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Utilization of biofloc meal as a feed ingredient for Nile tilapia and common carp

Pemanfaatan tepung limbah bioflok pada ikan nila dan ikan mas

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ABSTRACT

This study was aimed to evaluate the utilisation of biofloc meal collected from biofloc-based catfish intensive culture as a mix ingredient for Nile tilapia *Oreochromis niloticus* and common carp *Cyprinus carpio* diet. A control diet containing 29.03% crude protein was used in this experiment. Experimental diet was made by mixing 30% biofloc waste meal with the control diet and repelleted after the addition of 2% of binder. To determine the experimental feed digestibility, 0.5% of Cr_2O_3 was added as a marker for digestibility. The feed was offered to satiation at a frequency of 3 times a day for 28 days of experimentation. Nile tilapia and common carp juveniles with an initial average body weight of 11.72±0.04 g and 8.81±0.04 g, respectively, were used as the experimental animals. Each fish species were randomly stocked with a density of 10 fish/aquarium (30x45x30 cm^3). The results showed that dry matter digestibility of diets with 30 % biofloc waste meal in both fish species were significantly lower than those of the controls ($P<0.05$). However, protein, lipid, and phosphorus digestibilities of diets containing biofloc waste meal were significantly higher than those of the controls ($P<0.05$). Feeding with biofloc waste meal mixed feed to tilapia resulted in lower growth rate compared to that to fed control feed. On the other hand, similar treatment to common carp resulted in comparable growth rate to the control treatment.

Keywords: biofloc meal, digestibility, growth performance, tilapia, common carp

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pemanfaatan tepung bioflok yang dikumpulkan dari limbah pemeliharaan ikan lele intensif berbasis teknologi bioflok sebagai campuran pakan untuk ikan nila O*reochromis niloticus* dan ikan mas *Cyprinus carpio*. Pakan kontrol yang digunakan adalah pakan komersial dengan kadar protein 29,03%. Pembuatan pakan uji dilakukan dengan mencampurkan tepung limbah bioflok (30%) dengan pakan kontrol (67,5%) dan dibentuk pelet kembali setelah dilakukan penambahan *binder* sebanyak 2% dan Cr_2O_3 sebanyak 0,5% sebagai penanda untuk menganalisis kecernaan pakan dengan tepung limbah bioflok. Pakan diberikan secara *at satiation* dengan frekuensi pemberian pakan 3 kali/hari selama 28 hari pemeliharaan. Bobot rata-rata ikan awal adalah 11,72±0,04 g untuk ikan nila, dan 8,81±0,04 g untuk ikan mas dengan kepadatan awal masing-masing 10 ekor/akuarium (30x45x30 cm^3). Hasil penelitian ini menunjukkan bahwa nilai kecernaan total pakan dengan tepung limbah bioflok baik pada ikan nila maupun ikan mas lebih rendah daripada pakan kontrol ($P<0,05$). Namun demikian, kecernaan protein, lemak, dan fosfor pakan dengan campuran tepung limbah bioflok lebih tinggi daripada kontrol ($P<0,05$). Pemberian tepung limbah bioflok sebanyak 30% sebagai campuran pakan menghasilkan laju pertumbuhan spesifik ikan nila yang lebih rendah ($P<0,05$), sedangkan perlakuan yang sama pada ikan mas memberikan laju pertumbuhan spesifik yang tidak berbeda nyata dengan kontrol ($P>0,05$).

Kata kunci: tepung limbah bioflok, kecernaan, kinerja pertumbuhan, ikan nila, ikan mas

INTRODUCTION

Feed is an aquaculture production input which strongly determine the fish growth, which, at the same time, is the highest cost component in the total production cost which may reach 50–60 % (Rana *et al.,* 2009). In Indonesia, the high cost of feed in aquaculture production is also affected by the progressively increasing feed price caused by the increase of the high price of the ingredients, which mostly are imported products. One of the strategies to reduce feed cost is by utilizing alternative feed ingredients which are locally based, originating from waste/by-product with less competitive with other human activities.

Biofloc is microbial aggregates that may be grown separately or in integration in a culture system, which can be used as an alternative ingredient for aquaculture feed. Biofloc meal may be produced separately in sequencing batch reactor (Kuhn *et al*., 2010; Kuhn *et al*., 2016) or grown naturally in a biofloc based aquaculture system by the addition of organic carbon source. The prime aim of biofloc based aquaculture system is to minimize nutrient waste in an aquaculture system by applying heterotrophic microbial conversion through the addition of organic carbon source (De Schryver *et al.,* 2008; Bossier & Ekasari, 2017). One of the problem in a biofloc-based intensive aquaculture system is the high accumulation microbial biomass, which requires high oxygen supply (Ekasari, 2014). Thus, the excess biofloc biomass should be removed out from the culture system. On the other hand, bioflocs have been reported to have sufficient nutritional composition to be used as an aquaculture feed ingredient (Kuhn *et al.,* 2010; Ekasari *et al.,* 2014; Matassa *et al.,* 2016, Dantas *et al.*, 2016; Kuhn *et al.,* 2016; Shao *et al.,* 2017; Valle *et al.,* 2015; Chen *et al.,* 2018). In this regard, excess biofloc biomass is potential to be developed as an alternative feed ingredient for aquaculture species. Previous research have done to explore the possibility of using biofloc meal as a feed ingredient for some aquaculture species such as shrimp and Nile tilapia (Kuhn *et al.*, 2010; Bauer *et al*., 2012; Van Den Hende *et al*., 2014; Kuhn *et al*., 2016; Kheti *et al.,* 2017; Gamboa-Delgado *et al.,* 2017).

In this study, bioflocs meal originated from biofloc-based intensive catfish production was added and mixed with commercial feed for Nile tilapia *Oreochromis niloticus* and common carp *Cyprinus carpio*, so that bioflocs meal maybe used directly by the farmer as a feed mixture. The fish used in this study was Nile tilapia and common carp, which are omnivorous fishes with relatively low protein requirement. The production statistics of these fish species have been increasing. In Indonesia, for instance, in a period from 2010 to 2013 the increase of tilapia and common carp productions have reached 34.85% and 9.09% respectively (KKP, 2014). Similar to other freshwater fish species, the profitability of tilapia and common carp is still limited by the high feed cost, which may account up to 30–60% of the total production cost. In this context, utilization of bioflocs meal as a feed ingredient may bring about some added values, including utilization of waste, and reduction of feed cost. The present study aimed to evaluate the utilization of biofloc meal collected from biofloc-based catfish intensive culture as a mix ingredient for Nile tilapia and common carp diet. In addition, a digestibility measurement was also carried out to assess the digestibility of bioflocs meal by Nile tilapia and common carp.

MATERIALS AND METHODS

Experimental diet preparation

This study compared the utilization of bioflocs meal as a feed mixture for Nile tilapia and common carp. For each species tested, two treatments (triplicates) of experimental diets were prepared, i.e. control and biofloc. Commercial feed for tilapia and common carp with a protein content of 29% was used as the reference diet. Biofloc diet was prepared by mixing 30% of bioflocs meal with 67.5% of grinded commercial feed. The commercial pellet (both control and biofloc) was grinded, mixed, and repelleted with an addition of a binder, carboxymethyl cellulose (2%) and Cr_2O_3 (0.5%) as an inert marker for digestibility assessment.

Bioflocs meal was collected from biofloc-based intensive catfish production using sieve with a mesh size of 200 µm. The bioflocs suspension was then oven dried at 40 °C for 24 h and milled into bioflocs meal by hammer mill.

Feeding experiment

A set of eight units of aquarium (30×45×30 cm^3) was used as the experimental containers for each fish and filled 33 L of water. An aeration line was provided in each aquarium for oxygenation. The containers and the water were disinfected with 30 mg/L chlorine solution for 24 h and neutralized by 15 mg/L of sodium thiosulfate.

Nile tilapia and common carp were used as the experimental fish with average body weight of 11.72±0.04 g and 8.81±0.04 g, respectively, and was previously acclimatized to laboratory condition for seven days. The fish was randomly distributed to each aquarium at a density of 10 fish/aquarium (250 fish/m^3). The fish was fed with the experimental diets to apparent satiation three times a day 08.00 am, 12.00 pm, and 16.00 pm for 28 days of culture period. The feed given to the fish was recorded daily.

To assess the digestibility of fecal materials were collected from each fish in each treatment. Feces collection was started after day-6 of culture by siphoning out the feces about 1–2 h after feeding. Fecal sample was collected and then stored in -20 °C until further analyses.

Proximate analyses and water quality maintenance

Proximate analyses were conducted on bioflocs meal, commercial feed, experimental diets and fecal material. Chromium analyses for digestibility assessment were performed to measure Cr content in experimental diets and fecal materials. Proximate, phosphorus, and chromium analyses were performed following the procedures described by Takeuchi (1988).

Water quality maintenance was done by daily water replacement of about 20%. To monitor the water quality, water quality parameters measurement were carried out at the initial and final day of the experiment, including temperature, dissolved oxygen (DO) concentration, pH and total ammoniacal nitrogen concentration (Table 1 and Table 2). Water quality analyses were conducted following the procedure described in Balai Penelitian Tanah (2009).

Data analyses

Apparent digestibility coefficient was calculated according to Glencross *et al.* (2007), including total, protein, lipid, and phosphorous. The total digestibility coefficient was calculated according to the following formula: total digestibility (%) = [1-(a/a')] ´ 100, with a is Cr_2O_3 concentration in feed and a' is Cr_2O_3 concentration in feces. Protein, lipid, and phosphorous digestibility were calculated by the following formula: Nutrient digestibility (%) = 100 ´ [1-(a/a' x b/b')], with a is Cr_2O_3 concentration in feed, a' is Cr_2O_3 concentration in feces, b is nutrient content in feed and b' is nutrient content in feces.

Fish survival was determined at the final day of experiment, which was the percentage of survived fish over the initial number of fish per aquarium. Fish specific growth rate (SGR) was calculated according to the following formula:

$$SGR\ (\%/day) = (\ln W_2 - \ln W_1)/T \times 100$$

with T is rearing period (day), W_1 is initial body weight and W_2 is final body weight.

Survival data was arcsin transformed. All parameters were compared using t-test for two independent samples using statistical software SAS version 9.1.3.

Tabel 1. Range of temperature, dissolved oxygen (DO), pH, and total ammoniacal nitrogen (TAN concentration in Nile tilapia rearing medium

Parameter	Biofloc	Control	Reference (Alabaster & Lloyd, 1982)
Temperature (°C)	25–28	25–28	22–29
DO (mg/L)	3.9–5.1	4.8–6.4	>2.0
pH	7.2–7.3	7.14–7.90	6–9
TAN (mg/L)	0.41–0.68	0.47–1.46	0.20–1.00

Tabel 2. Range of temperature, dissolved oxygen (DO), pH, and total ammoniacal nitrogen (TAN) concentration in common carp rearing medium

Parameter	Biofloc	Control	Reference (Alabaster & Lloyd, 1982)
Temperature (°C)	25–28	25–28	25–30
DO (mg/L)	3.9–5.1	5.0–6.5	>4
pH	7.20–7.30	7.28–7.30	6.5–8.5
TAN (mg/L)	0.41–0.68	0.40–0.67	<0.2

RESULTS AND DISCUSSION

Results

Proximate compositions of bioflocs meal and experimental diets were presented in Table 3. Both bioflocs meal and bioflocs experimental diets were lower in protein and energy contents, but higher in lipid, fiber, ash contents than those of the control diet.

Nile tilapia experiment

Daily feed offered to the fish during 28 days of feeding experiment is presented in Figure 1. It can be seen that daily feed consumption by Nile tilapia in biofloc diet was generally higher than that of the control diet.

Apparent digestibility of control diet in Nile tilapia was higher than that of bioflocs meal ($P<0.05$). However, feed mixed with bioflocs meal showed higher protein, lipid, and phosphorus digestibilities than those of the control diet ($P<0.05$) (Figure 2).

Fish survival was not significantly different between the treatments with a range of 93% to 96%. However, specific growth rate of Nile tilapia in bioflocs diet treatment was significantly lower than that of the control (Figure 3).

Table 3. Proximate composition (% dry weight) of bioflocs meal and experimental diets

	Moisture (%)	Protein (%)	Lipid (%)	Crude fiber (%)	Ash (%)	NFE*(%)	GE** (kcal/kg)
Bioflocs meal	11.41	9.11	8.70	19.07	3.95	59.17	3754
Commercial feed	10.80	29.03	4.37	5.36	7.61	53.63	4235
Biofloc diet	10.43	22.93	6.61	7.76	5.42	57.28	4254
Control diet	10.23	28.83	5.45	4.26	6.87	54.58	4365

*NFE = nitrogen free extract

**GE (*Gross energy*) was calculated according to Watanabe (1988) with the following assumption: 5.6 kcal/kg protein, 4.1 kcal/kg NFE and 9.4 kcal/kg lipid.

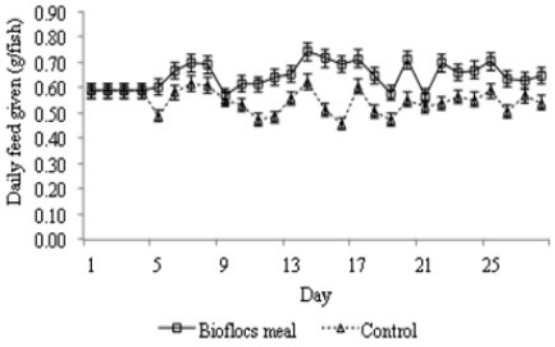

Figure 1. Daily feed (g/fish) offered to Nile tilapia *Oreochromis niloticus* for 28 days of culture.

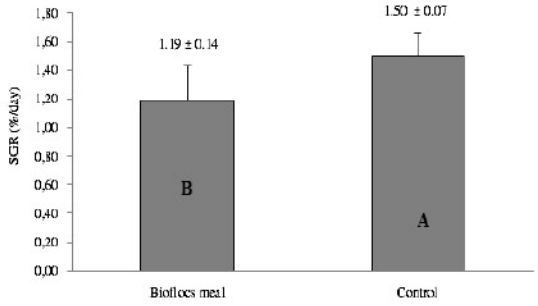

Figure 3. Specific growth rate (SGR) of Nile tilapia *Oreochromis niloticus* fed with biofloc and control diets. Different letters in the bars indicate significant difference ($P<0.05$).

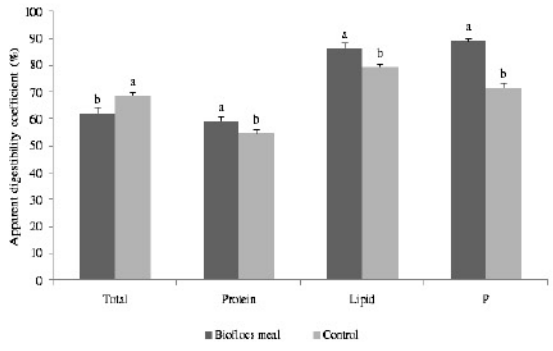

Figure 2. Total, protein, lipid, and phosphorous (P) apparent digestibility coefficients of bioflocs and control diets in Nile tilapia *Oreochromis niloticus*. Different letter in bars in the same parameter indicate significant difference ($P<0.05$).

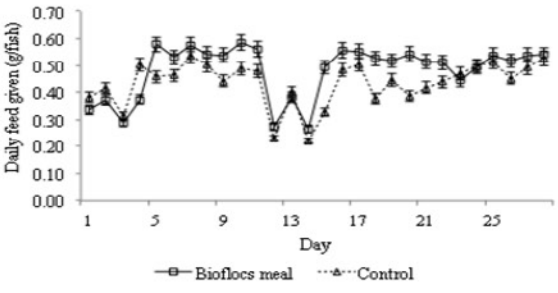

Figure 4. Daily feed (g/fish) offered to Nile tilapia *Oreochromis niloticus* for 28 days of culture.

Common carp experiment

Daily feed consumption of diet by common carp is presented in Figure 4. Daily feed consumption of bioflocs diet was generally higher than that of the control diet (Figure 4).

Similar to Nile tilapia, apparent digestibility of control diet by common carp was also higher than that of bioflocs meal ($P<0.05$). However, bioflocs diet also showed higher protein, lipid, and phosphorus digestibilities than those of the control diet ($P<0.05$; Figure 5).

Fish survival and specific growth rate were not significantly different between treatments ($P>0.05$). Fish survival was the same at a level of 96%, whereas the specific growth rate was in a range of 1.35 to 1.69%/day (Figure 6).

Discussion

Feed consumption is one of the indicators that can be used to assess feed digestibility. The present study demonstrates that both Nile tilapia and common carp consumption on diets with bioflocs meal addition were higher than those of the control ($P<0.05$). High feed consumption may be caused by two factors, i.e. feed palatability and energy requirement. Ju *et al.* (2008) reported that bioflocs contained free amino acids such as alanine, glycine, and proline that may function as feed attractant. In this regard, it is possible that the feed containing bioflocs meal has a higher attractant than that of the control diet. Higher feed consumption may also be caused by the effort of the fish to fulfill their energy requirement. This possibility, however, might not be the case in the present study, as the energy content of the experimental diets was within the range of energy requirement of Nile tilapia, 2500–4300 kcal/kg and common carp 3100–3600 kcal/kg (NRC, 2011).

Digestibility measurement is one of the most important evaluation tools in assessing the quality of a feed ingredient in aquaculture diet (Glencross *et al.,* 2007; Liu *et al.,* 2016). The present study showed that bioflocs meal addition to commercial diet significantly affected the total and nutrients digestibility of the feed by both Nile tilapia and common carp. The total digestibilities of bioflocs diets were lower than those of the control in both Nile tilapia and common carp ($P<0.05$). This might be explained by the high fiber content in bioflocs meal that affected the total digestibility of the feed. Nonetheless, the digestibilities of protein, lipid, and phosphorus of bioflocs diets in both Nile tilapia and common carp were considerably higher than those of the control diets. This may be caused by the contribution of exogenous digestive enzymes (protease and lipase) secreted by the microorganisms associated in bioflocs that improved the digestibilities of the bioflocs meal (Ju *et al.,* 2008; Xu & Pan, 2013; Wang *et al.,* 2016; Anand *et al.,* 2017). Other studies also showed that dietary addition of bioflocs meal could stimulate the production of endogenous digestive enzyme by the cultured organism (Anand *et al.,* 2014; Zhang *et al.*, 2016). With regard to phosphorus digestibility, Ekasari (2014) suggested that the microorganisms associated in bioflocs might also produce phytase that improve the digestibility of phosphorus by the fish.

The fish survival in all treatments for both Nile tilapia and common carp was not significantly different, indicating that bioflocs diet did not

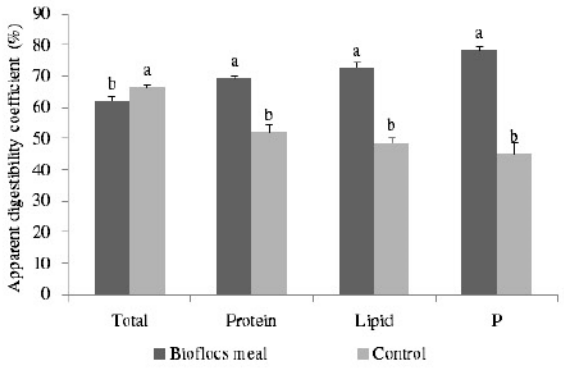

Figure 5. Total protein, lipid, and phosphorous (P) apparent digestibility coefficients of bioflocs and control diets in common carp *Cyprinus carpio*. Different letter in bars in the same parameter indicate significant difference ($P<0.05$).

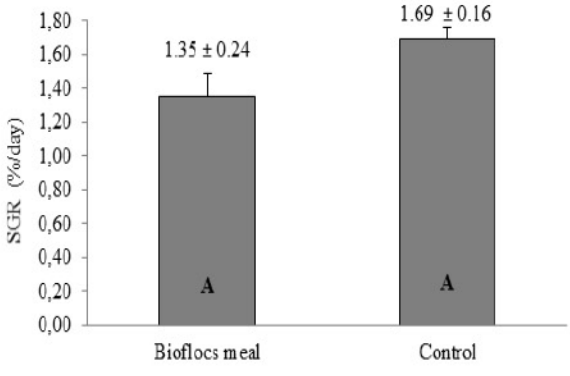

Figure 6. Specific growth rate (SGR) of common carp *Cyprinus carpio* fed with biofloc and control diets. Same letter in the bars indicates no significant difference ($P>0.05$).

negatively affect the fish survival. The addition of bioflocs meal in feed of about 30% was not significantly affect the growth of common carp, but significantly reduced the growth of Nile tilapia. This may be due to the difference in the feed total digestibility by the tilapia and common carp. The higher the feed digestibility, the higher the nutrient bioavailability used for metabolic processes to support optimal growth of the fish. Furthermore, the lower growth in Nile tilapia in bioflocs diet treatment may also caused by the lower protein content of the feed which was about 23% compared to the control which was about 29%. Although tilapia and common carp are naturally an omnivorous fish with relatively low dietary protein requirement at about 20–25%, maximum growth of these fish may only be achieved at dietary protein level of 26–30% for tilapia and 28–32% for common carp (NRC, 2011). The low dietary protein content in bioflocs diet was mostly caused by the low protein content of bioflocs meal used in the present study (9.11%). Indeed this level of protein in bioflocs meal was lower than that reported in previous study, which may range from 15 to 40% (Ekasari, 2014). The differences in bioflocs protein content may be affected by various factors including carbon source, C/N ratio, biological composition, fish species, and other operational parameters (Ekasari, 2014).

CONCLUSION

The digestibility of the feed with bioflocs meal by Nile tilapia and common carp was lower than that of the control feed. However, protein, lipid, and phosphorus digestibilities of bioflocs feed were higher than those of the control feed. The addition of bioflocs meal of about 30% did not affect the survival and growth rate of common carp, but significantly affected the growth of Nile tilapia.

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