0.01) exposed treatment (Fig. 5A). The feed intake of control treatment and 3% CO_2 treatment was 2.93 % and 3.35%, respectively (Fig. 5B). After 60 days, FCR and FI of CO_2 exposed treatments were significantly higher than the control treatment ($p<0.05$). FCR of the control and 3% CO_2 treatment was 1.07±0.01 and 1.38±0.01, respectively.

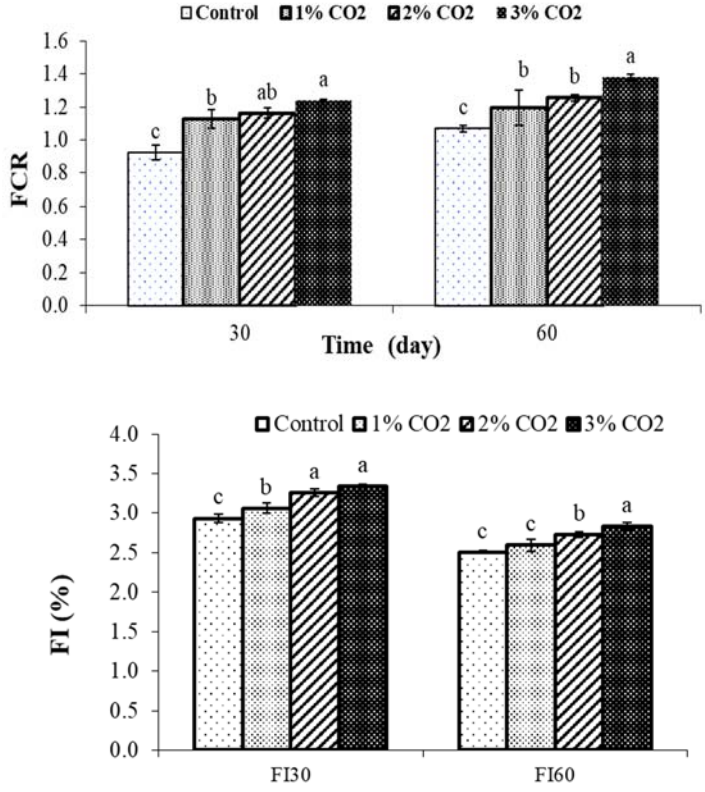

Fig. 5: FCR (A) and FI% (B) of *P. bocourti* exposed to different CO_2 concentrations at 30 and 60 days

Different letters (a, b, c) in a sampling period signify a significant difference ($p<0.05$)

3.2.3 Survival rate

The survival rate of fish at day 60 decreased with the increase of CO_2 concentrations. Survival rate of 3% CO_2 treatment (83.3%) was significantly lower than that of the control treatment (98.9%) ($p<0.05$) (Fig .6).

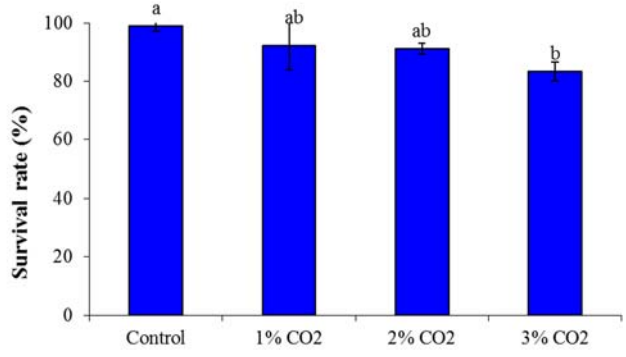

Fig. 6: Effect of CO_2 on survival rate of *P. bocourti*

Different letters (a, b, c) in a sampling period showed a significant difference ($p<0.05$).

4 DISCUSSIONS

4.1 Acid-base regulation

The results of the study showed that pH_e of fish exposed to CO_2 decreased at the beginning of experiment (1 and 6 hrs). It means that the blood of fish was acidified (Fig. 3A) leading to the acid-base regulation in the blood of fish was influenced. The plasma pH in this experiment was regulated after 24 hours for the exposed groups because of increased bicarbonate plasma concentration (Fig 3B). The CO_2 exposed fish could accumulate more [HCO_3^-] to neutralize the acid ion in blood (Heisler, 1986). The accumulation of [HCO_3^-] is indicated by the increase of the blood ions after CO_2 exposure. This might result in the increase of pH_e in the period of 24 and 168 hours. The increase of pH_e was also described in *Salmo salar* which exposed to CO_2 at the concentration of 1.3 mg/L (control); 10.6; 26 and 44 mg/L; the result of this study showed that the pH_e of fish exposed to 26 and 44 mg/L CO_2 increased from 1 to 41 days of exposure (Fivelstad *et al*., 1998). Dimberg and Høglund (1987) stated that bicarbonate concentration in freshwater was much higher than that in marine water so the increase of pH_e of high CO_2 exposed fish may be acclimated character of freshwater fish.

The study of Petochi *et al*. (2011) also showed the similar results, the blood pH of seabass increased after 3 hrs. of CO_2 exposure at the concentration of 15-20, 30-35 and 50-55 mg CO_2/L due to the increase of [HCO_3^-] but the difference was not considerable. However, the pH_e of 30-35 and 50-55 mg/L treatments was significantly higher than that of control. In addition, pCO_2 concentration increased with the increase of CO_2 in water environment. The pCO_2 and [HCO_3^-] of CO_2 exposed fish increased in most of fish species, but the acid-base regulation was different from species to species. Some studies indicated that the pH of some species did not increase, whereas it decreased in CO_2 exposed fish such as, armoured catfish and striped catfish (Brauner *et al*., 2004 and Damsgaard *et al*., 2015). Basa catfish and other species control acid-base balance of blood by accumulating ion HCO_3^-. For instance, the concentration of HCO_3^- of sea bass blood increased with CO_2 concentration exposure (Petochi *et al*., 2011), striped catfish also showed the similar results in the study of Damsgaard *et al.* (2015). The result of current study shows that the concentration of Cl^- decreased during CO_2 exposure, it may due to the Cl^-/HCO_3^- ion exchange mechanism (Cameron and Iwama, 1987). The uptake of bicarbonate-equivalent ions from the water was accompanied by a net release of [Cl^-], suggesting a 75% contribution of the Cl^-/HCO_3^- exchange mechanism (Fivelstad, 2013). Fivelstad (2013) stated that to respond to pCO_2 increase in blood, fish had to accumulate many ion [HCO_3^-] and 1 mM [HCO_3^-] increase would correspond to 1 mM [Cl^-] decrease. The author also indicated that there were correlations of water CO_2 concentration and [HCO_3^-] and [Cl^-], the results of basa catfish of this study were also similar.

4.2 Growth performance

The results showed that growth performance of fish was considerably affected by CO_2 concentration. The present of CO_2 resulted in low weight gain, DWG and SGR of basa catfish after 60 days of exposure at the concentration of 1% CO_2, 2% CO_2 and 3% CO_2. Besides, physiological parameter (pCO_2 and [HCO_3^-]) changed just after exposure and lasted to the end of experiment. The lower growth performance might result from acid-base regulation process, which utilizes energy (Evans *et al*., 1999). The effect of CO_2 on growth performance of basa catfish was similar to that of turbot (*Scophthalmus maximus*), the weight gain and SGR of turbot exposed to 26 and 42 mg/L CO_2 was low if compared with that of fish exposed to 5 mg/L (Stiller *et al.,* 2015); in addition, this authors also indicated that the utilizing of accumulated protein was 3 times as high as that of control fish. Stiller *et al.* (2015) concluded that the slow growth rate was due to the reduction of feed intake and the increase reliance on protein as a fuel source. The effect of CO_2 on fish was also similar to salmon (*Salmo salar L.*) (Fivelstad *et al*., 2015) and mykiss (*Oncorhynchus mykiss*) (Hafs *et al*., 2012). Fivelstad *et al*. (2015) stated that the relationship of CO_2 concentration and SGR was second order equation. At low CO_2 concentration (15-20 mg/L), the SGR of salmon was slightly decrease. However, when the concentration of CO_2 was higher than 20 mg/L, the SGR of exposed fish was noticeably decreased. The FCR and FI of basa catfish of CO_2 exposed treatments were significantly higher than those of control treatment (Fig 5A and 5B). This indicated that exposed fish utilized more feed compare to the control but the lower growth was obtained. The high FCR of exposed fish might result from low metabolize rate or the fish had to use energy for responding to bad environmental condition.

The survival rate of basa catfish in this experiment was significantly affected by the increase of CO_2 concentration. The survival rate was lower in exposed treatments which indicated that the survival rate considerably decreased when prolonging exposure CO_2 duration. This result was similar to the study of Fivelstad *et al.* (1998), i.e. mortality of salmon in the medium and high CO_2 groups (12 and 20 mmHg) were increased if compared to control treatment, 1.1% and 4.3%, respectively.

5 CONCLUSIONS

The results of this study showed that acid-base regulation of basa catfish was affected by high CO_2 exposed. Growth performance of basa catfish reduced when exposed with CO_2 concentration of above 1%. It was suggested that study on effect of CO_2 on other important species in the Mekong Delta should be taken for knowing of tolerance and adaptation of these species to environmental changing.

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