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Biofloc technology on the intensive aquaculture of bronze corydoras ornamental fish *Corydoras aeneus* with different stocking densities

Teknologi bioflok pada budidaya intensif ikan hias koridoras *Corydoras aeneus* dengan padat tebar berbeda

Iis Diatin[1*], Muhammad Agus Suprayudi[1], Tatag Budiardi[1], Enang Harris[1], Widanarni[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), West Java, Indonesia 16680
*Corresponding author: iisdiatin@yahoo.co.id

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ABSTRACT

Ornamental fish is non consumption fish which is an important source of Indonesian foreign exchange. The objective of this study is to analyze the productivity of bronze corydoras *Corydoras aeneus* ornamental fish through increased stocking density with biofloc technology. The average weight of the experimental corydoras was 0.61–0.72 g with 2.32–2.40 cm standard length. This study used a randomized design method with biofloc technology treatment in 3000, 4500, and 6000 fish/m^2 stocking densities. The results showed that the daily length and weight-growth rate among treatments were not significantly different (P>0.05), while survival rate and the number of fish production on all treatments were significantly different (P<0.05). The water quality during the rearing period, such as temperature, pH, alkalinity, ammonia, nitrite, and nitrate, were in a tolerable range for corydoras culture. The total suspended solids tended to be higher due to higher stocking density. The best productivity using biofloc technology obtained from 6000 fish/m^2 stocking density.

Keywords: Biofloc technology, *Corydoras aeneus*, growth rate, stocking density, survival rate.

ABSTRAK

Ikan hias merupakan produk perikanan non konsumsi yang menjadi sumber devisa Indonesia yang cukup penting. Penelitian ini bertujuan untuk menganalisis produktivitas ikan hias koridoras melalui peningkatan padat tebar dengan teknologi bioflok. Ikan yang digunakan adalah ikan hias koridoras *Corydoras aeneus* berbobot 0,61–0,72 g dan panjang baku 2,32–2,40 cm. Penelitian ini menggunakan rancangan acak lengkap dengan perlakuan teknologi bioflok pada padat tebar 3000, 4500, dan 6000 ekor/m^2. Hasil penelitian menunjukkan bahwa laju pertumbuhan harian panjang dan bobot antar perlakuan tidak berbeda nyata (P>0,05), sedangkan kelangsungan hidup dan jumlah produksi ikan pada semua perlakuan berbeda nyata (P<0,05). Nilai kualitas air selama pemeliharaan yakni suhu, pH, alkalinitas, amonia, nitrit, dan nitrat yang berada pada kisaran yang cukup baik untuk budidaya ikan. Total padatan tersuspensi cenderung tinggi akibat dari semakin tinggi padat tebar. Produktivitas terbaik pada budidaya ikan koridoras dengan teknologi bioflok adalah pada padat tebar 6000 ekor/m^2.

Kata kunci: *Corydoras aeneus*, kelangsungan hidup, padat tebar, pertumbuhan, teknologi bioflok

INTRODUCTION

Indonesian ornamental fish production continues to increase for years. In 2016, Indonesian ornamental fish production had reached 1.3 billion fish and expected to reach 2.5 billion fish in the coming year (KKBK, 2017). Indonesia has proclaimed the national action plan of the ornamental fish industry to achieve this target (KKBK, 2017). Ornamental fish nowadays is a very prospective business with a great opportunity for development, thus has been grown into the fisheries industry in Indonesia. An effort to increased production was through the intensive fish culture system due to the limited natural resources such as land and water availability (Emerenciano *et al*., 2012; Gutierrez *et al*., 2016; Ahmad *et al*., 2017). This intensive culture system performed by maintaining fish production with high density (Avnimelech, 2012).

Corydoras is one of the export commodities that has been cultured in urban areas. The size of this fish is relatively small (5–8 cm) with an attractive shape, therefore it is possible to be maintained in the aquarium. There are approximately 170 species of corydoras fish widespread in the world (Tencatt *et al*., 2016). The low stocking density has been an issue that prevents the boost in the productivity. Performing an effective and efficient land and water usage, including increasing productivity and stocking density, could solve the problem. However, high stocking density causes declined water quality, resulting poor water environment, therefore requiring high control of environment, nutrients, predators, competitions, and disease agents (Appleford *et al*., 2012). One effort to reduce water quality degradation is applying the biofloc technology. This technology was generally applied to the intensive cultivation system with minimum or without water exchange (zero water discharge) and utilized the bacterial activity to degrade the organic material residue accumulation in water (Avnimelech, 2012). This technology increased production and contribute to sustainable fish culture support (Bossier & Ekasari, 2017).

Biofloc technology had been applied in shrimp and fish culture. Nile tilapia culture with biofloc technology produced better growth rate than the control treatment (Widanarni *et al*., 2012; Luo *et al*., 2014; Long *et al*., 2015; Zhang *et al*., 2016; Pinho *et al*., 2017; Mansour & Esteban, 2017) also improved the fingerling quality and production performance (Ekasari *et al*., 2015; Perez-Fuentes *et al*., 2016; Alves *et al*., 2017; Garcia-Rios *et al*., 2019). Biofloc technology also increased the growth rate and fish feed efficiency in *Labeo victorianus* (Magondu *et al*., 2013), Pangasius catfish (Sutama *et al*., 2016), African catfish (Green *et al*., 2014; Bakar *et al*., 2015; Dauda *et al*., 2018a), and goldfish (Bakhshi *et al*., 2018). Biofloc technology enhances the survival rate of *Pseudotropheus saulosi* fish (Harini *et al*., 2016) and nile tilapia (Fleckenstein *et al*., 2018), as well as increasing nile tilapia immunity (Liu *et al*., 2018). Biofloc technology application in ornamental fish had been reported on goldfish (Faizullah *et al*., 2015; Wang *et al*., 2015), gibel carp *Carassius auratus gibelio* (Qiao *et al*., 2018), *Pelteobagrus vachelli* fish (Deng *et al*., 2018), and fin barb hybrid lemon fish (Dauda *et al*., 2018b). Previous experiment of the intensive corydoras fish culture with high density has been performed on the water exchange system with 30%/day and produces the best survival rate with 25 fish/L stocking density, equivalent to 3750 fish/m^2 (Diatin *et al*., 2014) and 4500 fish/m^2 using 100%/day water exchange (Diatin *et al*., 2015).

Based on the survey results on the corydoras ornamental fish culture center in Bogor and Depok, the ornamental fish farmers keep maintaining the application of low stocking density environment conditions (extensive system) with 80–400 fish/m^2, resulting in relatively low production of the fish. An experiment of biofloc technology application ornamental fish culture with high stocking density never performed. Therefore, this study aimed to analyze the productivity of bronze corydoras ornamental fish (*C. aeneus*) with biofloc technology on different stocking densities.

MATERIALS AND METHODS

Fish rearing

The fish used in this study was bronze corydoras fish (*C. aeneus*) with 0.61–0.72 g weight and 2.32–2.40 cm standard length. The fish obtained from the hatchery in Bekasi, West Java. This study located in the laboratory of aquaculture production and management engineering, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University Bogor. The experiment performed by a randomized design method with three treatments and three replications. Treatments given in this study were different stocking densities of corydoras fish on the fish culture using biofloc technology, namely 3000 fish/m^2 (BFT3000), 4500 fish/m^2 (BFT4500),

Table 1. Daily length and weight growth rate, survival rate, and total production number of bronze corydoras fish on different stocking densities using biofloc technology

Parameter	Treatment		
	BFT3000	BFT4500	BFT6000
Daily weight growth rate (%/day)	1.02 ± 0.06[a]	0.96 ± 0.03[a]	0.97 ± 0.08[a]
Daily length growth rate (%/day)	0.30 ± 0.01[a]	0.30 ± 0.01[a]	0.29 ± 0.01[a]
Survival rate (%)	67.50 ± 2.20[c]	62.04 ± 1.60[b]	50.42 ± 3.00[a]
Total production (fish/m^2)	2025 ± 66[c]	2792 ± 72[b]	3025 ± 180[a]

*The same letter on the same line shows insignificant different value with 5% confidence level (Duncan test)

Table 2. The range value of DO, temperature, and pH during the rearing period of bronze corydoras culture using biofloc technology with different stocking densities

Parameter	BFT3000	BFT4500	BFT6000
DO (mg/L)	4.2–5.9	4.1–5.9	4.2–5.6
Temperature (ºC)	25.2–26.6	25.3–26.6	25.3–26.5
pH	7.7–8.7	7.6–8.8	7.5–8.4

and 6000 fish/m^2 (BFT6000), referred to Diatin *et al*. (2015). The fish density on each aquarium treatment was 120, 180, and 240 fish.

The fish placed on a pre-treatment condition of 10 days emplacement in aquarium sized 100 cm × 50 cm × 40 cm with 1500 fish/m^2. At the same time, the fish were fed sludge worm with *ad libitum* method during this adaptation period. Sequentially, the ornamental fish were kept for 40 days in aquarium sized 20 cm × 20 cm × 20 cm, then aerated and filled with water as high as 15 cm. Water volume total of each aquarium was 6 L. Biofloc technology used C/N ratio 12 with molasses as the carbon source (45.27% carbon level). Molasses were given daily on 2 hours after feeding. N content in feed and molasses requirements on each day was calculated based on the formula of De Schryver *et al*. (2008).

This fish fed of live sludge worm containing 8.12% protein, 4.26% lipid, 0% crude fiber, 82.29% water, 1.97% ash, and 4.26% NFE (nitrogen free extract). Feed was given twice a day at 07.00 and 18.00 GMT+7 as much as 5% of fish biomass per day. Water was added every two weeks on all treatments to maintain the water quality and reduced water volume due to evaporation.

Parameters

Growth sampling performed every ten days by measuring the fish length and weight from 30 fish on each treatment. Survival rate sampling performed concurrently with growth sampling by calculating the total number of living fish. Water quality was measured daily during the fish rearing containing dissolved oxygen, pH, and temperature. Water quality measurement performed in the morning (at 07.00 GMT+7). The measurement of ammonia, nitrite, nitrate, alkalinity, TSS (total suspended solid), VSS (volatile suspended solid), and floc volume performed weekly. Bacterial abundance and content in water and fish intestinal fish determined at the beginning and end of the study.

The fish weight was measured using a 200 g capacity digital scale, while the fish length was measured using vernier caliper. Growth and survival rates were calculated using Huisman (1987) formula. Temperature measurement used thermometers, dissolved oxygen used DO meter, pH used pH meter, and turbidity level used Turbidimeter. Alkalinity was measured using a volumetric method, whereas TSS and VSS value was measured using the gravimetric method. The measurements of ammonia, nitrite, and nitrate were performed using spectrophotometer with APHA (1989) method. The floc volume was measured using 15 ml conical tube and calculated based on Avnimelech (2012) formula. Bacterial abundance was analyzed using the spread plating method. Bacteria that dominantly grew on each sample were identified based on the physiology and biochemistry characteristics (Cowan & Steel, 1974).

Data analysis

Data on the observation results were presented based on the average value and standard deviation. Data were processed and analyzed using Ms. Excel 2007 software and SPSS 20.0. The daily

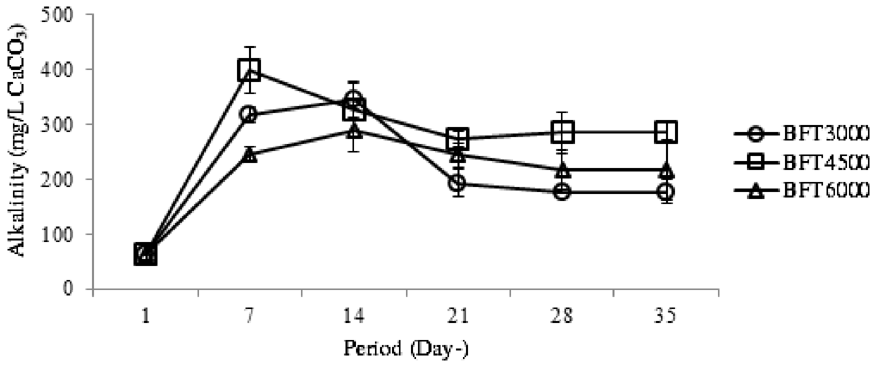

Figure 1. The alkalinity level of water media for bronze corydoras culture using biofloc technology with different stocking densities.

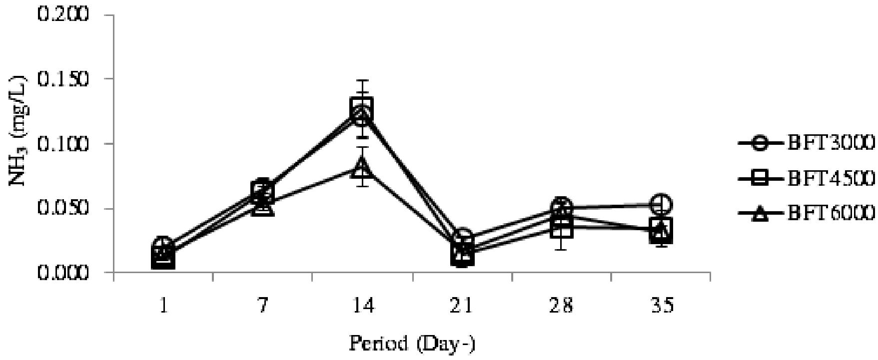

Figure 2. The ammonia (NH_3) level of water media for bronze corydoras culture using biofloc technology with different stocking densities.

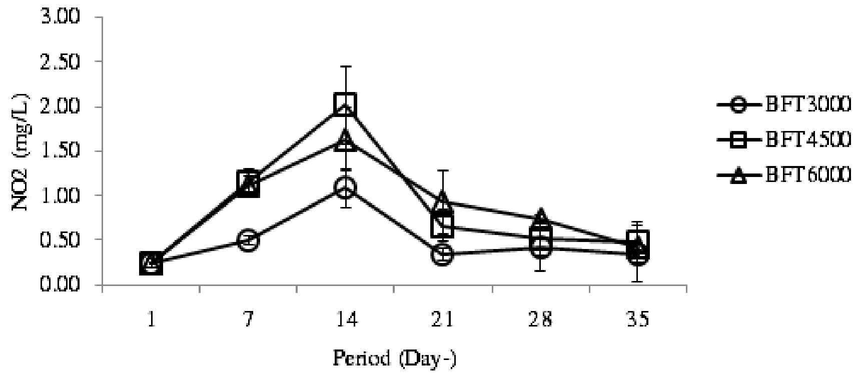

Figure 3. The nitrite (NO_2) level of water media for bronze corydoras culture using biofloc technology with different stocking densities.

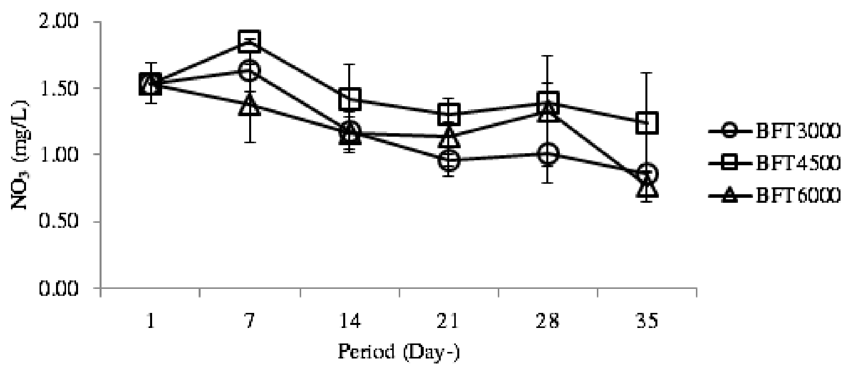

Figure 4. The nitrate (NO_3) level of water media for bronze corydoras culture using biofloc technology with different stocking densities.

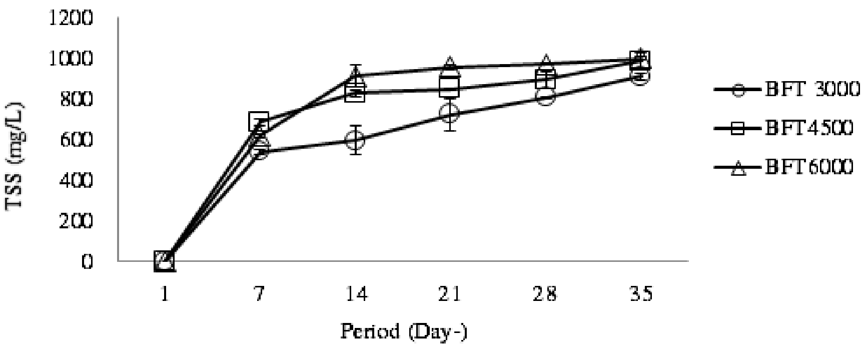

Figure 5. The TSS (total suspended solids) level of water media for bronze corydoras culture using biofloc technology with different stocking densities.

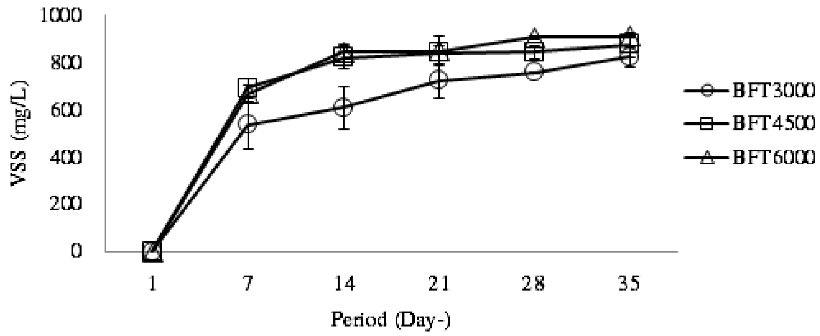

Figure 6. The VSS (volatile suspended solids) level of water media for bronze corydoras culture using biofloc technology with different stocking densities.

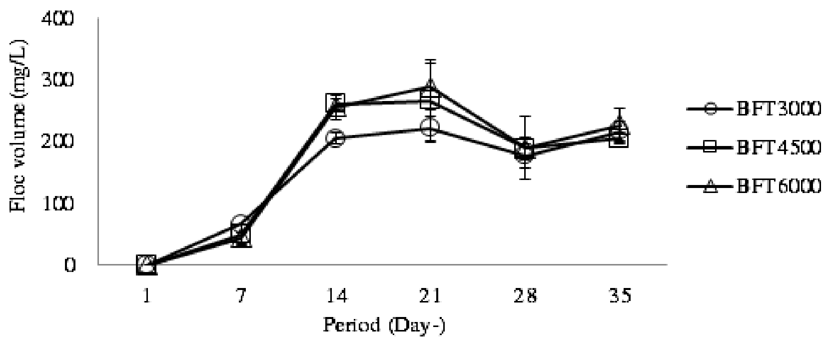

Figure 7. The floc volume of water media for bronze corydoras culture using biofloc technology with different stocking densities.

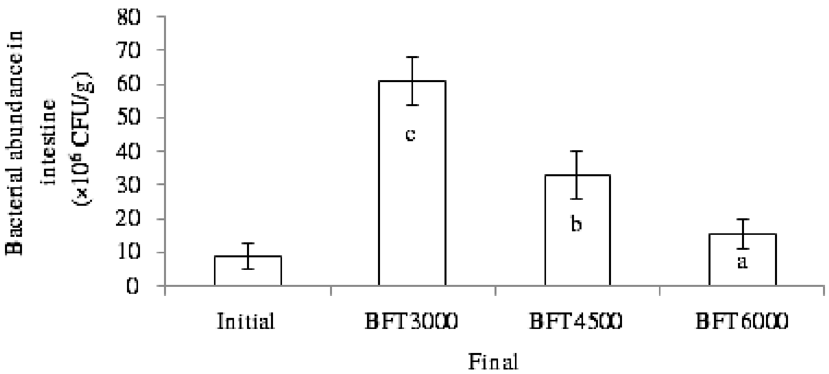

Figure 8. Bacterial abundance on the intestine of bronze corydoras fish on biofloc technology with different stocking densities.

Table 3. Bacterial type on the rearing media and intestine of bronze corydoras fish on the biofloc technology with different stocking densities

Treatment period		Bacterial type	
		Water	Fish intestine
Initial		*Bacillus* sp.	*Streptobacillus* sp.
		Kurthia sp.	*Kurthia* sp.
		Enterobacteria sp.	*Enterobacteria* sp.
Final	BFT3000	*Streptococcus* sp.	*Enterobacteria* sp.
		Enterobacteria sp.	*Alcaligenes* sp.
		Kurthia sp.	*Bacillus* sp.
		Acinetobacter sp.	*Kurthia* sp.
	BFT4500	*Streptococcus* sp.	*Enterobacteria* sp.
		Enterobacteria sp.	*Alcaligenes* sp.
		Kurthia sp.	*Bacillus* sp.
		Acinetobacter sp.	*Aeromonas* sp.
	BFT6000	*Kurthia* sp.	*Pseudomonas* sp.
		Enterobacteria sp.	*Enterobacteria* sp.
		Streptococcus sp.	*Kurthia* sp.
		Acinetobacter sp.	*Alcaligenes* sp.
			Bacillus sp.

growth, survival, and fish production rate were analyzed using analysis of variance (ANOVA) at 95% confidence interval, then continued with Duncan significant different test.

RESULT AND DISCUSSION

Result

Daily growth rate

The daily weight and length growth rate, as well as survival rate and total production of bronze corydoras fish on each treatment presented in Table 1.

Statistical analysis results (Table 1) indicated that the value of daily length and weight growth rate on all treatments were insignificantly different ($P>0.05$). However, the survival rate of bronze corydoras ornamental fish produced significantly different value on each treatment. The highest survival rate showed in 3000 fish/m^2 stocking density, however, the highest total production showed in 6000 fish/m^2 stocking density.

The daily water quality parameters measured were DO, temperature, and pH which are presented in Table 2. Other water quality parameters, such as alkalinity, ammonia, nitrite, and nitrate, presented in Figures 1–4.

The study result showed that the TSS and VSS value increased after 14th days of rearing, while the floc volume extremely increased on 21st days of rearing, as presented in Figure 5, 6, and 7. The bacterial abundance level and the types were shown in the following chart (Figure 8). Bacterial on the rearing media using biofloc technology obtained five dominant types, while seven dominant types were obtained from the bronze corydoras fish intestine, as presented on Table 3.

Discussion

The daily length and weight growth rate (Table 1) among treatments were insignificantly different as the stocking density of bronze corydoras fish culture using biofloc technology did not affect the length and weight growth of fish. This condition suggests that bronze corydoras fish on all treatments acquired adequate feed intake. Corydoras fish usually form flock (school and shoal) to find food and produced a sound to draw attention so that when fish are schooling, the feed will be utilized efficiently by all fish (Ithurralde *et al.*, 2014; Hadjiaghai & Ladich, 2015).

High stocking density caused limited fish movement, causing space competition, increased aggressiveness among fish, and cannibalism that leads to death (Manley *et al.*, 2014; Barros *et al.*, 2019; Xi *et al.*, 2017). Fish aggressiveness will result in increased friction among fins and movements that will injure other fish. Fish aggressiveness will also produce a dominant fish in their group, making the lost fish will divide, stressed, then dead (Huntingford & Damsgard, 2012). The survival rate of bronze corydoras fish using biofloc technology yield a significant difference among treatments. The highest survival rate was found on 3000 fish/m^2 stocking density, while the lowest found on 6000 fish/m^2 stocking density (Table 1). Higher stocking density of fish cultured with biofloc technology results in a low survival rate. This result was in line with Magondu *et al*. (2013) on *Labeo victorianus*, Widanarni *et al*. (2012) on red tilapia, Schveitzer *et al*. (2013) on vaname shrimp, and Ardiansyah and Fotedar (2016) on snapper juvenile. However, the total production of bronze corydoras fish produced at the end of the study showed that the highest total production discovered on a very high intensive treatment (BFT6000). This was presented by the total production of fish at the end of the study on all treatments, i.e., BFT3000, BFT4500, and BFT6000 with 2025 ± 66 fish/m^2, 2792 ± 72 fish/m^2, and 3025 ± 180 fish/m^2 respectively (Table 1).

Increased stocking density in an intensive culture can lead to decreased quality of culture water and increased waste metabolites in the culture environment (Emerenciano *et al*., 2012; Luo *et al*., 2013). *C. aeneus* live optimally at 24–28°C temperature, pH 6–7 (Alderton, 2008), pH 6.2–8.5 (Mahapatra & Dutta, 2014), and about 3 mg/L DO (Boyd, 2010). Corydoras fish lives in the water base (Davis *et al*., 2014) with additional breathing apparatus in intestines, making it more tolerant to low oxygen level (Satora *et al*., 2017). The temperature level during corydoras culture was 25.2–26.6°C, pH 7.5–8.8, and DO 4.1–5.6 mg/L (Table 2), which was still in the tolerance for corydoras fish culture.

Biofloc system can cause alkalinity fluctuation (Furtado *et al*., 2015). This study showed increased alkalinity value on the 7th day of rearing, then stabilized on the 21st day until the end of the study (Figure 1). The same result found on the vaname shrimp with biofloc technology, showing the relatively stable alkalinity value after the 21st day (Furtado *et al*., 2015). Corydoras fish prefer water with a neutral or slightly alkaline condition (Alderton, 2008). Previous studies with biofloc technology in vaname shrimp acquired alkalinity value reached 300 mg/L (Furtado *et al*., 2015) and 200 mg/L (Maica *et al*., 2018), then goldfish reached 260 ± 4.2 mg/L (Faizullah *et al*., 2015).

Ammonia concentration increased on 14th day, then decreased at the end of the study with the highest value was found on BFT4500 treatment of 0.128 ± 0.022 mg/L. However, the overall value could still within the range of toleration of corydoras fish. The ammonia reduction on African catfish (*Clarias gariepinus*) culture with C/N 15 began on 9th to 12th day, until 30th day reaching 98.7%, while C/N 10 increased ammonia reduction occurred from 18th to 21st day, reaching 98.51% (Bakar *et al*., 2015). This study used C/N 12 ratio, therefore, the increasing ammonia value to the maximum on the 14th day was most likely occured due to the conversion of ammonia by the heterotrophic bacteria, which performed from the 14th day until the end of the study.

Ammonia is generally toxic to fish (Shiwanand & Tripathi, 2013) when reaching out of 1.5 mg/L (Yusoff *et al*., 2011; Avnimelech, 2012). The concentration of 2 mg/L nitrite causes the slow growth rate of fish and 4 mg/L causes acute death (Yusoff *et al*., 2011). Acute concentration of nitrite in yellow catfish (Pelteobagrus fulvidraco) sized 0.029 ± 0.049 g was 8.74 mg/L (Zhang *et al*., 2012), while nitrite content of 3.92 mg/L lowered the daily growth rate below 2% in african catfish (*C. gariepinus*) (Roques *et al*., 2015). Nitrates are relatively non-toxic to fish, but the concentrations of 75 mg/L will reduce the growth rate on fish juvenile (Yusoff *et al*., 2011). The concentration of lethal ammonia (LC_{10}) is 0.120 mg/L and LC_{50} is 0.146 mg/L for corydoras fish (Souza-Bastos *et al*., 2017), while nitrite concentration reaches 0.4 mg/L and nitrate 2.5 mg/L (Casenave *et al*., 2005). Corydoras fish includes in the ornamental catfish with ammonia-tolerant (Santos & Esteves, 2015; Souza-Bastos *et al*., 2017), whereby the concentration of ammonia, nitrite, and nitrate acquired in this study were still in the tolerance range for corydoras culture.

The existence of biofloc was detected through TSS, VSS, and floc volume value (De Schryver *et al*., 2008; Avnimelech, 2012). One main character of biofloc system was high suspended solid value as an indicator of the high water suspended organic material (Crab *et al*., 2012). The TSS value range recommended for biofloc technology should be 200–1000 mg/L (De Schryver *et al*., 2008). Tilapia culture obtained TSS value with 643 mg/L (Avnimelech, 2012) and 421–457 mg/L in shrimp cultivation integrated with tilapia (Poly *et al*., 2019). Moreover, channel catfish (*Ictalurus punctatus*) culture produced the lowest survival rate with TSS value of 759.4 ± 44.5 mg/L (Green *et al*., 2014). South American catfish (*Rhamdia quelen*) acquired TSS value with 425 ± 2.38 mg/L (Rocha *et al*., 2017) reaching 1000 mg/L (Poly *et al*., 2015), while on African catfish (*C. gariepinus*) reached 701.25 ± 48.17 mg/L (Dauda *et al*., 2018b). The results of this study showed that the highest TSS value (Figure 5) found in BFT6000 treatment. The height of suspended solid causes the gill interference that causes the fish to breathe hard and lead to death, therefore lowering the survival rate. According to Schveitzer *et al*. (2013), gill disturbances in vaname shrimp due to high suspended solid (800–1000 mg/L TSS value) can reach 60%. VSS is used to measure bacterial biomass (De Schryver *et al*., 2008; Avnimelech, 2012). The heterotrophic system generates approximately 406 VSS/kg feed with a 35% feed protein level (Ebeling & Timmons, 2008). Other studies on vaname shrimp resulted VVS value was 408 mg/L (Kim *et al*., 2013) and on channel catfish (*I. punctatus*) was 641 ± 42.9 mg/L (Green *et al*., 2014). The value of VSS on all treatments at the end of the study ranged 823 ± 3.28 – 910 ± 4.24 mg/L (Figure 6), showing heterotrophic bacterial biomass on this study.

Fish can utilize biofloc as a protein source and feed for growth enhancement, however the certain types of fish in biofloc density should be at the optimum range due to fish feeding behavioral interference, whether given too much biofloc and cause fish stress (Bakar *et al*., 2015). Biofloc is an aggregate composed of floc-forming bacteria, filamentous bacteria, microalgae (phytoplankton), protozoa, organic matter, bacterial eaters (Avnimelech, 2012), zooplankton, fungi and viruses (Browdy *et al*., 2012). Corydoras fish can utilize biofloc as feed evidenced from the bacterial abundance as one of the forming floc aggregates in the fish intestine. The bacterial abundance in the fish intestine at the end of the study increased compared to the initial period as there was an improved bacterial abundance occurred on BFT3000 treatment, reaching six times (Figure 8). The utilization of biofloc as feed sources depends on the species, feeding habits, size, floc density, and floc size (Avnimelech, 2012). The result of this study showed that floc formed from 7th day, then the floc volume increased until 14th day (Figure 7). The floc volume after the 14th day ranged between 206–289 mL/L, indicating floc is available in the water utilized as feed source by corydoras fish.

The bacterial density in the fish intestine during the initial period was 8.9 ± 3.81 × 10^{6} CFU/g, then increased on each treatment differently (Figure 8). Increased bacterial density in fish intestine suggests that corydoras fish can utilize biofloc as a feed source. The bacterial density in BFT3000 treatment increased almost six times, BFT4500 increased almost three times, and BFT6000 increased about 1.5 times. This condition suggests that biofloc can be utilized as a feed source to support the growth of corydoras fish.

Bacterial type in biofloc rearing media (Table 3) obtained five types of dominant bacteria, namely *Bacillus* sp., *Kurthia* sp., *Enterobacteria* sp., *Streptococcus* sp., and *Acinetobacter* sp., while seven types of dominant bacteria found in the fish intestine, namely *Bacillus* sp., *Kurthia* sp., *Enterobacteria* sp., *Alcaligenes* sp., *Streptobacillus* sp., *Aeromonas* sp., and *Pseudomonas* sp. Five types of bacteria found in this study was the same as obtained by Widanarni *et al*. (2013) on red tilapia culture with biofloc technology, discovering six types of dominant bacteria, namely *Bacillus* sp., *Kurthia* sp., *Listeria* sp., Alcaligenes sp., *Enterobacteria* sp., and *Acinetobacter* sp. The biofloc technology could increase the probiotic bacterial abundance that was beneficial for fish growth and immunostimulatory agent (Gutierrez *et al*., 2016; Ahmad *et al*., 2017). The results of the bacterial identification found in this study generally are heterotrophic bacteria. Heterotrophic bacteria grow faster and produce more than 40 times bacterial biomass than autotrophic bacteria (Ebeling & Timmons, 2008), therefore decreased water quality problems due to high density can be more quickly solved by heterotrophic bacteria role in biofloc technology.

CONCLUSION

Bronze corydoras ornamental fish culture using biofloc technology can be applied with 6000 fish/m^{2} stocking density.

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