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The Growth, Feed Efficiency, and Survival Rate of Bonylip Barb (*Osteochillus hasselti*) in Biofloc Media C/N Ratio 10 with Different Stocking Densities

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Abstract

Bonylip barb (*Osteochillus hasselti*) is a favorite freshwater fish, especially in West Java, Indonesia. Cultivation of bonylip barb is carried out in a conventional method; therefore, it is necessary to apply new cultivation technology, namely biofloc. The purpose of this study was to know the growth, feed efficiency, and survival rate of Bonylip barb in biofloc system cultivation (C/N ratio 10) with different stocking densities. This research was conducted in March – July 2021, and the experiment was conducted at the Laboratory of Aquaculture, Faculty of Agriculture, Djuanda University, Bogor. The object of research is bonylip barb seed (size 5.0 ± 0.5 cm). The research design was a completely randomized design with three treatments of stocking density {A (10 fish/21 L), B (20 fish /21 L), and C (30 fish /21 L)} with four replications. Parameters observed were specific growth rate, feed efficiency, survival rate, and water quality. Furthermore, data were analyzed using analysis of variance (ANOVA) and LSD test. The results showed that treatment A (stocking density 10 fish/21 L) had the best performance with a specific weight growth rate ($2.03 \pm 0.15\%$), feed efficiency (78.7%), and survival rate (100%). Water quality during the study was feasible for bonylip barb life.

INTRODUCTION

Bonylip barb (*Osteochilus hasselti*) is a favorite freshwater fish, especially in West Java, Indonesia. Fish farmers have cultured this fish conventionally (Jubaedah and Hermawan, 2010), so it is necessary to apply new technology to rear bonylip barb. At least the new technology must meet good criteria, such as simple (Maher *et al.*, 2021), successfully applied (Pullanikkatil *et al.*, 2014; Dahooie *et al.*, 2021), energy efficiency (Saygin *et al.*, 2011),

environmentally friendly and sustainable (M. Guo *et al.*, 2020; Starkl *et al.*, 2018), and economically feasible (Pullanikkatil *et al.*, 2014; Starkl *et al.*, 2018).

Biofloc Technology (BFT) is an alternative technology for the development of aquaculture (Emerenciano *et al.*, 2013; Bossier and Ekasari, 2017), even industrial-scale (Kasan *et al.*, 2018), as well as for the improvement of bonylip barb aquaculture. On the eye-catching side, biofloc

technology has many advantages, including high productivity (Bossier and Ekasari, 2017), optimal survival rate and growth (Kasan *et al.*, 2018), suitable water quality control capability, producer of additional feed, eco-friendly, and sustainability. Moreover, biofloc technology is socially and economically feasible for sustainable aquaculture (Crab *et al.*, 2012; Rangka and Gunarto, 2012). Based on these advantages, the cultivation of bonylip barb using biofloc will have the potential to increase production and sustainable economic benefits.

Various studies have shown that the C/N ratio plays a vital role in determining the performance of biofloc; the right C/N ratio will support high fish production in aquaculture. Where the C/N ratio significantly affects the growth of heterotrophic bacteria in biofloc media, and then these bacteria will decompose organic matter (feed waste) and produce good water quality, significantly lower levels of toxic ammonia (Rosmawati and Muarif, 2017; Panigrahi *et al.*, 2018). In addition, the C/N ratio also has an impact on growth performance and the immune system. Furthermore, many studies have shown that C/N ratio 10 has a high resistance to pathogens and provides a high survival rate of *Litopenaeus vannamei* (Panigrahi *et al.*, 2018), better growth of *L. vannamei* and *Pangasius* sp. (H. Guo *et al.*, 2020; Das and Mandal, 2021; Meritha *et al.*, 2018), lower feed conversion ratio of *L. vannamei* and *Pangasius* sp. (Meritha *et al.*, 2018; Das and Mandal, 2021), and resistance to stress of *Pangasius* sp. (Meritha *et al.*, 2018). Successful application of biofloc (C/N ratio 10) to increase the yield of several fish provides an opportunity to use it as a beneficial medium for bonylip barb culture.

In fish farming, apart from culture media, stocking density also influences fish culture production. Fish stocking density would impact growth performance (M'balaka *et al.*, 2012; Ani *et al.*, 2022), water quality (Ani *et al.*, 2022), feed conversion ratio (M'balaka *et al.*, 2012), mean yields, and profitability (Chowdhury

et al., 2020). For the traditional culture of bonylip barb in ponds, one study suggested a low stocking density of 10 fish/m³ (Rizaqi *et al.*, 2016), and conversely, another suggested a high stocking density of 50 fish/m³ (Aryani *et al.*, 2017). One more study showed that the best bonylip barb rearing in an aquarium was a stocking density of 1-3 fish/L (Ismayadi *et al.*, 2016).

The status of bonylip barb has several problems, including its population in inland waters is decreasing (Fauzi *et al.*, 2018), its cultural technology being traditional (even just a by-product of other fish cultivation) (KKP, 2018), and low aquaculture production (Subagja, 2011). The development of barb bonylip culture requires new technology for these problems. Bonylip barb culture on biofloc media and proper stocking density will be the best solution. For this reason, this study aims to determine the growth, feed efficiency, and survival rate of bonylip barb in biofloc system (C/N ratio 10) with different stocking densities.

12 METHODOLOGY

Place and Time

This research was conducted in March - July 2021 at the Aquaculture Laboratory, Faculty of Agriculture, Djuanda University, Bogor.

Research Materials

This study used bonylip barb seed measuring 5.0±0.5cm and other materials of this study feed and molasses. The aquarium of 30 cm x 30 cm x 30 cm as a research container added lamp (5 w) for the stability of temperature and aerator system. The research instrument included a digital scale (for weight), millimeter block (for length), water quality tester (for temperature and pH), DO meter (for DO), and Hanna Checker Ammonia LR (for TAN).

Research Design

13 This study used a completely random design (CRD) with three stocking

density treatments of Bonylip barb with four replications. These treatments are Treatment A (stocking density of 10 fish/21 L), Treatment B (stocking density 20 fish/21 L), and Treatment C of 30 fish/21 L). This stocking density modifies the research results by Ismayadi *et al.* (2016), who found the best stocking density of 1 fish/liter of bonylip barb.

Work Procedure

Bonylip barb seed cultured in the aquarium with a volume of water is 21 liters for 40 days. This study maintains temperature stability using a lamp (to maintain a high, ideal, and stable temperature) and keeps sufficient dissolved oxygen using a blower for water aeration (one spot in each aquarium). Feeding using the *ad libitum* method with twice/day frequency, at 08.00 and 16.00. The feed given is pellets measuring 0.5-0.7 mm with a protein content of 34.25%. This study used molasses (29.18% C) as a carbon source in the rearing medium and was given one hour after feeding in the afternoon. The procedure for determining the C/N ratio follows the method of De Schryver *et al.* (2008).

11 Sampling to measure weight & water quality was carried out at the beginning of the study and continued every ten days. This study observed three fish production parameters: specific growth rate, feed efficiency, and bonylip barb survival and measured water quality during the experiment.

Calculation of specific growth rate data using the formula from Lugert *et al.* (2016):

$$SGR = \frac{\ln W_t - \ln W_o}{t} \times 100\%$$

Where:

SGR = specific growth rate (%/day)

Wt = average final weight (g)

Wo = average initial weight (g)

t = time (day)

Calculation of feed efficiency data using the modified formula from NRC (2011) is as follows:

$$FE = \frac{(W_t + D) - W_o}{F} \times 100\%$$

Where:

FE = feed efficiency (%)

Wt = final weight (g)

D = dead fish weight (g)

Wo = initial weight (g)

F = total amount of feed (g)

The survival rate data was obtained using the formula from Krebs (2014):

$$SR = \frac{N_t}{N_o} \times 100\%$$

Where:

SR = survival rate (%)

Nt = final number of fish

No = initial number of fish

Water quality measurements include temperature and pH using a water quality tester, dissolved oxygen (DO) using a DO meter, and total ammonia (TAN) using the Hanna Checker Ammonia LR.

Data Analysis

Initial data analysis used analysis of variance (ANOVA). Further data analysis used LSD test to determine the response between each treatment.

RESULTS AND DISCUSSION

Specific Growth Rate

The analysis of variance showed that the specific growth rate (SGR) of bonylip barb was significantly different between treatments ($P < 0.05$). The LSD test also showed that the data between treatments A, B, and C were significantly different from each other ($P < 0.05$). The specific growth rate (SGR) of bonylip barb has an average range of 1.33%/day to 2.02%/day. Treatment A had the highest mean SGR and was significantly different from other treatments (Table 1).

Table 1. Mean specific growth rate, feed efficiency, and survival rate of bonylip barb.

Treatment	Variable		
	Specific growth rate (%)	Feed efficiency (%)	Survival rate (%)
A	2.02 ^c	78.77 ^a	100 ^b
B	1.33 ^a	64.28 ^a	96 ^b
C	1.67 ^b	54.05 ^a	78 ^a

Note: Different superscript shows the significantly different data (P <0.05).

1 The specific growth rate describes the percentage of additional weight daily of fish bonylip barb. Many factors were affecting fish growth, such as the quality of brood fish, feed (Hermawan *et al.*, 2015; Triyanto *et al.*, 2016)), feed efficiency (Costa-Bomfim *et al.*, 2017), water quality (Boyd, 2017), fish diseases (Rahman *et al.*, 2019), and stocking density (Ismayadi *et al.*, 2016; Verawati *et al.*, 2015). The stocking density will affect the growth is not directly, that is through the mechanism of energy demand, feed competition, and water quality. In this study, differences in stocking density affect growth through the mechanism of energy demand and competition for feed. Water quality in all treatments is relatively the same so all treatments will provide the same support to the growth rate.

Treatment A had the lowest density and gave the highest SGR, and was significantly different from other treatments. Low stocking density has the highest growth rate because fish have more expansive space to move than higher stocking density treatments. This activity requires energy, so increased activity will increase energy requirements and further increase metabolism. Increased metabolic rate will increase the specific growth rate. The energy generated from metabolic processes is used for basal metabolism, activity, growth, and reproduction (Putra, 2015).

Lower stocking density also gives more space for a fish to move to get feed, resulting in lower competition for a feed so that each fish has a high chance of getting the full feed. Obtaining full feed will provide a good source of energy for fish to grow better. In contrast, the stress arising from the higher stocking density

increases energy maintenance and reduces growth energy (Rahmawan *et al.*, 2020).

1 This research uses biofloc media and provides support for fish growth. According to Rosmawati and Muarif (2017), biofloc media, although no water changes, support fish's better growth than non-biofloc media. The ability of biofloc C/N ratio 10 to increase the growth rate can be seen from the SGR value in this study which was higher than in the non-biofloc study. The specific growth rates of bonylip barb in this study were 2.02% (stocking density 10 fish/21 L), 1.33% (stocking density 20 fish/21 L), and 1.67% (stocking density 30 fish/21 L) is higher than the study of (Rizaqi *et al.*, 2016), who kept bonylip barb in media without biofloc and obtained SGR of 1.25% (stocking density of 10 fish/m³), 1.22% (stocking density 15 fish/m³) and 1.09% (stocking density five fish/m³). 1 The better SGR value in this study was the impact of biofloc as an additional feed. Several studies stated that floc volume formed using a C/N ratio of 10 for 45 days of rearing was 4.5 mL/L (Panigrahi *et al.*, 2018) and 3.50-4.63 mL/L (Duy and Khanh, 2018).

Feed Efficiency

8 The average feed efficiency (FE) bonylip barb ranged from 54,05% up to 78,77%. The analysis of variance on the feed efficiency data showed that the feed efficiency of bonylip barb was not significantly different between treatments (P>0.05). Table 1 presents the distribution of the average feed efficiency for each treatment in this study.

Feed efficiency shows how much fish use feed for body growth. The more excellent feed efficiency value shows that the fish uses maximum feed for its growth (Noval *et al.*, 2019). Several factors that

affect feed efficiency are feed quality, genetics, environment, fish health, size, and body composition (Robinson and Li, 2015). Feed efficiency affects fish production in an aquaculture system, where the low efficiency of feed causes the production to be low and vice versa efficiency that is high supports the production of fish (Muarif *et al.*, 2021a).

Fish as cold-blooded animals have high feed efficiency because the maintenance energy is lower for fish than for warm-blooded animals. There are four reasons for low energy requirements : (1) fish do not have to maintain a constant body temperature ; (2) they expend less energy to maintain their position ; (3) energy loss in urine and lower gill excretion; and (4) increased energy costs associated with the assimilation of more minor digested food (Robinson and Li, 2015). The feed efficiency in this study ranged from 54.05 to 78.77%, so it was classified as high.

The CN 10 ratio biofloc media provides support in providing additional feed for fish. Growing flocks can be a source of feed protein so that feed is more efficient (Wijayanti *et al.*, 2019). Biofloc media increase the availability of feed by providing biological floc so that fish feed needed for high growth are available. Fish used the flocks as feed, and biofloc also triggers digestive enzymes' activity (Rosmawati and Muarif, 2017), thus generating high energy for growth.

The efficiency of feed fish bonylip barb on research is not significantly different between treatments ($P > 0.05$), as well as research on the efficiency of feed fish carp is not significantly different among treatments (Rahmawan *et al.*, 2020). Provision of feed is *ad libitum* already provides sufficient feed that is uniform to the whole fish in each treatment. Uniformity adequacy feed causes the amount of feed consumed to increase the growth of the same, so feed efficiency is also similar in each treatment.

Survival Rate

Based on statistical tests (analysis of variance), the survival rate of bonylip barb was significantly different between treatments ($P < 0.05$). Survival rates ranged from 78%-100%, and treatment A had the highest survival rate. LSD tests declared SR in treatment A (100%) of significantly different treatment C (78%) ($P < 0.05$) (Table 1).

The survival rate is the percentage of fish on the last day of reared and the number of fish at the beginning of reared (Muarif *et al.*, 2021b). The survival rate is the reciprocal of the mortality rate and means that the survival rate is high when the mortality rate is low. Factors that affect the survival rate are equal to the mortality that is stocking densities (Ronald *et al.*, 2014), water quality (Rosmawati and Muarif, 2017), pathogenic bacteria (Wijayanti *et al.*, 2019), feed (Nazhiroh *et al.*, 2019), pests (predators), and disease (Rosmawati and Muarif, 2017; Dzakiy *et al.*, 2017). The application of stocking density which is different in research affects the survival rate of fish bonylip barb as an object of research.

Treatments A and B have the highest survival rates and significantly differ from treatment C ($P < 0.05$). The fish population in the treatment of A (lower density) can live fully until the end of the study (SR=100%). The condition shows increasingly low stocking density will be an increasingly high survival rate of living fish bonylip barb.

Biofloc media provide power support that is much better for the survival rate of live fish than the non-biofloc media, although during maintenance of the fish in biofloc media is no change of water (Rosmawati and Muarif, 2017). Similar to this reason, the level of survival of live fish bonylip barb in the research is also high, namely treatment A 100%, Treatment B 96 %, and Treatment C 78%. Other studies also showed that biofloc media with a CN 10 ratio had a higher survival rate than non-biofloc media, including *Platydoras costatus* (100%)

(Teduh *et al.*, 2017), *L. vannamei* (88.5%) (Panigrahi *et al.*, 2018), and *Scatophagus argus* (80.8%) (Duy and Khanh, 2018). The survival rate on biofloc media is higher because biofloc can maintain good water quality (low toxic ammonia concentration) and support higher fish immunity. Therefore, it is more resistant to disease and able to survive well. The population of heterotrophic bacteria that grows in the biofloc system can reduce inorganic nitrogen from feed waste into its energy source to keep the ammonia concentration low in the culture media. Biofloc containing poly- β hydroxybutyrate on immunity (Qiao *et al.*, 2020) and inhibiting the growth of pathogens (Laranja and Bossier, 2019) was shown to be able to prevent infection with Cyprinid herpesvirus 2 (CyHV-2) in *Carassius auratus gibelio* immunity (Qiao *et al.*, 2020) and this contributes to increased resistance to pathogenic infections. The high growth of the population of heterotrophic bacteria can also suppress the growth of the population of pathogenic bacteria so that fish are

protected from disease and death (Rosmawati and Muarif, 2017).

The lower stocking density able to provide conditions that favor the survival of live fish was good, and a higher stocking density indicates the cause of death is high. The high density in this study causes high oxygen competition. The presence of high TAN causes the oxygen demand of each fish to be high, so competition for sufficient oxygen becomes essential. High competition has an impact on stress and can cause fish death. At low densities, the available oxygen concentration is sufficient to meet the needs of fish life, so this treatment has an SR of 100%.

Water Quality

The characteristics of water quality in each treatment have relatively the same range. Distribution water quality during the study are as follows temperature ranged 26.2-29.1 °C, pH ranging 5-6, the solubility of oxygen (DO) 6.7-7.9 mg/L, and ammonia ranged 0-0.0002 mg/L (Table 2).

9 Table 2. Water quality during the study.

Parameter	Treatment		
	A	B	C
Temperature (°C)	26.3-29.1	26.3-28.8	26-28.6
pH	5	5-6	5-6
DO (mg/L)	6.7-7.9	6.8-7.8	6.8-7.9
Ammonia (mg/L)	0-0.0002	0.0001-0.0002	0.0001-0.0002

Water quality greatly determines the production of fish in aquaculture (Muarif *et al.*, 2019). In this experiment, the water quality in all treatments was relatively the same and classified as good. This study used a biofloc system so that during the experiment, there was no water change. This condition shows that the biofloc system can control water quality so that the water quality in the experimental media is in good and stable condition. Biofloc can reduce the N content in fish culture media so that the water quality is good for fish growth even though no water changes were done (Rosmawati and Muarif, 2017).

The temperature in this study of bonylip barb ranged from 26.3-29.1 °C in treatment A, 26.3-28.8 °C in treatment B, and 26.0 -28.6 °C in treatment C. Water temperature of the pond fish farming in the range of 22-30 °C is at a decent value for fish (Muarif, 2016) so that the temperature is quite feasible in this research. In this temperature range, biofloc bacteria can grow but not optimally because the optimal growth of bacteria is at 37 °C (Yang *et al.*, 2018).

DO at the time of observation in treatment A, ranging from 6.7-7.9 mg/L in treatment B, 6.8-7.8 mg/L, and 6.8-7.1 in treatment C. According to (Boyd, 2015)

that in fish farming, the availability of DO in waters must be > 2 mg /L. In fish farming, using biofloc technology requires a high concentration of DO, because dissolved oxygen is needed for fish life and good performance of heterotroph bacteria. In biofloc media, DO affect nitrification bacteria that play a role in converting food waste and fish waste into nutrients, and low DO can damage the biofloc structure (Deswati *et al.*, 2020).

The pH level of the bonylip barb rearing media in treatment A was 5 in treatment B 5-6 and in treatment C 5-6. The pH value obtained is still at normal pH levels. According to (Boyd, 2015), the pH value is less than 4, and more than 11 is deadly for fish. pH 4-6 lead growth was a little more slowly. The degree of pH is related to the ammonium ionization process. In this study, the pH value was less than 7, the ammonium would be ionized, and the ammonia (NH₃) was low, so it was non-toxic and harmless to fish.

Ammonia which toxins have levels that are very low in every treatment between 0-0.0002 mg/L. Ammonia levels were very low, safe for the fish's life, and the ammonia does not have the effect of harmful at levels below 0.05 mg/L (Boyd, 2015). Biofloc can control ammonia through the mechanism of protein decomposition through heterotroph bacteria. An increase in the population of heterotroph bacteria in the biofloc system through using nitrogen from organic compounds, feces, and feed residues will reduce nitrogen in the culture media so that the ammonia level in the culture media is low (Rosmawati and Muarif, 2017).

CONCLUSION

Stocking density of bonylip barb in media biofloc CN ratio of 10 is significantly different to specific growth rate and survival rate, and no significantly different to the efficiency of feed fish bonylip barb. Treatment A (stocking density of 10 fish /21 L) gave the best results for specific growth rate, feed efficiency, and survival rate. Biofloc CN

ratio of 10 to support growth, survival rate, and water quality is suitable for this research.

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