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Submission date: 31-May-2022 03:51PM (UTC+0700)
Submission ID: 1847700678
File name: 10._Biofloc_Consumption,_Growth_Performance_And.pdf (790.71K)
Word count: 4457
Character count: 23080

IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Biofloc Consumption, Growth Performance And Water Quality Of African Catfish (*Clarias gariepenus*) And Tilapia (*Oreochromis Niloticus*) Cultured In Biofloc System Without Water Exchange

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Abstract: This current work aimed to analyze consumption of biofloc by fish samples (catfish and tilapia) and investigate the effects on fish growth performance under biofloc culture system. The experiment was carried out separately, according to fish species, including catfish with biofloc (BC), catfish without biofloc (NC), tilapia with biofloc (BT) and tilapia without biofloc (NT). All experiments were made at triplicates. The results showed comparable level TSS and TAN in BC to be higher than in NC. Similarly was found higher in BT than in NT. Biofloc treatment resulted in lower nitrite level in catfish groups, but contrast result occurred in tilapia groups. Furthermore, biofloc fish groups had higher biofloc volume than non biofloc groups. In all groups, all planktonic matters found in fish gut corresponded to those in biofloc. For bacteria, the similarity between bacteria composition in fish gut and culture medium varied greatly as follows: 66.67% (BC), 45.46% (NC), 21.43% (BT) and 30.00% (NT). These findings brought noticeable evidence, that catfish and tilapia consumed biofloc during experiment. In terms of SGR, no significant difference was found between biofloc and non biofloc groups for catfish and tilapia groups. Biofloc treatment significantly increased survival rate and biomass gain in catfish, but they did not differ significantly in tilapia.

Keywords: bacteria, biofloc consumption, food habit

INTRODUCTIONS

Intensive fish farming with high density and commercial feeding is known to provide high productivity. However, it has remained a great challenge on massive amount of effluents especially organic matters and nitrogen-containing compounds which may adversely affect fish and environment. regarding the development of more sustainable farming practices, biofloc technology has been applied, enabling to use less water in the fish culture. Principally, it enables to retain water quality with aid of the aggregates of microbes, algae, protozoa, as well as detritus and organic particles. The microbial communities in this system improve water quality, while the content of microbial protein acts as essential source of nutrition for cultured fish. In short, bioflocs are defined as the aggregates of algae, zooplankton, bacteria, protozoa, and other kinds of particulate organic matters such as feces and uneaten feed (Hargreaves et al, 2013), live or dead organic particulate (Irshad et al, 2017). The existing bacteria can convert nitrogen in the culture medium to form biofloc aggregate, which in turn enhancing the quality of culture medium. With addition of heterotroph bacteria and molase as source of carbon, sample quantity of nitrogen in medium can be converted into floc, enabling to reduce ammonia level in the culture medium (Nootong et al, 2011), (Zhao et al 2012) revelead that concentration of ammonia ions and nitrite in the treated ponds was lower than that in control ponds. (Rajkumar et al 2016) the addion of carbone–hydrate significantly reduced the total ammonia-N, nitrite-N and nitrate-N in water and significantrly increased the total heterotrophic bacteria population in the biofloc treatments.

Biofloc importantly acts as additional source of nutrition, estimated to reach 30 – 45% of protein (dry basis), 1 – 5% of fat. Specifically, protein as one of the major feed components accounted for 30.4% of biofloc (Emerciano et al, 2012). For this reason, biofloc is a promising source of nutrition for improving growth and reducing Feed conversion ratio (FCR) (Nootong et al, 2011)]. Former study also found that shrimps cultured in biofloc system with or without commercial feed treatments showed higher growth in comparison with those cultured in conventional clear ponds (Emerciono et, 2012) In addition, culture of nile tilapia in biofloc system with 20% less feeding rate showed similar growth compared with those treated with 100 feeding doses (Fuentes et, 2016). The use of biofloc was reported capable of increasing individual weight, growth rate, survival rate, while lowering FCR in comparison with fish cultured in recirculated system (luo et al (2014). (Zhao et, 2012) reported that biofloc system increased production of shrimp up to 41.3%, with lower FCR up to 7.22%, in which *Bacillus* sp. dominated the biofloc system.

Although many studies have been made on discussing the advantageous effects of biofloc system on nutritional source and growth performance, However, the types of fish, especially catfish and tilapia that are able to be to biofloc consumption have not been widely reported. Tilapia belongs to member of herbivore, strongly believed to receive more benefits in biofloc system, compared with catfish which tends to be carnivorous. For this reason, this present work aims to compare consumption of biofloc between catfish and tilapia and its effects on growth performance.

MATERIAL AND METHODS

Experimental Animal and Materials

Catfish and tilapia seeds (average weight of 6 g and 15 g, respectively) were reared in 12 experimental containers (each 1.5 × 1 × 1) 06 m^3. The 40 days-experiment was conducted in Department of Fisheries Extension, Jakarta Technical University of Fisheries.

Experimental Design

The experiment was divided into two groups, according to fish species. The first one was carried out for catfish, arranged with completely randomized design with two factors: biofloc (BC) and non biofloc (NC). The second one was carried out for tilapia, consisting of biofloc (BT) and non biofloc (NT). All experiments were performed at triplicates.

The experiments were carried out for 40 days in JFU, Indonesia. Fish were randomly maintained in 6 ponds (each 1.5 × 1.0 × 1.0 m^3, 60 cm depth) with aeration, but without water exchange. The density was set at 500 individuals/m^3 for catfish and 100 individuals/m^3 for tilapia. The fish were fed twice a day with commercial feed containing protein of 30% at dose of 5% of fish biomass.

Biofloc culture system was also enriched with probiotics (20 g/m^3) and palm sugar (150 g/m^3) given at initial period of experiment, then they were added to the pond each 5 day.

Sampling and analytical methods

The fish were analyzed for growth rate, survival rate and biomass gain. All fish were individually weighed at the beginning of the experiment.

Specific growth rate = ln (final body weight/initial body weight)/days of of the experiment) × 100

Survival rate = (number of fish harvested × 100) /number of fish stocked

Biomass gain = final weight – initial weight

Feed conversion ratio = amount of feed consumed / fish weight gain

Determination of water and biofloc consumption

Dissolved oxygen was measured by using DO meter, while other parameters (pH, TSS, TAN, nitrite) were analyzed by using APHA (2017). Biofloc consumption is measured by analyzing the types of plankton and bacteria in the intestines and in the maintenance media water, then their similarity is analyzed to ensure that the types of plankton and bacteria consumed come from the rearing water media. Analysis of plankton and bacteria was conducted in Laboratory of Production and Environment (in Faculty of Fisheries and Marine Sciences, IPB University). Plankton Net size 25 micron to filter phytoplankton and size 150 micron to filter zooplankton, bucket used for sample container on plankton net, 100 ml sample bottle for sample container, label paper, drop pipette, digital camera and stationery. The tools used in research in the laboratory are the SedgwickRafter used as a phytoplankton counting tool and the Bogorov dish is used as a zooplankton counter, inverted microscopes with 4 × 10 accuracy are used to observe phytoplankton, binocular microscopes are used to observe zooplankton, cover glass is used to cover the Sedgwick-Rafter. In the microscope, the dropper pipette is used to collect phytoplankton sample solution, the pipette stamp is used to collect the zooplankton sample solution, the plankton identification form and stationery to record the plankton identification results, the identification book used to identify phytoplankton and zooplankton, and the camera is used for documentation. Bacterial composition was identified using total plate count (TPC) analysis in Laboratory of Aquatic Organism Health (in Faculty of Fisheries and Marine Sciences, IPB University). In general, bacterial tests were carried out inoculation of the bacteria from the target organ into agar media for TSA dishes using the spread method, then incubated for 18-24 hours. The next step is the bacterial colonies that grow are then selected based on colony shape and color. Purification is carried out by inoculating the selected bacteria on Nutrient Agar (NA) agar slant media, and incubating for 18-24 hours. The next stage is the biochemical test and the gram test to obtain the bacterial genus

Statistical analysis

Experimental design followed completely randomized design with two treatments. Data were then statistically evaluated using analysis of variance. The comparison between means was performed with least significant difference , followed by Duncan's multiple range tests (for Post hoc test). The results were considered statistically significant when *p-values* were below 0.05 ($p<0.05$). All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 22.0 for windows).

RESULTS AND DISCUSSIONS

Quality of Water and Biofloc

Table 1 presents parameters for quality of water, including dissolved oxygen, pH, TSS, TAN, and nitrite.

Table 1. Comparison of water quality parameters in treatment

Treatments	Dissolved oxygen (mg/L)	pH	TSS (mg/L)	TAN (mg/L)	Nitrite (mg/L)
BC	5.67±0.32	7.04±0.15	733,00±512,78	11,71±0.76	0.04±0.01
NC	5.43±0.42	7.12±0.23	462,33±130,48	8,14±1,50	0.10±0.10
BT	6,50±0,26	7.06±0.26	149,00± 65,59	2,17±0,01	0.33±0.31
NT	6,36±0,21	6.90± 0.09	90.67± 32,15	1,75±1,16	0.19±0.08

The results revealed that level of DO and pH in biofloc system was comparable compared with that in non biofloc system, these indicators were at acceptable range for fish. Afterwards, two other parameters, i.e. TSS and TAN, were higher in BC than NC and BT than in NT. In terms of nitrite, the compound was lower in BC than in NC; conversely, it was found higher in BT than NT.

The rise of biofloc volume was observed each 10 days, and the results were depicted in Figure 1. In general, the volume increased over the period of experiment. Biofloc volume in BC was higher than in NC, as well as in BT being higher than in NT. In this case, we highlighted that biofloc was also present in NC and NT, though existed at small quantity.

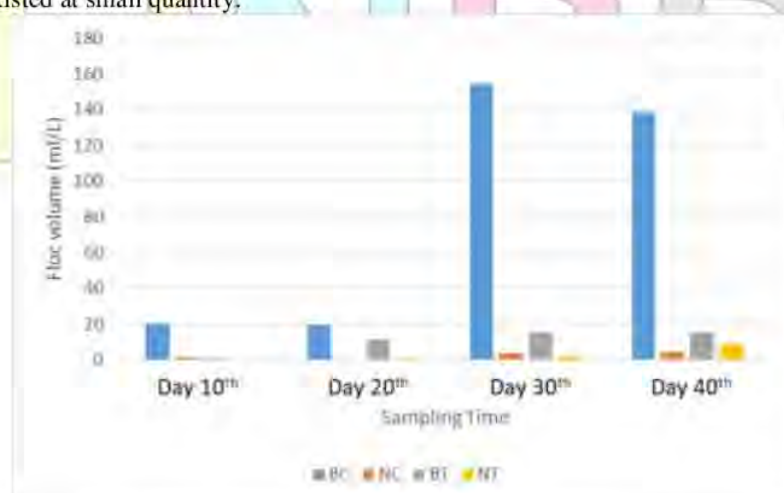

Figure 1. Changes in biofloc volume during 40 days of experiment

As presented in Table 1 and Figure 1, concentration of TSS, TAN and biofloc volume in BC was higher than in NC. Those parameters were also higher in BT than in NT. Addition of carbon in biofloc system can improve C/N, enabling to support growth of heterotroph bacteria in utilizing nitrogen to form biofloc. Biofloc volume in BC was higher than in NC. The greater volume was also found in BT than in NT. This finding supports the previous studies of (Dauda et al, 2018), [(Xu et, 2012), reporting that carbon enrichment into culture medium remarkably brings beneficial impacts to biofloc volume. [12] reveals that addition of carbon into the medium positively correlates to formation of biofloc. Interestingly, though not enriched with carbon, formation of biofloc also exists in NC and NT, reaching up to 4.3 ml/L and 8.4 ml/L, respectively. This means that source of carbon in non biofloc system is reliant to feed containing carbohydrate, resulting in C/N level which is insufficient for growth of heterotroph bacteria. The effect of C/N on biofloc production was reported by (Dauda et al 2018), concluding that the highest volume of biofloc occurred at C/N 20, C/N 15 and C/N 10; on the contrary, the lowest one was achieved on control group. (Fuentes et al, 2016) showed that C/N 10 was more desirable over other ranges, including C/N 12.5, 15.0, 17.5 and 20.0.

TSS level in BC was higher than in NC, while it was also higher in BT than NT. This means that TSS may correspond to concentration of biofloc: higher TSS represents higher concentration of biofloc. Nevertheless, presence of biofloc in BC and BT is unable to reduce TAN and nitrite concentration in comparison with that in non biofloc system. A result of previous investigation by (Xu et al 2012) also reports this finding, that addition of carbon results in the rise of C/N, but it unalter content of TAN and nitrite. Similarly, (Chen et al (2020) find that carbohydrate supplementation into biofloc system has no effects on level of TAN, nitrite and dissolved organic matters. However, other studies show discrepancies, as reported (Wang et al, 2015) and (Zhao et al, 2012), finding that concentration of ammonia ions and nitrite was lower in biofloc groups compared with control. Content of nitrite and nitrate in treated groups was also lower than control (Long et al 2015).

Biofloc Consumption

a. Consumption of plankton

Table 2 shows the results of habit identification, successfully finding variety of planktonic items in catfish and tilapia gut. Planktons in biofloc seemed to be much more varied than in fish gut. In all groups, we detected similarities of plankton present in gut and biofloc, suggesting that plankton in fish sample originated from biofloc.

Table 2. Types of plankton in fish gut and biofloc

Groups of treatment	Fish gut	Biofloc
BC	*Notholca sp, Arcella sp Euglypha sp*	*Notholca sp , Arcella sp, Euglypha sp, Chlococcus sp, Diatomae sp, Microcytus p, Chlamydomonas sp, volvox sp, Nostoc, Nitzchia sp*
NC	*Diatome sp, Synedra sp , Scenedesmus sp, Euglypha sp Nitzchia sp, Fragillaria sp,*	*Diatomae, Synedra sp, Scenedesmus, Euglypha sp, Nitzschia, Fragillaria sp Arcella sp, , Oocystus sp, Cymatoleura sp, , Parameceum, Euchlanus sp, Nostoc sp*
BT	*Notholca, Nitzchia sp, Euglypha sp Diatomae sp, Synedra sp, Oscillaria sp,*	*Nottholca sp, Nitzchia , Euglypha sp , Diatomae sp, Synedra sp, Oscillatoria sp, Arcella sp, Gloecystus sp, Spirulina sp, Paramecium sp, , Euchalamus sp, Anabaena sp, Oocystus sp, Microcystis sp, Polyodrium sp*
NT	*Nitzchia sp, Arcella sp, Euglypha sp, Synedra, Diatome sp, Chlococcus sp, Microcystis sp*	*Nitzschia, sp, Arcella sp , Euglypha sp synedra sp , Diatomae, Chlococcus sp, Microcytis, Scenedesmus sp, Diatomae sp, Paramecium sp, , Oocystus sp, Vorticellasp, Nostoc sp, Pediatrum sp, Notholca sp,*

b. Consumption of Bacteria

Table 3 shows diversity of bacteria in all groups of fish culture. The results demonstrated that bacteria in each fish culture medium were more diverse in comparison with those in fish gut. In BC, 8 of 12 bacteria present in fish pond were found in catfish intestine (66,67%), while in NC, 5 of 11 bacteria were detected

(45,46%). For tilapia, 3 of 14 bacteria were found in tilapia gut under biofloc treatment (21.43%); while, 3 of 10 bacteria were detected in tilapia gut under non biofloc group (30.00%). It is noteworthy that similarity of bacteria between biofloc and fish gut was higher in catfish than in tilapia.

Table 3. Number of bacterial varieties in culture medium and fish gut

Groups of treatment	Variety of bacteria in culture medium	Variety of bacteria in fish gut	Similarity of bacteria between medium and gut	Proportion of bacteria consumption (%)
	A	B	C	C/A × 100
BC	12	11	8	66.67
NC	11	11	5	45.46
BT	14	6	3	21.43
NT	10	7	3	30.00

Table 2 and 3 present the abundance of plankton and bacteria present in fish gut and culture medium. In this regard, both fish were evidenced to consume biofloc as additional nutrition.

The similarity of bacterial composition between culture medium and fish gut, though at different percentages, revealed the ingestion of biofloc in fish samples. Besides bacteria, biofloc also contains plankton. Bacteria and plankton constitute major component of biofloc, as investigated by (Xu et al, 2016), reporting that algae and autotropic bacteria dominated the biofloc. In addition, (Hargreaves, 2013) found that biofloc shows the aggregates of algae, zooplankton, bacteria, protozoan and other kinds of particulate organic matter such as as feces and uneaten feed. (Irshad et al, 2017) explained that organic particulates, either viable or not, also existed as significant component in biofloc. The relationship between bacteria diversity in fish gut and culture water was reported by (Fuentess et al, 2016), which revealed that most bacteria in culture medium (15 of 17 bacteria) occurred similarly in fish gut.

Growth Performance

Table 4 shows growth performance of fish in all groups. The results demonstrated that no significant difference was found between BC and NC, as well as between BT and NT ($P>0.05$). However, there was significant difference in survival rate and biomass gain between BC and NC ($P<0.05$). However, these parameters did not differ significantly between BT and NT ($P>0.05$).

Table 4. Growth performance of fish cultured in biofloc and non biofloc system

Groups of treatment	SGR (%)	Survival rate (%)	Biomass gain (kg)
BC	3.33±0.41	86.65±9.49[a]	4.98±0.40[a]
NC	2.93±0.06	61.43±5.83[b]	2,12±0.43[b]
BT	2.67±0.42	86.67±8.08	1.17±0.38
NT	2.63± 0.06	95.33±3.03	1.31±0.08
P value			
BC and CC	0.47	0.02	0.01
BT and CT	0.39	0.56	0.16

According to research results, biofloc volume increased in presence of additional carbon compared with control (see Figure 1). Regarding the similarity of plankton and bacterial composition between fish gut and culture medium, our present work revealed the consumption of biofloc by all studied fish. However, we need to note the presence of much higher biofloc in BC and BT did not alter SGR of the fish. This suggests that high protein in feed (reaching up to 30%) given at dose of 5% over fish weight is sufficient to achieve optimum fish growth; thus, biofloc consumption may not affect the growth. As argued by (Dauda et al, 2018), the supply of extraneous carbon into catfish culture medium did not affect SGR. (Xu et al, 2016) also found the unaltered growth of catfish as increase in biofloc volume. The further report by (Dauda et al, 2018) support previous evidence, in which growth performance of fish fed with 80%, 90% and 100% diet in biofloc ponds showed no difference in comparison with those fed with 100% diet. (Fuentes et al, 2016) investpigated growth of tilapia cultured under biofloc system with 20% less feed, showing that their growth was comparable to control group (100% feed dose). In short, it is important to note, under less feed supply, the fish can fulfill their nutritional requirement from biofloc. Different results may also occur. (Nootong et al, 2011) reported that biofloc treatment as additional nutrition could also increase growth of fish. Biofloc which contains plankton and bacteria as a source of protein and essential fatty acids for fish (Kzebietke et al, 2017) that chlorophyta and cryptophytes provide more polyunsaturated fatty acids (PUFA> SAFA> MUFA), while cyanobacteria SAFA> MUFA> PUFA.

Survival rate of fish in BC was higher than that in NC, while it did not differ significantly between BT and NT. Catfish and tilapia culture at density of 2.10 kg/m^3 and 0.75 kg/m^3 was fed with 5% of weight; therefore, the catfish received higher quantity of feed than tilapia. TAN value in BC was higher than that in NC, but we could see the fish in BC group had higher survival rate than that in NC. This may show the considerable effects of probiotic in BC culture system, allowing to enhance the fish immunity. The rise of immune status is essential to induce resistance towards external stressors. This is in accordance with report of (Irshad et al, 2017) finding the increase in survival rate due to the stronger defense and immune system induced by biofloc treatment.

Furthermore, BC resulted in higher biomass gain compared with NC. SGR for both treatment groups did not differ significantly; hence, the difference in biomass gain resulted from different survival rates between BC and NC. (Zhao et al, 2012) reported the yield of shrimp farming up to 41.3%, while (Long et al, 2015) reported the higher production up to 19.46% compared to control. Additionally, (Sganaulin et al, 2018) reported the increase of final biomass weight in comparison with control. Biofloc contains crude protein of 30.4%, being a noticeable source of protein for fish (Emerciano et al, 2011).

Conclusion

Supplementation of carbon into culture medium resulted in higher biofloc volume than control. The analysis results also revealed similarity of planktonic and bacterial profile present in fish gut and culture medium, meaning that the fish (catfish and tilpia) consumed the biofloc. Growth performance was the same for both catfish and tilapia reared in biofloc and control ponds. The biomass gain of catfish in the biofloc pond was higher than that of the control pond, while for tilapia the biomass gain was the same.

ACKNOWLEDEMENTS

The authors would like to acknowledment the support of Jakarta Techical University of fisheries, and also we would like to thank the management of Deparment of Fisheries Extension.

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