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Original Article

Effect of different stocking densities on growth performance and gut villi histology of endemic ornamental fish (*Betta rubra*) in the biofloc technology

Fazril Saputra*[1], Zulfadhli[1], Muhammad Arif Nasution[2], Ahmad Fahrul Syarif[3], Maftuch[4], Mu'amar Abdan[5], Sofian[6], Dini Islama[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Teuku Umar. Jl. Alue Peunyareng, Gunong Kleng, Meureubo, West Aceh 23681, Aceh, Indonesia.
[2]Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Teuku Umar. Jl. Alue Peunyareng, Gunong Kleng, Meureubo, West Aceh 23681, Aceh, Indonesia.
[3]Department of Aquaculture, Faculty of Agriculture, Fisheries and Marine Sciences, Universitas Bangka Belitung, UBB Integrated Campus, Balunijuk, Merawang, Bangka Regency 33172, Bangka Belitung Islands Province, Indonesia.
[4]Program Study of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.
[5]Department of Aquaculture, Faculty of Agriculture, Universitas Gajah Putih, Jl. Simpang Kelaping-Lukup Badak-Belang Bebangka, Pegasing, Takengon, Central Aceh, 24560, Aceh, Indonesia.
[6]Program Study of Fisheries Science, Faculty of Fisheries and Marine Science, Univeritas PGRI Palembang. Jl. Ahmad Yani. Lr. Gotong Royong 9/10 Ulu Palembang, 30251, South Sumatra Province, Indonesia.

Abstract: The endemic ornamental fish *Betta rubra*, known locally as serban malem, is a high-value non-consumptive fishery product. This study aims to evaluate the growth performance and histological structure of gut villi of *B. rubra* fish maintained in a biofloc system with different stocking densities. This study used a completely randomized design with four treatments: 1 fish/liter (control, no biofloc system), 1 fish/liter (biofloc system), 2 fish/liter (biofloc system), and 4 fish/liter (biofloc system). Treatments showing significant differences were further evaluated using Duncan's test. The results showed that the survival rate, length increase, and feed conversion ratio in the biofloc system were significantly different from those of the control ($P<0.05$). In contrast, daily growth rate and weight gain were not significantly different between treatments. Fish intestinal villi in the biofloc system were significantly different compared to those in the control. In conclusion, the optimal rearing density of *B. rubra* is 2 fish/liter in the biofloc system (P2).

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Introduction

Ornamental fish are non-consumptive fishery products with high economic value. The demand for ornamental fish has continued to increase at the local, national, and international levels over the past few years (Tarihoran et al., 2023; Peh and Azra, 2025). The increase in demand for ornamental fish in Indonesia began in 2020, when the COVID-19 pandemic hit the world and the implementation of work-from-home policies caused many employees to experience boredom and stress (Iskandar et al., 2022; Fu'adi et al., 2022). The Ministry of Maritime Affairs and Fisheries stated that Indonesia achieved positive results in ornamental fish exports in 2023, with first-semester exports amounting to USD 20.5 million (Fauzan et al., 2024). One ornamental fish species that has economic and export value is the betta fish (Saputra and Efianda, 2018; Saputra et al., 2020; Shahputeri and Nurmalina, 2023). There is an endemic ornamental betta fish species, *Betta rubra*, in Aceh Province that has high economic value and export quality. This fish has begun to be domesticated, but domestication also presents its own challenges, including a slow growth rate (Saputra et al., 2024). Abroad, *B. rubra* is priced at USD 54.95 or IDR 906,675 (based on an exchange rate of USD 1 to IDR 16,500), while domestically, it is priced at around IDR 150,000 to IDR 250,000 per pair (Saputra et al., 2024; Henry, 2025).

The high market demand has made this ornamental betta fish increasingly rare in its natural habitat, threatening its survival due to increased fishing,

*Correspondence: Fazril Saputra
E-mail: fazrilsaputra@utu.ac.id

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deforestation, and land conversion to plantations (Nur et al., 2022). Data from the IUCN Red List show that the status of *B. rubra* is currently endangered (EN) (Low, 2019). Initial domestication research on *B. rubra* was conducted by observing fish growth when fed different natural feeds (Saputra et al., 2025), but conventional domestication yields low production performance. This is thought to be due to the limited use of technology in the domestication of *B. rubra*. The integration of technology into fish farming aims to increase productivity, with the ultimate goal of shortening the fish farming cycle so that farmers can harvest faster and increase their income (Jiang et al., 2023; Segun et al., 2023). One technology that can improve production performance is biofloc technology.

Biofloc technology is a fish farming system that continues to develop towards sustainable aquaculture. This technology employs water quality management by utilizing and controlling heterotrophic bacteria in cultivation systems without water exchange (Bossier et al., 2017; Dauda et al., 2018; Melaku et al., 2023). It provides a carbon source to the cultivation system, stimulating the growth of heterotrophic bacteria that convert toxic ammonia into nitrogen gas (Jiménez-Ojeda et al., 2018; Khanjani et al., 2023). Biofloc technology can improve fish production performance (Oliveira et al., 2021; Amalia et al., 2023; Soaudy et al., 2024). Therefore, the aim of this study was to improve production performance and examine gut villi histology in *B. rubra* fish at different stocking densities using biofloc technology.

Materials and Methods

This research was conducted at the Laboratory of Aquaculture Systems, Technology, and Environment, Faculty of Fisheries and Marine Sciences, Teuku Umar University, West Aceh Regency, Aceh Province. Fish were maintained for 40 days, from August to September 2025, to test the effects of biofloc technology on *B. rubra*. The fish used in this study were domesticated specimens originating from Aceh Jaya Regency, Aceh Province.

Research design: The experimental design was a completely randomized design with four treatments, each repeated three times. The treatment is the difference in stocking density in biofloc media: 1 fish/liter (without biofloc media/ control) (P0), 1 fish/liter (biofloc system) (P1), 2 fish/liter (biofloc media) (P2), and 4 fish/liter (biofloc media) (P3). The stocking density in this study matches that used in previous studies of *B. rubra* without a biofloc system (Saputra et al., 2024).

Biofloc-based systems preparation: Biofloc technology uses a C/N ratio of 12 with molasses as the carbon source (45.27% carbon content) (Diatin et al., 2019). Molasses is administered daily 2 hours after feeding. The N content in the feed and the daily molasses requirement are calculated based on De Schryver et al. (2008). Floc formation was achieved by adding commercial bacteria from Biocare Biofloc. The bacteria were cultured in a container containing 100 liters of water. The water used was well water. Floc culture was established by adding 1 g of commercial bacteria, 20 mL of molasses, and 60 g of fish pellets per 100 liters of water. The floc culture was maintained for 10 days, after which it was transferred to the aquarium.

Fish maintenance procedures: This study used 240 fish with an average initial length of 2.718±0.025 cm and an average initial weight of 0.250±0.005 g. A digital vernier caliper was used to measure the length. A Joil digital scale with a capacity of 500 grams and a precision of 0.01 grams was used to weigh the fish. The fish were kept in a 10-liter volume aquarium with a stocking density appropriate to the treatment. Feeding was given at 5% of the fish's body weight. Juvenile *B. rubra* were fed commercial feed (44.14% crude protein, 3.63% fat, 29.14% carbohydrate, 4.08% water content, 10.61% ash content) twice daily (at 09:00 AM and 04:00 PM) until satiated. Food consumption of each experimental unit was measured every 10 days by recording the difference in the weight of the food in the aquarium. The length and weight of the fish were also measured every ten days. No water changes were performed; the only water added was water that evaporated during the biofloc system.

Study parameters: This study primarily assessed growth performance, gut villi histology, and water quality. The measured parameters were based on Saputra et al. (2025). Growth performance indicators, including survival rate (SR), specific growth rate (SGR), increase in length (IL), weight gain (WG), feed conversion ratio (FCR), and Feed efficiency (FE), were assessed. Data were collected to examine growth parameters every 10 days.

Survival rate (SR): It is the proportion of fish that survive (Saputra and Mahendra, 2019), and was calculated using the formula of SR = Ni/Nf x 100, where SR = Survival Rate (%), Ni = Final number of fish maintenance, and Nf = Initial number of fish kept.

Specific growth rate (SGR): It is the average growth of fish from the beginning to the end of the study, calculated using the formula of (Gabriel et al., 2019) SGR = (LnWf-LnW0)/t x 100, where, Wf = Average weight of fish at the final of the study (g), W0 =Average weight of fish at the start of the study (g), and t = Maintenance time (days).

Increase in length (IL): The increase in length can be calculated using the formula from Saputra et al. (2016) as follows: IL = Lf-L0, where IL = Increase in length (cm), Lf = Average length of fish at the final of treatment (cm), and L0 = Average length of fish at the initial of treatment (cm).

Weight gain (WG): The weight gain can be calculated using the formula from Saputra et al. (2016) as follows: WG=Wf-W0, where WG = Fish weight gain, Wf = Average weight of fish at the final of the study (g), and W0 = Average weight of fish at the initial of the study (g).

Feed conversion ratio (FCR): The feed conversion ratio is calculated based on Rodde et al. (2021) as follows: FCR = F/((Wf+Wd)-W0), Where: Wf = Final fish Weight (g), W0 = Initial Fish Weight (g), F = Feed Given (g), and Wd = Weight of Fish Dead during Maintenance (g).

Feed efficiency (FE): Feed efficiency is the comparison of the increase in fish weight obtained with the amount of feed consumed. Feed efficiency of fish is calculated using the formula (Gabriel et al., 2019) of FE = ((Wf+Wd)-W0)/F x 100, Where FE = Feed efficiency (%), Wt = Final fish Weight (g), and W0 = Initial Fish Weight (g), Wd = Weight of Fish Dead during Maintenance (g), and F: Feed Given (g).

Histology of gut villi: The histological preparation followed the procedures outlined by Humason (1979), including sampling, fixation, dehydration, paraffin infiltration, sectioning, and staining. The gut tissue architecture was then analyzed using a microscope equipped with a digital camera (Zulfahmi et al., 2022). The mean height and width of the villi across several treatments were determined using the equations (German and Horn, 2006) of TRV = (T_{VIg} + T_{Vrg} + T_{Vbg} + T_{Vbg}) / 4, and LRV = (L_{VIg} + L_{Vrg} + L_{Vbg} + L_{Vbg}) / 4, where: TRV = Average length of gut villi (μm), LRV = Average width of gut villi (μm), TVlg = Length of the left gut villi (μm), TVrg = Length of the right gut villi (μm), TVug = Length of the upper gut villi (μm), TVbg = Length of the bottom gut villi (μm), LVlg = Width of the left gut villi (μm), LVrg = Width of the right gut villi (μm), LVug = Width of the upper gut villi (μm), and LVbg = Width of the bottom gut villi (μm).

Bacterial diversity and abundance: A 50 mL sample of the rearing water was collected using a sterile sample bottle. This water sample bottle was stored in a cool box. Sample testing was conducted at the Ujung Batee BPBAP Fish Health Laboratory.

Water quality: Temperature, dissolved oxygen, pH, ammonia, nitrite, and nitrate were tested to assess water quality. Water quality was checked every 10 days. A thermometer was used to measure temperature; a pH meter to measure pH; a DO meter to measure dissolved oxygen; and a spectrophotometer to measure ammonia, nitrite, and nitrate.

Data analysis: The data were compiled and tabulated using Microsoft Excel. Statistical analysis was performed using SPSS version 27.0. Analysis of variance was used to determine whether the experimental treatments differed significantly in the measured parameters. When ANOVA showed significant variation among treatments, Duncan's multiple range test was applied to compare treatment means and identify specific groups that differed

Table 1. Growth performance and survival data of *Betta rubra* during 40 days experiment.

Treatments	Survival Rate [%]	Specific growth rate [%/days]	Increase in length [Cm]	Weight gain [g]	Feed conversion ratio	Feed efficiency [%]
P0	100.00±0.00^{a}	1.42±0.09^{a}	0.93 ± 0.54 ^{ab}	0.19±0.01^{a}	2.62±0.18^{a}	38.29±2.70^{a}
P1	100.00±0.00^{a}	1.67±0.23^{a}	1.14 ± 0.05 ^{a}	0.24±0.02^{a}	2.13±0.21^{b}	47.20±4.85^{b}
P2	86.67±2.89^{b}	1.55±0.49^{a}	0.96 ± 0.14 ^{ab}	0.21±0.06^{a}	1.09±0.04^{c}	92.16±3.56^{c}
P3	85.00±2.50^{b}	1.33±0.16^{a}	0.72 ± 0.22 ^{b}	0.18±0.02^{a}	1.17±0.04^{c}	85.22±2.71^{d}

Description: P0 = 1 fish/liter (without biofloc system), P1 = 1 fish/liter (biofloc system), P2 = 2 fish/liter (biofloc media) and P3 = 4 fish/liter (biofloc media). Different superscript letters in the same row show a significant difference (<0.05). Different superscript letters in the same column show a significant difference (P<0.05).

Table 2. Bacterial abundance (CFU/mL) in maintenance water media of *Betta rubra* fish using biofloc technology with different stocking densities.

Treatments	Bacterial density (CFU/mL)		
P0 = 1 fish/liter (without biofloc media)	R1: 1.4 x 10^{3}	R2: 1.8 x 10^{5}	R3: 2.3 x 10^{4}
P1 = 1 fish/liter (biofloc system)	R1: 1.4 x 10^{3}	R2: 2.0 x 10^{5}	R3: 2.0 x 10^{5}
P2 = 2 fish/liter (biofloc system)	R1: 1.2 x 10^{6}	R2: 2.8 x 10^{5}	R3: 2.8 x 10^{5}
P3 = 3 fish/liter (biofloc system)	R1: 6.9 x 10^{5}	R2: 2.7 x 10^{6}	R3: 2.7 x 10^{6}

Table 3. Types of bacteria in the maintenance water media and intestines of *Betta rubra* fish using biofloc technology with different stocking densities.

Bacterial type	
Water	Fish intestine
Corynebacterium sp.	*Bacillus* sp.
Klebsiella sp.	*Aeromonas* sp.
Acinetobacter sp.	*Klebsiella* sp.
	Pseudomonas sp.

significantly (P<0.05).

Results

Growth performance: Table 1 summarizes the growth performance parameters of *B. rubra* during the experiment. Survival rates were significantly different (P<0.05) between treatments. Treatments P0 and P1 had the highest survival rates. Specific growth rates did not differ significantly between treatments (P>0.05). Length and weight gain rates were significantly different (P<0.05) between treatments. The highest rates of length and weight gain were observed in treatment P1. Feed conversion ratio and feed efficiency rates were significantly different (P<0.05) between treatments. The highest feed conversion ratio and feed efficiency rates were found in treatment P2.

Average value of gut villi measurements in *B. rubra*: At the end of the study, the dimensions of the intestinal villi of *B. rubra* fish were measured for each stocking density treatment in the biofloc system. The villi measurements are depicted in Figures 1-3. The results indicated that the stocking density treatment using biofloc media at 2 fish/liter (P2) significantly (P<0.05) increased the length and width of the fish intestinal villi.

Abundance and types of bacteria in rearing water and intestines: The abundance and types of bacteria in the maintenance water and intestines of *B. rubra* fish are shown in Tables 2 and 3. The highest bacterial density was found in the biofloc media treatment, namely 4 fish/liter (P3). There were six types of bacteria found in the maintenance water and intestines of *B. rubra* fish.

Water quality monitoring: Water quality evaluation for *B. rubra* fish maintenance with different stocking densities included temperature, pH, dissolved oxygen (DO), ammonia, nitrate, and nitrite. Table 4 presents the results of the water quality measurements. The findings indicate that water quality during the experiment remains within the proper range for its survival (Table 4).

Table 4. Water quality data of *Betta rubra* fish during the experiment.

water quality	unit	p0	p1	p2	p3
Temperature	°C	26.2-31.1	26.7-30.9	27.1-31.6	27.6-30.8
pH		6.4-7.7	6.1-7.6	6.4-7.9	6.5-7.6
DO	mg/l	4.63-5.65	5.46-5.79	5.38-5.87	5.36-5.59
NH_3	mg/l	0.06-0.30	0.04-0.13	0.04-0.11	0.01-0.20
NO_2	mg/l	0.027-0.134	0.036-0.085	0.022-0.074	0.016-0.112
NO_3	mg/l	4.7-32.7	6.7-16.9	6.3-12.11	5.2-16.5

Description: P0 = 1 fish/liter (without biofloc media), P1 = 1 fish/liter (biofloc system), P2 = 2 fish/liter (biofloc media) and P3 = 4 fish/liter (biofloc media).

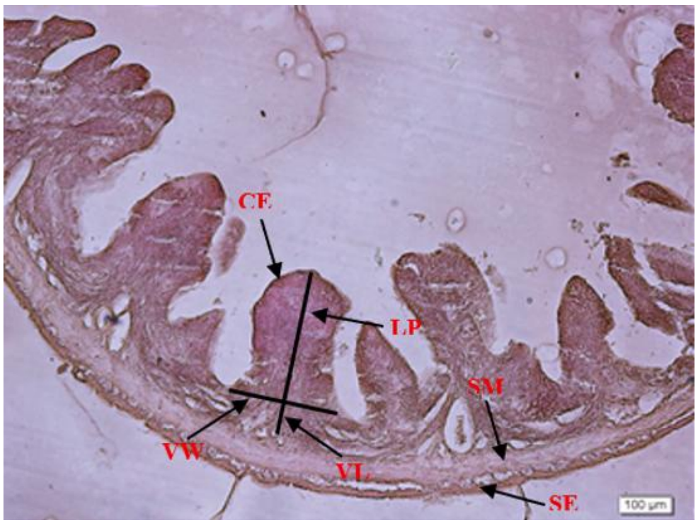

Figure 1. Histological structure of the gut villi in *Betta rubra*: P0 = 1 fish/liter (without biofloc system), P1 = 1 fish/liter (biofloc system), P2 = 2 fish/liter (biofloc media), and P3 = 4 fish/liter (biofloc media). SE: serous layer, SM: submucosal layer, LP: lamina propria, VL: villi length, CE: columnar epithelium, VW: villi width.

Discussions

Growth performance: The higher the stocking density, the lower the survival rate of *B. rubra*. This is due to increased stress, aggressive behavior, and competition for food. However, with biofloc media, the *B. rubra* survival rate (SR) remains high. This is because the biofloc system can control and recycle toxic nitrogenous waste, provide protein-rich natural food, and ensure optimal oxygenation and agitation (Raza et al., 2024). The SGR of B. rubra, which did not differ significantly, is thought to be due to the fish still experiencing stress and being unable to fully adapt to the high stocking density. Lingam et al. (2019) found that the higher the stocking density in milkfish, *Chanos chanos* (Forsskal, 1775), the lower the growth rate. The increase in IL and WG in the biofloc culture system for *B. rubra* fish is due to the presence of biofloc clumps (aggregates of microorganisms, algae, bacteria, protozoa, and detritus) suspended in the water and continuously consumed by the fish, i.e., fish receive food almost 24 hours a day. According to Matishov et al. (2025), the biofloc system contains numerous microorganisms, including photoautotrophic (algae), chemoautotrophic (nitrifying bacteria), and heterotrophic (fungi, infusoria, protozoa, and zooplankton). Based on the results, FCR and FE were better in cultivation using biofloc system because the biofloc system creates an ideal environment for fish growth through two pathways: (1) extra nutrition: fish get additional protein and nutrients from the floc, allowing for faster and more efficient growth, (2) energy efficiency: well-controlled water quality reduces the energy expenditure of fish to deal with stress and toxins, so that more energy is allocated to physical growth.

Average value of gut villi measurements in *B. rubra*: The intestinal villi of *B. rubra* consist of several main components: serous layer, submucosal layer, lamina propria, and columnar epithelium. All of these components were detected in the intestinal villi of *B. rubra* in all treatments, both in biofloc media and media without biofloc. The difference in intestinal villi of *B. rubra* lies only in their dimensions. This variance is caused by differences in the stocking density. Excessively high stocking density is a strong environmental stressor that can significantly damage the morphology of fish intestinal villi, ultimately reducing absorptive function and inhibiting fish growth performance. According to Liu et al. (2022), environmental stress can damage intestinal morphology, including significantly shortening intestinal villi, thereby affecting intestinal immunity

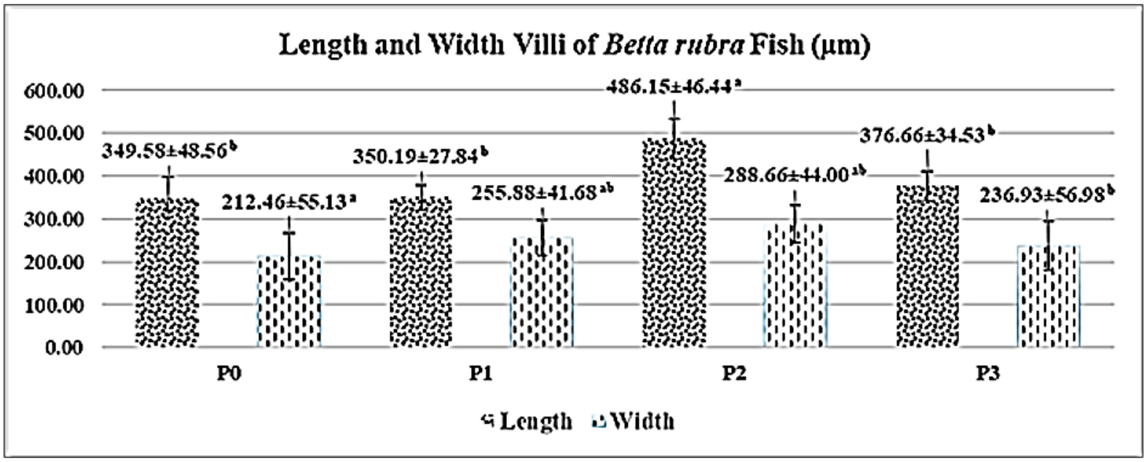

Figure 2. Average value of gut villi measurements in *Betta rubra*. Description: P0 = 1 fish/liter (without biofloc media), P1 = 1 fish/liter (biofloc system), P2 = 2 fish/liter (biofloc media), and P3 = 4 fish/liter (biofloc media). Different superscript letters in the same row show a significant difference (P<0.05).

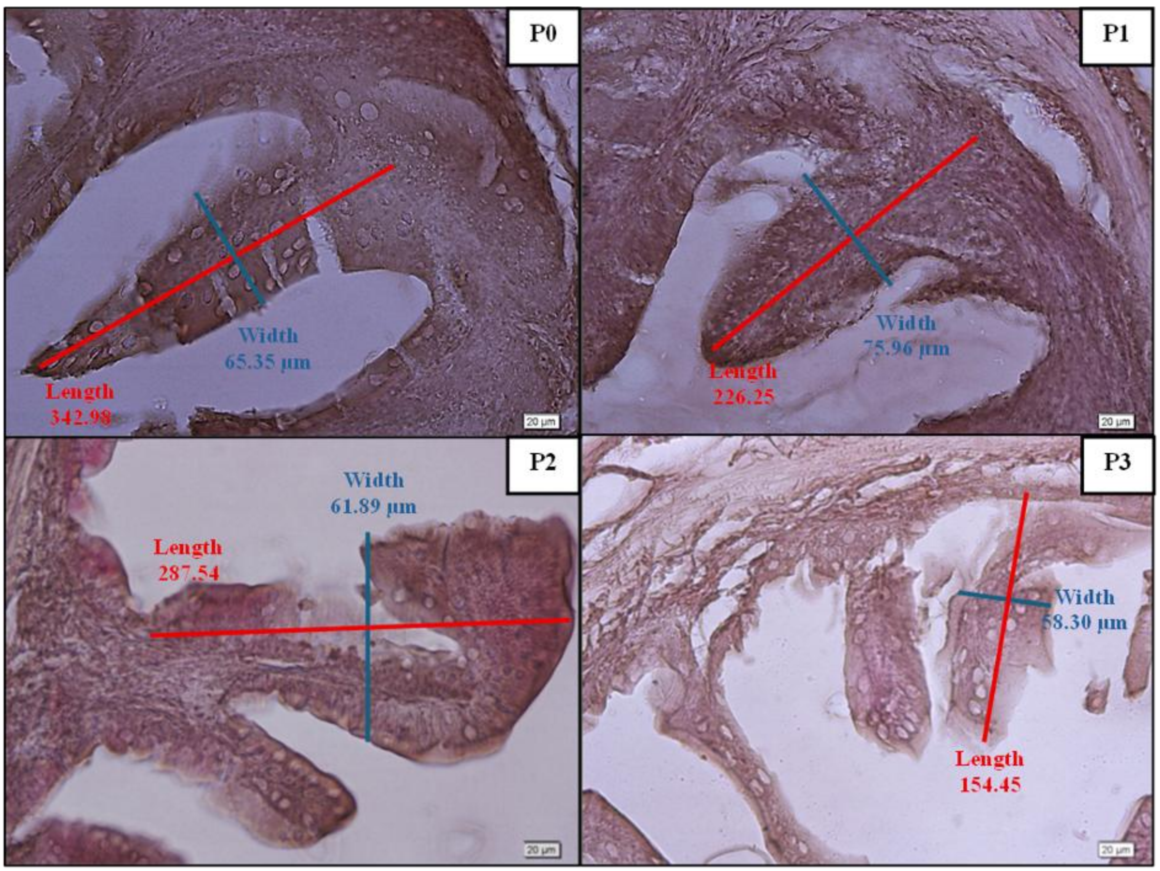

Figure 3. Length and width of intestinal villi in *Betta rubra* in each treatment: P0 = 1 fish/liter (without biofloc media), P1 = 1 fish/liter (biofloc system), P2 = 2 fish/liter (biofloc media), and P3 = 4 fish/liter (biofloc media).

and digestive function during food absorption.

Abundance and types of bacteria in rearing water and intestines of *B. rubra*: The higher the stocking density in biofloc media, the higher the bacterial density. This trend is thought to be due to increasing stocking density, which affects waste production, specifically feces and ammonia. This results in higher nitrogen availability in the culture medium for *B. rubra* fish, leading to higher bacterial density and activity. Eid et al. (2020) stated that higher stocking density is associated with higher bacterial density in biofloc media. Of the 6 most dominant species found in maintenance water and fish intestines, 2 types of bacteria are often found forming flocs in maintenance media: *Bacillus* sp. and *Pseudomonas* sp, which are heterotrophic. Heterotrophic bacteria are the dominant group and play a major role in floc formation, especially when the Carbon: Nitrogen (C/N) ratio is maintained at a high level. These bacteria convert ammonia into their own cellular proteins (floc biomass). According to Khanjani et al. (2022), one of the most dominant floc components is from the genus *Bacillus* sp. and *Pseudomonas* sp.

Water quality monitoring: Lichak et al. (2022) showed that the optimal temperature range for the growth of *Betta* sp. is 27-32°C, with an optimal pH range of 5-9. DO values for *B. rubra* during rearing remained within the normal range. DO value for *B. rubra* in its natural habitat ranges > 5 mg/l (Matielo et al., 2019). Ammonia concentrations were within the acceptable range for ornamental fish (0.0-0.9 mg/liter) (Oliveira et al. 2008). Concentrations exceeding 1.5 mg/l can be toxic (Wahyuningsih and Gitarama, 2020). Xu et al. (2022) reported that nitrite becomes toxic at ≥29.36 mg/L. Nitrate concentrations were also within acceptable levels for freshwater culture. Concentrations exceeding 10 mg/l may be harmful (Deswati et al., 2023).

Conclusion

In conclusion, this study suggests that the best stocking density for *B. rubra* is 2 fish per liter using a biofloc system. This conclusion can be seen from the results of growth performance, gut histology, and bacterial abundance and types in the water and gut of *B. rubra*.

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