Micro/Nano Bubble Technology : Characteristics and Implications Biology Performance of Koi *Cyprinus carpio* in Recirculation Aquaculture System (RAS)

Henry Kasmanhadi Saputra[1], Kukuh Nirmala[2], Eddy Supriyono[2], and Nurul Taufiqu Rochman[3]

[1]*Graduate School of Aquaculture Science, Bogor Agricultural University, Bogor 16680, West Java, Indonesia*
[2]*Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor 16680, West Java, Indonesia.*
[3]*Centre for Innovation, Indonesian Institute of Sciences, Cibinong 16916, West Java, Indonesia.*

[*]*Corresponding author*: h3nrykhs@gmail.com

ABSTRACT

Technology for aquaculture is growing with the high demand of fish. Micro / nano bubble is one of technology that is developed to increase dissolved oxygen in water. This technology is used to increase Koi fish production in high density via RAS. The purpose of this study was to determine the characteristics of micro / nano bubble technology and its effect on biology of koi fish (*Cyprinus carpio*). Lutor was used to introduce micro / nano bubble in the fish tank by using fish length (e.g. 7.49 ± 0.29 cm). This research used completely randomized design with one factor (fish density) and three replications. This factor was three densities of fish at 15 fish.60 L^{-1} (A), 30 fish.60 L^{-1} (B), and 45 fish. 60 L^{-1} (C). The result showed that there was the absolute length of each treatments was (0.52 ± 0.03^{a}) cm for A, treatment B (0.36 ± 0.07^{ab}) cm and C treatment (0.29 ± 0.08^{b}) cm. The best treatment is A treatment (15 fish. 60 L^{-1}) and different significant in statistic with C treatment (45 fish. 60 L^{-1}) but not significant in statistic with B treatment (30 fish. 60 L^{-1}).

Keywords: Lutor, koi, bubble, micro / nano

1. Introduction

Aquaculture is developed due to the high demand of fish either for consumption or ornamental fish. Increasing dissolve oxygen water is valuable to increase the fish production. Micro / nano bubble is a technique to increase dissolve oxygen in the short period. This technology is already developed in Japan for more than 50 years. In 1950 (Alheshibri et al., 2016), this technology applied to assist the degradation of fluid waste (Yamasaki et al., 2010) and patented in 2005 in Japan (Yamasaki et al., 2009). In principle, micro / nano bubble particles are generated from submerge pump generators plus air pressure and water discharge (Agarwal et al., 2011).

According to Tsuge (2015) smaller of particle size and greater the surface area of the particle so that dissolved oxygen solubility in water can be increased. Therefore, it can be applied to fish culture with high stocking density. High density production that produce through recirculation systems (Abbink et al., 2011) is beneficial to fish farmers due to land constraints and environmental issues (Martins et al., 2010) and increasing demand.

Koi fish *C. carpio* is one of ornamental fish that has high demand. The demand of Koi fish has risen. However, the Koi fish production did not fulfill the demand by increasing only 9.43% between 2014 and 2015. Koi fish accounted for 26.8% of the total production of national ornamental fish (KKP 2015). The micro / nano bubble technology is expected to improve the yield of koi fish by increasing oxygen dissolve in the water that is suitable for fish growth and (Crab et al., 2012) and the outcome is sustainable integrated aquaculture (Wang and Lu, 2015). Hence, our study was to determine the the characteristics of micro / nano bubble from Lutor and its effect on koi fish *C. carpio* biology performance.

2. Materials and Methods

Time and place

This micro / nano bubble research activity was held in cooperation with Instrumentation Development Center (BPI) LIPI - Bandung. This research was conduct in June - October 2017 at Pathology Laboratory - PSSP (Pusat Studi Satwa Primata) IPB Cilibende and Laboratory of Fishery Pond Experiment of Babakan, FPIK – IPB.

Basic characteristics test of micro / nano bubble Lutor

In this section was purpose to measure the performance of Lutor (LIPI Ultrafine Bubble Generator) was used to introduce micro / nano bubble in the fish tanks. This prototype has patented with patent register number P00201706248. This tool is a series consisting of a water pump and nozzle. Its tool in plain water without biota and more fundamental toward physics and chemical (water quality). These activities were done in the container so it can determine the characteristics of the tool. The prototype of Lutor were shown in Figure 1.

Figure 1. Lutor (LIPI Ultrafine Bubble Generator) with Capacity 120 L . 80 watt^{-1} and Sprayer Bubble Distance 1.5 m.

Micro / nano bubble Lutor test

We got three the prototype unit from BPI LIPI – Bandung, so their used in three container water stock for fish culture (120 L) and every volume container for 3 replay water culture media. In micro / nano bubble Lutor test, there were three variations of test tool, namely: 1st Lutor vertical (nozzle and water pump in vertical position, like Figure 1), 2nd Lutor horizontal (nozzle and water pump in horizontal position) and

3rd water only used air stone (without Lutor). The parameters tested were dissolved oxygen for some time. The value bubble size was measured with Particle Image Velocimetry (Figure 2), dissolved oxygen was measured every 20 minutes by means of DO meters and observed the greatest probability of increasing the dissolved oxygen value from the three variations of the tool. The largest value of dissolved oxygen used to research biological performance research.
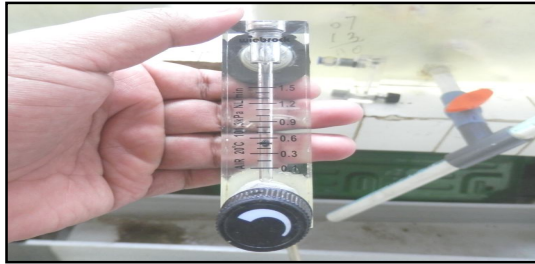
Figure 2. Particle Image Velocimetry (with specification Air 20^0C 101.3kPa NL . min^{-1}) for manual sizing of micro / nano bubble was used in research with principle gas pressure.

Micro / nano bubble experiment design

The experiment design was used completely randomized design with one factor (fish density) three treatments and three replications. Treatment A (15 fish.60 L^{-1}), B (30 fish.60 L^{-1}) and C (45 fish.60 L^{-1}). The test parameters include : daily water quality (dissolved oxygen, temperature, water pH) and 14 daily (total ammonia nitrogen, nitrite, and total organic requirement or TOM) according to the methods in Muangkeow et al., (2011) that physiological response parameters: oxygen consumption level, and cholesterol measured from the amount of fish blood taken from the base of a koi tail with a 1 ml plastic syringe.The blood sample was preparation then transferred to the tube, fed into the ice box then measured by clinical method. Measured koi fish biology performance parameters: survival rate, absolute length, specific growth rate weight and final biomass rate.

Preparation of koi fish sample

The fish was used in this observation was koi fish that has 7.49 ± 0.29 cm length with a weight of 11.43 ± 1.92 g.fish^{-1}. Koi fish was collected from koi fish farmers at Parung, Bogor, West Java which are maintained in a recirculation system in plastic containers with volume of 120 L. Change water pond for quality management by 2 times a week before feeding. According in Rahman (2015) commercial feed was applied to fish during experiment for aquaculture with high stocking density, so we used commercial feed PF 800 product by PT. Matahari Sakti. Feeding was carried 3 times a day (07.00 am, 13.00 pm and 19.00 pm) and *ad satiation*.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to examine difference of parameters among treatments. Post hoc test used with Duncan Multiple Range. Probability level at α = 0.10 was considered significant. All statistical analysis was performed using IBM SPSS Statistics version 24, and microsoft office excel 2010.

3. Results and Discussion

Physical characteristics of micro / nano bubble Lutor

The material used was PVC, the bubble result from Lutor vertical position in culture water stock : horizontal bubble spray distance maximum 150 cm, furthest distance vertical spray is 50 cm. The micro or nano size formed is unstable, for the size of a micro meter (dominated) formed 25 µm and nano meter nm 800 nm. The stability of the 80 % of bubble results is influenced by the magnitude of the pumping power (watts) and the type of nozzle used and the air impacts 20 %.

Chemical characteristics of micro/nano bubble Lutor

The use of Lutor in different treatments of Koi fish density was affect the value of water quality, such as temperature (27.02 ± 0.44), dissolved oxygen (5.20 ± 0.79), pH (6.32 ± 0.46), TAN (0.19 ± 0.15), nitrite (0.89 ± 0.40), and TOM (3.93 ± 1.60). Only nitrite in Lutor container did not quality standard for koi fish aquaculture. Stirring speed with high organic waste conditions has an impact on the chance of bacteria that convert nitrites to nitrate very low because bacteria could not associate object in water, and its ecology (Boyd 2015) and finally had impact mortality of fish. The list of chemical result in water stocks of Lutor were shown in

Table 1. Chemical characteristic (water quality) of Lutor vertical

Parameter	Unit	Water Container by Lutor	Quality Standar
Temperature	^{0}C	27.02 ± 0.44	25-30 [1]
Dissolved Oxygen	mg.L^{-1}	5.20 ± 0.79	>3 [2]
pH	degree	6.32 ± 0.46	6 – 9 [3]
TAN	mg.L^{-1}	0.19 ± 0.15	< 5 [4]
Nitrite	mg.L^{-1}	0.89 ± 0.40	< 0.1 [5]
TOM	mg.L-1	3.93 ± 1.60	< 10 [2]

1) SNI : 01- 6133 - 1999
2) Boyd 2015
3) Boyd and Nevin 2015
4) Boyd and Gautier 2000
5) Wedemeyer 1996

Best position of Lutor in water

The result of the experimental test showed that the initial DO to treatment was 4.33 mg.L^{-1} from all treatments and this value increased after 20 minutes of Lutor application. The highest dissolved oxygen value from the initial 20 minutes was present in the Lutor vertical position at 37.39 % (6.10 mg.L^{-1}) followed by the horizontal position Lutor at 31.64 % (5.70 mg.L^{-1}) and the aerator 13.16 % (4.90 mg.L^{-1}) as shown in Figure 3. This showed that Lutor positioned vertical was better than other; therefore this position was applied on the next research.
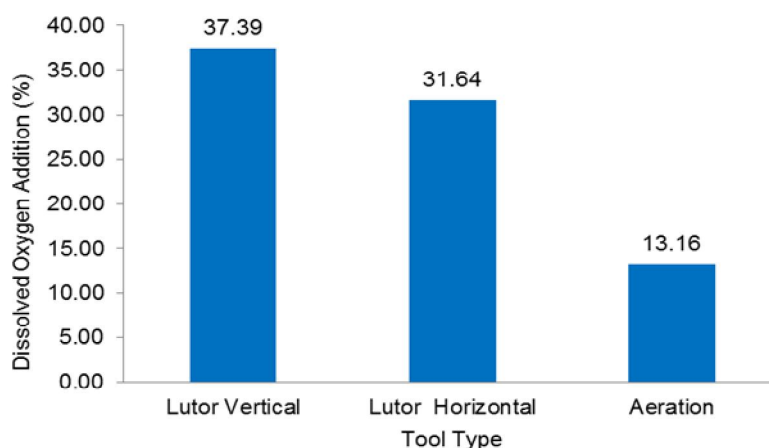

Figure 3. The mean result of dissolved oxygen addition the from 0 - 20 minutes firstly from different kind of tools (%). Lutor vertical position with higher score (37.39%) than other Lutor horizontal position (31.64%) dan Aeration (13.16%).

Fish biological performances of Lutor (physiological response)

There were several parameters of physiological response measured, cholesterol and total oxygen consumption. The highest final cholesterol value was C treatment (135.67 ± 30.83[a] mg.dL^{-1}) with 52.59 % mortality (highest mortality), treatment A (131.67 ± 16.07 [a] mg.dL^{-1}) with 37.78 % mortality (lowest mortality) and B treatment (124.00 ± 11.14[a] mg.dL^{-1}) with 48.89 % mortality (middle mortality). The cholesterol value of all treatments was likely to be higher than the first treatment (112.00 ± 0.00 mg.dL^{-1}). Nevertheless, treatment C was almost similar the quality standard for cholesterol 151.7 mg.dL^{-1} (Wedemeyer 1996).

Cholesterol value in treatment C was very high because at the beginning of the study there are many deaths due to high nitrite, so stress decreases and affects the formation of more cholesterol. The value of cholesterol of fish is influenced by the process of taking blood samples (Tripathi et al., 2004). Therefore, the best cholesterol value is treatment A due to the lowest fish density and lowest mortality rate from initial treatment, and all treatment not significant different in statistic.

The lowest difference in the rate of oxygen consumption between the final and the initial of the study sampling was on treatment A (0.06 mg.L^{-1}) is the lowest treatment than C treatment (0.09 mg.L^{-1}). It was happened because the mortality rate at treatment A (37.78 %) was lower than the treatment of (48.89 %) and C (52.59 %), in addition the low amount of fish density encourages the need for low dissolved oxygen and all treatment not significant different in statistic. Peoples et al., (2008) were showed that low oxygen consumption levels result in a decrease in energy requirements for the adjustment of fish to the environment derived from the reversal of glucose and oxygen, so that energy can be used for stored in glycogen The list physiological response parameters of koi fish is shown in Table 2.

Table 2. Physiological response (Cholesterol and Oxygen Consumption Rate) parameter koi *Cyprinus carpio*

Parameter	A (15 fish.60 L^{-1})		B (30 fish.60 L^{-1})		C (45 fish.60 L^{-1})		Quality Standar
	Initial	Final	Initial	Final	Initial	Final	
Cholesterol (mg.dL^{-1})	112± 0.00	131.67± 08.06[a]	112± 0.00	124± 5.07[a]	112± 0.00	135.67± 15.42[a]	151.7 [1]
Oxygen Consumption Rate (mg.L^{-1})	0.19± 0.00	0.13± 0.01[a]	0.19± 0.00	0.11± 0.003[a]	0.19± 0.00	0.10± 0.03[a]	$(DO)_{t-0} = <$ [2]

[1] *Wedemeyer 1996*
[2] *Peoples et al 2008*

Fish biological performances of Lutor (survival rate)

The survival rate of koi fish during the observation calculated at the end of the study indicated that the highest value was A treatment with 62.22 ± 5.09[a] % and the lowest treatment C with value 47.41 ± 7.88[a] %, but all treatment have not significant different in statistic. The value of survival rate has shown in Figure 4.
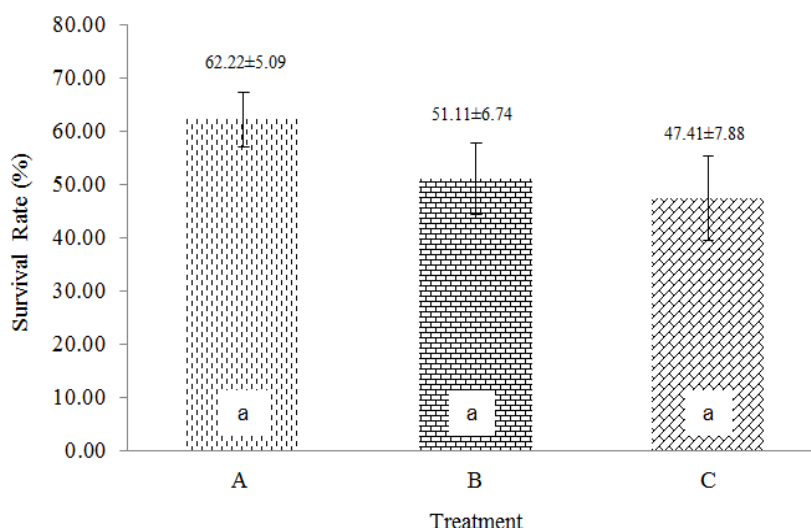

Figure 4. Survival rate of koi fish with Lutor. The A treatment (15 fish. 60 L^{-1}) was highest score 62.22 ± 5.09[a] % but not significant in statistic and lowest C treatment 47.41 ± 7.88[a] % (15 fish. 60 L^{-1}) was not significant in statistic.

Fish biological performances of Lutor (absolute length)

The highest score for the absolute length during the study was treatment A (15 fish. 60 L^{-1}) with a score of 0.52 ± 0.07[a] cm, and the lowest treatment was C treatment (45 fish. 60 L^{-1}) with a score of 0.29 ± 0.16[b] cm and B treatment (30 fish. 60 L^{-1}) with score 0.36 ± 0.07[ab]. Treatment A significant different with C treatment in statistic. The value of absolute length has shown in Figure 5.

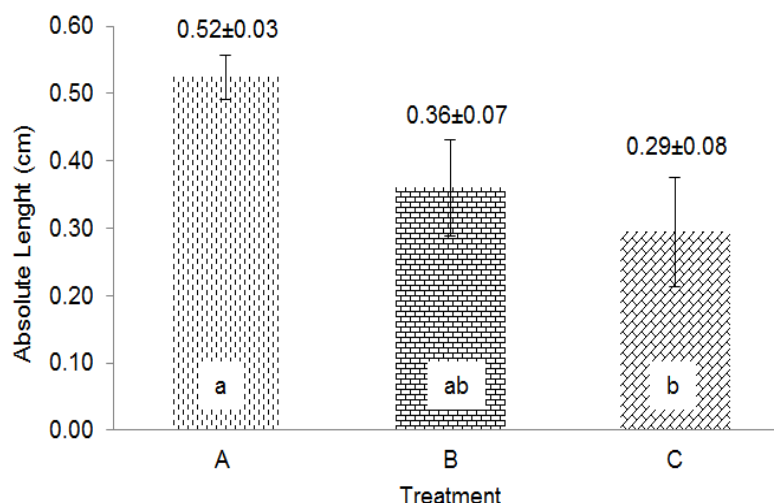**Figure 5**. Absolute length of koi fish with Lutor. The highest score for the absolute length during the study was treatment A (15 fish. 60 L^{-1}) with a score of 0.52 ± 0.07^a cm, and the lowest treatment was C treatment (45 fish. 60 L^{-1}) with a score of 0.29 ± 0.16^b cm. Treatment A significant different with C treatment in statistic.

Fish biological performances of Lutor (specific growth rate-weight)

The other biological status of koi fish observed was specific grow rate (SGR weight). The absolute length growth of koi fish was the growth rate of koi fish. Evident from the highest value of koi fish weight was treatment A with the percentage of SGR weight at 2.35 ± 0.07^a % and the lowest was at treatment C with score of 1.17 ± 0.05^b % and B treatment with score 1.19± 0.12^b %. Treatment A significant different in statistic with treatment B and C. The value of absolute length has shown in Figure 6.
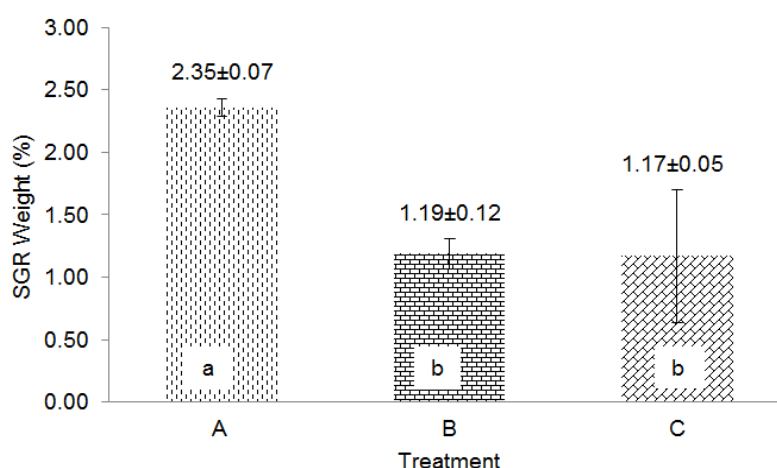

Figure 6. SGR weight of koi fish with Lutor. The highest value was treatment A w 2.35 ± 0.07^a % (15 fish. 60 L^{-1}) and the lowest was at treatment C with score of 1.17 ± 0.05^b % (45 fish. 60 L^{-1}). Treatment A significant different in statistic with treatment B and C.

Fish biological performances of Lutor (final biomass rate)

The highest koi fish biomass parameter was treatment A with score 13.67 ± 0.33^a g and the lowest was treatment C with score 13.09 ± 0.49^a g and treatment B with score 13.27 ± 0.47^a g, but all treatments not significant in statistic. The value of absolute length has shown in Figure 7.
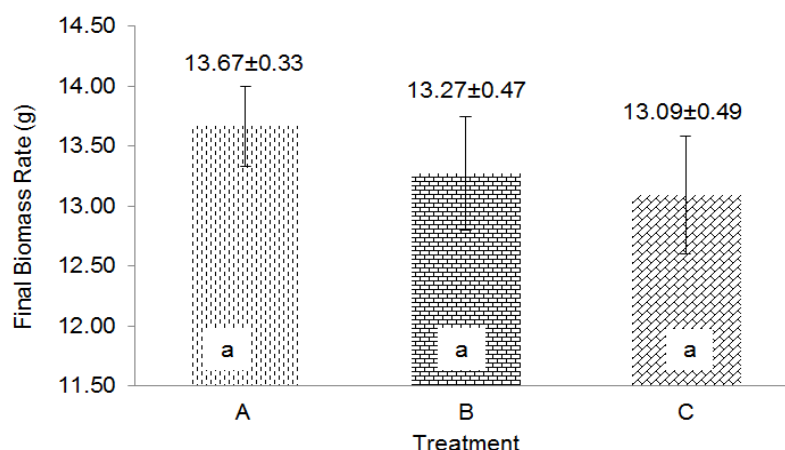
Figure 7. Final biomass rate of koi fish with Lutor. The highest score was treatment A 13.67 ± 0.33[a] g (15 fish. 60 L^{-1}) and the lowest was treatment C with score 13.09 ± 0.49[a] g (45 fish. 60 L^{-1}), but all treatments not significant in statistic.

All of them, there could be seen that long growth is linearly applied with weight growth. In accordance with the statement of Abbink et al., (2011) that growth (length and weight) is proportional to the optimum condition of water quality (quality standard) influenced by fish density. In addition, high oxygen solubility has an impact on the lack of competition to obtain food, so that with low fish density (A treatment) koi fish has optimal growth (Barreto et al., 2010).

4. Conclusion

The size of bubble generated by Lutor is unstable bubble produce, between 800 nm - 25 μm (dominated). The spread of bubble is far so that the stirring of fish culture is high mortality (up welling) rate of fish. Lutor vertical position optimizes the addition of the fastest dissolved oxygen. The power of the submersible pump (watt) on the Lutor becomes the main factor determining the stability of the bubble size compared to the nozzle. A treatment (15 fish.container[-1]) showed the best result due to highest data in of biological performance : survival rate, and final biomass rate (not significant different in statistic), also absolute length, and SGR-weight (significant different in statistic).

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