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Different effects of swamp probiotics application frequency as a biofloc-forming agent on the production of catfish (*Clarias gariepinus*)

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ABSTRACT

Catfish (*Clarias gariepinus*) that are reared with probiotics as biofloc-forming agent is thought to increase the fish production. Applying swamp probiotics to the water media has never been studied to ensure the flocks' availability in the rearing media. This study aimed to determine the appropriate frequency of probiotics application collected from swamps for biofloc formation to improve the catfish production. This study used a completely randomized design with two treatments and three replications. The treatments were composed of different application frequency of swamp probiotics: (P1) once in 42 days of rearing and (P2) twice in 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth rate, feed efficiency, survival rate, and water quality were analyzed by T-test with a 95% confidence level. Meanwhile, the flock composition data were analyzed descriptively. The results showed that P2 obtained the best treatment with a floc volume of 68.33 ± 10.41 mL/L, absolute length growth of 8.18 ± 1.03 cm, absolute weight growth of 19.30 ± 3.12 g, feed efficiency of 135.24 ± 7.98%, survival rate of 89.33 ± 6.21%, biomass production of 24639.50 ± 1344.51 g, temperature of 28.85-29.59ºC, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg/L, ammonia of 0.45-1.15 mg/L, and total dissolved solids (TDS) of 717.33-885.50 mg/L. Therefore, swamp probiotics should be applied to catfish culture media twice for 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias gariepinus*) is a widely-cultivated freshwater fish commodity due to high market demand. In Indonesia, the Ministry of Marine and Fisheries Affairs of the Republic of Indonesia (2022) claimed, that the catfish production in grow-out phase in 2019 was 289,000 tons, then reached to 384,000 thousand tons in 2020, before falling to 360,000 tons in 2021. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and minimizing the waste disposal in the surrounding waters (Fauzi *et al.*, 2022). According to Putra *et al.* (2017), biofloc technology with probiotics application as heterotrophic bacteria can produce natural feed from the flocs to increase the feed efficiency and improve the water quality of the rearing media.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the rearing media. Microorganisms found in swamp contain *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti *et al.*, 2018). The application of swamp probiotics (*Bacillus* sp. and *Streptomyces* sp.) has been studied either as an aquafeed supplement (Tanbiyaskur *et al.*, 2022) or a biofloc-forming agent in catfish rearing (Wijayanti *et al.*, 2021). Swamp probiotics have also been proved to increase growth, feed efficiency, fish survival rate, and water quality of the rearing media (Wijayanti *et al.*, 2020).

Several types of swamp probiotics, such as *Bacillus* sp. and *Streptomyces* sp. can be used as a biofloc-

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forming agent. Previously, Wijayanti et al. (2021) applied the swamp probiotics once in 42 days of rearing, which resulted in a floc volume of 40 mL/L on the final day of rearing (42nd day). Using these flocs for 60 days of rearing resulted in an improved weight and length growth from 7.16 g to 36.95 g and 9.50 cm to 18.50 cm, although producing a relatively low floc volume. According to Agusta *et al.* (2022), the addition of a carbon source (molasses) in catfish culture with biofloc system produced a floc volume of 111 mL/L. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water at about 70-80% of the water volume. According to Feroza *et al.* (2021), flocs in the rearing media will decrease due to frequently consumed by fish every day, thus requiring a sufficient floc formation to control the microorganisms blooming due to excess floc volume. Therefore, it is necessary to evaluate the different application frequency of swamp probiotics as a biofloc-forming agent on the catfish production.

Materials and Methods

Location and time

This study was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University on December, 2022–January, 2023.

Materials and Tools

The materials used were catfish fingerlings (7 ± 0.5 cm in length), swamp bacteria (*Bacillus* sp. and *Streptomyces* sp.), molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tools used were a tarpaulin pool with a diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, Imhoff cone, loop needle, Erlenmeyer flask, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter, and ammonia test kit.

Study Design

This study used a completely randomized design with two treatments and three replications. The treatments were composed of different frequency application of swamp probiotics, namely:
P1 = once in 42 days of rearing
P2 = twice in 42 days of rearing

Procedures

Swamp Probiotic Culture

Previously, a pure culture of the probiotics contained *Bacillus* sp. and *Streptomyces* sp. was successfully isolated from swamps (Wijayanti *et al.*, 2018) in liquid media. The *Bacillus* sp. were cultured in Erlenmeyer flasks filled with 20 mL of Nutrient Broth (NB) at one Ose needle. In contrast, *Streptomyces* sp. was cultured on the 20 mL of Yeast Malt (YM) liquid media in Erlenmeyer flasks at one Ose needle. These bacteria were agitated with a magnetic hot plate stirrer for three days (*Bacillus* sp.) and five days (*Streptomyces* sp.). The bacterial density was calculated using the Total Plate Count (TPC) method. Then, bacteria were mixed with 5% molasses for biofloc formation. For stock culture, bacteria were mixed with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish (*Clarias gariepinus*) were reared in six rounded tarpaulin tanks with a diameter of 2 m and a height of 1.2 m. The tank was cleaned by brushing the entire tank and drying for a day to kill the pathogens. Then, the tank was filled with water as high as 0.7 m (volume 2198 L) and incubated for three days (Ma'ruf, 2016). Then, the tank was added with salt at 1 kg m^{-3} and camphor dolomite at 50 g m^{-3} (Sucipto *et al.*, 2018) and incubated for a day (Wijayanti *et al.*, 2021). Aeration was installed at four points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process used catfish at 7 ± 0.5 cm with a stocking density of 500 fish m^{-3} (BSN, 2018) and reared for 42 days. Fish stocking was carried out in the morning when water conditions were normal and fish were acclimatized for seven days to reduce stress. Probiotics were applied during the rearing period at a density of 10^5 CFU/mL for each bacteria (Wijayanti *et al.*, 2020). The application frequency followed the treatments, namely (P1) once in 42 days of rearing and (P2) twice in 42 days of rearing. A carbon source in the form of molasses was also administered at 200 mL m^{-3} with a frequency of once every seven days (0, 7, 14, 21, 28, 35 days) (Putra *et al.*, 2017). Fish were fed daily using a commercial feed with 39-41% protein content. The feeding frequency was three times, namely at 08.00 am, 12.00 am, and 04.00 pm under apparent satiation. Dead fish were weighed, when existed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental tank unit.

Parameters

Floc Volume and Composition

Floc volume was measured using an Imhoff cone by taking the water at 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on 7th, 14th, 21st, 28th, 35th, and 42nd day of rearing. After measuring the floc volume, the precipitated floc was taken to observe the composition of floc microorganisms. Floc composition was microscopically observed with microscope at 40x magnification on 0, 1st, 21st , and 42nd day of rearing.

Total Bacterial Colonies

The total bacterial colonies formed in the rearing media were counted using a colony counter. After The water sample was diluted at 10^{-5}, 10^{-7}, and 10^{-9}, the bacterial density was calculated on 0th (before probiotics application), 1st, 21st, and 42nd day of rearing. The bacterial colonies proliferation were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth Rate

The formula used to measure the absolute weight growth, according to Hopkins (1992) was:

$$W = Wt - Wo$$

Note:

W = Absolute Weight Growth Rate (g)
Wt = Average fish weight at the final period of the study (g)
Wo = Average fish weight at the initial period of the study (g)

The formula used to measure the absolute length growth, according to Hopkins (1992), was:

$$L = Lt - Lo$$

Note:

L = Absolute length growth rate (cm)
Lt = Average length of fish at the final period of the study (cm)
Lo = Average fish length at the initial period of the study (cm)

Feed Efficiency

Feed efficiency (FE) was calculated based on a formula of Afrianto & Liviawaty (2005):

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

Note:

FE = Feed efficiency (%)
Wt = Fish biomass at the end of the study (g)
Wo = Fish biomass at the start of the study (g)
D = Fish dead biomass during the study (g)
F = Amount of feed applied during the study (g)

Survival Rate (SR)

The survival rate was calculated using a formula based on Aliyu-Paiko*et al.* (2010):

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Note:

SR = Survival rate (%)
Nt = The final quantity of fish at the end of rearing (g)
No = The initial quantity of fish at initial rearing (g)

Biomass Production

The biomass production was calculated, according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Note:

P = Biomass production (g)
W = Average weight of fish at the end of rearing (g)
N = The final number of fish at the end of rearing (fish)

Water quality

Water quality parameters, including temperature and pH, were measured every day at 08.00 a.m. and 04.00 p.m. Meanwhile, dissolved oxygen level, TDS, and ammonia were measured every seven days (0, 7, 14, 21, 28, 35, and 42 days). Temperature (°C) was measured using a thermometer, pH was measured using a pH meter, Dissolved oxygen (DO) was measured using a DO meter (mg/L), the Total Dissolved solid (TDS) (mg/L) was measured using a TDS meter, and ammonia (mg/L) was measured using a spectrophotometer method.

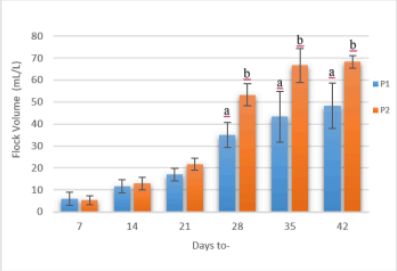
Data analysis

Data on flock volume, total bacterial colonies, absolute growth rate, feed efficiency, survival rate, and water quality were analyzed with T-test at 95% confidence level. Meanwhile, the flock composition data were analyzed descriptively.

Results and Discussion

Floc Volume

The T-test analysis results of flock volume are presented in Figure 1.



Note: Different superscript letters in the same days show significant differences at 5% level of T-test

Figure 1. Flock volume

The floc volume in the P1 treatment was significantly different from the P2 treatment on 28th, 35th, and 42nd days. The floc volume is the amount number of suspended solid over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment at 68.33 mL/L. The floc volume obtained in this study was higher than in Wijayanti *et al.* (2021) at 40 mL/L (42nd day of rearing period). This was presumably due to the influence of the application frequency of probiotics to the rearing medium. According to De Schryver *et al.* (2008), factors that influence the biofloc formation are the administration of probiotics as a floc-forming agent, agitation intensity by aeration device, organic carbon source, and water quality. Malaputra *et al.* (2016) reported, that the administration of commercial probiotics for 14 times (70 days of rearing) produced a floc volume of 120 mL/L, which was higher than for seven times at 80 mL/L. Therefore, the more often probiotics applied to the rearing medium, the more floc volume obtained. The maximum floc volume for catfish culture is 100 mL/L. According to Agusta *et al.* (2022), the addition of molasses as a carbon source in the catfish culture with biofloc system produces a floc volume of 111 mL/L. When the floc volume exceeds the limit threshold, partial water removal should be performed at about 70-80% of the total water volume (Zaidy, 2022). High floc volume can cause fish death (Rofianingrum *et al.*, 2022).

In addition to providing swamp probiotics as a biofloc starter that contain heterotrophic bacteria, the floc volume increase in this study was thought to be due to the C/N ratio, which affected the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), when the C/N ratio >10, the formation of biofloc reaches its optimum level. According to Avnimelech *et al.* (1999), the C/N ratio required for biofloc formation should be >15. Therefore, essential organic carbon addition is necessary such as molasses, which can be added every seven days at 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The floc composition in this study is presented in Table 1.

Table 1. The floc composition of catfish (*Clarias gariepinus*) reared in biofloc system

Floc-forming microbes	Treatment							
	H0		H1		H21		H42	
	P1	P2	P1	P2	P1	P2	P1	P2
Chlorophyta	✓	✓	✓	✓	✓	✓	✓	✓
Cyanophyta	✓	✓	✓	✓	✓	✓	✓	✓
Protozoa	✓	✓	✓	✓	✓	✓	✓	✓
Coelenterates	-	✓	-	✓	-	✓	-	✓
Rotifers	✓	✓	✓	✓	✓	✓	✓	✓
Arthropods	-	✓	-	✓	-	✓	-	✓

Information: (✓) present, (-) absent

Based on the observations of floc composition, the P2 treatment presents more diverse microbes than P1 treatment, namely Chlorophyta, Cyanophyta, protozoa, coelenterate, rotifers, and arthropods. In P1, the microbes were only Chlorophyta, Cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), diverse microbes in the floc composition was thought due to the effect of probiotics application, which was in line with the increased floc volume in the rearing medium, so the microbes could proliferate properly. Based on Hargreaves (2013), the biofloc system consists of algae, bacteria, protozoa, zooplankton, and other microorganisms. Previously, Wijayanti *et al.* (2020) produced a combination of Chlorophyta and swamp probiotics, containing bacteria *Bacillus* sp. In addition, De Schryver *et al.* (2008) mentioned, that the floc is composed of heterogeneous combination of

microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach <1000 µm in size.

Total Bacterial Colonies

The total bacterial colonies on each rearing medium are presented in Table 2.

Table 2. The total bacterial colonies in the biofloc system on each tank

Days (-th)	Total bacterial colonies (× 10^9 CFU/mL)	
	P1	P2
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19[a]	19.73±6.19[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The total bacterial colonies in the P1 treatment were only significantly different on the 42nd day. In this study, the bacterial density range was among 2.77-19.73 × 10^9 CFU/mL. After applying the swamp probiotics on the rearing media, the total bacterial colonies increased on the 1st day, then decreased on the 21st day. This condition was thought due to a lack of nutrients (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020), which could occur as nutrients, like carbons in molasses, have not been added. According to Nasmia and Rifai (2020), the number of bacterial colonies decreased due to carbon source reduction. On the 42nd day, the bacterial density increased again with a higher bacterial density was obtained from the P2 treatment at 19.73 x10^9 CFU/mL, while the P1 treatment only produced the bacterial density of 8.42 x10^9 CFU/mL. . This condition was occurred due to probiotics re-administration on the 21st day of rearing period. Similarly, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish culture by applying probiotics up to 10^{14} CFU/mL.

According to Sitorus *et al.* (2019), the bacterial density in floc formation can reach approximately 10^9 CFU/mL. Widnyana (2016) stated that the total bacterial colonies in flocs ranged from 10^3-10^4 CFU/mL, while the system has a bacterial range of 10^8-10^{10} CFU/mL. This was because the system had a high intensity of sunlight as one of the supporting factors in bacterial growth. High density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, assisted by their effectiveness in breaking down organic matters (Adharani *et al.*, 2016).

Absolute Growth Rate, Feed Efficiency, Survival Rate, and Biomass Production

The absolute growth rate, feed efficiency, and survival rate of catfish for 42 days of rearing period are presented in Table 3.

Table 3. The absolute growth rate, feed efficiency, survival rate, and biomass production of catfish (*Clarias gariepinus*) in the biofloc system

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24[a]	151.90 ± 7.98[b]
Survival rate (%)	81.54 ± 1.59	89.33 ±6.21
Biomass production (g)	17439.14 ± 1346.55[a]	24639.50 ± 1344.51[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The absolute length and weight growth rates of catfish in P1 treatment was insignificantly different from P2 treatment. The absolute length and weight growth rates in P2 treatment were higher than P1 treatment. These results indicate that catfish can utilize the available floc in the rearing medium as additional feed due to high protein content, that can increase the fish growth (Putra *et al.*, 2017). In addition, Wijaya *et al.* (2016) reported that a floc contains 42.42% protein, 92.15% moisture, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacteria such as *Bacillus* sp., can accumulate in the rearing medium and form flocs that can be used as an aquafeed source for fish.

Furthermore, *Bacillus* sp. and *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. *Streptomyces* sp. in fish culture can also be applied as a growth-promoter (Cruz *et al.*, 2012).

The feed efficiency in P1 treatment was significantly different from P2 treatment. The feed efficiency of P2 treatment was higher at 151.90%, while P1 treatment was 104.97%. If the feed efficiency value is converted to feed conversion ratio (FCR) value, then the FCR value for P1 is 0.96 while P2 is 0.66. The FCR values in this study were better than Wijayanti *et al.* (2021) at 0.97. Putra *et al.* (2017) administered probiotics to the catfish rearing media

with biofloc technology also resulted in high feed efficiency at 88.17-110.86%. In this study, high feed efficiency value was suspected as fish used flocs as additional feed. De Schryver *et al.* (2008) stated that flocs as additional feed is important in increasing the feed efficiency. This shows that swamp probiotics can form flocs, which fish then consume them as additional feed.

The survival rate of catfish culture in P1 treatment was insignificantly different from P2 treatment. The survival rate in P2 treatment was higher than P1 treatment (89.33% vs 81.54%). These results were closed to Wijayanti *et al.* (2021) at 87.57% with a rearing period of 90 days. The administration of swamp probiotics in the form of a *Bacillus* sp. and *Streptomyces* sp. combination provides a defense mechanism against pathogens by increasing the non-specific immunity in the fish immune system and maintaining a water quality balance, so the fish can survive (Wijayanti *et al.*, 2020). Growth and survival rates will affect the production value of fish biomass.

The catfish biomass production in P1 treatment was significantly different from P2 treatment. The biomass production of P2 biomass was higher than P1 (24,639.50 g vs 17,439.14 g). Probiotics application is thought to increase the biomass production of catfish in the biofloc technology. The biomass determines the production value at the end of culture period and the survival rate of fish at the end of culture period, so the more fish survives, the more fish production obtained (Anam *et al.*, 2017). Based on the results of absolute growth rates and feed efficiency in this study, the swamp probiotics can form floc used by fish as additional feed. Applying swamp probiotics is also useful in preventing pathogenic bacteria to prevent mass fish death. Therefore, swamp probiotics can increase the fish growth and survival rate, thus increasing the fish biomass production.

Water quality

Temperature and pH

The temperature and pH of the rearing media are presented in Table 4.

Table 4. The temperature and pH of rearing media with biofloc system

Treatment	Temperature (°C)	pH
P1	28.85-29.22[a]	7.27-7.40[a]
P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The temperature and pH of the catfish-rearing media in P1 treatment were insignificantly different from P2 treatment. The temperature of the rearing medium in this study was 28.85-29.59°C. According to BSN (2014), the temperature standard for catfish rearing is 25-30°C. Catfish will experience slow growth when the temperature is 16-24°C or 31-32°C, while catfish will die if the temperature is below 16°C or above 32°C (Pujiharsono & Kurnianto, 2020). The degree of acidity or pH was among 7.27-7.42. BSN (2014) stated that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth, when the pH is 4-6.4 or 8.6-11, and the fish will die if the pH is below 4.0 or above 11.0 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The DO value during the rearing period is presented in Table 5.

Table 5. The DO contents of the rearing tank with the biofloc system

Days (-th)	DO (mg/L)	
	P1	P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]
21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The Dissolved Oxygen (DO) in P1 treatment was significantly different from P2 treatment on 14th, 21th, and 28th days. High frequency of swamp probiotics application as presented in P2 treatment (twice in 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study were 3.91 -5.95 mg/L. According to BSN (2014), the DO value for catfish culture is >3 mg/L. Therefore, the DO value in this study is still relatively safe for catfish, because the rearing container is equipped with four aeration source points, besides photosynthesis from phytoplankton and oxygen diffusion from the air (Putra *et al.*, 2014). Thus, the increased DO value in P1 treatment is better than P2 treatment. Furthermore, *Bacillus* sp. is suspected to regulate the dissolved oxygen concentrations in fish culture media up to > 3 mg/L. The results of Kurniawan and Utama (2018) also yielded DO values for catfish rearing >6 mg/L by administering *Bacillus* sp. treatment, which was in accordance to Prihanto *et al.* (2021) as *Bacillus* sp. bacteria can improve the water quality.

Ammonia

The results of the ammonia contents in the rearing tank are presented in Table 6.

Table 6. The ammonia contents in rearing tanks with the biofloc system

Days (-th)	Ammonia (mg/L)	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The T-test analysis showed that the ammonia in treatment P1 was significantly different on only 28th and 35th days. The administration frequency of swamp probiotics in P2 treatment (twice in 42 days of rearing) is proven to reduce the ammonia levels in rearing media, compared to P1 treatment (once in 42 days of rearing). The ammonia values in this study were high, namely at 0.45 - 1.62 mg/L, while Wijayanti *et al.* (2021) reported the ammonia content was only 0.27 mg/L. The ammonia content for catfish culture with biofloc system should be <1 mg/L (Wijaya *et al.*, 2016). High ammonia value can cause fish death due to the toxic nature of ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion matter, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). High ammonia value in this study is likely due to the lack of C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in the water. Meanwhile, Hargreaves (2006) stated that a C/N ratio <10 causes heterotrophic bacteria to release ammonia into the environment. Bakar *et al.* (2015) also stated that ammonia reduction in catfish culture using biofloc technology using a C/N ratio of 15 could eliminate ammonia by 93.56% on the 12th day, while the C/N ratio of 10 on the 12th day was only 11.01%, and the maximum ammonia elimination level was found on the 30th day at 98.51%.

Total Dissolved Solid (TDS)

The TDS analysis results in the rearing tanks are presented in Table 7.

Table 7. The TDS analysis results in the rearing tank with the biofloc system

Days (-th)	TDS (mg/L)	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76[b]	872.67±41.55[a]
28	804.17±59.93	757.67±136.93
35	810.00±57.38[b]	717.33±21.37[a]
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

Based on the results of the T-test analysis, the Total Dissolved Solid (TDS) in P1 treatment was only significantly different on 21th and 35th days. The administration of swamp probiotics with P2 treatment can highly reduce the TDS values, compared to P1 treatment. The TDS values in this study was among 717.33 - 1014.83 mg/L. Catfish could still tolerate the TDS values in this study. BSN (2018) stated that TDS standard for water quality of fish culture activities is 1000 mg/L. The high TDS level on the 21st day, especially in the P1 treatment, is thought due to the administration of commercial drugs to prevent dead fish, which contained organic and inorganic compounds. This condition followed the statement of Rinawati *et al.* (2016), that large amounts of organic and inorganic compounds could result in high levels of TDS in the water.

Conclusion

This study concludes that swamp probiotics application twice in 42 days of rearing (P2) provides the best results on flock volume, fish growth rate, feed efficiency, survival rate, biomass production, and water quality. We suggest to evaluate further regarding the swamp probiotic applications in other types of fish with the biofloc system.

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