The use of rubber microporous tubing as an aeration diffuser and the effect on blood gas in hybrid catfish *Pangasius* sp.

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Abstract. The purpose of this study was to evaluate the use of micro-porous rubber tubing as an aeration diffuser at lengths of 40 cm and 90 cm on aeration velocity and bubble diameter, and feed consumption and blood gas of hybrid catfish *Pangasius* sp. The treatment was compared to a stone aeration diffuser as the control, and each experiment was done triplicate. Each diffuser was attached to the bottom of the tank with a blower as the source of air, all having the same air pressure (90 mbar). Aeration velocity was measured by catching the air blown out of each diffuser using a beaker glass. The bubble diameter was measured using the *imageJ* image processing software. The catfish were reared for 60 days. The experimental catfish (78.30±2.34 g) were reared at a density of 12 individuals per tank. The catfish were fed twice a day. There was a significant difference in the aeration volume between treatments. The microporous diffuser (40 and 90 cm long) provides smaller volumes compared to the control, 0.20 L minute^{-1}, 0.21 L minute^{-1} and 1.73 L minute^{-1} ($p < 0.05$), respectively. However, the treatment using micro-porous rubber tubing demonstrated 9.77% smaller bubble diameter and 50.24% higher feed consumption compared to the control. The blood gas results demonstrated no significant difference among all treatments.

Key Words: bubble diameter, blood gas, feed input, microporous tube, *Pangasius* sp.

Introduction. The importance of fish cultivation in supplying protein for the global demand is rapidly developing in Asian countries as one of the motors (FAO 2007). The global demand for white fish such as the Nile tilapia (*Oreochromis niloticus*) and Siamese catfish (*Pangasianodon hypophthalmus*) has grown rapidly (Globefish 2011, 2012). Indonesia is currently the second largest exporter with productions exceeding 400 thousand tons (FAO 2015). Indonesia has been cultivating Siamese catfish since the early 1970s (Subagja et al 1999). The quality of this fish is not in accordance with the export standards because of its yellowish meat, while the market demands a white-coloured meat (Sørensen 2005). Djambal catfish (*Pangasius djambal*) is an indigenous fish species from Indonesia, and since 1996 several sampling studies have provided detailed information about the geographical distribution of pangasiid species. Legendre et al (2000) stated that Djambal catfish is found in Java (Brantas and Bengawan Solo Rivers) and Kalimantan (Barito, Mendawai, and Kahayan Rivers) and Sumatera (Musi, Batang Hari, and Indragiri Rivers). Djambal catfish has white meat which is suitable for export (Tahapari & Nurlaela 2016).

Gunadi et al (2006) stated that one of the efforts to combine the superior qualities of the Djambal catfish and Siamese catfish is through crossbreeding (hybridisation). This activity needs to be done judiciously and requires extensive research so that the expected goal, a superior breed of catfish, could be achieved. Hybrid catfish (*Pangasius* sp.) is the result of breeding between a female Siamese catfish (*P. hypophthalmus*) with a male Djambal catfish (*P. djambal*) (Setijaningsih et al 2006). The superiority of this breed can be seen in its relatively fast growth compared to Djambal catfish and its white meat, unlike the Siamese catfish. Besides being superior, this hybrid also has less a number of desirable traits such as intolerance to less than optimum environmental conditions; at dissolved oxygen of < 3 mg L^{-1} hybrid catfish die.

This study aimed to evaluate the effect of rubber microporous tubing diffuser on aeration velocity, the size of the bubbles produced, feed consumption, and also to confirm the presence of oxygen in the blood via blood gas analysis.

Material and Method. This study was conducted between November 2015 and March 2016. The activities in this study were equipment and material preparation, rearing, and analysis of the results. The study was conducted at the Cibalagung Cultivation Fishery Environment and Toxicology Research Installation, Bogor Freshwater Aquaculture Research Station. The water quality analysis was conducted in the Laboratory of Aquaculture Environment, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, and blood gas analysis in the Laboratory of Haematology, Indonesian Red Cross Hospital Bogor.

This study was conducted using an experimental method. The experiment design employed was the Completely Randomised Design (CRD) with three treatments, triplicate. The treatments were the use of different lengths of the microporous tubing as diffusers: 40 cm, 90 cm, and aeration stone diffuser as the control.

The fish used in this study were hybrid catfish (*Pangasius* sp.) weighing 78.3±2.34 g $fish^{-1}$ and measuring 22.89±1.85 cm in total length. The experimental fish were acclimatized in a rearing tank before being exposed to the treatments. The microporous tubing used was imported from China, produced by Tianjin Zeguang Rubber and Plastic Products Co., Ltd, with the specifications as follows: pore diameter 0.03-0.06 mm, pore density 700-1200 pore m^{-1}, and the diameter of bubbles produced 0.5-2 mm. The microporous tubing was shaped as dictated by the treatments.

The fish were reared in 9 fiberglass tanks with a diameter of 160 cm and a height of 40 cm each. The density of the fish reared was 12 fish $tank^{-1}$. Feeding was done with pelleted semi-buoyant commercial grower feed at 5% of the total fish biomass. Feed was given twice a day in the morning and in the evening, *ad satiation*. The fish were reared for 60 days from 15 November, 2015 to 14 January, 2016. The adaptation period was one week before the experiment.

The measurement of aeration velocity was conducted using a modification of the system mentioned by Nakagawa et al (2011), by collecting the bubbles in a beaker glass per time unit. The measurement of the bubble diameter was done using a photographic method and the analysis used the imageJ software (Gaillard et al 2015). Based on Gallagher et al (2010), the measurement of the blood gas parameters was conducted using a model ABL80 FLEX® blood gas analyzer. The amount of feed consumed was calculated from the beginning until the end of the experiment.

The results of the bubble size, flow velocity and feed consumption rate were analyzed using one-way ANOVA followed by Duncan's multiple range test at a 95% confidence. The results of the blood gas measurement were analyzed descriptively.

Data analysis. All data were presented as the average ± standard errors of replicate measurements (n = 3). Statistical analyses of data were carried out with SPSS 22.0 software. The significance of results was tested by an analysis of variance (ANOVA) and Duncan's Multiple-Range Test. The significance of differences was defined at $p < 0.05$.

Results and Discussion. The size of the bubbles and aeration velocity were measured before the rearing period; the bubbles were measured using a photographic method and digital image processing. The results of the bubble diameter observation for the control (stone diffuser) are presented in Figure 1. The results of the bubble diameter observation for the microporous diffuser are showed in Figure 2. The measurements of blood gas included the blood pH, HCO_3, total CO_2, and O_2 saturation. These measurements could illustrate the blood's ability to bind oxygen and remove carbon dioxide and could demonstrate the hybrid catfish's respiration performance. The results of the blood gas measurements are presented in Tables 1 and 2.

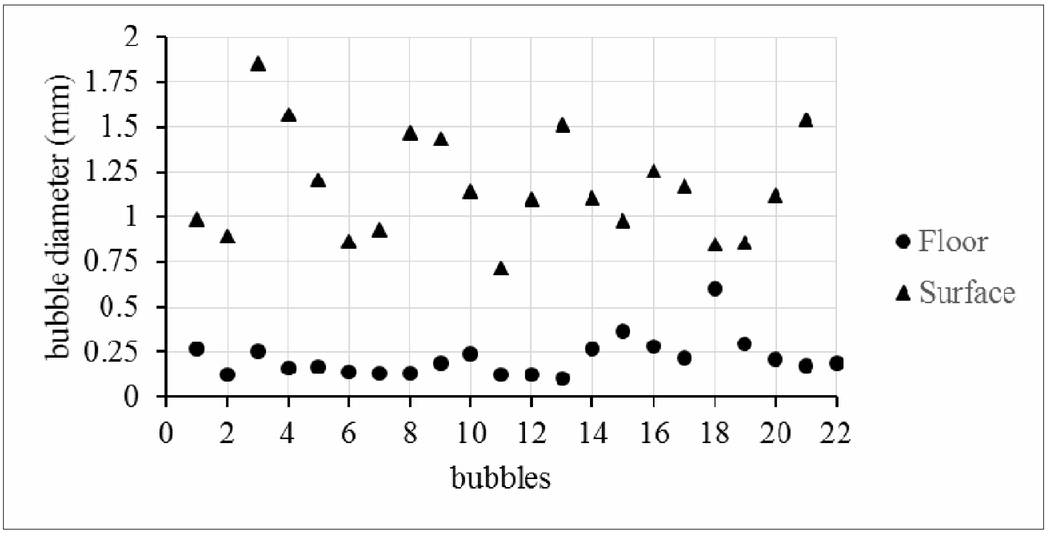

Figure 1. The measurements of bubble diameter at the floor (●) and the surface (▲) of the rearing tank for the control (K).

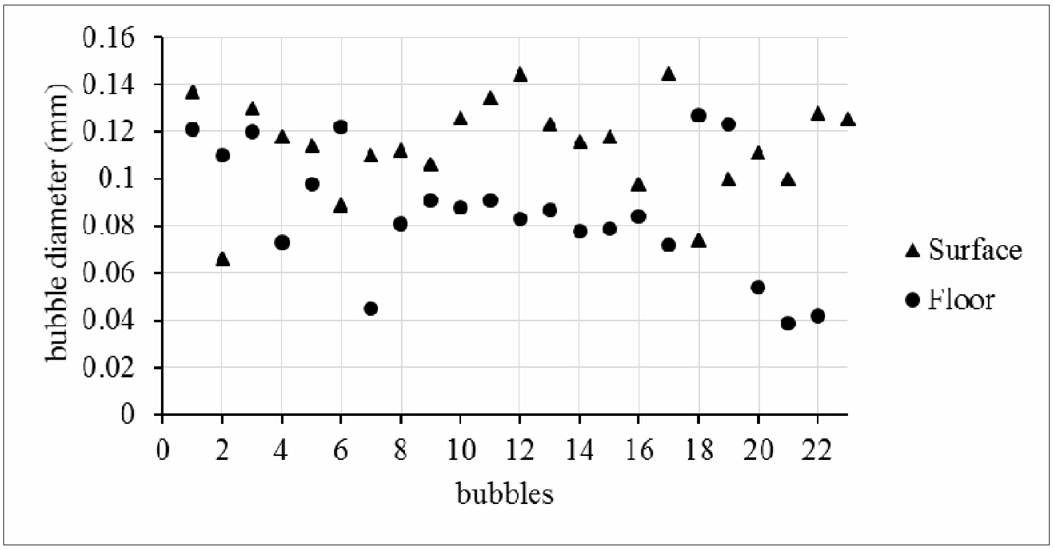

Figure 2. Measurements of bubble diameter at the floor (●) and surface (▲) of the microporous treatment tank.

Table 1

Blood gas parameters of the hybrid catfish aerated using different diffusers on day 0 of the experiment

Parameter	*Initial values*
pH	6.77±0.22
HCO_3 (mmol L^{-1})	6.20±2.32
Total CO_2 (mmol L^{-1})	7.63±2.30
Saturation O_2 (%)	11.85±12.75

Table 2

Blood gas parameters of the hybrid catfish aerated using different diffusers on day 60 of the experiment

Parameter	*Type of diffuser*		
	Control	*Micro-porous 40*	*Micro-porous 90*
pH	$7.30±0.06^{b}$	$7.36± 0.02^{a}$	$7.38±0.06^{a}$
HCO_3 (mmol L^{-1})	$14.25±3.18^{b}$	$16.00±1.98^{ab}$	$17.10±6.08^{a}$
Total CO_2 (mmol L^{-1})	$15.20±3.54^{a}$	$16.90±1.98^{a}$	$18.05±6.58^{b}$
Saturation O_2 (%)	$43.50±0.23^{c}$	$58.60±0.02^{d}$	$36.90±0.07^{b}$

Different letters on the same row show significant difference at a confidence interval of 95%.

The results of the aeration velocity are presented in Figure 3. The amount of feed consumed by the hybrid catfish during the 60 days of observation is presented in Figure 4.

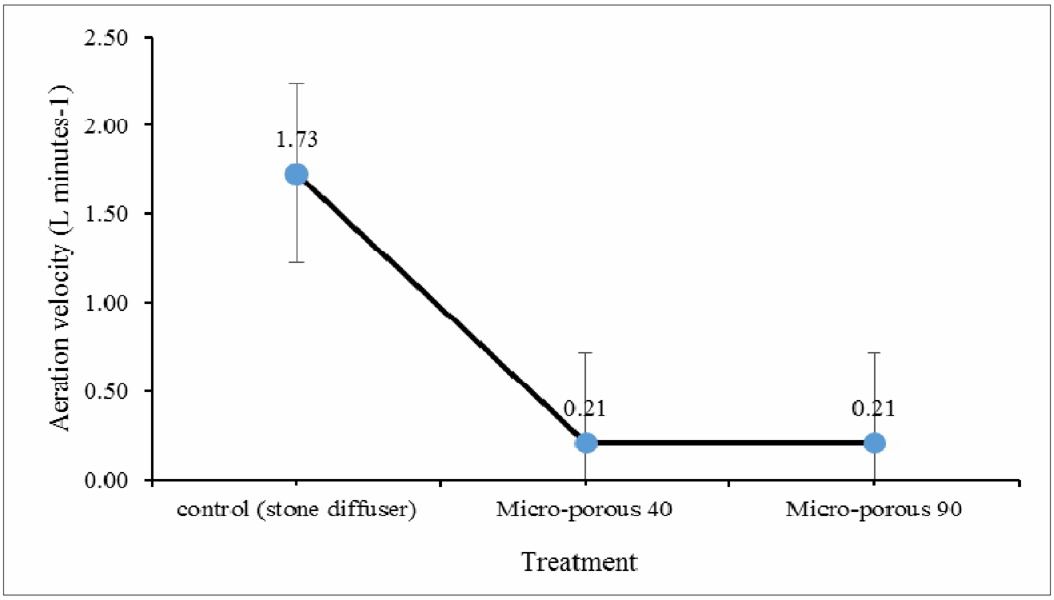

Figure 3. Measurements of aeration velocity, a comparison between the control and micropore diffuser.

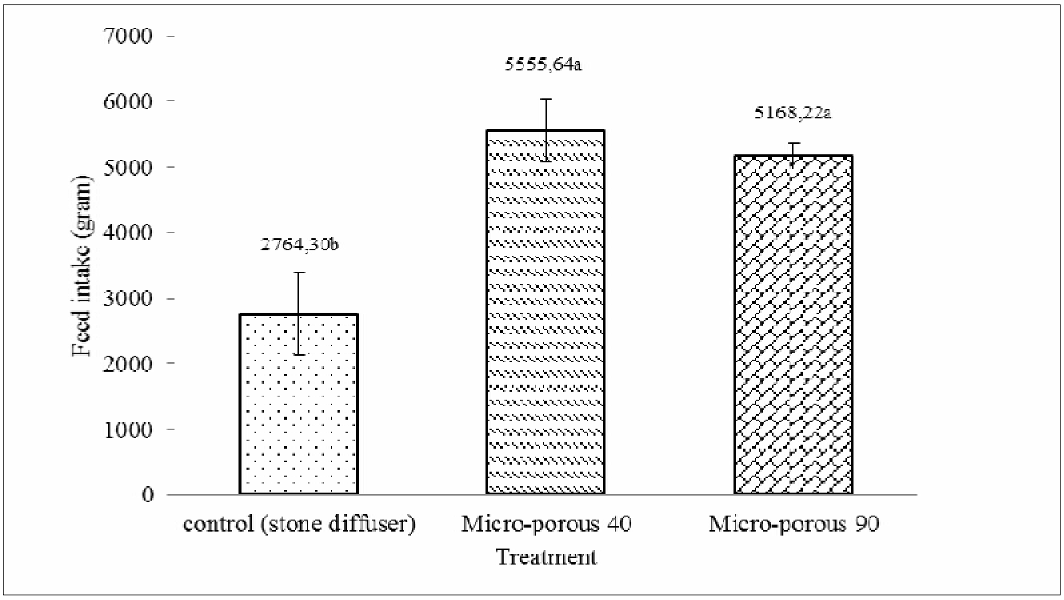

Figure 4. The amount of feed consumed by the hybrid catfish during the 60 days of rearing.

The measurement results of the control demonstrated that the diameter of bubbles produced varied (Figure 1), ranging between 0.098 and 0.6 mm at the floor and between 0.7 and 1.85 mm on the water surface. These results determined that there was a fairly significant difference between the diameter of the bubbles on the bottom of the tank and those on the surface ($p < 0.05$). The significant difference between the size of the bubbles on the bottom and on the surface show that the use of the control diffuser could increase the size of the bubbles.

The measurement results of the micro-porous treatments demonstrated that the diameter of the bubbles produced (Figure 2) ranged between 0.039 and 0.127 mm on the floor and ranged between 0.066 and 0.145 mm on the water surface. These results showed that the difference in bubble diameter on the floor and on the surface was not significant ($p > 0.05$). Small bubble diameter increases the surface of the contact area between water and air, facilitating diffusion of air to the water. The smaller the bubble diameter and the longer the bubble is in the water, the better the gas transfer and the higher dissolved gas concentrations are (Navisa et al 2014).

The measurement of aeration velocity demonstrated that the control diffuser was faster per time unit at an average of 1.731±1.036, whereas the micro-porous 40 and micro-porous 90 were 0.210±0.035 and 0.208±0.044 L minute^{-1}, respectively. The different pore sizes and density of each diffuser at a similar pressure (90 mbar) caused the flow of air to differ. A diffuser with a smaller pore size would produce smaller, more numerous bubbles. The total bubble surface area is inversely proportional to bubble size at similar air flow rates (Yoon 2015).

The amount of feed consumed in this study was calculated based on the amount of feed consumed by the fish from the start until the end of the rearing period. The total feed consumed value of each treatment, 2.76±0.64 kg in the control, 5.17±0.19 kg in the micro-porous 90 treatment, and 5.56±0.21 kg in the micro-porous 40 treatment. The results of the total feed consumed were significantly different ($p < 0.05$) from the control; this was caused by, among others, a high mortality rate in the control group at beginning of the rearing period. Decreased feed consumption could be caused by hypoxia in fish as stated by Chua et al (2010) who determined that one of the effects of chronic hypoxia is refusing to feed.

The results of the initial blood gas are presented in Table 1. The results of the initial measurement demonstrated that the blood pH was 6.77±0.22, HCO_3 6.20±2.32 (mmol L^{-1}), total CO_2 7.63±2.30 (mmol L^{-1}), and O_2 saturation 11.85±12.75 (%). The blood pH blood was normal on the day 0. The observation at the end of the rearing period showed pH values of 7.30±0.06 in the control, 7.38±0.06 in the micro-porous 90 treatment, and 7.36±0.02 in the micro-porous 40 treatment. The optimum pH is seven, and if it drops below seven, the animal will experience acidosis. There are two types of acidosis: the respiratory acidosis which is caused by the accumulation of CO_2 in the body and metabolic acidosis which is caused by the failure of the kidney in regulating acid-base in the body. In this study, the blood pH was still within the normal range, so it could be said that different diffusers did not affect the hybrid catfish's blood pH.

HCO_3 is the concentration of hydrogen carbonate in the blood. This is used to determine the presence of acid-base imbalance, and regulation parallel to that of pH and CO_2 (Mohammed & Abdelatief 2016). The results of the hybrid catfish's blood bicarbonate initial observation were 6.20±2.32 (mmol L^{-1}). Observations made on day 60 demonstrated that the HCO_3 value fluctuated; the control's increased to 14.25±3.18, the micro-porous 90 treatment increased to 17.10±6.08, and the micro-porous 40 treatment increased to 16.00±1.98. HCO_3 concentration is influenced by the concentration of CO_2 in the blood, in accordance to the reversible equation $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$ (Perry & Gilmour 2006).

Observation of the total blood CO_2 parameter yielded a value of 7.63±2.30 on day 0 of the experiment. Observation on day 60 revealed that the value in the control increased to 15.20±3.54, in the micro-porous 90 treatment increased to 18.05±6.58, and increased to the micro-porous 40 treatment at 16.90±1.98. The total carbon dioxide (CO_2) is a measurement that encompasses all forms of carbon dioxide, bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and carbonic acid ($H_2CO_3^-$). Most carbon dioxide is present as bicarbonate. Carbon dioxide (CO_2) is released by cells as a metabolic waste material which is then absorbed by red blood cells (Hb) and converted into carbonic acid with help from the carbonic anhydrase enzyme. In this form, carbon dioxide is carried to the gills to be excreted (Mah & Cheng 2015).

Based on the results of the blood gas assessment, the oxygen saturation value fluctuated from the beginning, middle, and end of the observation period. O_2 saturation is the percentage of hemoglobin fully bonded with oxygen. The blood oxygen saturation (SO_2) value at the beginning of the observation period was 11.85±12.75%, while at the end the SO_2 value was between 23.30-58.60%. The highest blood O_2 saturation was found in the micro-porous 40 diffusers, 58.60%. Blood O_2 saturation could be influenced by the high or low oxygen saturation in the medium. This analysis was used as a method to confirm the presence of gasses in the blood that was influenced by aeration. As confirmation of the presence of oxygen in the blood, the parameter used for interpretation was oxygen saturation in the blood (SO_2). As a comparison, Eatwell et al (2013) in their study demonstrated that oxygen saturation in the blood of rabbits (*Oryctolagus cuniculus*) ranged between 88.8-98.0%. Blood oxygen saturation in terrestrial and aquatic animals differs due to the lower dissolved oxygen levels in water (Gallagher et al 2010).

Conclusions. The use of microporous rubber tubing as an aeration diffuser in rearing hybrid catfish (*Pangasius* sp.) could produce smaller bubbles ($p < 0.05$) and a smaller air flow, increase feed consumption and increase the blood oxygen saturation level.

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