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# Combination of Biofloc and Sex Reversal Technology in Red Tilapia Intensive Culture (*Oreochromis niloticus*): performance of culture and water quality profile

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**ABSTRACT**

The purpose of this study is to analyze the application of biofloc technology using male monosex larvae to the growth rate, survival rate, feed conversion ratio, feed efficiency, and water quality profile of red tilapia with different stocking densities. The method of this research was monosex red tilapia production (masculinization) using immersion of tilapia larvae aged 10 days after hatching using the hormone 17á-metiltestosterone at a dose of 2 mg/l, stocking density of 250 individuals/m$^3$ at 36° C for 4 hours. Then the biofloc treatment with a density of 50 fish/m$^3$ and 100 fish/m$^3$ without biofloc and with biofloc C/N ratio 15. The results obtained that the pond with biofloc treatment has specific growth rate, average daily gain, feed efficiency, and survival rate higher than control. In addition it has a lower FCR value for biofloc treatment, especially in ponds with stocking densities of 100 fish/m$^3$. In this study, ponds with biofloc treatment at 100 fish/m$^3$ stocking densities gave the best results on aquaculture performance.

***Key words :*** *Monosex, Densities, Survival rate, C/N ratio*

## Introduction

Tilapia culture has developed over the past few decades as a source of high-quality protein. The advantage of tilapia cultivation is the ability to breed and produce new generations quickly and resistant to high-level disease and is more flexible for cultivation in various systems. This fish also tolerate on shallow and turbid water (Silva *et al.,* 2013). Intensive aquaculture is the maintenance of fish with high density per unit area and requires support from production inputs such as seeds and high protein feed. Intensive aquaculture also requires good management including water quality management and other aquatic environments that support optimal growth (Ekasari *et al*., 2013). Increasing the production intensity of juvenile fish is the most possible way to increase the number of fish. However, the increase in density has the potential to lead to high accumulation of waste originating from uneaten feed, feces, and metabolic byproducts which ultimately leads to a decrease in water quality (Fauzi *et* *al*., 2018)

One solution that can be used to overcome the decline in water quality and aquaculture environment is by applying biofloc technology (De Schryver, 2008).

Biofloc technology (BFT) is one of the technologies currently being developed in aquaculture that aims to improve water quality and improve the efficiency of nutrient utilization (Ekasari, 2009). This technology is used to degrade organic waste by microorganisms and produce floc microbial (Najdegerami, 2015). Microbial biomass will then gather with other microorganisms and particles suspended in water to form what is called "biofloc", which can eventually be consumed in situ by cultured animals or harvested and processed as feed ingredients (Avnimelech, 1999; Avnimelech, 2007). Previous studies also have found that bioflocs contribute to the growth and production of tilapia, which are known to use in situ food particles such as suspended bacteria (Avnimelech, 2007; Azim and Little, 2008; Beveridge and Baird, 2000; Little *et al*., 2008; Luo *et al*., 2014). .

Biofloc technology for intensive aquaculture of red tilapia has been investagated by Widanarni *et al.* (2012) with different fish density. The result showed that BFT application in higher fish density resulted in higher production but lower fish survival and growth. The uncontrolled reproduction process however interrupted fish growth because it used mixed sex. Srisakultiew and Komonrat (2013) revealed that mixed population in tilapia culture can inhibit growth because energy for growth is used for gonadal maturation.

The problem of transferring growth energy in mixed cultivation can be overcome using male monosex seed. The selection of male monosex seeds is due to the growth of male tilapia faster almost twice than female tilapia (Firdous *et al.,* 2011; Silva 2013). This seed can be produced by sex transfer using the hormone testosterone (Megbowon and Mojekwu, 2014). The production of male monosex tilapia seed has been done by Fauzan *et al.* (2017), which uses a soaking method with the hormone 17a-metiltestosteron at a temperature of 36 $^{0}$C at a dose of 2 mg/l for 4 hours resulting in a male sex ratio of 83%. So we need male monosex seed for intensive culture of tilapia using biofloc technology. The application of a combination of sex reversal technology for monosex seed production and biofloc technology in intensive culture of red tilapia is expected to be a solution to increase the productivity of red tilapia and the management of aquaculture waste so as to create sustainable and environmentally friendly aquaculture. The aim of the study was to analyze the application of biofloc technology using male monosex fish to growth, survival rate, feed conversion ratio, feed efficiency and water quality profile of red tilapia *Oreochromis niloticus* which was intensively maintained at different densities.

## Materials and Methods

The first step in this study was produced monosex tilapia.The larvae of male monosex red tilapia are produced using a combination of sex reversal methods and increased temperature. The method of masculinization of red tilapia used is larvae aged 10 days of soaked red tilapia using the hormone 17a-methyltetosterone (MT) with an MT dose of 2 mg / L at 36$^{0}$C for 4 hours (Fauzan *et al.,* 2017). After immersion in larvae the next stage is the maintenance of larvae. The maintenance of larvae is done during forty days, after that larvae are ready to be used in the biofloc treatment.

Monosex red tilapia with an average body weight 1.11 g and average body length 2.80 cm was used as the experimental animal for biofloc treatment. Twelve units of outdoor tarpaulin pond were using in this study (2.52 m$^2$). Aeration was provided by an air blower. This study consisted of two densities of fish (50 fish/m$^3$ and 100 fish/m$^3$) with 3 replications respectively, and for each density there were control (without external C input) and BFT (with external C input) treatments. The treatment is given in this study include K1: density of 50 fish/ m$^3$without biofloc, K2: density of 100 fish/m$^3$ without biofloc, P1: density of 50 fish/m$^3$ with BFT C / N ratio of 15 and P2: density of 100 fish/m$^3$ with BFT C / N ratio 15. As an external organic C source, molasses was added weekly to the BFT treatments with a C/ N ratio of 15. The amount of molasses addition per day was determined based on the calculation described in Avnimelech (1999). This research used C : ratio 15 because Avnimelech (1999) and Asaduzzaman *et al.* (2008) recommend a 15:1 to 20:1 C:N ratio for good biofloc cultivation.

Feeding was done twice a day at satiation with feeding rate was 3% of fish biomass.Water quality measurement data is carried out every day including morning, afternoon, and evening (temperature and pH), DO measurements (morning and evening), TAN, flock volume, nitrite, and nitrate once a week.

The experiment period of fish was carried out for 35 days, by sampling the weight and body length of the fish carried out once a week until the end of the maintenance period. A sample of 30 fish was taken to measure the fish body length and body weight.

Specific growth rates were calculated according to the formula:

$$SGR\ (\%) = [\sqrt[t]{Wt/Wo} - 1] \times 100$$

SGR = specific growth rate (% $day^{-1}$), Wt = final average fish body weight (g), Wo = initial average fish body weight (g), t = experimental period (day).

Average daily gain were calculated according to the formula:

$$ADG(g/day) = [Wt-Wo]/t$$

ADG= Average Daily Gain, Wt = final average fish body weight (g), Wo = initial average fish body weight (g), t = experimental period (day).

Survival rate were calculated according to the formula:

$$SR\ (\%) = [N_t/N_o] \times 100$$

SR = survival rate, $N_t$ = final number of fish, $N_0$= initial number of fish

The feed conversion ratio (FCR) was calculated by dividing the total amount of feed given in each replicate by the total fish biomass gain. Feed efficiency was calculated by 1/FCR.

For volume of floc were obtained from 1000 ml of water sample was deposited for 30 minutes in a 1000 mL conical tube. The volume of flocking that settles is recorded and then calculated using the formula :

Volume of floc (ml/L) = Volume of deposition/ volume of water sample x 1000

Spesific growth rate, average daily gain, survival rate, feed conersion ratio, feed efficiency, and volume of floc were analyzed by one-way analysis of variance, using SPSS Statistics Base 22.

## Results

### Spesific growth rate

The specific growth rate of red tilapia in general both P1 and P2 is higher than the control (Fig. 1). The specific growth rate value in the P2 treatment (2.16%) exceeds the P1 treatment (1.33%), while for the control in P1 and P2 respectively 1.26% and 1.01%. However, the specific growth rate of biofloc treatment at 50 fish / $m^3$ (P1) stocking density was not significantly different from control (K1) ($p > 0.05$), whereas 100 fish / $m^3$ (P2) stocking density was significantly different from control (K2) ($p < 0.05$).

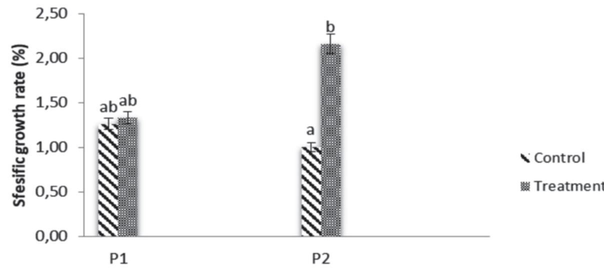


**Fig. 1.** Specific growth rate of red tilapia as a result of biofloc treatment with a C/N ratio of 15. P1 = stocking density of 50 fish/$m^3$, P2 = stocking density of 100 fish/$m^3$. Different lowercase letters above the standard deviation bar show a marked difference ($p < 0.05$)

### Average daily gain

Average daily gain is also the same as the specific growth rate where the value of the two treatments both 50 fish/$m^3$ (P1) stocking density and 100 fish/ $m^3$ stocking density are higher than the controls (Fig. 2). Biofloc treatment on stocking density of 100 fish/$m^3$ has the highest average daily gain value was 0.035 g/day (Figure 3). However, average daily gain of biofloc treatment at 50 fish/ $m^3$ (P1) stocking

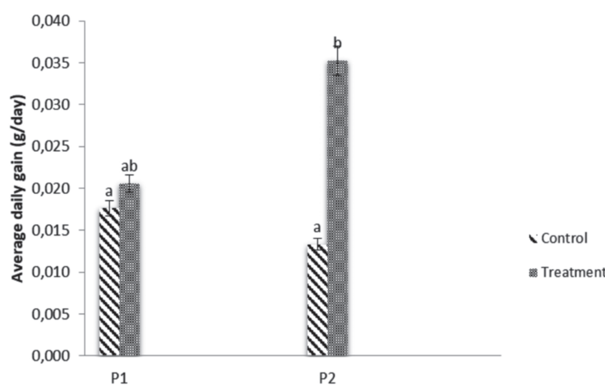


**Fig. 2.** Average daily gain of red tilapia as a result of biofloc treatment with a C / N ratio of 15. P1 = stocking density of 50 fish / $m^3$, P2 = stocking of 100 fish / $m^3$. Lowercase letters that differ above the standard deviation bar show significant differences ($p < 0.05$).

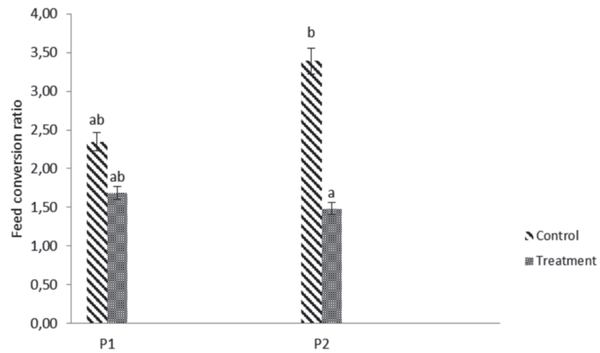


**Fig. 3.** Feed Convertion ratio (FCR) of red tilapia as a result of biofloc treatment with a C / N ratio of 15. P1 = stocking density of 50 fish / m$^3$, P2 = stocking of 100 fish / m$^3$. Lowercase letters that differ above the standard deviation bar show significant differences (p <0.05).

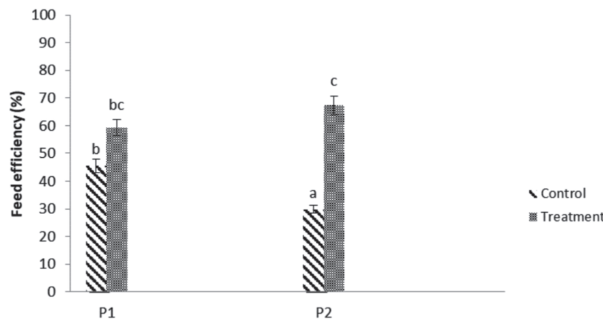


**Fig. 4.** Feed efficiency of red tilapia as a result of biofloc treatment with a C / N ratio of 15. P1 = stocking density of 50 fish / m$^3$, P2 = stocking of 100 fish / m$^3$. Lowercase letters that differ above the standard deviation bar show significant differences (p <0.05).

density was not significantly different from control (K1) (p> 0.05), whereas 100 fish / m$^3$ (P2) stocking density was significantly different from control (K2) (p < 0.05)

### Feed Corvertion Ratio (FCR)

Based on the results it was found that the FCR value in the biofloc treatment in both stocking densities was lower than each control. The lowest FCR value was found in biofloc treatment with stocking density of 100 fish / m$^3$, which was 1.48 (Fig. 3). However, the FCR of biofloc treatment at 50 fish/ m$^3$ (P1) stocking density was not significantly different from the control (K1) (p> 0.05), whereas the 100 fish / m$^3$ (P2) stocking density was significantly different from the control (K2) (p <0.05) .

### Feed efficiency

Feed efficiency on biofloc treatment on both stocking densities was higher than the control. In general, biofloc treatment on stocking density of 100 fish / m$^3$ has the highest feeding efficiency value of 67% (Fig. 4). However, feed efficiency on biofloc treatment at 50 fish/m$^3$ (P1) stocking density was not significantly different from control (K1) (p> 0.05), whereas 100 fish/m$^3$ (P2) stocking density was significantly different from control (K2) (p <0.05) .

### Survival rate

Survival rate in biofloc treatment with stocking density of 50 fish/ m$^3$ and 100 fish/m$^3$ is higher than the control. Biofloc treatment on stocking density of 100 fish/m$^3$ has the highest survival rate of 99% (Fig. 5). However, the viability of biofloc treatment at 50 fish/ m$^3$ (P1) stocking density was not significantly different from control (K1) (p> 0.05), whereas 100 fish / m$^3$ (P2) stocking density was significantly different from control (K2) (p <0.05).

### Volume of floc

Volume of floc during 35 days of maintenance showed an increase in ponds P1 and P2, while the control did not show floc. At the end of maintenance for treatment P1 has an average value of 8 ml / L whereas for P2 it has an average floc volume value of 14 ml/l (Fig. 6).

### Water quality

In general, the average values of water quality range of temperature, pH, DO, nitrite, nitrate, and TAN are still in the optimal range. Temperatures range from 24-26 °C, pH ranges between 7-8, dissolved

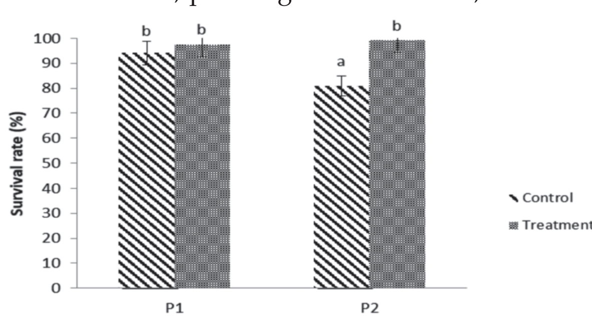


**Fig. 5.** Survival rate of red tilapia as a result of biofloc treatment with a C / N ratio of 15. P1 = stocking density of 50 fish / m$^3$, P2 = stocking of 100 fish / m$^3$. Lowercase letters that differ above the standard deviation bar show significant differences (p <0.05).

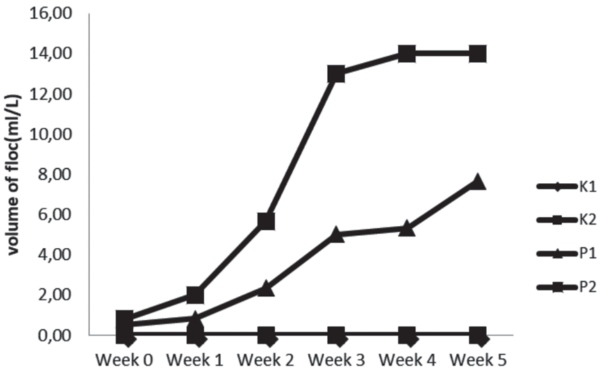


**Fig. 6.** Volume of floc in water media for maintenance of red tilapia aquaculture with a biofloc system with a C / N ratio of 15. K1 = Control of stocking density of 50 fish/m$^3$, K2 = Control of stocking density of 100 fish/m$^3$, P1 = treatment of stocking densities of 50 fish/m$^3$, P2 = biofloc treatment at 100 fish/m$^3$.

oxygen ranges between 4-6 mg/l. For nitrogen, the nitrite values in 0.31-0.78 mg/l and for nitrates in all treatments it was 0 mg/l. For the TAN value all treatments have a value of 0-0.13 mg/L (Table 1).

## Discussion

Based on the results of the study it was found that the specific growth rate and average daily gain of red tilapia also indicate that the presence of a biofloc system can increase the growth rate of red tilapia. P2 pond, which is biofloc treatment on stocking density of 100 fish/m$^3$ has the highest specific growth rate compared to the others. The specific growth rate value of biofloc treatment using this monosex juvenile was 1.33% for the density of 50 fish/m$^3$ and 2.16% for the stocking density of 100 fish/m$^3$. This is still said to be higher than the research of Maryam (2010) who examined the culture of biofloc systems using mixed red tilapia juvenile with SGR values ranging from 0.51-0.71%. According to Fauzan (2017), culture performance on male monosex juvenile using immersion of the MT hormone can increase growth rate. This is due to the presence of proteolytic activity in the intestine to increase the rate of growth. Avnimelech (1999) also said that the addition of molasses as a carbon source in aquaculture can increase the C / N ratio of the water which will further reduce inorganic nitrogen waters by increasing the growth of heterotrophic bacteria will form floc that can be used as high protein fish feed. According to Caldini *et al.* (2015) biofloc can increase the digestion and utilization of feed, so that it can increase growth.

**Table 1.** Water quality of this experiment

| Parameter | Unit | Measurement results |
|---|---|---|
| Temperature | $^0$C | 24-26 |
| pH | - | 7-8 |
| DO | mg/L | 4-6 |
| Nitrite | mg/L | 0,31-0,78 |
| Nitrate | mg/L | 0 |
| TAN | mg/L | 0-0,13 |

Feed conversion ratio is the ratio of the amount of feed needed to produce 1 kg of cultured fish meat. The lower the FCR value the better, this means the lower the conversion value of the amount of feed to the production of meat produced (Rozi *et al.*, 2018). The average FCR value of tilapia ranges between 1.5-3 (Maryam, 2010). The FCR value is inversely proportional to the value of feed efficiency. The low FCR value means that the more efficient feeding. The good FCR value for this research was in P2, because has the lowest FCR than other treatment (1.48) and also P2 have the highest feed efficiency (67%). The low FCR value and the high feed efficiency value in the biofloc treatment in both stocking densities is suspected due to the presence of biofloc in the culture media which is a source of additional food for fish. This causes the amount of artificial feed eaten by fish with biofloc treatments to be less than fish in the control treatment.

Survival rate for biofloc treament of this research was 97% for P1 and 99% for P2. It was higher than other research such as Pérez-Fuentes (2016) which found survival rate for biofloc treatment was 94.60 ± 2.03% and also Widanarni *et al.,* (2012) which found for biofloc treatment 93-97.78 ± 0.77%. Survival rates in treatments P1 and P2 have higher values than controls. It is suspected that biofloc is a natural food provider (Asaduzzaman *et al.,* 2009), and the presence of microbes in floc can improve fish health (Azim and Little, 2008). This can increase the survival rate of fish. Tilapia with a density of 100 fish / m$^3$ has the highest survival rate of 99%. This is thought to be in line with the high volume of floc obtained, so that feed preparation and its role in improving fish health are increasingly optimal. Flock volume is one indicator of flocculation on maintenance media. According to Crab *et al.* (2010), a bacterial community that accumulates in a heterotrophic aquaculture system will form a floc that can be used as a food source for fish.

In general, water quality values including temperature, pH, and DO in tilapia rearing media was in an optimal range. The presence of nitrite in both control and BFT treatments indicates the occurrence of nitrification processes in both culture systems. According to (Erlania *et al.,* 2010) nitrite is a compound that is toxic to aquatic organisms, but generally in nitrite levels water is unstable because nitrite is a transitional product obtained through the nitrification process of ammonia to nitrate. Watenpaugh *et al.* (1985) states that the optimal nitrite for maximum fish growth is 1. Based on Indonesia National Standard(2009) the optimal temperature for the growth of tilapia is 25-32$^0$C, while the pH is 6.5-8.5. For optimal dissolved oxygen which is more than 3 mg/l.

## Conclusion

1. The treatment of biofloc systems in redtilapia by using male monosex juvenile can increase specific growth rate, average daily gain, feed efficiency, and survival rate and also reduce FCR value.
2. The treatment of biofloc systems with a C / N ratio of 15 and ponds with a stocking density of 100 fish/ m3 produced the best values among other treatments, namely the specific growth rate was 2.16%, average daily gain was 0.035 g / day, FCR was 1.48, feed efficiency was 67%, and 99% survival rate.

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