The effect of fermented and non fermented biofloc inoculated with bacterium *Bacillus cereus* for catfish (*Clarias gariepinus*) juveniles

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Abstract. Biofloc technology has increased additional values in aquaculture, both in feeding management and water quality management. Molasses is considered as one of the safest way to increase the water quality. As an optional feed, biofloc is capable to enhance growth due to high protein content. The aim of the study was to evaluate the effect of molasses addition for catfish (*Clarias gariepinus*) growth and feed conversion. The biofloc was fermented for 24h using molasses, fish meal and grains. The experiment used three treatments of trial (no biofloc, fermented biofloc and non fermented biofloc). The results showed that molasses treatment on the application of probiotics has the higher growth and the lowest feed conversion. The water qualities during the experiment were stable. The catfish growth in no biofloc, fermented biofloc and non fermented biofloc were 0.10; 0.30±0.060; and 0.12±0.02 respectively. Feed conversion of catfish in fermented biofloc was 0.45±0.07, while for the no biofloc and non fermented biofloc were 0.95 and 0.81±0.07 respectively. This indicates that probiotics were used as the optional feed and that molasses treatment in fermenting probiotics was able to increase the nutritional values of the biofloc and therefore increase the growth of catfish.
Key Words: catfish, biofloc, biofloculation, molasses, probiotics.

Introduction. Biofloc technology (BFT) was introduced in Indonesia since 2003 and getting more interested as proved to provide positive impact on aquaculture production (Yuniartik et al 2015). BFT is based upon the production with zero or minimal water exchange, resulting in accumulation of organic substrates and subsequent development of dense microbial population, mostly aggregated in bioflocs (Avnimelech 2012). Bioflocculants are macromolecules secreted by microorganisms, solid particles, bacteria, cells and colloidal particles in a liquid suspension to flocculate and sediment (Hargreaves 2006). These macromolecules are co-culture of heterotrophic bacteria and algae which is grown in flocs under controlled conditions within the culture pond. Microbial biomass is grown on fish excreta resulting in a removal of these unwanted components from the water. Several studies have shown that the application of BFT plays a role to improve water quality, biosecurity, productivity, feed efficiencies and reduce production costs through lowered feed expenses (Burford et al 2004; Wasielesky et al 2006; Avnimelech et al 1994; Hari et al 2004).

Floc biomass formed in bodies of water may be consumed by fish as additional sources of feed and as water purifier (Aiyushirota 2009). Bacteria as main component of biofloc is capable to produce polyhydroxybutyrate (PHB) which is useful in aquaculture. The advantages of PHB are an energy reserve for fish, digestible in intestine, increasing unsaturated fatty acid, and increasing growth of fish (De Schryver et al 2012).

According to Ekasari et al (2010), the feed efficiency of BFT increased due to the presence of additional nutrition source for the fish in the form of increased biofloc microbe biomass. The feed conversion ratio of the fish farmed using BFT is better compared to conventional methods. Megahed (2010) also reported that by adding biofloc can reduce total feed cost and also increase the water quality of green tiger shrimp (*Penaeus semisulcatus*). Molasses is biologically and economically considered as one of the safest way to increase the water quality. Molasses are produced from a byproduct of sugar production industry and it contains a high carbohydrate that can be utilized by the probiotic bacteria (Avnimelech 1999).

Molasses are used to increase the nutrition of the probiotic bacteria and can be used to increase the bacteria ability in improving water quality. Sartika et al (2012) reported that by adding 2.4 g L^{-1} molasses to probiotic which contain *Bacillus* sp. has increased water quality and common carp (*Cyprinus carpio*) growth. Therefore, the aim of this study was to evaluate growth rates and feed efficiency of African catfish, *Clarias gariepenus* juveniles treated with fermented and non fermented bioflocs supplemented with molasses. This species is one the very popular species for aquaculture in Indonesia (Muchlisin et al 2010).

Material and Method. The experiment was carried out at Jakarta Fisheries University, Indonesia on 12 January – 15 February 2014. Juvenile catfish were stocked into concrete tanks (vol. 1 m^3). Each tank contained 1000 juveniles (mean body weight 2.5 g). The high density farming was applied based on C-First 250 (Catfish Farming in Recirculation System Tank 250 kg m^{-3}), where the main goal is to harvest 250 kg of catfish in only 1 m^3 water and this technique is successfully done since 2014 in Jakarta Fisheries University. The experiment consisted of two treatments (non fermented bioflocs and fermented bioflocs systems) and one control (conventional clear water system). All treatments were done by two replicates. During experiment, juvenile were fed with artificial feed with 32% crude protein (CP) three times a day (06:00, 14:00 and 21:00) at satiation for 35 days.

Preparation of biofloc. A control treatment in the absence of bioflocs was challenged against two bioflocs treatments, fermented and non fermented bioflocs. Fermented biofloc was prepared by adding 1.5 kg fish meal, 1.5 kg grains, 250 mg L^{-1} molasses, and 5 mg L^{-1} probiotics (*Bacillus* sp.) in 30 L water for 24 h. After 24 h, the fermented probiotics were spread into every replicate bin. While for the non fermented biofloc, 5 mg L^{-1} probiotics and 250 mg L^{-1} molasses were added into every replicate. The mixture was aerated and maintained for another 7 days prior to use as bioflocs.

Sampling. Sampling on individual body weight was done one week intervals. A total of 10 fish were randomly selected in each bin and weighed using digital scale (Sartorius, US). Variations in body weight were then used to determine the effects of fermentation on probiotics.

At the end, total biomass was measured to calculate feed conversion ratio (FCR), survival rate (S), and average daily growth (ADG). Daily water quality parameters were monitored as follows: temperature (°C) using thermometer, dissolved oxygen (mg L^{-1}) using DO-meter, pH using digital pH-meter, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) using test kit.

Performance evaluation*.* Survival rate was estimated based on equation: S = (Nt/N0)*100%, where Nt is total live fish at the end of experiment, N0 is total fish stocked (Xu et al 2012). FCR was determined as follows: FCR = feed intake/weight gain (Zhao et al 2012). ADG was estimated based on: ADG = [(Wt – W0)/t], where: Wt = average individual body weight at the end of culture period; W0 = average initial body weight, and t = experiment duration (days) (Schram et al 2009).

Statistical analysis*.* All data were analyzed by one way analysis of variance (ANOVA) and Duncan's multiple comparison test using a statistic program of SPSS version 21 for Windows. All probability values were set at 0.05 level of significance.

Results and Discussion. Growth performance, feed utilization, survival rate after 35 days of culture using fermented and non fermented biofloc are summarized in Table 1. Average initial juvenile weight of each experimental group were similar ($p < 0.05$), being 2.5 g $fish^{-1}$. In this experiment, the highest final weight (FW) was found in the fish fed the fermented biofloc (8.7±1.27).

Table 1
Growth response, feed intake, feed conversion (FCR), and survival rate (SR) of juvenile *Clarias gariepinus* cultured in biofloc system for 35 days

Parameters	*Treatment*		
	Control	*Non fermented biofloc*	*Fermented biofloc*
Initial weight (g)	2.5 ± 0.00^{a}	2.5 ± 0.00^{a}	2.5 ± 0.00^{a}
Final weight (g)	4.7 ± 0.35^{a}	4.9 ± 0.57^{a}	8.7 ± 1.27^{b}
Weight gain (g)	2.2 ± 0.13^{a}	2.4 ± 0.57^{a}	6.2 ± 1.27^{b}
Average daily growth (g day^{-1})	0.10 ± 0.17^{a}	0.12 ± 0.02^{a}	$0.30 \pm 0,06^{b}$
FCR	0.95 ± 0.12^{a}	0.81 ± 0.07^{ab}	0.45 ± 0.17^{c}
SR (%)	64.5 ± 3.42^{a}	85.8 ± 5.80^{b}	91.4 ± 8.06^{bc}

The different superscript letter in common per factor, in the table above indicates significant difference ($p < 0.05$) with Duncan's test.

FW between the control and non fermented biofloc were not significantly different ($p > 0.05$). Although the FW value of fish treated with non-fermented biofloc was higher than those treated in control, they were not significantly different ($p > 0.05$). In the same way, the weight gain of fish which treated by fermented biofloc was significantly higher than that of fish treated in non fermented biofloc and control ($p < 0.05$). However, the ADG of catfish juvenile treated with fermented bioflocs (0.30 g day^{-1}) was significantly higher than that which treated with non fermented biofloc and control ($p < 0.05$). Bioflocs consist of several microorganisms including bacteria, fungi, plankton and these microorganisms are natural fish feed contain high nutrition. Increased growth was also reported in catfish (*C. gariepinus*) with reduced fed up to 25% has similar ADG compared to control (Rostika et al 2014). Azim & Little (2008) reported that biofloc use in tilapia (*Oreochomis niloticus*) showed no difference in fish growth between 35% and 24% CP feed under BFT, but both were higher than control without biofloc with 35% CP. These findings may indicate that fermented biofloc has the potential to improve growth performance of cultured fish.

However, there was no difference in growth rate on Brazilian endemic tropical fish tambaqui (*Colossoma macropomum*) treated with BFT system and did not improve fish growth compared to control (without BFT) (Emeranciano et al 2011). These discrepancies might be due to the metabolize system in Brazilian endemic fish and it need further research to clarify the effect of BFT in those fish.

In the present experiment, fermented biofloc treatment could improve the FCR; highest FCR was found in juvenile catfish (0.45) treated with fermented biofloc. This shows the fish ability to efficiently digest and convert the given feed into a lean mass. According to Avnimelech (1999), the protein and single–cell protein which synthesized by the heterotrophic bacterial can be directly utilized as feed source for the cultured fish, therefore lowering the demand for supplemental feed protein. Crab et al (2007) pointed out that BFT system in aquaculture can be used as alternative feed source for fish. Burford et al (2004) and Wasielesky et al (2006) showed that daily food intake consisting in microbial flocs can decrease FCR and reducing cost in feed compared to clear water conditions. Fermented biofloc had better utilization than non fermented biofloc treatment. This indicates that by fermenting using grain and fish meal in biofloc enhances the nutrition of the biofloc and therefore increases the utilization of the feed. By adding by product carbon, a high carbon concentration in water could supersede the carbon assimilatory capacity of algae, and contributing to bacteria growth (Emeranciano et al 2011). De Schryver et al (2012) described that bacteria as the main component of biofloc is capable of producing polyhydroxybutyrate which is useful in aquaculture. He also elucidated that the beneficial effects of polyhydroxybutyrate include providing energy reserve, increasing unsaturated fatty acid as well as improving growth for several fish species.

During the feeding experiment, it can be seen that the fish juvenile in all the biofloc treatments were able to consume flocs. This was observed by during the experiment the FCR in biofloc treatments were better than control, especially the fermented biofloct treatment.

Interestingly, although the survival of juvenile catfish cultured in fermented biofloc was significantly higher than those of the control treatment, it was not significantly different compared with those cultured in non fermented biofloc. This clearly indicated that biofloc, whether fermented or non fermented play a significant role in enhancing the survival of the fish. BFT is a natural probiotic internally and externally against *Vibrio* sp., ectoparasites, and infections and stimulate growth and survival of shrimp and fish larvae due to its biodegradable polimer (polyhydroxybutyrate/PHB) which synthesized by bacteria inside the biofloc (De Schryver et al 2012). Thus, fish cultured in fermented biofloc seems to have a high tolerance to internal or external stressors, which might have been the case in the present experiment.

The floc which accumulate in fermented biofloc were much dense and big, compared to non fermented biofloc (Figure 1). This indicates that by using fermented method can enriched the biofloc.

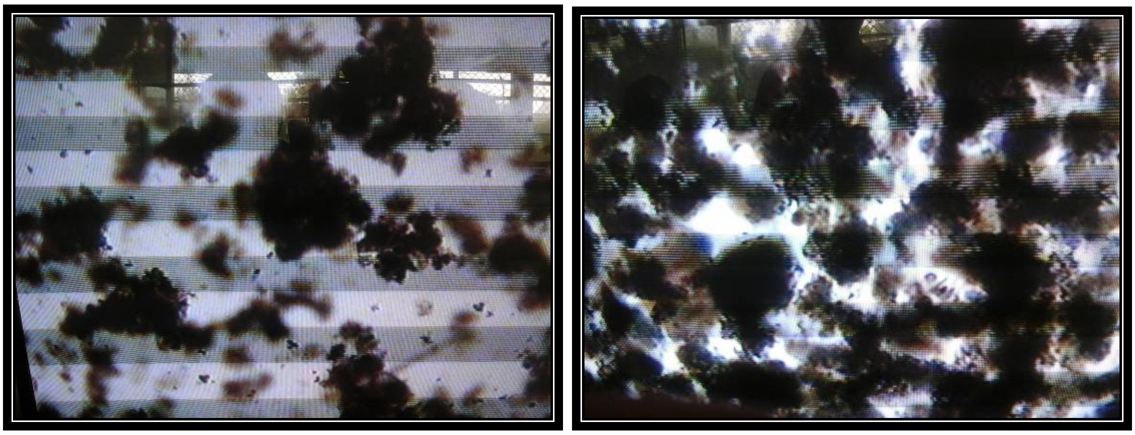

Figure 1. Non fermented biofloc (left); and fermented biofloc (right).

The average value of temperature, pH, ammonia, nitrite and nitrate during the course of the experiment are displayed in Table 2. There were no significant differences in water quality parameters (temperature, pH, ammonia, nitrite and nitrate) between control and biofloc systems ($p < 0.05$). Yuniartik et al (2015) reported that ammonia-nitrogen accumulation is depending on feeding level where, when more feed were given to the system will resulted in more ammonia-nitrogen accumulated in the system, this will effect to unbalanced environment. The artificial feeds that were fed during this experiment were in a small amount. This may be the reason all the water quality between all treatments were not significantly differ.

Table 2

Summary of water quality observed over 35 days growing period during the experiment

Treatment	*Parameters*				
	Temperature (^oC)	*pH*	*Ammonia ($mg\ L^{-1}$)*	*Nitrite ($mg\ L^{-1}$)*	*Nitrate ($mg\ L^{-1}$)*
Control	28.4 ± 1.7^a	7.6 ± 0.55^a	2.51 ± 0.08^a	0.2 ± 0.57^a	39.3 ± 11.33^a
Non fermented biofloc	28.7 ± 1.2^a	7.4 ± 0.63^a	2.01 ± 0.37^a	0.1 ± 0.04^a	38.1 ± 17.04^a
Fermented biofloc	28.7 ± 1.4^a	7.8 ± 0.37^a	2.25 ± 0.04^a	0.4 ± 0.63^a	37.3 ± 18.02^a

The different superscript letter in common per factor, in the table above indicates no significant difference ($p > 0.05$) with Duncan's Test.

Conclusions. In summary, the fermented bioflocs can enhance growth and feed utilization of juvenile catfish *C. gariepinus*. However, nutritive value of fermented biofloc and non fermented biofloc might have played an important role in promoting growth and feed utilization, however this deserve further investigations.

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