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EFFECTS OF FEED REDUCTION ON GROWTH PERFORMANCE, WATER QUALITY, AND HEMATOLOGY STATUS OF AFRICAN CATFISH, *Clarias gariepinus* REARED IN BIOFLOC POND SYSTEM

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ABSTRACT

Biofloc technique is a method of fish culture using minimal water exchange and microbial aggregates formed within the culture media as fish supplementary feed. Biofloc as a protein source is currently not being considered part of the feeding dosage in catfish culture. This present work aimed to determine the effects of commercial feed reduction on growth performance, water quality, and hematology of catfish cultured in biofloc ponds. Fish with an average body weight of 7.53 ± 0.47 g were stocked in nine ponds with a stocking density of 100 fish/pond and reared for nine weeks. The experiment was arranged in a completely randomized design, with the following treatments: full feed + non-biofloc (C100) as control; 10% less feed + biofloc (B90); 20% less feed + biofloc (B80). As such, the total feed doses given daily per each treatment fish biomass were: 5% for C100, 4.5% for B90, and 4.0% for B80. Each treatment was arranged in triplicates. The results showed that all water quality parameters (dissolved oxygen, pH, TAN, and nitrite) and biofloc concentrations did not differ significantly among the treatments ($P>0.05$). Specific growth rate, survival rate, weight gain, and feed conversion ratio of the fish were also not significantly different ($P>0.05$). The weight gain of biofloc-treated fish in B90 and B80 reached 2.71% and 12.65%, respectively; which were higher than the control treatment during the treatment period. The feed conversion ratios of B90 and B80 were 14.39% and 7.58%, respectively; lower than the control treatment. The biofloc treatment did not adversely alter the fish's blood cell profiles. This study revealed that feed reduction did not affect water quality in the ponds and blood cell profiles of the fish. Moreover, fish in the biofloc-treated ponds had higher weight gain and lower food conversion ratios compared to fish cultured in the non-treated biofloc ponds. This study suggests that using the biofloc system in catfish rearing can reduce the use of feed up to 10%-20% with similar biomass yield compared to the non-biofloc system.

KEYWORDS: **biofloc; feeding rate; food habit; catfish**

INTRODUCTION

In fish culture, feed contributes for over half of total variable operating cost (Tacon & Jackson, 1985). Unfortunately, feed is often not eaten by cultured fish due to several factors, such as excessive feed application and low feed quality. Biofloc technology has been developed in the past two decades as a supplementary feed that can effectively reduce feed costs in fish farming. Biofloc serves as a feed additive sourced from the development of microbial flocs formed by bacteria, algae, and protozoa, as well as detritus and aggregates of organic particles (Dauda *et al*., 2018).

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With a protein content of up to 30.4%, biofloc might serve as a dietary protein supplement (Emerenciano *et al*., 2012) to support the growth of cultured fish. Nootong *et al*. (2011) demonstrated that biofloc enhanced the growth of cultured fish while also reducing the feed conversion ratio. Another finding showed that shrimps grew more rapidly in a biofloc culture system than in a clear water pond (Emerenciano *et al*., 2012). Additionally, biofloc technology improved individual weight, growth rate, survival rate, and feed conversion, suggesting that the technology is better than the recirculation pond system (Luo *et al*., 2014). Furthermore, some studies have attempted to use dry biofloc with high protein content as a supplementary feed for cultured fish. For example, the addition of dry biofloc between 4%-8% in feed could improve the growth and diges-

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tive enzyme activities in shrimp (Anand *et al*., 2014).

Biofloc technology does not only reduce water usage in fish farming but also improve water quality condition. The formation of biofloc aggregation by a microbial community is directly responsible for removing nitrogen, leading to improving water quality in the cultured media. When heterotrophic bacteria and molasses are added as additional carbon sources, the microbes use nitrogen in water to form flocs, thereby reducing ammonia concentration (Nootong *et al*., 2011). Zhao *et al*. (2012) reported that ammonia-N and nitrite-N concentrations in water treated with biofloc were significantly lower than in non-treated biofloc ones.

Biofloc is also reported to be able to strengthen the fish's immune system (Lee *et al*., 2017). Feed supplemented with 4% biofloc improved growth performance, immune, and resistance to diseases of white shrimp in comparison with control. Mansour & Esteban (2017) reported that biofloc technology in tilapia farming could enhance the hematology profile, immune response, antioxidant status, and enzymatic activity in the cultured fish. For catfish, research on biofloc application in catfish farming is quite extensive with different subjects of research focus. However, there were few and far between studies regarding the effects commercial feed reduction on the growth of catfish reared in biofloc systems. Therefore, this research investigated the effects of feed dose reduction on the growth performance and hematology status of catfish cultured in biofloc ponds. This research also sought to determine the effects of feed reduction on the biofloc ponds' water quality.

MATERIALS AND METHODS

Experimental Design

The experiment followed a completely randomized design consisting of three treatments: full feed + non biofloc (C100) as control; 10% less feed + biofloc (B90); 20% less feed + biofloc (B80). Each treatment was performed in triplicate.

The nine weeks experiment was carried out at the experimental unit of the Department of Fisheries Extension, Jakarta Technical University of Fisheries. Catfish seeds with an average weight of 7.53 ± 0.47 g were randomly stocked in nine earthen ponds at a stocking density of 100 fish/pond. Each pond had a dimension of 1.5 m x 1.0 m x 1.0 m and was lined with high-density polyethylene (HDPE) plastic sheets. The ponds were previously filled with water with a volume set at 0.90 m^3 and well aerated. The fish were fed twice a day using a commercial pellet containing 30% protein at a dose of 5% total biomass for control (C100), 4.5% (B90), and 4% (B80), respectively.

At the beginning of the experiment, probiotics were added to the biofloc ponds at 20 g/m^3. After five days, the fish was introduced to the experimental ponds. During the experiment, cane sugar was regularly added to the ponds to maintain C/N above 10. The aerator was set on each experimental unit.

Analytical Procedures

Growth performance

The measured parameters of growth performance included specific growth rate (SGR), survival rate (SR), biomass production (BP), and food conversion ratio (FCR). Each parameter was calculated using the following equation:

$$SGR\left(\%\right) = \frac{\ln\left(\frac{\text{final weight}}{\text{initial weight}}\right)}{\text{time}} \times 100$$

$$SR\left(\%\right) = \frac{\text{fish harvested}}{\text{fish stocked}} \times 100$$

$$BP\left(kg\right) = \text{final weight} - \text{initial weight}$$

$$FCR = \frac{\text{total feed given}}{\text{fish weight gain}}$$

Water quality, feeding habit, and hematology

The measured water quality parameters consisted of dissolved oxygen, pH, total ammonia, nitrogen, and nitrite. The measurement procedures followed APHA (2017) (23^{nd} Edition, 4500). Observation of the fish feeding habit was conducted in the Laboratory of Biomakro, Division of Ecology and Marine Resource Conservation, Faculty of Fisheries and Marine Science, IPB University. The feeding habit of fish was determined by dissecting the stomach and counting its contents. For the counting purpose, the stomach content was diluted with 2 mL of distilled water. Diluted gastric contents were placed on a petri dish and observed under a microscope with a magnification of 100x. Blood analysis was carried out in the Laboratory of Aquatic Organism Health in the Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University. Blood samples were taken at the base tail using a 1 mL syringe as much as 1 cc and then put into a sample bottle that had been given

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ethylenediaminetetraacetic acid (EDTA) to prevent the blood from clotting. The experimental procedures for quantifying erythrocytes and leucocytes, hemoglobinand hematocrit adopted the method published by Blaxhall & Daisley (1973), Wedemeyer & Yasutake (1977), and Anderson & Siwicki (1993).

Biofloc volume

One liter of water sample was put into the Imhoff cone to measure the floc volume. The floc volume, expressed in mL/L, was measured after 30 min allowing the water to settle in the cone (Dauda *et al*., 2018).

Determination of protein and fat contents

The contents of protein and fat were quantified according to the method of AOAC (2005) at the Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor.

Statistical Analysis

Statistical analyses of the collected data were performed using analysis of variance following the research design of a completely randomized design with three replications. The means were compared using the least significant difference (Steel & Torrie, 1980), followed by a posthoc analysis. The *p-values* less than 0.05 ($P<0.05$) indicated significance. The statistical analysis was conducted using Statistical Product and Service Solutions (SPSS) version 16.0 (IBM Corp., USA) for Windows 10 (Microsoft, USA).

RESULTS AND DISCUSSION

Water Quality and Biofloc

Ranges of water quality parameters and biofloc volume in each treatment are presented in Table 1. The results showed that all water quality parameters did not differ significantly among the treatments ($P>0.05$). This is in accordance with the findings of Chen *et al*. (2020), who found that carbon addition in biofloc ponds resulted in similar concentrations of TAN, nitrite, and organic carbon compared with the control ponds. Xu & Pan (2012) also reported similar findings that the application of external carbon sources to increase C/N had no effects on the level of TAN and nitrite. In contrast, Long *et al*. (2015) and Zhao *et al*. (2012) found that the use of extraneous carbon lowered the concentrations of ammonia ions, nitrite, and nitrate in comparison with a control treatment.

The statistical analysis also provided evidence that there was no significant difference ($P>0.05$) among treatments regarding biofloc volume. Despite no significant differences among the treatments, treatment B90 produced the highest biofloc volume (58.57 mL/L). This finding is similar to other studies in which carbon supplementation in media positively affected biofloc volume (Dauda *et al*., 2018; Xu & Pan, 2012). Moreover, Xu *et al*. (2013) reported increased biofloc volume as more carbon was added. Even though carbon was not added to C100, biofloc still formed due to the role of carbohydrates supplied from the commercial feed serving as the carbon source. A similar finding was reported by Zaki *et al*. (2020), who stated that the production of biofloc was supported by carbon from carbohydrates contained in the fish feed. On the other hand, treatment B90 and B80 received a considerable amount of carbon from different sources (feed and sugar cane). The relationship between carbon supply and biofloc volume was investigated by Dauda *et al*. (2018), who determined that different levels of C/N affected biofloc volume.

Food Habit

Table 2 presents the results of catfish feeding habits, in which various planktons were present in the catfish intestines. There were 6-8 types of plankton found in the fish intestine cultured under treatments C100, B90, and B80. The plankton originated from the consumed biofloc. As Xu *et al*. (2016) reported, algae and autotrophic bacteria frequently dominated the composition of biofloc. The evidence of biofloc consumption by fish has also been reported on nile tilapia, shrimp, and shellfish (Ekasari *et al*., 2014).

Table 1. Water quality and biofloc

Treatments	Dissolved oxygen (mg/L)	pH	TAN (mg/L)	Nitrite (mg/L)	Biofloc volume (mL/L)
C-100	5.233 ± 0.153^a	6.980 ± 0.053^a	0.209 ± 0.020^a	0.102 ± 0.024^a	20.00 ± 11.53^a
B-90	4.933 ± 0.252^a	6.957 ± 0.040^a	0.404 ± 0.270^a	0.111 ± 0.043^a	58.67 ± 34.44^a
B-80	4.267 ± 0.493^a	6.953 ± 0.015^a	0.244 ± 0.020^a	0.115 ± 0.035^a	50.33 ± 27.06^a

Description: The same superscript letters in each column indicated no significant difference at a 0.05 confidence level

Table 2. Plankton variety detected in catfish intestine

Treatments	Plankton
C-100	*Nitzchia* sp. *Euglypha* sp. *Difflugia* sp. *Oedogonium* sp. *Lecane* sp. *Arcella* sp. *Achnantes* *Navicula* sp.
B-90	*Diatome* sp. *Nitzchia* sp. *Oedogonium* sp. *Lecane* sp. *Arcella* sp. *Euglypha* sp.
B-80	*Navicula* sp. *Nitzchia* sp. *Oscillatoria* sp. *Arcella* sp. *Euglypha* sp. *Notolca* sp. *Lecane* sp. *Stautrum* sp.

Growth Performance

Specific growth rate, survival rate, weight gain, and feed conversion are provided in Table 3. These growth performance parameters did not significantly differ among the treatments ($P>0.05$). However, the highest weight gain was achieved by the fish in B90, followed by B80 at 25.71% and 12.65%, respectively. All treatments were higher than the control treatment. The feed conversion ratio of B90 and B80 treatments were lower than the control, reaching up to 14.39% (B90) and 7.58% (B80), respectively.

The results of these growth performance parameters are similar to that of nile tilapia cultured under a biofloc system reported by Fuentes *et al*. (2018). In their experiment, although the feed dose was reduced to 20%, the growth of fish in the biofloc system was comparable with that of the control ponds fed with 100%. This result suggests that the feed reduction of 10%-20% can be replaced by consuming biofloc, as evidenced by the presence of plankton in the fish intestine. The research evaluating the nutritional abundance of biofloc was also carried out by Anand *et al*. (2014). They reported that the primary nutrition in-

Table 3. Growth performance

Treatments	Specific growth rate (%)	Survival rate (%)	Weight gain (kg)	Feed conversion
C-100	3.83 ± 0.12^a	91.67 ± 9.29^a	2.451 ± 0.480^a	1.323 ± 0.046^a
B-90	4.20 ± 0.36^a	95.67 ± 2.88^a	3.082 ± 0.479^a	1.130 ± 0.174^a
B-80	3.97 ± 0.15^a	95.67 ± 1.53^a	2.768 ± 0.259^a	1.223 ± 0.083^a

Description: The same superscript letters in each column indicated no significant difference at a 0.05 confidence level gredients of dry biofloc included protein (24.30%) and essential fatty acids such as palmitic (46.54%), cis-vaccenic (15.37%), linoleic (10.67%), and oleic (9.19%).

Studies on the impacts of biofloc on growth performance have been carried out by various researchers. Anand *et al*. (2014) used dry biofloc (4% and 8%) in feed, resulting in higher final weight and lower food conversion than the control. Lee *et al*. (2017) reported the enhanced growth performance of white shrimp after being treated with biofloc at a dose of 4%. Dry biofloc for pellet supplementation of up to 15% could increase the growth of sea cucumber in comparison with control (Chen *et al*., 2018), while the addition of 12% biofloc into feed was revealed to be effective in supporting the growth of *Rhynchopris lagowskii* Dybowski (Yu *et al*., 2021). Luo *et al*. (2014) also described the desirable impacts of biofloc on weight gain, growth rate, survival rate, and feed conversion ratio of tilapia compared to that of fish cultured in a non-biofloc-recirculation system. Meanwhile, Zhao *et al*. (2012) reported an increase in shrimp production up to 41.3% cultured under a biofloc system. Similarly, Sgnaulin *et al*. (2018) reported higher final biomass of shrimp cultured under biofloc compared to the control treatment. Furthermore, a study by Dauda *et al*. (2018) revealed that a biofloc pond system with a variety of feed doses (100%, 90%, and 80%) produced a similar growth rate of African catfish compared with the control pond with a 100% feed dose.

In this current work, the feed conversion ratios of fish in B90 (14.59%) and B80 (7.56%) were lower than that of C100. Although not significant, the quantities of biofloc in B90 and B80 were higher than that of C100, which arguably means that B90 and B80 had more nutritious and complete feed for the catfish. Zhao *et al*. (2012) reported similar findings, in which the biofloc system successfully produced lower food conversion, reaching down to 7.22% in comparison with the control treatment in shrimp farming. In summary, our present experiment confirmed that using biofloc with feed reduction of up to 10%-20% successfully lowered the feed conversion ratio and subsequently reduced the production cost.

Hematology Response

Numerous researches have been carried out to examine the quality of blood using qualitative and quantitative parameters, including the number of red blood cells, white blood cells, hematocrit, and hemoglobin (Michael *et al*., 2019). In this study, the blood cell of the tested fish was evaluated, focusing on the number of erythrocytes, leukocytes, hemoglobin, and hematocrit (Table 4). The hematology analysis showed no significant differences among the treatments ($P<0.05$). In this work, the number of erythrocytes was within the normal range, with the highest achieved by B90 treatment. The result is supported by Kumar *et al*. (2013), who confirmed that erythrocytes mainly comprise the blood ranging from 1.05-3.0 x 10^6 cell/mL.

Leukocyte constitutes a key component of the non-specific immune system in fish, comprising several components, i.e., monocyte, granulocyte, and non-specific cytotoxic cells (Fraser *et al*., 2012). Total leukocyte cells in control and B90 treatments are comparable, reaching up to 2.9 ´ 10^4 cell/mm^3. Leukocyte plays a crucial role in the fish immune system and serves as a bio-indicator of fish health status. Hemoglobin (Hb) is a part of the erythrocyte capable of distributing oxygen to other parts of the fish body. In this study, the quantities of hemoglobin and erythrocyte demonstrated an identical pattern, with the highest amount measured in B90. The highest hematocrit was observed in catfish in B90 (43.14%), which is usually expected considering that a higher percentage of erythrocytes directly corresponds to a higher hematocrit level.

CONCLUSION

This recent study successfully confirms that feed reduction does not affect the growth and hematology parameters of catfish and the general conditions of water quality in the biofloc ponds. Despite being statistically not different, the weight gain and feed conversion ratio of biofloc-treated fish are higher than the control. This study could safely recommend that catfish farmings using biofloc application with a similar culture arrangement to this study could reduce

Table 4. The quantity of erythrocytes, leukocytes, hemoglobin, and hematocrit in fish blood

Treatments	Total erythrocyte (cell/mm³)	Total leukocyte (cell/mm³)	Hemoglobin (g/%)	Hematocrit (%)
C-100	2.95 x 10^6	2.9 x 10^4	8.23	35.97
B-90	3.98 x 10^6	2.9 x 10^4	9.03	43.14
B-80	3.5 x 10	2.06 x 10^4	7.56	34.99

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between 10%-20% of commercial feed application while producing catfish akin to non-biofloc ponds.

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