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RESILIENCE AND PHYSIOLOGICAL RESPONSES OF THE DOMESTICATED ASIAN REDTAIL CATFISH *Hemibagrus nemurus* TO HYPOXIA CONDITION

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ABSTRACT

Hypoxia is one of the critical issues in aquaculture production systems as it can lead to physiological disturbances in cultured fish. This research aimed to evaluate the tolerance level and physiological responses of domesticated Asian redtail catfish *Hemibagrus nemurus* reared in various hypoxia conditions. A total of 12 fish/treatment were acclimated to gradually decreased dissolved oxygen treatments until fish experienced aquatic surface respiratory (ASR) and loss of equilibrium (LOE). Cortisol, haemoglobin, and glucose levels were detected in the blood plasma to evaluate the stress response of the fish to hypoxia. The result showed that ASR of *H. nemurus* was identified at 2.17 ± 0.14 ppm of dissolved oxygen (DO) concentration with the percentage of ASR was 77.67 ± 9.53%, while LOE critical of *H. nemurus* happened at 0.63 ± 0.15 ppm of DO where 55.56 ± 4.81% of the fish experienced LOE. There were significant differences in the values of physiological parameters (blood cortisol, haemoglobin, and glucose) between control and treatments as fish experienced LOE ($P<0.05$). In the present study, it was found that the Asian redtail catfish is classified as a hypoxia-sensitive fish group. This finding is valuable information for the rearing and growing of the fish to provide an optimal DO concentration for their growth and survival.

KEYWORDS: ASR; LOE; stress; cortisol; haemoglobin; glucose

INTRODUCTION

The Asian redtail catfish *Hemibagrus nemurus* has been developed as a new candidate aquaculture species in Indonesia (Gustiano *et al.*, 2018; Kurniawan *et al.*, 2021). During the domestication programme, this species showed excellent performance regarding growth, survival, and nutritional value (Kusmini *et al.*, 2020; Subagja *et al.*, 2015). As the new aquaculture species, evaluation of the effects of different water quality on their optimal growth and survival would be necessary before the domesticated fish would be officially released to end users.

Water quality parameters play an essential role in aquaculture production systems and affect metabolic rate, physiological functions, behaviour, growth, survival, health, and production performance (Svobodova *et al.*, 1993; Boyd, 2017; Prakoso *et al.*, 2019; Prakoso *et al.*, 2021; Pyanuth *et al.*, 2020). The effects of changes in water quality in cultured media can be higher or lower depending on each parameter. Low dissolved oxygen (DO) levels or high concentrations of ammonia, nitrogen, nitrite, and sulphide can lead to stress and even mortality in cultured fish. Research to evaluate the resilient performance of cultured fish on various water quality parameters is beneficial for the successful aquaculture production of *Oreochromis niloticus* (Makori *et al.*, 2017). Water quality parameters in aquaculture systems can be affected by the input of uneaten feed, which leads to poor water quality and impacts the stress of cultured fish and

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the change of physiological responses in golden pompano larvae (Allais *et al.*, 2019). The use of artificial feed and high-density aquaculture systems can cause local aquatic hypoxia, which negatively impacts aquaculture development (Dong *et al.*, 2011; Douxfils *et al.*, 2012).

Hypoxia refers to the depletion of DO concentration in aquaculture ponds where DO levels remain below 1-2 mg/L for a few hours (Abdel-Tawwab *et al.*, 2019). Hypoxia causes physiological and immune disturbances in Atlantic salmon, which can reduce appetite, growth, and activity (Hvas & Oppedal, 2019). Each species of fish has various responses and resilience to hypoxia. *Oreochromis niloticus* and *Clarias batrachus* experience stress with dissolved oxygen (DO) of about 0.7 ± 0.1 mg/L and 0.98 ± 0.1 mg/over six hours, respectively (Li *et al.*, 2018; Tripathi *et al.*, 2013). To investigate the effects of hypoxia on fish, the *loss of equilibrium* (LOE) of Atlantic cod can be used as an indicator of its critical tolerance in response to bad water quality (Gollock *et al.*, 2006). LOE can be indicated when the chinook salmon is unable to maintain position or does not respond to a physical stimulus (Del Rio *et al.*, 2019). Before LOE happened, aquatic surface respiration (ASR) could be used as the initial sign of the decrease in DO concentration in gulf killifish (Rees & Matute, 2018). The stress response of fish can also be identified from changes in the levels of haemoglobin, haematocrit, cortisol, and blood glucose (Cho *et al.*, 2015; Prakoso *et al.*, 2016; Pastore *et al.*, 2018). This present research aims to evaluate the tolerance and physiological responses of domesticated Asian redtail catfish to hypoxia.

MATERIALS AND METHODS

Fish acquisition and care

The second generation of domesticated fish fingerlings of Asian redtail catfish (*Hemibagrus nemurus*) was obtained from the breeding facility at the Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Bogor, Indonesia. The fingerlings were transported to the rearing tanks in the RIFAFE laboratory. Fish were acclimatised for a week in the rectangular tanks (80 cm × 60 cm × 60 cm) prior to treatments.

Experimental design

The effects of hypoxia on the fish fingerling were performed with three replications. An investigation on fish responses to hypoxia conditions was carried out in the rectangular tanks following a procedure conducted by Ferrari *et al.* (2016). A total of 12 fish per treatment (control and hypoxia) were measured for their total length and weight. In the control tanks, the fish were maintained in normoxia. Meanwhile, in the hypoxia treatment tanks, dissolved oxygen (DO) concentrations were gradually reduced by flowing nitrogen into the water in the rectangular tanks (40 L, 40 cm × 30 cm × 30 cm; DO: 5 to 7 ppm) to obtain hypoxia conditions. To prevent the fish from accessing the oxygen around the water surface, mesh screens were installed about 5 cm below the water surface. The DO level was gradually decreased from 5 ppm to 4 ppm in 30 minutes and kept stable in the next 30 minutes. The same procedure was carried out from DO 4 ppm to 3 ppm, and from 3 ppm to 2 ppm. As the DO concentration reached 1.5 ppm, DO gradually decreased every 0.5 ppm in 30 minutes until the fish rose to the water surface to breathe as an indication of aquatic surface respiratory (ASR) and was followed by a loss of equilibrium (Wu *et al.*, 2017). DO concentration and temperature were measured using a dissolved oxygen meter (Lutron DO-5510, Taiwan) and pH was measured using a digital pH meter (Lamotte pH 5 plus, USA). The measurement of DO, temperature, and pH were continuously monitored by placing those equipments under the water surface of hypoxia treatment and control tanks.

Calculation of ASR and LOE critical

Observed parameters e in this study included abnormality of behaviour, ASR and LOE critical. These parameters were determined and recorded by visual observation. The critical DO value of fish in response to hypoxia conditions was measured as the fish breathed to the water surface (identified as aquatic surface respiratory-ASRcrit) and the fish identified loss of equilibrium (LOEcrit) (Dhillon *et al.*, 2013). ASRcrit was calculated using a formula explained by He *et al.* (2015):

$$\mathrm{ASR}_{\mathrm{Crit}} = [\mathrm{PO}_2] + \frac{t}{T} + \Delta \mathrm{PO}_2 \text{ ....(1)}$$

PO_2 = DO value before ASR, ΔPO_2 = the change of DO (1 ppm ASR every 30 minutes), t = times as fish resilience in 30 minutes, T = times for holding DO value in 30 minutes.

while LOEcrit was estimated following Dhillon *et al.* (2013):

$$\mathrm{LOE}_{\mathrm{Crit}} = [\mathrm{O}_2]2\mathrm{i} - \frac{\mathrm{ti}}{\mathrm{tii}} + [\mathrm{O}_2]2\mathrm{ii} \text{ ....(2)}$$

$[O_2]2i$ = DO value before LOE, $[O_2]2ii$ = decreasing DO (0.5 ppm of LOE every 30 minutes), ti = times as fish resilience in 30 minutes, tii = times for holding DO value in 30 minutes.

Blood sampling procedure

To evaluate the physiological response of fish to different DO levels, blood samples were collected in the ventral venepuncture of the vertebral artery at the end of the study as the fish lost equilibrium. Blood parameters were analysed for glucose, haemoglobin, and cortisol. The blood samples were collected from three fish in each control tank (DO level: 5) and treatment tank (three fish at each DO level during hypoxic conditions). Prior to collecting blood samples, fish fingerlings were anaesthetized using an anaesthetic solution (2-phenoxyethanol, dose of 0.5 mL/L water). From each fish, 0.5 mL of blood was collected using a 1 mL syringe (Terumo, Japan). Glucose levels were measured using a blood glucose reader (ACCU-CHEK Active, Roche, Germany). Haemoglobin levels were measured using the Sahli method (Haemometer Marienfeld), and cortisol was analysed using the ELISA method (Biosan HiPo MPP-96). All analytical procedures were done according to the manufacturer's instructions. Abnormalities of the fish exposed to different DO were also observed, including abnormal swimming behaviour, abnormal ventilatory function, and abnormal skin pigmentation (OECD, 2019).

Statistical data analysis

Statistical analysis was performed using RStudio, on all collected data. Fish length and weight, blood, and water quality parameters were analysed using One-way ANOVA and Duncan's test (confidence interval: 95%) to obtain the significant differences within treatments.

RESULTS AND DISCUSSION

The Asian redtail catfish fingerlings challenged to hypoxia showed an increase in stress response with a decrease in DO concentration. The decrease in DO concentration from 5 ppm (5.00 ± 0.74 ppm) to 4 ppm (3.84 ± 0.15 ppm) and 3 ppm (3.04 ± 0.11) did not negatively impact behavioural abnormalities in fish (Table 1). However, as DO was reduced to 2 ppm, the fish started to rise to the surface area to breathe, searching for a higher oxygen concentration, known as the Aquatic Surface Respiratory (ASR) condition. The average ASR value was obtained at a concentration of DO of 2.17 ± 0.14 ppm, with the percentage of fish observed experiencing ASR conditions was 77.67 ± 9.53%. Furthermore, as DO continued to decline, at the certain concentration (the critical point), the fish experienced a loss of equilibrium known as LOE critical. The LOE critical of the Asian redtail catfish occurred as DO was reduced from 1 ppm (1.09 ± 0.08 ppm) to 0.5 ppm (0.51 ± 0.06 ppm). Based on the calculation, the LOE critical of *H. nemurus* was predicted to occur at DO 0.63 ± 0.15 ppm, where 55.56 ± 4.81% of the fish experienced LOE.

Table 1. Dissolved oxygen (DO) tolerance of the Asian redtail catfish *Hemibagrus nemurus* challenged in gradually decreased DO

Repetition	Fish number	Fork Length (cm)	Total Weight (g)	ASR* (%)	DO at ASR* (ppm)	LOE** (%)	Critical DO at LOE** (ppm)
Control	12	9.97 ± 1.76[a]	10.62 ± 4.53[a]	-	-	-	-
1	12	10.39 ± 0.90[a]	12.28 ± 2.50[a]	83.33	1.98	58.33	0.57 ± 0.11
2	12	10.08 ± 1.2[a]	11.85 ± 3.39[a]	66.67	2.30	50.00	0.69 ± 0.18
3	12	10.53 ± 1.30[a]	12.28 ± 3.84[a]	83.33	2.23	58.33	0.65 ± 0.14
Mean ± STDV (1-3)				77.67 ± 9.53	2.17 ± 0.14	55.56 ± 4.81	0.63 ± 0.15

Note: Different superscripts in the same columns indicated significant differences (confidence interval: 95%); *ASR = Aquatic Surface Respiratory; **LOE: Loss of Equilibrium

The effects of hypoxia on the physiological performance of the fish were observed based on cortisol, haemoglobin, and glucose levels. Cortisol levels increased with a decrease in DO concentration indicating that a decrease in DO concentration can impact on the stress of the fish. Cortisol levels at DO 5 and 4 ppm were significantly different from DO 3 and 1 ppm ($P < 0.05$; Figure 1a). In contrast, haemoglobin concentration decreased with the decrease in DO concentration. The haemoglobin level did not significantly differ among three levels of DO at 5, 4, and 3 ppm ($P > 0.05$). However, there was a significant difference between haemoglobin level and DO levels of 5 to 1 ppm ($P < 0.05$; Figure 1b). Furthermore, the glucose level increased with decreasing DO concentration. A significant difference in glucose level was detected between two DO levels at 1 and 5 ppm ($P < 0.05$; Figure 1c). Water quality during the study showed optimal values for the growth and survival of fish except for DO (Table 2).

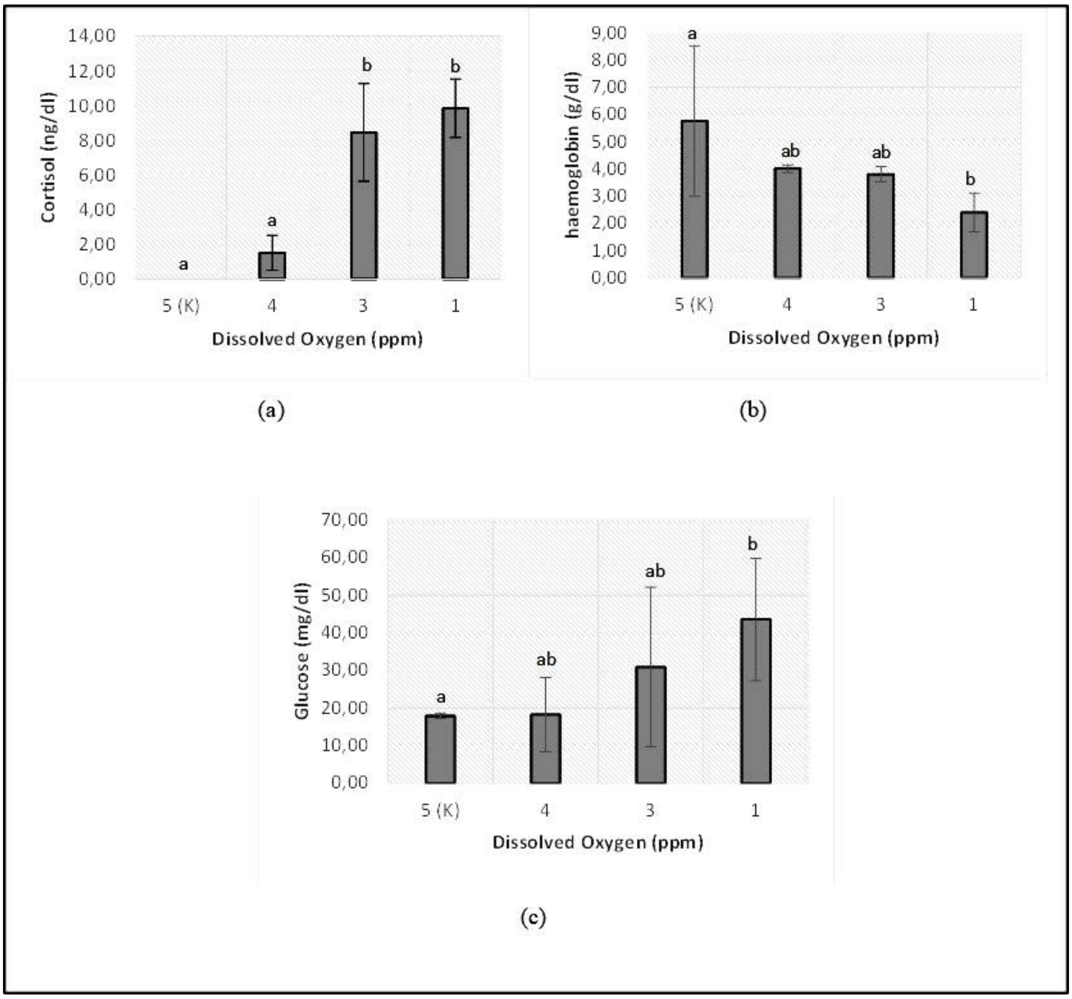

Figure 1. Stress responses of the Asian redtail catfish *Hemibagrus nemurus* fingerlings based on cortisol (a), haemoglobin (b) and glucose (c) challenged in different dissolved oxygen levels (K = control) (Note: Different superscripts indicated significant differences at confidence interval 95%)

Table 2. Water quality parameter of dissolved oxygen tolerance test of Asian redtail catfish *Hemibagrus nemurus* fingerlings in experimental tanks

Water quality	Dissolved oxygen (ppm)					
	Control	4	3	2	1	0.5
Dissolved oxygen (ppm)	5.00 ± 0.74^{f}	3.84 ± 0.15^{e}	3.04 ± 0.11^{d}	2.05 ± 0.18^{c}	1.09 ± 0.08^{b}	0.51 ± 0.06^{a}
Temperature (°C)	27.20 ± 0.68^{a}	27.00 ± 0.07^{a}	27.48 ± 0.10^{a}	27.13 ± 0.06^{a}	28.13 ± 0.05^{a}	28.13 ± 0.06^{a}
pH	7.26 ± 0.32^{a}	7.20 ± 0.11^{a}	7.14 ± 0.03^{a}	7.20 ± 0.40^{a}	7.36 ± 0.03^{a}	7.34 ± 0.01^{a}

Note: Different superscripts in the same rows indicated significant differences at confidence interval 95%

Water quality showed no significant differences in either temperature or pH during the experiment. Therefore, the changes in fish behaviour in the tanks occurred due to the decrease in dissolved oxygen concentration.

Hypoxia in aquaculture ponds has frequently happened because of feed and faecal accumulation, temperature fluctuation, and low photosynthetic activity, especially in intensive aquaculture systems (He *et al.*, 2015; Schafer *et al.*, 2021). As hypoxia occurred in aquatic systems, fish responded with various behaviours and movements, such as moving to the water surface to contact air, becoming more active to escape from hypoxic area, or becoming less active to reduce oxygen demand (Rogers *et al.*, 2016).

In this present study, ASR was obtained at DO 2.17 ppm. A lower ASR value of approximately 0.3 mg/L was found in a previous study on southern catfish *Silurus meridionalis* (Yang et al., 2013), while a higher ASR value (3 mg/L) was found on juvenile armored catfish *Pterygoplichthys* sp. (Gibbs et al., 2021). Aquatic surface respiration can be used as the first indicator of fish's response to environmental stress in relation to hypoxic conditions (Urbina *et* *al*., 2011). The ASR values indicated by the decrease in DO concentration in the environment. The decrease in DO concentration in the aquatic environment resulted in ASR values that convert to the normal fish respiratory mechanism (Takasusuki *et al.*, 1998). The differences in ASR values found in the present and previous studies indicated that the values are species- and size-dependent.

Fish having different tolerance responses to hypoxia conditions depends on the temperature of their habitat. The common carp *Cyprinus carpio* has excellent hypoxia tolerance, with the LOE critical occurring at DO 0.1 mg/l at 12°C (Dhillon *et al.*, 2013). The LOE critical of the Southern catfish. *Silurus meridionalis* was identified to be 0.08 mg/L at 25 °C (Yang *et al.*, 2013). However, several fish species are identified to have sensitive responses to hypoxic conditions. The LOE critical of the bream *Megalobrama pellegrini* was 0.60 mg/L at 12°C (Dhillon *et al.*, 2013), white mullet *Mugil curema* was reported to be 1.5 mg/L at 25°C (Fangue *et al.*, 2001), and blunt snout bream *Megalobrama amblycephala* was 1.53 mg/L at 30°C (Wu *et al.*, 2017). Based on the present finding, the Asian redtail catfish is classified as a hypoxia-sensitive fish group. This information is crucial to provide an optimal DO concentration for their growth and survival during rearing and growing the fish.

The present study showed that the cortisol level of Asian redtail catfish fingerlings increased significantly under hypoxic conditions. This result was similar to previous studies on yellow catfish *Pelteobagrus fulvidraco* (Wang et al., 2021) and hybrid yellow catfish (*Pelteobagrus fulvidraco* × *P. vachelli*) juveniles (Dagoudo *et al*., 2021). Cortisol is an essential hormone for the body with several functions, including a role in glucose metabolism, the immune system, blood pressure regulation, and the response to stress conditions (Crosby *et al.*, 2006). Changes in plasma cortisol levels are often used as a major indicator of stress. The stress response will stimulate the hypothalamus to release cortisol hormone, which plays a significant role in metabolism processes, immunity mechanisms, blood pressure, and the response to stress (Syawal *et al.*, 2011). Hypoxic conditions resulted in a lower haemoglobin level in Asian redtail catfish fingerlings as demonstrated by this study. However, previous studies on hybrid yellow catfish (*Pelteobagrus fulvidraco* × *P. vachelli*) juveniles (Dagoudo *et al*., 2021) and adult *Clarias batrachus* (Rao *et al*., 2014) showed the increasing of haemoglobin under hypoxia treatments. The different results on hypoxia treatment related to haemoglobin indicated the variation in each species' ability to cope with stress conditions. As the result of the decreased haemoglobin in the present study, the availability of oxygen content in the blood cells was reduced (hypoxia). Therefore, the metabolism process was disrupted, which caused the fish to experience a lack of energy. More than 90% of the oxygen carried by haemoglobin through the gill epithelium by diffusion through haemoglobin in the red blood capillaries (Evans & Claiborne, 2005). Moreover, glucose levels in the present study showed a significant increase under hypoxia. Glucose can be used as a biomarker for stress and hypoxia responses. The result from the present study was also similar to previous studies on the same species (Prakoso *et al*., 2018) and hybrid yellow catfish (*Pelteobagrus fulvidraco* × *P. vachelli*) juveniles (Dagoudo *et al*., 2021). Glucose levels also increase in the Oscar *Astronotus ocellatus* as a response to decreasing dissolved oxygen (Li *et al.*, 2018). Similarly, the glucose level of the perch *Scorpaena porcus* increases two-fold at hypoxia compared to a normal condition (Silkin & Silkina, 2005). When exposed to stress and low oxygen levels, fish experience an increase in their blood glucose levels due to the activation of their hypothalamic-pituitary-interrenal (HPI) axis, which further triggers the release of stress hormones. These hormones, cortisol and catecholamines, cause the release of glucose from glycogen stores in the liver and muscles into the bloodstream. This released glucose provides energy for the fish against the stressor and survive in a hypoxic environment (Lepic *et al.*, 2014; Cofiel & Mattioli, 2009).

CONCLUSION

The Asian redtail catfish (*H. nemurus*) is categorised as hypoxia-sensitive fish group. The critical DO for Asian redtail catfish was found at DO 0.63 ± 0.15 ppm, indicated by stress and physiological responses detected in the increase in cortisol level. Information on optimal DO concentration in this study is essential for the growth and survival of Asian redtail catfish during rearing.

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