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A STUDY ON THE GROWTH AND PHYSIOLOGICAL RESPONSE OF JUVENILE TINFOIL BARB *Barbonymus schwanenfeldii* (Bleeker, 1854) UNDER THE INFLUENCE OF pH CHANGES

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ABSTRACT

Tinfoil barb *Barbonymus schwanenfeldii* is recognized as one of the potential aquaculture commodities in Indonesia. Nonetheless, lack of data on their environmental tolerance causing this issue to become essential to be investigated. The present study was carried out to assess the pH influence on growth and physiological response of juvenile tinfoil barb. Juvenile tinfoil barbs (TL: 5.5 ± 0.8 cm; BW: 2.4 ± 1.0 g) were treated with four pH level treatments (5, 6, 7, and 8) and three replications with the stocking density of 10 fish each aquarium. Fish were kept for 21 days and fed with commercial fish feed. In this study, pH 8 exposure resulted to lower growth of juvenile tinfoil barb compared to pH treatment 5 to 7 ($p < 0.05$). In terms of survival rate, the results showed significantly lower value at pH 8 treatment compared to those of pH 6 and 7 treatment ($p < 0.05$), while it is not significantly different with pH 5 treatment ($p > 0.05$). Additionally, this study found the highest value of glucose, cortisol, and aspartate aminotransferase (AST) on pH 8 treatment ($p < 0.05$). On the other hand, pH 8 exposure led to the lowest creatinine, blood urea nitrogen (BUN), and alkaline phosphate (ALP) level ($p < 0.05$). In conclusion, the optimal pH for juvenile tinfoil barb rearing ranged around 6-7.

KEYWORDS: *Barbonymus schwanenfeldii*; pH; physiological response; specific growth rate; fish stress

INTRODUCTION

Tinfoil barb *Barbonymus schwanenfeldii* is a native freshwater fish species from the family Cyprinidae (Isa *et al.*, 2012) which distributed in most of Southeast Asian countries (Gante *et al.*, 2008; Nafees *et al.*, 2022). This omnivorous species has become a common protein source (Jaffar *et al.*, 2019) and accounted 1.3% of world's total carp production (Miao & Wang, 2020). In Indonesia, tinfoil barb is acknowl-

edged as one of potential aquaculture commodities (Kusmini *et al.*, 2021). However, its quantity and productivity are still low. This species is appropriate for various farming techniques (Dewantoro *et al.*, 2018; Kusmini *et al.*, 2020; Nurfadillah *et al.*, 2021).

The degree of acidity or pH is an index of H^+ in water, which is essential on affecting most chemical and biological processes (Boyd *et al.*, 2011; Boyd, 2017; Radkhah & Eagderi, 2021). Non-optimal water pH could be toxic for fish (Wurts & Durborow, 1992). It could also inhibit fish growth and make fish more sensitive to parasites and bacteria (Terech-Majewska, 2016). Changes in pH could increase fish mortality due to disruption of the respiratory process (Esbaugh

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et al., 2012; Miller *et al.*, 2016) and disease susceptibility (Liu *et al.*, 2016). In addition, low pH causes disturbances in fluid volume distribution, hematology, and acid-base homeostasis (Goss *et al.*, 1995; Mathan *et al.*, 2010).

As habitat degradation and river pollution increase, tinfoil barb populations in their natural habitat are also threatened (Radkhah & Eagderi, 2021). On this basis, the domestication program is necessary to preserve its sustainability. Fish domestication is an adaptation process in captive and controlled environments, including human intervention on artificial breeding (Teletchea, 2019; 2021). In Indonesia, the development on captive rearing and breeding techniques have been done by previous studies in some native freshwater fish species (Tang *et al.*, 2017; Gustiano *et al.*, 2020; Prakoso *et al.*, 2020; Prakoso & Kurniawan, 2020; Dewi *et al.*, 2021; Jubaedah *et al.*, 2023; Prakoso *et al.*, 2024), including tinfoil barb (Kurniawan *et al.*, 2021; Kusmini *et al.*, 2020, 2021, 2023). In this process, good water quality is needed for the success of domestication (Lorenzen *et al.*, 2012; Boyd, 2017; Radkhah & Eagderi, 2023).

Previous studies related to the effect of pH on physiological aspects in fish have been carried out on several species, including Atlantic salmon *Salmo salar* (Lacroix, 1989), common carp *Cyprinus carpio* (Mathan *et al.*, 2010), zebrafish *Danio rerio* (Kwong *et al.*, 2014), mahseer *Tor soro* (Pambudi, 2019), Roho labeo *Labeo rohita* and Mrigal carp *Cirrhinus cirrhosus* (Mukherjee *et al.*, 2019), and Asian redtail catfish *Hemibagrus nemurus* (Prakoso *et al.*, 2020). Research on physiological response of *Barbonymus schwanenfeldii* subjected to various pH exposure has never been carried out. Changes in water pH level could potentially reduce the growth and survival of this species, which generally has a slow growth rate. The information regarding their response on pH variability is essential for domestication and aquaculture program of tinfoil barb in Indonesia. Therefore, this

study aims to determine the effects of various pH exposure on the growth and physiological responses of juvenile tinfoil barb.

MATERIALS AND METHODS

Study Location and Source of Fish

This study was carried out from May to October 2021 at the Research Station for Freshwater Fisheries Germplasm, which is part of Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor, West Java, Indonesia. Fish from this study are the second generation of domesticated tinfoil barb from West Kalimantan population.

Experimental Design

Fish (TL: 5.5 ± 0.8 cm; BW: 2.4 ± 1.0 g) were treated and maintained on the aquaria (L \times W \times H: 30 \times 25 \times 20 cm) with 10 fish for each aquarium. Complete randomization design was performed in this study with four pH treatments (5, 6, 7, and 8) and three replications for each treatment. The pH level on each aquarium was adjusted by adding 10% nitric acid (HNO₃) to lower the pH and 0.4% sodium hydroxide (NaOH) to increase the pH. The experiment was carried out for 21 days. During the experiment, in order to maintain pH level in each aquarium, similar treatment of HNO₃ or NaOH addition was also carried out after daily tank cleaning and water changes. Fish were fed with commercial feed (MS Prima Feed PF1000; nutrition facts are shown in Table 1) in the morning (09:00) and evening (16:00).

Weight measurement and fish mortality were observed at the initial and final day of experiment. In addition, blood sample collections (from three fish in each treatment) were also performed at initial and final stage of experiment to assess the physiological responses of juvenile tinfoil barb. Prior to collecting blood samples, fish were anesthetized using 2-phenoxyethanol (0.5 ml/L water). The blood samples

Table 1. Nutritional composition of commercial feed given to juvenile tinfoil barb (*Barbonymus schwanenfeldii*) in this study

Parameters	Level (%)
Crude protein	39
Lipid	5
Fiber	6
Ash	12
Moisture	10

were taken from the fish caudal part. To measure blood glucose, one drop of blood from each fish was dripped to the glucose check strip and read using glucometer (Accu-Chek Active, Roche, Germany). Meanwhile, hemoglobin was measured by adding a drop of blood to hemoglobin strip and read using multi parameters monitoring pack (Fora® 6 Plus, Suisse AG). Thereafter, fish samples were ground by using pestle and then centrifuged (10,000 rpm for 10 min at 25°C) to obtain the serum. Serum samples were stored at refrigerator (-20°C) and analyzed using an ELISA Cortisol Kit (EIA-1887, DRG, Germany) and microplate photometer (Biosan HiPo MPP-96, Latvia) for whole body cortisol parameter and chemistry analyzer (Riele Photometer 5010 V5+, Germany) for creatinine, blood urea nitrogen (BUN), total protein, aspartate transaminase (AST), alkaline transaminase (ALT), and alkaline phosphate (ALP).

The observed growth parameters are weight gain, specific growth rate in weight, average daily growth, total feed consumed, and survival rate. Weight gain (WG, g) was calculated using the following equation: $WG = BW_t - BW_0$, where BW_0 and BW_t are the initial and final body weight, respectively. The specific growth rate in weight (SGR_w , %/day) was calculated according to the following equation: $SGR_w = ((\ln BW_t - \ln BW_0) / t) \times 100$, where BW_0 and BW_t are the initial and final body weight, respectively and t is the duration of the experiment (days). The average daily growth (ADG, g/day) was calculated according to the following equation: $ADG = (BW_t - BW_0) / t$, where BW_0 and BW_t are the initial and final body weight of fish, respectively while t is the duration of the experiment (days). Total feed consumed (TFC, g) was calculated using the following equation: $TFC = F_t - F_0$, where F_0 and F_t are the initial and final weight of the feed, respectively. Survival rates (SR, %) were calculated by

comparing the number of juvenile at final day of experiment (N_t) with the initial (N_0) using the following equation: $SR (\%) = (N_t / N_0) \times 100$. For supporting parameters, some water quality parameters were measured twice per day (08:00 a.m. and 03:00 p.m.), such as temperature, pH, dissolved oxygen, conductivity, alkalinity, and ammonia. The temperature and dissolved oxygen in each aquarium were observed by using dissolved oxygen meter (Lutron DO-5510, Taiwan). Meanwhile, pH level was measured by pH meter (Lutron BPH-231, Taiwan) and conductivity was measured using conductivity meter (LaMotte Con 6 Plus, US). Alkalinity level was analyzed by using the titration method (Salifert KH/Alkalinity Test Kit) and ammonia was measured by using colorimeter (LaMotte SMART3, US).

Data Analysis

The collected data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's test. All data were analyzed using RStudio version 4.0.2 (RStudio Team, 2022) with a 95% confidence level.

RESULTS AND DISCUSSION

In this study, the average of pH level in each pH treatment was significantly different ($p < 0.05$), while the average level of conductivity showed significant decrease with increasing pH ($p < 0.05$). On the other hand, no significant differences ($p > 0.05$) were found on average temperature, dissolved oxygen, alkalinity, and ammonia within treatments (Table 2).

No significant differences were found on temperature, dissolved oxygen, alkalinity, and ammonia. This indicates all treatments were on similar environmental condition except the pH level (as an explanatory variable), resulting in different conductivity levels.

Table 2. Water quality parameters in the aquariums during pH exposure treatment to juvenile tinfoil barb (*Barbonymus schwanenfeldii*) for 21 days

Parameters	pH treatment			
	5	6	7	8
pH	5.01 ± 0.36 ^a	6.10 ± 0.37 ^b	7.20 ± 0.21 ^c	7.96 ± 0.25 ^d
Temperature (°C)	23.80 ± 1.24 ^a	24.10 ± 1.32 ^a	24.01 ± 1.29 ^a	23.94 ± 1.31 ^a
Dissolved Oxygen (mg/L)	7.5 ± 0.39 ^a	7.5 ± 0.37 ^a	7.5 ± 0.38 ^a	7.5 ± 0.40 ^a
Conductivity (µS)	170.40 ± 43.26 ^c	155.73 ± 35.26 ^b	133.97 ± 25.37 ^a	129.70 ± 27.06 ^a
Alkalinity (mg/L)	94.33 ± 3.51 ^a	90.83 ± 9.39 ^a	93.67 ± 7.18 ^a	100.73 ± 14.49 ^a
Ammonia (mg/L)	0.19 ± 0.11 ^a	0.16 ± 0.06 ^a	0.11 ± 0.02 ^a	0.12 ± 0.09 ^a

Description: The number followed by different superscript letter on the same row shows significant difference based on Duncan's test with a 95% confidence interval ($p < 0.05$).

Water quality parameters need to be maintained at stable condition to keep the fish at suitable environmental conditions (Rojas-Tirado *et al.*, 2018). In this study, different conductivity levels were found within treatments. However, these levels are still a suitable range for juvenile tinfoil barb. The conductivity levels in this study were still in the range of 20-1500 mhos/cm, which is the range of natural water conductivity (Effendi, 2003). Conductivity has a strong relationship with pH because it is related to ions made from organic and dissolved salts (Mieftawati, 2014).

In terms of growth parameters (Table 3), the results showed that pH 8 treatment had a significantly lower value of mean final weight, weight gain, average daily growth, and specific growth rate in weight of juvenile tinfoil barb compared to those of in pH 5-

7 ($p < 0.05$). In the meantime, no significant differences were found in initial weight and total feed consumed within pH treatments ($p > 0.05$).

In terms of survival rate (Figure 1), the results showed significantly lower value at pH 8 treatment compared to those of pH 6 and 7 treatment ($p < 0.05$), while it is not significantly different with pH 5 treatment ($p > 0.05$). Concurrently, the survival rate is not significantly different within pH treatment 5 to 7 ($p > 0.05$).

Significant results on juvenile tinfoil barb's growth occurred in the treatment with a pH range of 5-7. On the other hand, with similar feed consumption, juvenile tinfoil barb in pH 8 treatment showed the negative results on growth performance. This phenomenon showed that unsuitable pH levels could cause an impairment on the growth of juvenile tinfoil barb.

Table 3. Growth parameters (mean \pm standard deviation (SD); $n = 3$) of juvenile tinfoil barb (*Barbonymus schwanenfeldii*) during pH exposure treatment for 21 days

Parameters	pH treatment			
	5	6	7	8
Initial Weight (g)	2.40 \pm 0.02 ^a	2.41 \pm 0.02 ^a	2.38 \pm 0.04 ^a	2.45 \pm 0.01 ^a
Final Weight (g)	2.53 \pm 0.04 ^b	2.49 \pm 0.04 ^b	2.52 \pm 0.06 ^b	2.21 \pm 0.10 ^a
WG (g)	0.12 \pm 0.02 ^b	0.08 \pm 0.02 ^b	0.14 \pm 0.01 ^b	-0.24 \pm 0.09 ^a
ADG (g/day)	0.01 \pm 0.001 ^b	0.004 \pm 0.001 ^b	0.004 \pm 0.002 ^b	-0.01 \pm 0.004 ^a
SGR _w (%/day)	0.24 \pm 0.04 ^b	0.16 \pm 0.05 ^b	0.27 \pm 0.02 ^b	-0.50 \pm 0.20 ^a
TFC (g)	4.58 \pm 0.21 ^a	4.49 \pm 0.30 ^a	4.32 \pm 0.12 ^a	4.45 \pm 0.17 ^a

Description: The number followed by different superscript letter on the same row shows significant difference based on Duncan's test with a 95% confidence interval ($p < 0.05$). WG: Weight gain; ADG: Average daily growth; SGR_w: Specific growth rate in weight; TFC: Total feed consumed.

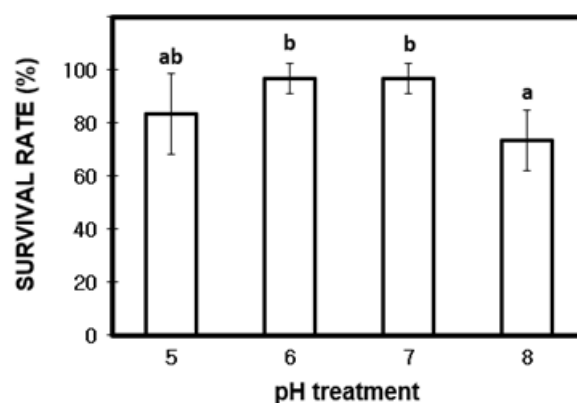


Figure 1. Survival rates (mean \pm SD; $n = 3$) of juvenile tinfoil barb (*Barbonymus schwanenfeldii*) during pH exposure treatment for 21 days.

This is supported by Parra & Baldisserotto (2019) who stated that pH level can be a factor that influence fish growth and survival. Previous study on striped snakehead *Channa striata* by Nisa *et al.* (2013) showed that the exposure of pH 4-6 reduced the growth rate because the energy obtained from feed consumption is not only needed for growth but also utilized to cope with environmental changes. The same pattern was found in da Paixao Lemos *et al.* (2018) on Nile tilapia juveniles which showed that at low pH (5.74) could alter fish growth. In the meantime, present study demonstrated lower survival rate of juvenile tinfoil barb at pH 5 and pH 8 treatment, indicating that 6-7 is the optimum pH range for juvenile tinfoil barb's survival. Previous study from Marimuthu *et al.* (2019) on catfish *Clarias gariepinus* showed similar pattern of survival rate at lower pH treatment. On the other hand, study from Reboucas *et al.* (2015) on tilapia *Oreochromis niloticus* demonstrated that tilapia can survive without mortality in the pH range of 4-8. According to those studies, survival is also influenced by species tolerance to pH. This statement is

supported by Copatti *et al.* (2019) who stated that each fish species has different tolerance and response to pH exposure.

In regard to physiological responses of juvenile tinfoil barb (Table 4), present study showed the highest value of glucose, cortisol, and AST from the pH 8 treatment ($p < 0.05$). On the other hand, it is apparent that the group of pH 8 treatment resulted in the lowest level of creatinine, BUN, and ALP ($p < 0.05$). No statistically significant differences were found in hematocrit, hemoglobin, total protein, and ALT levels of juvenile tinfoil barb within pH treatments ($p > 0.05$).

Lower pH exposure on fish could potentially cause hematocrit and hemoglobin dysfunction, resulting slower growth and lower survival (Baldisserotto, 2011). Meanwhile, higher pH exposure will potentially increase ammonia levels, which can be harmful for fish. Acidic and alkaline water conditions can interfere with metabolism and respiration in fish (Esbaugh *et al.*, 2012; Bolner *et al.*, 2014).

Table 4. Blood chemical parameters (mean \pm SD; n = 3) of juvenile tinfoil barb *Barbonymus schwanenfeldii* exposed with different pH level

Parameters	Initial	pH treatment			
		5	6	7	8
Hematocrit (%)	31.33 \pm 10.02 ^a	35.00 \pm 1.00 ^a	29.33 \pm 6.66 ^a	30.00 \pm 6.24 ^a	36.00 \pm 9.85 ^a
Hemoglobin (g/dL)	10.60 \pm 3.40 ^a	11.87 \pm 0.33 ^a	9.93 \pm 2.30 ^a	10.17 \pm 2.08 ^a	12.20 \pm 3.32 ^a
Glucose (mg/dL)	33.00 \pm 13.75 ^a	29.00 \pm 6.00 ^a	33.33 \pm 8.62 ^a	41.00 \pm 7.00 ^a	75.33 \pm 25.20 ^b
Cortisol (ng/mL)	28.35 \pm 9.83 ^a	18.70 \pm 3.28 ^a	26.71 \pm 11.77 ^a	25.64 \pm 5.69 ^a	55.48 \pm 13.04 ^b
Creatinine (mg/dL)	2.00 \pm 0.52 ^b	1.80 \pm 0.67 ^b	2.10 \pm 1.85 ^b	1.98 \pm 0.24 ^b	1.37 \pm 0.29 ^a
BUN (mg/dL)	39.33 \pm 10.07 ^b	34.67 \pm 18.01 ^b	31.67 \pm 29.74 ^b	30.67 \pm 5.86 ^b	15.33 \pm 2.08 ^a
Total Protein (g/dL)	1.73 \pm 0.67 ^a	1.20 \pm 0.36 ^a	1.43 \pm 1.45 ^a	1.53 \pm 0.59 ^a	1.27 \pm 0.12 ^a
AST (U/L)	17.00 \pm 6.56 ^b	12.00 \pm 11.14 ^b	10.67 \pm 11.72 ^b	3.00 \pm 2.00 ^a	23.33 \pm 27.79 ^b
ALT (U/L)	14.67 \pm 10.26 ^b	5.00 \pm 3.61 ^a	8.33 \pm 9.29 ^a	2.33 \pm 1.53 ^a	7.33 \pm 5.03 ^a
ALP (U/L)	682.67 \pm 142.31 ^b	433.67 \pm 290.03 ^b	547.33 \pm 575.66 ^b	515.67 \pm 200.76 ^b	289.33 \pm 14.57 ^a

Description: The number followed by superscript on the same row shows significant difference based on Duncan's test with a 95% confidence interval. BUN: Blood Urea Nitrogen; AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alkaline Phosphate

Present study shows that pH is a main factor influencing stress response of juvenile tinfoil barb. The pH value greater than 7 contributes to significant alterations on some blood chemical parameters of juvenile tinfoil barb, except in hematocrit, hemoglobin, total protein, and ALT. Stressed fish can cause physiological conditions and hormones to be unbalanced so that blood components also experience changes (Afonso, 2020).

Results from this study displayed a significant increase of glucose, cortisol, and AST level at pH 8. Conversely, pH 8 exposure contributes significantly to lowering creatinine, BUN, and ALP. The pattern on glucose parameter is similar with Bolner *et al.* (2014) on silver catfish *Rhamdia quelen* and Copatti *et al.* (2018) on pacu *Piractus mesopotamicus* which showed an increase in blood glucose after alkaline pH exposure. In the present study, increasing glucose level

was found at pH 8 exposure, showing that juvenile tinfoil barbs are under stress. Changes in glucose levels could reveal fish stress due to various treatments (Hastuti *et al.*, 2003). In fish, changes in glucose level could be an indicator of shifting the energy allocation on activation of physiological systems to deal with stress (Schreck & Tort, 2016). Stress fish will experience an increase in blood glucose as a secondary response (Barton, 2002). The release of the cortisol hormone by the hypothalamus into the blood and towards the liver will break down glycogen into glucose. This process increased the blood glucose on fish body. In distress conditions, glucocorticoids will trigger the glucose to increase (Faught & Vijayan, 2016).

In addition, cortisol level in this study was significantly higher at pH 8 treatment compared to pH 5, 6, and 7. In da Paixao Lemos *et al.* (2018), low pH (5.74) causes an increase in cortisol levels of juvenile Nile tilapia *Oreochromis niloticus*. Increased cortisol levels at a low pH (4) also occurred in brown trout *Salmo trutta* (Brown *et al.*, 1989). Changes in cortisol levels on present study and previous studies above is the indicator of various stress response from different fish species subjected to non-optimal pH exposure. Fish stress levels can be determined by measuring cortisol as a primary stress indicator (Shreck & Tort, 2016). Cortisol levels in fish have a role to play in dealing with stressors through acid-base balance, metabolism, respiration, immunity, and minerals (Ciji & Akhtar, 2021). According to Barton (2002), the increase in cortisol levels caused by stress is in the range of 30-300 ng/mL. In accordance with those statements above, the results of present study showed that pH 8 exposure resulted stress on juvenile tinfoil barb. Cortisol is a glucocorticoid compound secreted by the internal tissue (steroidogenic cells) located in front of the kidneys in fish. This hormone is released by activation of the hypothalamus. When the organism is under stress, the hypothalamus releases corticotropin into the blood vessels (Gorissen & Flik, 2016). If cortisol is exposed to an acidic pH, osmoregulation adaptation will occur (Copatti & Baldisserotto, 2021). Cortisol levels have a relationship with glucose on the stress response in fish. Cortisol can stimulate glycogen to become glucose which is a source of energy in the active transport process and homeostatic processes (Schreck & Tort, 2016).

Our findings show an insignificant difference in total protein after higher pH exposure to juvenile tinfoil barb. Total protein in fish is vital for determining the plasma volume, which can be altered in conditions, such as liver disease and nephrotic syndrome (Chen *et al.*, 2003; Öner, 2008). This parameter can

be one of nutritional indicators which provide information about current available energy stores for fish activities (Congleton & Wagner, 2006). In this study, insignificant difference in total protein of each treatment shows that juvenile tinfoil barbs still have available energy to maintain internal stability from harmful effect of pH.

Current findings also showed lowering levels of BUN and creatinine in the blood of juvenile tinfoil barb exposed to higher pH. Previous study on *Notopterus notopterus* (Kulkarni & Barad, 2015) showed a similar lowering pattern after acidic pH exposure. BUN and creatinine serve as indicators for kidney injury. Elevated level of BUN and creatinine in the blood reveal renal tissue injury (Nelson *et al.*, 1999; Mahmood *et al.*, 2023). Results from present study indicated that higher pH exposure may contribute to the renal tissue damage of juvenile tinfoil barb.

The present findings indicated that elevated pH exposure negatively affected liver function by causing lower level of ALP. In the meantime, fluctuated levels on AST and insignificant changes on ALT were shown after increasing pH from 5 to 8. Congleton & Wagner (2006) reported the presence of AST, ALT, and ALP in several fish tissues. These biomarkers have been recognized as liver tissue injury indicators in fish (Javed *et al.*, 2016; Huang *et al.*, 2021; Ghelichpour *et al.*, 2020). Regarding the present study, the AST, ALT, and ALP levels in response to pH exposure can be correlated and attributed to stress, which resulting tissue injury of juvenile tinfoil barb.

CONCLUSION

Juvenile tinfoil barb experienced reduced growth and survival rate at high pH exposure (pH 8). Most of blood parameters observed in this study demonstrate that this species experienced stress at pH 8. This study obtained the optimal pH range around 6-7 for juvenile tinfoil barb rearing. Further study is needed to assess the effect of longer-term pH exposure (low and high pH) on survival of this species.

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