Growth performance and survival rate of Asian swamp eel in biofloc systems with different stocking density

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Abstract. The cultivation of Asian swamp eel (*Monopterus albus*) has been known for a long time in Asia and the biofloc system is widely applied in the cultivation of some fish species in this region. However, so far, this technology has not been developed for Asian swamp eel cultivation. This study aimed to determine the growth performance and survival rate of this eel reared in a biofloc system with different stocking densities. The work was conducted from April to June 2020, by using a completely randomized design. The animals were reared in 100 L containers under different densities: treatment A (10 fish tank^{-1}), B (20 fish tank^{-1}), C (30 fish tank^{-1}), and D (40 fish tank^{-1}). The animals were fed with earthworms twice a day, 5% of bodyweight. The results showed that stocking densities affected the absolute weight (Wg) and specific growth rate (SGR) of Asian swamp eel significantly ($p<0.05$). Treatment C was the best treatment compared to others, with an average absolute weight gain of 9.32±0.461 g and an average daily growth rate of 2.69±0.238%. The best survival rate was in treatment C (93.33±0.19%), although it was not significantly different from other treatments. The hematological profile (total erythrocytes, hemoglobin, hematocrit and total leukocytes) were also affected by the treatments ($p<0.05$). The stocking density of 30 fish tank^{-1} (C) yielded the highest total erythrocytes ($2.88\text{x}10^6$ cells mm^{-3}), hemoglobin (17.4 g dL^{-1}), hematocrit (31.66%), and total leukocytes ($3.57 \text{ x } 10^4$ cells mm^{-3}). The difference in stocking density did not have an effect on blood glucose levels ($p>0.05$). Glucose levels ranged from 33.33 to 54.66 mg dL^{-1}, with the highest glucose levels in treatment D.

Key Words: absolute weight, blood profiles, daily growth rate, hematological profiles, ricefield eel.

Introduction. The Asian swamp eel (*Monopterus albus*) is a commercially important, air-breathing species of fish from the family Synbranchidae. The animal is also known as rice eel, rice field eel, or rice paddy eel. The native range includes tropical, subtropical and temperate climates, but it is mainly found in East and Southeast Asia, where it is a very common fish sold throughout the region (Matsumoto et al 2010; Chen et al 2021). Eels are a good protein source, with a protein content of 66.7%, fat content of 10.74%, and an ash content of 7% (Vishwanath et al 1998).

The demand for eels increased through the years, and it is estimated that only 10% can be provided (Suzuki et al 2003). The eel cultivation is still using mud media with a mixture of coarse and fine bran, straw, banana stems, and manure as a substrate (Herawati et al 2018). The stocking density is one of the factors that determines the growth of fish in a rearing system. Determination of the proper stocking density will not only provide information on optimal growth for fish, but will also increase business efficiency. Such information, especially for the Asian swamp eel, is still limited.

Biofloc is a particle that is stirred by aeration and circulation, which consists of a collection of autotrophic and heterotrophic organisms (in the form of bacteria, phytoplankton, fungi, ciliates, nematodes and detritus) and non-living materials (Avnimelech et al 2015). This management system converts organic and inorganic compounds containing carbon compounds (C), hydrogen (H), oxygen (O), nitrogen (N) with little available phosphorus (P) into sludge mass in the form of bioflocs by using flocs-forming bacteria, which synthesize poly hydroxy alkanoate biopolymers, namely as bioflocs bonds (Abakari et al 2021).

Biofloc in fish culture is applied by adding a starter in the form of carbon and bacteria. The addition of organic carbohydrates into the medium could stimulate bacterial growth (de Lima et al 2019; Hargreaves 2006; Rajkumar et al 2015). Biofloc technology provides natural feed for domestic fish, is feasible, and does not pollute the environment (Emerenciano et al 2017; Supriatna et al 2019). The application of biofloc technology has been widely used in fish and shrimp culture (Ekasari et al 2015; Putra et al 2017; Serra et al 2015), but the information in application of the technology to Asian swamp eel is still limited. This study aimed to determine the growth performance and survival rate of this eel reared in biofloc system under different stocking densities.

Material and Method

Time and place. The research was carried out from April to June 2020, in the Aquaculture Technology Laboratory, Faculty of Fisheries and Marine Sciences, Riau University, Indonesia. The animals were purchased from local an Asian swamp eel hatchery in Riau.

Experimental design. The experiment was conducted within the ethical guidelines in animal research developed by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). A completely randomized design with four levels of treatment and three replications was used in this study. The Asian swamp eels were reared in 100 L container under different densities, namely treatment A (10 fish tank^{-1}), B (20 fish tank^{-1}), C (30 fish tank^{-1}), and D (40 fish tank^{-1}).

Biofloc cultivation system. The biofloc cultivation systems were set up in a 100 L canvas containers. Each container was filled with 25 L of water. The system was prepared by mixing 10 mL of probiotics *Bacillus* sp. (5×10^{10} CFU mL^{-1}) and 0.48 mL L^{-1} water molasses into the containers and aerated continuously for seven days to grow the flock. The Asian swamp eels (average weight of 5.85±0.75 g) were stocked into the system, according to the above treatments. Additional inoculants, namely 10 mL of probiotics *Bacillus* sp. (5×10^{10} CFU mL^{-1}) were added to each tank at every 5-day interval. The animals were fed with earthworms (*Lumbricus rubellus*) twice a day, 5% of total body weight. Fish weight gain was measured every ten days for 40 days.

Growth of Asian swamp eel. Growth performance of Asian swamp eel was measured by weighing the animals at the beginning and at the end of experiment. The weight gain (Wg) was calculated as follows:

$$Wg = Wt - Wo$$

Where: Wg - weight gain (g); Wt - the weight of the eel at the end of the experiment (g); and Wo - the weight of the eel at the beginning of the experiment (g).

Specific growth rate (SGR) was calculated by using the following formula;

$$SGR\ (\%/day) = [(\ln Wt - \ln Wo)/t] \times 100\ \%$$

Where: Wt - the weight of the eel at the end of the experiment (g); Wo - the weight of eel at the beginning of the experiment (g); and t - time or duration of cultivation (days).

Feed efficiency. The efficiency of feed utilization consumed by the Asian swamp eel was measured referring to de Verdal et al (2017):

$$FE = \frac{((Wt+D)-Wo)}{F} \times 100$$

Where: FE - feed efficiency (%); Wt - weight of the fish biomass at the end of the experiment (g); Wo - weight of the fish biomass at the beginning of the experiment (g); D - weight of the fish that died during the study (g); F - amount of feed provided to the fish during the study (g).

Hematological profile. Blood samples were collected from 3 animals per container at the end of the study (40 days). The Asian swamp eel was anesthetized with 0.1 mL L^{-1} clove oil before blood collection. A set of clean and sterile syringe and Eppendorf tube were rinsed with blood anti-coagulant 10% EDTA. Blood sample (as much as 1 mL) was collected with a 1 mL syringe from the caudal vein at a slope of 45^o to pierce the vein. A volume of 0.5 mL of blood was placed into an Eppendorf tube. Hematological profiles of blood included total erythrocyte cells mm^{-3} (Blaxhall 1972), hemoglobin (%) (Wedemeyer & Yasutake 1977), hematocrit levels (%) (Mauel et al 2007), total leukocytes (cells mm^{-3}) (Blaxhall 1972), leukocytes and blood glucose (mg dL^{-1}) (Papazafiropoulou et al 2010).

Water quality. The main water quality parameters such as dissolved oxygen (DO), pH, and temperature were measured using a digital water checker (YSI-550 A, ASTM, Alla, (France) at 10-day intervals, while total ammonia nitrogen (TAN) was measured every ten days using spectrophotometric method (APHA 2008).

Total ammonia nitrogen. Total ammonia nitrogen (TAN) was measured every 10 days for each treatment for 40 days. It was measured using a spectrophotometer (Optima SP300), with a wavelength of 630 nm. The measurements were carried out by placing 50 mL of the sample into a 100 mL Erlenmeyer flask, then adding 1 mL of Nessler's solution, then shaking it and allowing the reaction process to last for at least 10 min. The solution was put into a cuvette on a spectrophotometer at a wavelength of 630 nm, read and recorded on the absorption scale. The levels of TAN were calculated using a calibration curve or a straight line equation (APHA 2008) and determined by the formula:

$$\text{TAN concentration (mg L}^{-1}\text{)} = (\text{Cst x As})/\text{Ast}$$

Where: Cst - concentration of the standard solution; Ast - absorbance value of standard solution; As - the absorbance value of the sample solution.

Data analysis. The data were subject to one-way analysis of variance (ANOVA) test to determine the effect of treatments on some parameters and followed by Newman-Keuls multiple range test, with a confidence level of 95%, while the water quality data was analyzed descriptively.

Results and Discussion

Growth and survival rates of Asian swamp eel. The results showed that the Wg of Asian swamp eel ranged from 6.96±0.695 to 9.32±0.461 g, SGR ranged from 1.9±0.379 to 2.69±0.238%, and survival rates ranged from 90 to 93.33±0.19%. ANOVA results showed that weight gain and SGR were significantly different between treatments ($p<0.05$), while survival rate was not significantly different ($p>0.05$). Treatment C (30 fish $tank^{-1}$) was the best one compared to others, with an average absolute weight of 9.32±0.461 g and an average SGR of 2.69±0.238%. The survival rate in treatment C was 93.33±0.19%, although it was not significantly different from other treatments. The best feed efficiency was also observed in treatment C (Table 1).

Hematological profiles. The cultivation of Asian swamp eel with different stocking densities had an effect on the hematological profile (total erythrocytes, hemoglobin, hematocrit and total leukocytes) ($p<0.05$). The stocking density of 30 individuals $tank^{-1}$ (C) yielded the highest total erythrocytes (2.88×10^6 cells mm^{-3}), hemoglobin (17.4 g dL^{-1}), hematocrit (31.66%), and total leukocytes (3.57×10^4 cells mm^{-3}). The hematological profile of the eels during the study was in the normal range. Siang et al (2007) reported that the normal hematological profiles of *Monopterus albus* range as follows: erythrocytes between 1.05 and 1.10×10^6 cell mm^{-3}, leukocyte between 47.58

and 48.56 x 103 cell mm^{-3}), hemoglobin between 20 and 21 g dL^{-1}), and hematocrit between 47.05 and 48.10 %. The difference in stocking density did not have an effect on blood glucose levels ($p>0.05$). Glucose levels ranged from 33.33 to 54.66 mg dL^{-1}, the highest glucose levels occurring in treatment D (Table 2).

Table 1

Growth performance and survival rate of Asian swamp eel

No	*Parameter*	*Treatment*			
		A	*B*	*C*	*D*
1	Weight gain (g)	7.38±0.500^a	8.32±0.429^b	9.32±0.461^c	6.96±0.695^a
2	SGR (%)	1.90±0.379^a	2.24±0.105^a	2.69±0.238^b	1.88±0.352^a
3	Survival rate (%)	90.00±0.00^a	90.00±0.00^a	93.33±0.19^a	92.50±0.00^a
4	Feed efficiency (%)	42.41±3.799^a	49.67±2.105^a	67.46±9.806^b	41.00±5.939^a

Note: SGR - specific growth rate; data represent mean and standard deviation; different superscripts in the same row show significant differences ($p<0.05$).

Table 2

Hematological profiles of Asian swamp eel

No	*Parameter*	*Treatment*			
		A	*B*	*C*	*D*
1	Erythrocyte (cell mm^{-3})	181.66x10^4±1.53^b	271.66x10^4±2.08^c	288.33x10^4±1.53^d	120.33x10^4±1.53^a
2	Hemoglobin (g dL^{-1})	13.46±1.28^a	14.8±0.72^a	17.4±1.44^b	13.4±0.52^a
3	Hematocrit (%)	25.33±1.52^b	27±2^b	31.66±1.52^c	20.33±0.57^a
4	Leukocyte	3.27±0.02^a	3.38±0.08^a	3.57±0.07^b	3.62±0.12^b
5	Glucose (mg dL^{-1})	33.33±4.16^a	41.33±1.52^a	46.33±2.51^a	54.66±3.05^a

Note: data represent mean and standard deviation; different superscripts in the same row show significant differences ($p<0.05$).

Water quality. In this study, water quality parameters were in normal ranges for aquatic organism life. The temperature ranged from 26.64±0.3°C to 26.95±0.51°C, pH ranged from 7.39±0.08 to 7.60±0.15, dissolved oxygen ranged from 7±0.57 mg L^{-1} to 7.53±0.21 mg L^{-1} and ammonia values from 0.001±0.012 mg L^{-1} to 0.003±0.018 mg L^{-1}. The results of the analysis on water quality were not significantly different ($p<0.05$) among the experimental treatments. The average values of the water quality parameters are presented in Table 3.

Table 3

Water quality of culture media of the biofloc system

No	*Parameter*	*Treatment*			
		A	*B*	*C*	*D*
1	Temperature (°C)	26.78±0.58	26.95±0.51	26.64±0.3	26.84±0.35
2	pH	7.39±0.08	7.45±0.09	7.6±0.15	7.45±0.04
3	Dissolved oxygen (mg L^{-1})	7.53±0.21	7.47±031	7±0.57	7.2±0.46
4	Ammonia (mg L^{-1})	0.001±0.012	0.002±0.018	0.003±0.017	0.003±0.018

The results showed that the best growth, highest survival and best level of feed efficiency in Asian swamp eels was in treatment C (the stocking density of 30 fish tank^{-1}). The increase of stock density to 40 fish tank^{-1} inhibited or slowed down the growth rate. It is thought that increased density was associated with stress levels, food competition, space availability for movement, appetite, and antagonistic interactions (Breine et al 1996; Ronald et al 2014; Yousif 2017). Stocking density does affect fish growth. Hastuti & Subandiyono (2020) reported that density of tilapia cultivated in an aquaponic system had an effect on biological parameters, such as growth, feed consumption and feed utilization efficiency. Billah et al (2019) also noted that the suitable stocking density was 6 m^{-2} for *C. carpio* and *O. niloticus* in rice fields for better growth and survival. However, stocking density does not affect the survival rates and hematological performances. This has been reported by several researchers; for example, El-Dahhar et al (2021) reported that growth performance indices of meagre (*Argyrosomus regius*) decreased with increasing fish density, but without significant difference ($p>0.05$). The growth of eels can be influenced by several factors, including feed quality, water quality, stocking density, and the application of biofloc used in the culture system (Hadiroseyani et al 2016; Putra et al 2020). The density of organisms maintained in the culture system also affected the growth, production, and space efficiency. Some authors reported that low stocking density may also cause a reduced growth (Ronald et al 2014).

Application of biofloc technology had a significant influence on the growth and survival of eels, since this technology is able to increase water quality parameters (Nurhatijah et al 2016; Stein & Klotz 2016), and can provide natural feed as additional feed, even though eels are fed with sufficient food (Avnimelech 1999; Putra et al 2017). The feed provided also plays a main role in the growth of eels. The feed used was earthworms with a high nutrient content, meeting the nutritional needs for Asian swamp eel. The dry matter of earthworms contains nutrients such as protein 60-72%, 7-10% fat, and 8-10% ash, making it suitable for carnivorous eels, including carnivorous Asian swamp eels that require high protein feed for growth and development (Hill & Watson 2007).

The stocking densities had an effect on the hematological profile (total erythrocytes, hemoglobin, hematocrit and total leukocytes) ($p<0.05$). The stocking density of 30 individuals per tank (C) provided the highest yield for total erythrocytes, hemoglobin, hematocrit, and total leukocytes. This data corresponds to the absolute weight gain and survival rate data of the Asian swamp eels. These animals grow better in these conditions and this can be seen in through their hematological profile.

The stocking density can be a trigger that affects movement and physiological activity, thereby affecting the blood profile. Some factors (species, sex, dietary nutrition, size, physical activity, and age of the fish) may influence the number of erythrocytes, hemoglobin, and hematocrit level. Overall, physiological status and environmental conditions do affect the number of erythrocytes (Fazio 2019; Syawal et al 2021).

The difference in stocking density in Asian swamp eel cultivation among the experimental treatments did not have an effect on blood glucose levels ($p>0.05$). Glucose levels ranged from 33.33-54.66 mg dL^{-1}, in which the highest glucose levels were in treatment D. Glucose levels in this study were lower compared to the findings of Hu et al (2018), where the levels ranged from 54 to 144 mg dL^{-1}. Peng et al (2019) reported that normal Asian swamp eel glucose levels range from 45 to 68 mg dL^{-1}, while Shi et al (2019) revealed that glucose levels ranged from 43 to 51 mg dL^{-1}. It can be said that Asian swamp eel glucose levels in treatment B, C, and D were within the normal range.

Water quality parameters also affect the growth of fish. Fish culture with biofloc technology provides several advantages, including improved water quality and additional feed (Stein & Klotz 2016; Putra et al 2017). The quality of rearing water for eels is in the optimal range for growth. Biofloc contributed to the growth and production of the cultured organism, as shown in this study. This technology's basic principle is using heterotrophic bacteria to manage the C:N ratio in the water media (Avnimelech 1999; Hargreaves 2006). Therefore, biofloc technology is considered suitable for the Asian swamp eel cultivation.

Conclusions. Cultivation in biofloc system with different stocking densities affected the weight gain and specific growth rate of Asian swamp eel significantly ($p<0.05$). Treatment C (30 fish $tank^{-1}$) was the best treatment compared to others, with an average absolute weight of eels of 9.32±0.461 g and an average specific growth rate of 2.69±0.238%. The survival rate in treatment C was 93.33±0.19 %, although it was not significantly different from other treatments. The hematological profile (total erythrocytes, hemoglobin, hematocrit and total leukocytes) revealed that the animals had a significantly better immunity status ($p<0.05$). The stocking density of 30 individuals $tank^{-1}$ (C) provided the highest total erythrocytes (2.88×10^6 cells mm^{-3}), hemoglobin (17.4 g dL^{-1}), hematocrit (31.66%), and total leukocytes (3.57×10^4 cells mm^{-3}).

Stocking density did not affect the blood glucose levels of these animals ($p>0.05$). Glucose levels ranged from 33.33-54.66 mg dL^{-1}, with the highest glucose levels in treatment D.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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