



## Optimization of striped snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria

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**Abstract.** Utilization of swamp areas as fish culture locations alters water quality. Therefore, it is necessary to improve the water quality with environmental friendly biological treatments, such as the addition of microbes as probiotics in media culture. The purpose of this study was to determine the combination of microbes from swamps that can improve the water quality of the swamp fish culture and production media. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and the second factor with four treatments and three replications. The first factor consisted of two scenarios: (1) without the addition of nitrification bacteria (N1) and (2) with the addition of nitrification bacteria (PROBAC)  $5 \times 10^6$  CFU mL<sup>-1</sup> (N2). The second factor consisted of four scenarios: (1) without the addition of swamp microbes (P1) and with the addition of: (2) Chlorophyta ( $3.43 \times 10^7$  sel L<sup>-1</sup>) and *Bacillus* sp. ( $10^5$  CFU mL<sup>-1</sup>) (P2); (3) Chlorophyta ( $3.43 \times 10^7$  sel L<sup>-1</sup>) and *Streptomyces* sp. ( $10^5$  CFU mL<sup>-1</sup>) (P3); (4) Chlorophyta ( $3.43 \times 10^7$  sel L<sup>-1</sup>), *Bacillus* sp. ( $10^5$  CFU mL<sup>-1</sup>) and *Streptomyces* sp. ( $10^5$  CFU mL<sup>-1</sup>) (P4). The result showed that the addition microbes from swamps in the combination of N1 and P4 treatment scenarios was able to improve the water quality value better than the other treatment scenarios, producing the best survival rate (63.94%), feed efficiency (59.65%), absolute weight growth (2.32 g) and absolute length growth of (2.27 cm).

**Key Words:** fish culture, biological treatments, probiotic, water quality.

**Introduction.** The swamp aquaculture must improve and maintain the water quality for fish rearing media, through environmentally friendly biological treatments, since the wastewater of fish rearing on swamps reduces the quality of swamps water. One of the treatments is adding probiotics in the rearing media. Irianto & Austin (2002) stated that environmental damaging can be prevented with probiotics able to degrade the organic materials in the habitat. Hartini et al (2013) showed that the addition of probiotics at a dose of  $10 \mu\text{L L}^{-1} \text{ week}^{-1}$  can improve and maintain optimal water quality. In other studies, the addition of effective probiotic microorganisms can reduce ammonia levels and suppress the population of pathogenic microorganisms from the culture media (Trisna et al 2013).

Swamps have high biodiversity, including sediment microbes able to improve the physical and chemical properties of their media. The identified swamp microbes include Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. (Wijayanti et al 2018). Bacteria (*Bacillus* sp.) with concentrations of  $10^5$  CFU mL<sup>-1</sup> (Khotimah 2018) and Chlorophyta microalgae with the optimum concentration of 10% of the maximum density (Utami 2019) are able to grow in the fish culture media and they can be used as environmental probiotics. Chlorophyta is a microorganism that can be used as green water in aquaculture media. Wijayanti et al (2018) showed that the use of Chlorophyta increased the level of dissolved oxygen in the pond culture media 60.52% and swamp water culture media 63.63%. The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. *Bacillus* sp. and *Streptomyces* sp. obtained are proteolytic bacteria that can increase the content of

NH<sub>3</sub>, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> into the media (Yuliani 2017; Saraswati 2018). Balcazar et al (2006) stated that *Bacillus* sp. is an example of efficient probiotic bacteria, used in aquaculture because it is able to convert organic matter into CO<sub>2</sub> used in cell metabolism. Gram-positive bacteria, such as *Bacillus* sp. can increase the animal immune system capabilities and also act favorably in improving the quality of the water system (Mohapatra et al 2013). Bernal et al (2017) stated that the combination of *Streptomyces* sp. and *Bacillus* sp. demonstrated a significant immunomodulatory activity by increasing the total number of hemocytes and the activity of Superoxide Dismutase (SOD), which provides a protective effect against *Vibrio harveyi* bacteria by increasing the immunological status of *Penaeus monodon*. Nitrifying bacteria are chemolitho-autotrophic bacteria (for example *Nitrosomonas* sp., *Nitrobacter* sp.), which are able to meet their carbon needs through CO<sub>2</sub> fixation (Calvin cycle), and their energy source comes from the oxidation process of reducing ammonia to nitrate. As an example, through the addition of nitrifying bacteria and denitrification, the molasses with a C/N ratio of 10 could decrease in ammonia levels by 28.5% (Yuniasari 2009).

Each of the swamp microbes and nitrifying bacteria has their own advantages and disadvantages, therefore a consortium of swamp microbes and nitrifying bacteria would be more effective. The emergence of a consortium, expected to form cooperative, commensal and mutualistic relationships between microbes, calls for an optimal combination of *Bacillus* sp., *Streptomyces* sp., Chlorophyta and commercial nitrification bacteria. The purpose of this study was to determine combinations of swamp microbes and nitrifying bacteria that can improve the water quality of media in swamp fish production.

## Material and Method

The experimental design used was a completely randomized factorial design (RAL) consisting of 2 factors: the first factor with 2 treatments and the second factor with 4 treatments and 3 replications. The first factor is the addition of nitrifying bacteria (PROBAC), namely:

N1- without the addition of nitrifying bacteria (PROBAC);

N2 – with the addition of nitrifying bacteria (PROBAC)  $5 \times 10^6$  CFU mL<sup>-1</sup>.

The second factor is the addition of swamp microbes, namely:

P1- without the addition of swamp microbes;

P2 - provision of 100 ml Chlorophyta ( $3.43 \times 10^7$  cell L<sup>-1</sup>) and *Bacillus* sp. ( $10^5$  CFU mL<sup>-1</sup>);

P3 - provision of 100 ml Chlorophyta ( $3.43 \times 10^7$  cell L<sup>-1</sup>) and *Streptomyces* sp. ( $10^5$  CFU mL<sup>-1</sup>);

P4 - provision of 100 ml Chlorophyta ( $3.43 \times 10^7$  cell L<sup>-1</sup>), *Bacillus* sp. ( $10^5$  CFU mL<sup>-1</sup>) and *Streptomyces* sp. ( $10^5$  CFU mL<sup>-1</sup>).

**Bacteria cultivation and propagation.** Pure bacterial colonies were obtained from the results of previous studies. The colony was cultivated using nutrient agar (NA) media for *Bacillus* sp. and yeast malt (YM) agar for *Streptomyces* sp. Bacterial colonies were scratched in a petri dish containing NA for *Bacillus* sp. and YM media for *Streptomyces* sp. using the quadrant streak method. Petri dishes were wrapped in wrapping paper and incubated for 2 days at room temperature (28-30°C). A single colony formed on a petri dish was transferred to the agar media and incubated until bacteria grew.

Swamp bacteria grown on NA and YM agar media were multiplied by nutrient broth (NB) media for *Bacillus* sp. and liquid YM for *Streptomyces* sp. As much as 5 mL of suspension were collected in a test tube in order to be cultured in the medium, and then homogenized with a shaker for approximately 2 days for *Bacillus* sp. and 5 days for *Streptomyces* sp., then multiplied from 5 mL to 500 mL. The concentrations of bacteria were measured by Mc Farlan spectrophotometry methods.

**Chlorophyta culture.** The culture media used for Chlorophyta was a technical fertilizer media consisting of ZA, Urea, TSP, and Gandasil B. All technical fertilizer ingredients were mixed in a 250 mL Erlenmeyer and added to 100 mL of distilled water and then

homogenized on a hot plate using a magnetic stirrer and sufficient heat, until all ingredients dissolved. The technical fertilizer media in the Erlenmeyer was sterilized using an autoclave at 121°C for 0.25 hour. Chlorophyta isolates (about  $10^7$  cell mL<sup>-1</sup> in 10 mL stock culture) were put into an Erlenmeyer containing a technical fertilizer media for liquid culture. They cultured during 9 days at the room temperature for scaling up to 1 L.

**Preparation of fish rearing media.** The container used in rearing was in the form of an aquarium with a size of 30 x 30 x 30 cm<sup>3</sup> as many as 24 units. The aquariums were cleaned using potassium permanganate to be sterilized of diseases or parasites. The aquarium was filled with 20 L of swamp water.

**Fish culture test.** The test organisms used in this study were 12 *Channa striata* specimens of 5±1 cm each for 20 L of water (Mulyadi 2016). Before stocking, acclimatization was done as an adaptation to the new environment in order to reduce stress on the test organism. After 7 days of stocking, a treatment was applied, consisting of a combination of Chlorophyta isolate ( $3.43 \times 10^7$  cell L<sup>-1</sup>), *Bacillus* sp. ( $10^5$  CFU mL<sup>-1</sup>), *Streptomyces* sp. ( $10^5$  CFU mL<sup>-1</sup>) as well as PROBAC nitrification bacteria ( $5 \times 10^6$  CFU mL<sup>-1</sup>).

**Rearing.** The fish culture was maintained for 40 days calculated after the addition of the treatment. During rearing, they were fed at satiation with a frequency of three times a day, by using commercial pellets with 40% protein content.

**Chlorophyta abundance.** Samplings were carried out at the beginning and end of the study by sub composite methods, in each treatment. Plankton net with 25 µm mesh size was used for 5 L of rearing media by experimental unit (sample of 25 mL). A microscope and "The Marine and Fresh Water Plankton" textbook were used for the observation of the Chlorophyta samples (Davis 1955). Chlorophyta abundance calculation was performed by using the Leackey Drop Microtransect method (American Public Health Association 1989) as follows:

$$N = Z \times \frac{X}{Y} \times \frac{1}{V}$$

Where:

N - total number (cell L<sup>-1</sup>);

Z - number of individuals found;

X - volume of filtered water (25 mL);

Y - volume 1 drop of sample water (0.05 mL);

V - volume of filtered water (5 L).

**Bacteria population.** The counting of bacterial populations was performed at the beginning and end of rearing with the plate count method on a multilevel dilution incubated at a temperature of 28-30°C for 24 hours. The growing population was determined in a Colony Forming Unit (CFU) and calculated using the following formula (Pepper & Gerba 2004):

$$\text{Total of Bacteria} = \text{Total of colonies} \times \frac{1}{\text{dilution factor}} \times \frac{1}{\text{mL sample}}$$

**Biofloc volume.** The biofloc volume measurements were done on the 10 and 40 days after rearing. The floc volume was obtained by collecting a rearing media, by using a glass cone 1L volume, then the floc in the water media was left to settle in the tube for 15-20 minutes.

**Survival rate.** The percentage of fish survival was calculated by using the following formula (Aliyu-Paiko et al 2010):

$$\frac{N_t}{N_0} \times 100$$

Where:

$N_0$  - number of fish at the beginning of rearing (individuals);

$N_t$  - number of fish at the end of rearing (individuals).

**Absolute weight growth.** Growth of fish weight during rearing was calculated by using the following formula (Hopkins 1992):

$$W = W_t - W_0$$

Where:

$W$  - growth of weight of fish for rearing (grams);

$W_t$  - weight of fish at the end of rearing (grams);

$W_0$  - weight of fish at the beginning of rearing (grams).

**Absolute length growth.** The absolute length growth of fish during rearing was determined by doing the following calculation (Hopkins 1992):

$$L = L_t - L_0$$

Where:

$L$  - growth of absolute length of fish for rearing (cm);

$L_t$  - length of fish at the end of rearing (cm);

$L_0$  - length of fish at the beginning of rearing (cm).

**Feed efficiency.** According to NRC (1977) feed efficiency can be calculated by using the formula:

$$EP = \frac{((W_t + D) - W_0)}{F} \times 100$$

Where:

EP - feed efficiency (%);

$W_t$  - weight of fish at the end of rearing (gram);

$W_0$  - initial fish rearing weight (gram);

$D$  - weight of fish that died during rearing (gram);

$F$  - amount of feed given (grams).

**Water quality.** Measurement of water quality data for *C. striata* rearing media included pH (pH meter), dissolved oxygen (DO meter), ammonia (spectrophotometry), and biological oxygen demand (DO meter) at the beginning and 40 days later, at the end of the rearing.

**Data analysis.** Research data including biofloc volume, survival, growth, feed efficiency, water quality was statistically processed by using the variance analysis. If the results of the variance analysis showed that the treatment has a significant effect, then it was continued with the LSD test (the Least significance difference) 5%. Chlorophyta abundance data and the total bacterial population were analyzed descriptively.

## Results and Discussion

**Chlorophyta abundance, total bacterial population and floc volume.** Chlorophyta abundance data on rearing media are presented in Table 1. *Chlorophyta* density at each treatment decreased after 40 days of rearing. *Chlorophyta* added in the rearing media experiences death or predation. In the rearing media, a food chain system occurred between Chlorophyta and zooplankton (Figure 1), resulting in a decrease in the population of Chlorophyta due to predation.

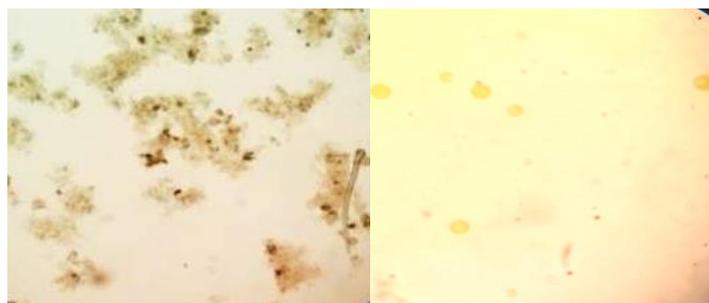


Figure 1. Biofloc and Chlorophyta profile in the rearing media of *Channa striata* culture in this study (40 magnification scale of microscope).

The pattern of the interactions between zooplankton and phytoplankton is a series of eating and prey relationships forming the path of the food chain. Phytoplankton as primary producers is eaten by zooplanktons, in turn zooplanktons are eaten by small fish at higher trophic levels (Bouman et al 2003).

Table 1  
Chlorophyta abundance in *Channa striata* rearing media at 0, 10, 40<sup>th</sup> day

Commercial nitrification bacteria	Swamp microbes	Chlorophyta abundance (cell L <sup>-1</sup> )		
		0 day	10 <sup>th</sup> day	40 <sup>th</sup> day
N1	P1	3.20×10 <sup>3</sup>	3.20×10 <sup>3</sup>	2.10×10 <sup>3</sup>
	P2	3.60×10 <sup>3</sup>	3.43×10 <sup>7</sup>	4.10×10 <sup>3</sup>
	P3	4.10×10 <sup>3</sup>	3.43×10 <sup>7</sup>	4.10×10 <sup>3</sup>
	P4	3.70×10 <sup>3</sup>	3.43×10 <sup>7</sup>	4.46×10 <sup>3</sup>
N2	P1	4.00×10 <sup>3</sup>	4.00×10 <sup>3</sup>	2.10×10 <sup>3</sup>
	P2	3.60×10 <sup>3</sup>	3.43×10 <sup>7</sup>	2.41×10 <sup>3</sup>
	P3	3.90×10 <sup>3</sup>	3.43×10 <sup>7</sup>	2.34×10 <sup>3</sup>
	P4	3.40×10 <sup>3</sup>	3.43×10 <sup>7</sup>	4.03×10 <sup>3</sup>

The total bacterial population in the rearing media is presented in Table 2, the results of the LSD test of the floc volume at ten and forty days of rearing are showed in Table 3 and Table 4, respectively.

Table 2  
Total bacterial population in the rearing media

Commercial nitrification bacteria	Swamp microbes	Total bacterial population (CFU mL <sup>-1</sup> )			
		0 day	1 <sup>st</sup> day	20 <sup>th</sup> day	40 <sup>th</sup> day
N1	P1	6.60×10 <sup>4</sup>	6.78×10 <sup>4</sup>	1.55×10 <sup>5</sup>	6.20×10 <sup>3</sup>
	P2	6.20×10 <sup>4</sup>	3.95×10 <sup>6</sup>	6.93×10 <sup>6</sup>	2.77×10 <sup>5</sup>
	P3	7.00×10 <sup>4</sup>	3.28×10 <sup>6</sup>	7.53×10 <sup>6</sup>	3.01×10 <sup>5</sup>
	P4	4.70×10 <sup>4</sup>	5.59×10 <sup>7</sup>	1.00×10 <sup>8</sup>	2.99×10 <sup>6</sup>
N2	P1	7.10×10 <sup>4</sup>	2.01×10 <sup>7</sup>	3.54×10 <sup>7</sup>	1.42×10 <sup>6</sup>
	P2	4.50×10 <sup>4</sup>	3.29×10 <sup>7</sup>	5.59×10 <sup>7</sup>	1.68×10 <sup>6</sup>
	P3	4.30×10 <sup>4</sup>	4.99×10 <sup>7</sup>	6.41×10 <sup>7</sup>	1.93×10 <sup>6</sup>
	P4	4.95×10 <sup>4</sup>	4.70×10 <sup>7</sup>	8.75×10 <sup>7</sup>	4.06×10 <sup>6</sup>

Based on Table 2, the total bacterial population increased on 20<sup>th</sup> day and decreased until the 40<sup>th</sup> day. The increase in population on the 20<sup>th</sup> day can be caused by adequate nutrients addition in the rearing media, stimulating the metabolic activity and growth of the bacteria and Actinomycetes, while the decline of bacteria population observed on the

40<sup>th</sup> day could be caused by the nutrient depletion (macronutrient and micronutrient) in the water.

Related to the factor of nitrification bacteria, the results of LSD at 10 and 40 days after rearing start showed that the volume of floc in the media without treatment was significantly higher compared to the treatment with nitrification bacteria. The addition of nitrifying bacteria cannot increase the volume of floc, because there were less exhausted bacteria in the media. The bacteria need more substrate from unconsumed feed of fishes, while the nitrifying bacteria accelerated the process of dissolving nitrogenous organic matter from the waste. Related to the factor of microbial addition from swamps, the volume of floc on the media treated with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. significantly showed higher levels compared to other treatments.

Table 3

The results of LSD test of the floc volume in the rearing media at 10 days of rearing

The single effect of nitrifying bacteria (N)	The single influence of swamps microbes (P) (not significant)				The main effects of nitrifying bacteria (N) (LSD <sub>0.05</sub> =4.386)
	P1	P2	P3	P4	
N1	11.111	16.666	13.332	26.668	16.944 <sup>b</sup>
N2	10.000	10.000	13.332	16.667	12.500 <sup>a</sup>
The main effects of swamp microbes (P) (LSD <sub>0.05</sub> =3.102)	10.556 <sup>a</sup>	13.333 <sup>a</sup>	13.332 <sup>a</sup>	21.667 <sup>b</sup>	

Table 4

The results of LSD test floc volume of rearing media at 40 days of rearing

The single effect of nitrifying bacteria (N)	The single influence of swamps microbes (P) (LSD <sub>0.05</sub> =6.631)				The main effects of nitrifying bacteria (N) (LSD <sub>0.05</sub> =3.315)
	P1	P2	P3	P4	
N1	11.112 <sup>a</sup>	26.667 <sup>b</sup>	16.667 <sup>a</sup>	38.889 <sup>c</sup>	25.834 <sup>b</sup>
N2	13.333 <sup>a</sup>	13.333 <sup>a</sup>	23.333 <sup>b</sup>	33.333 <sup>c</sup>	20.833 <sup>a</sup>
The main effects of swamp microbes (P) (LSD <sub>0.05</sub> =4.689)	12.223 <sup>a</sup>	20.000 <sup>b</sup>	20.000 <sup>b</sup>	41.111 <sup>c</sup>	

It is presumed that certain types of microorganisms are predisposed for flocs forming. Related to the influence of interactions between factors at 40 days of rearing, the observations showed that the treatment combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. without nitrifying bacteria determined the highest volume of floc, 38.89 mL L<sup>-1</sup>, but it was not significantly different from the treatment combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. with nitrifying bacteria. The volume of floc in this study was lower than the study from Mulyadi et al (2016), where in treatment with stocking density of 450 *C. striata* m<sup>-3</sup> which was kept for 41 days resulted in a floc volume of 40.7 mL L<sup>-1</sup>. It is presumed that the rearing media lacks a carbon source used by bacteria for the floc formation. According to Panigrahi et al (2019), *Litopenaeus vannamei* cultivation without a biofloc system can produce a volume of floc of 4.53 mL L<sup>-1</sup>, which is lower than the cultivation of *L. vannamei* with a biofloc system, by adding molasses. Floc thickness will continue to increase along with the provision of bacteria and carbon sources that are continuously balanced with feed metabolic waste producing ammonia. The bacteria could bind to ammonia and will form a biofloc (Sitohang et al 2018).

The results of the analysis of variance showed that the effect of the interaction between factors and the effect of the factor defined by the addition of swamp originated microbes on the survival of *C. striata* varied significantly between treatments, but the factor defined by the adding of commercial nitrification bacteria had no significant effect.

Based on further testing of LSD on microbial factors from swamps, in the rearing media treated with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp., the survival rate was significantly higher than for the other treatments. Interactions between factors showed that in the rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, the survival rate reached 63.94%, a significantly higher performance compared to the other treatments.

Based on the results of the survival percentage, it is showed that the combination of swamp microbes is able to suppress unfavorable microbes, to improve the water quality in the rearing media and to increase the *C. striata* survival rate. This is shown in the treatment of rearing media consisting in a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, which provided a *C. striata* survival rate of 63.94%. The combination of *Bacillus* sp. and *Streptomyces* sp. was able to provide more protection against unfavorable microbes in the media, the presence of *Bacillus* sp. giving effect to *Streptomyces* sp. to produce antimicrobial compounds. Luti & Mavituna (2011) explained that *Bacillus* cultured together with *Streptomyces* increased the production of antimicrobial compounds when it was compared with the single genus culture.

Table 5

LSD test of survival rate of *Channa striata*

The single effect of nitrifying bacteria (N)	The single influence of swamps microbes (P) (LSD <sub>0.05</sub> =6.02)				The main effects of nitrifying bacteria (N) (not significant)
	P1	P2	P3	P4	
N1	26.06 <sup>a</sup>	36.91 <sup>b</sup>	28.03 <sup>a</sup>	63.94 <sup>d</sup>	38.74
N2	31.75 <sup>ab</sup>	28.03 <sup>a</sup>	35.16 <sup>b</sup>	48.20 <sup>c</sup>	35.79
The main effects of swamp microbes (P) (LSD <sub>0.05</sub> =4.25)	28.91 <sup>a</sup>	32.47 <sup>a</sup>	31.59 <sup>a</sup>	56.07 <sup>b</sup>	

The N1P1 treatment (without the addition of microbes from swamps, neither of commercial nitrification bacteria) was the treatment with the lowest survival value of 26.06% compared to other treatments. These results prove that the addition of swamp origin microbes to rearing media can increase the survival rate of *C. striata*. This is in line with the results of Hartini et al (2013), suggesting that the addition of EM-4 probiotics to the rearing media had a significant influence on the survival of *C. striata*. The average survival of *C. striata* with 10 µL L<sup>-1</sup> week<sup>-1</sup> EM-4 probiotics 96.66% tended to be higher, compared to control treatments (without EM-4 probiotics) which was 8.89%. Budiando & Heny (2017) stated that the bacteria *Bacillus* sp. has bacteriocin compounds with specific inhibiting action on the growth of *S. iniae* and *P. fluorescens*. *Streptomyces* bacteria has the potential to control pathogenic bacteria by means of competition, parasitism or by producing secondary metabolite compounds (Lutfi 2018). The combination of the two microbes can provide a high percentage of survival compared to the no combination scenario. According to Irianto & Austin (2002), probiotic bacteria can increase the survival or reduce mortality through the development of the immune system due to an increasing phagocyte and lysozyme activity, thereby suppressing pathogenic bacterial colonies. Sanchez et al (2014) stated that probiotics can increase immune stimulation in fish, as a protection against pathogenic bacteria that causes death in fish culture.

**Feed efficiency, absolute weight and length growth.** The results of the analysis of the efficiency of various feed on *C. striata* showed that the interaction between factors, the microbial addition factors from swamps and the addition of commercial nitrification bacteria, can increase the value of fish feed efficiency, which is significantly affected by the treatment type and concentration. LSD test results of the efficiency of fish feed for 40 days of rearing are presented in Table 6.

Table 6

LSD test result of the efficiency of *Channa striata* feed for 40 days of rearing

The single effect of nitrifying bacteria (N)	The single influence of swamps microbes (P) (LSD <sub>0.05</sub> =3.32)				The main effects of nitrifying bacteria (N) (LSD <sub>0.05</sub> =1.66)
	P1	P2	P3	P4	
N1	18.93 <sup>a</sup>	47.34 <sup>d</sup>	37.97 <sup>c</sup>	59.65 <sup>e</sup>	40.97 <sup>b</sup>
N2	22.00 <sup>a</sup>	29.52 <sup>b</sup>	34.89 <sup>c</sup>	44.11 <sup>d</sup>	32.63 <sup>a</sup>
The main effects of swamp microbes (P) (LSD <sub>0.05</sub> =2.35)	20.47 <sup>a</sup>	38.43 <sup>b</sup>	36.43 <sup>b</sup>	51.88 <sup>c</sup>	

Based on these results, the effects of the different combinations on the *C. striata* feed efficiency could be observed. The value of the *C. striata* feed efficiency in the treatment of the media of rearing was: (1) significantly higher without commercial nitrification bacteria than for the treatment with nitrification bacteria; (2) significantly higher with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp., compared to other treatments and interactions between factors; (3) significantly higher with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria than the results of other treatments. It is thought that swamp microbes added to the media enter the digestive tract of *C. striata* during the process of respiration and while eating. Chlorophyta that enters the digestive tract could be a natural food source, while *Bacillus* sp. and *Streptomyces* sp. which entered the digestive tract could be working together to improve their digestibility. High digestibility in the feed will increase the absorption of nutrient, which at optimal levels will increase the value of feed efficiency, favoring fish growth. *Bacillus* sp. is a bacterium that can increase digestibility because it can secrete protease, lipase and amylase enzymes (Singh et al 2016).

The bacteria which are members of the genus *Bacillus* are known to produce a wide variety of antimicrobial substances and bacteriocins that can suppress pathogenic bacteria (Deghhanifar et al 2019). *Streptomyces* sp. is a genus of actinomycetes that can produce various antibiotic compounds. Common antibiotic compounds produced by *Streptomyces* have restrictions such as narrow range spectrum, low permeability to specific tissues, and toxicity for the live organisms, as human body (Dehghanifar et al 2019). It has the potential to control pathogenic bacteria by conducting competition, parasitism or by producing secondary metabolites (Lutfi 2018). The bacterium *Bacillus* sp. increases the value of feed efficiency by secreting enzymes that can stimulate digestion, while *Streptomyces* sp. secretes antibiotics able to suppress pathogens. Both bacteria work together to improve the digestibility and immunity of *C. striata*, which ultimately results in high feed efficiency. Bacterial activity in the digestive tract will experience rapid fluctuations with microbes entering through feed or water and causing changes in the intestinal microbial balance. The entry of these microbes is antagonistic to the pathogenic microbes in the digestive tract, improving growth, protein efficiency ratio and feed efficiency (Midhun et al 2018; Nargesi et al 2019).

Swamp microbial addition factor, commercial nitrification bacteria addition factor, and interactions between factors influenced the feed efficiency in a significantly different manner, depending on the treatment. Based on the results of the LSD<sub>0.05</sub> test (Table 7) on the main effect of the addition of commercial nitrification bacteria, the increase of the absolute weight of *C. striata* following the treatment of the rearing media was: (1) significantly higher without commercial nitrification bacteria than with commercial nitrification bacteria; (2) significantly higher with the addition of a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. than with the other treatments; (3) significantly higher than the other treatments' results with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, but (4) not significantly higher from the treatment results given a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrifying bacteria.

Table 7

LSD 0.05 test results of growth in absolute weight of *Channa striata*

The single effect of nitrifying bacteria (N)	The single influence of swamps microbes (P) (LSD <sub>0.05</sub> =0.08)				The main effects of nitrifying bacteria (N) (LSD <sub>0.05</sub> =0.04)
	P1	P2	P3	P4	
N1	1.30 <sup>a</sup>	2.26 <sup>f</sup>	1.70 <sup>c</sup>	2.32 <sup>f</sup>	1.90 <sup>b</sup>
N2	1.73 <sup>c</sup>	1.41 <sup>b</sup>	1.88 <sup>d</sup>	2.08 <sup>e</sup>	1.78 <sup>a</sup>
The main effects of swamp microbes (P) (LSD <sub>0.05</sub> =0.05)	1.51 <sup>a</sup>	1.84 <sup>b</sup>	1.79 <sup>b</sup>	2.20 <sup>c</sup>	

Based on the results of the variance analysis, swamps microbial addition factor and interactions between factors significantly influenced the increase of the absolute length, but the factor of adding commercial nitrification bacteria has no significant effect. The LSD results of growth in absolute length of *C. striata* are presented in Table 8.

Table 8

LSD test results for growth in the absolute length of *Channa striata*

The single effect of nitrifying bacteria (N)	The single influence of swamps microbes (P) (LSD <sub>0.05</sub> =0.10)				The main effects of nitrifying bacteria (N) (not significant)
	P1	P2	P3	P4	
N1	0.69 <sup>a</sup>	1.79 <sup>e</sup>	0.91 <sup>b</sup>	2.27 <sup>f</sup>	2.12
N2	1.13 <sup>c</sup>	1.08 <sup>c</sup>	1.60 <sup>d</sup>	1.74 <sup>e</sup>	2.09
The main effects of swamp microbes (P) (LSD <sub>0.05</sub> =0.07)	0.91 <sup>a</sup>	1.44 <sup>c</sup>	1.26 <sup>b</sup>	2.00 <sup>d</sup>	

The main influence of the addition of microbes on the increase of the absolute length of snakehead fish in the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. was significantly different than in other treatments. The influence of interactions between factors on the increase of the absolute length of *C. striata* was significantly higher in the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria than the in the results of the other treatments.

The addition of a combination of swamp microbes and commercial nitrification bacteria gave significant results on the increase of absolute weight and absolute length. The highest absolute weight and length increase was produced by the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, and the lowest by the treatment without the addition of microbes. The treatment without the addition of microbes is thought to be the less effective in terms of absorption of nutrients in the feed, causing a sub-optimal growth, compared to the other treatments. In the treatment of N1P4, swamp microbes added to the media could be entered into the digestive tract of *C. striata* during the respiration process and when eating. These microbes break down complex compounds into simpler ones and increase digestibility of feed, by accelerating the process of absorption of the food. The work of probiotics in aquaculture is the ability of microbes to break down proteins, polysaccharides, lipids and stress resistance in aquaculture system (de melo Pereira et al 2018). The addition of 10<sup>4</sup> CFU mL<sup>-1</sup> probiotics to the rearing media increased the length and weight of pacific white shrimp (*L. vannamei*) larvae, compared to the controls (Widarnani et al 2010). *Bacillus licheniformis* at 10<sup>5</sup> CFU mL<sup>-1</sup> in the rearing media of *Pangasius hypophthalmus* showed a significant increase in the growth, immune and antioxidant responses compared to 10<sup>7</sup> CFU mL<sup>-1</sup> (Gobi et al 2016).

**Dissolved oxygen and ammonia in the rearing media.** The results of the analysis of variance on day 0 showed that the factor of commercial nitrification bacteria, swamp microbes and the interaction between the factors did not significantly influence the dissolved oxygen content (Table 9). At 5 and 10 days after rearing start, the factor of commercial nitrification bacteria and swamp microbes significantly affected the dissolved oxygen content, but the interaction between the factors had no significant effect.

The results of dissolved oxygen in the rearing media at 5 and 10 days after rearing start showed that the main effect of the addition of commercial nitrification bacteria was significantly lower than without commercial nitrification bacteria.

The main influence of the addition of microbes from swamp on the dissolved oxygen in the rearing media was the best for dissolved oxygen concentration in culture media with a combination of Chlorophyta, *Bacillus* sp., and *Streptomyces* sp. (P4), than in other treatments.

Table 9

LSD test result of dissolved oxygen in the rearing media

		<i>Dissolved oxygen (mg L<sup>-1</sup>)</i>								
		<i>Days after rearing</i>								
		0	5	10	15	20	25	30	35	40
LSD					0.18	0.14	0.14	0.18	0.18	0.18
N1P1		3.60 ±0.1	3.53 ±0.1	3.53 ±0.1	3.40 ±0.1 <sup>c</sup>	3.10 ±0.1 <sup>c</sup>	3.00 ±0.1 <sup>c</sup>	2.97 ±0.1 <sup>c</sup>	2.77 ±0.1 <sup>c</sup>	2.67 ±0.1 <sup>c</sup>
N1P2		3.63 ±0.1	3.53 ±0.1	3.53 ±0.1	3.23 ±0.1 <sup>ab</sup>	2.93 ±0.1 <sup>ab</sup>	2.83 ±0.1 <sup>ab</sup>	2.77 ±0.1 <sup>ab</sup>	2.57 ±0.1 <sup>ab</sup>	2.47 ±0.1 <sup>ab</sup>
N1P3		3.67 ±0.1	3.57 ±0.2	3.47 ±0.2	3.33 ±0.1 <sup>bc</sup>	3.03 ±0.1 <sup>bc</sup>	2.93 ±0.1 <sup>bc</sup>	2.87± 0.1 <sup>bc</sup>	2.67± 0.1 <sup>bc</sup>	2.57 ±0.1 <sup>bc</sup>
N1P4		3.57 ±0.1	3.70 ±0.1	3.60 ±0.1	3.60 ±0.1 <sup>d</sup>	3.50 ±0.1 <sup>e</sup>	3.40 ±0.1 <sup>e</sup>	3.40 ±0.1 <sup>e</sup>	3.20 ±0.1 <sup>e</sup>	3.10 ±0.1 <sup>e</sup>
N2P1		3.63 ±0.1	3.43 ±0.1	3.43 ±0.1	3.13 ±0.1 <sup>a</sup>	2.83 ±0.1 <sup>a</sup>	2.73 ±0.1 <sup>a</sup>	2.67 ±0.1 <sup>a</sup>	2.47 ±0.1 <sup>a</sup>	2.37 ±0.1 <sup>a</sup>
N2P2		3.57 ±0.1	3.47 ±0.1	3.47 ±0.1	3.33 ±0.1 <sup>bc</sup>	3.03 ±0.1 <sup>c</sup>	2.93 ±0.1 <sup>bc</sup>	2.87 ±0.1 <sup>bc</sup>	2.67 ±0.1 <sup>bc</sup>	2.57 ±0.1 <sup>bc</sup>
N2P3		3.53 ±0.1	3.43 ±0.1	3.33 ±0.1	3.27 ±0.1 <sup>ab</sup>	3.10 ±0.1 <sup>c</sup>	3.00 ±0.1 <sup>c</sup>	2.90 ±0.1 <sup>bc</sup>	2.70 ±0.1 <sup>bc</sup>	2.60 ±0.1 <sup>c</sup>
N2P4		3.67 ±0.1	3.63 ±0.1	3.53 ±0.1	3.40 ±0.1 <sup>c</sup>	3.30 ±0.1 <sup>d</sup>	3.20 ±0.1 <sup>d</sup>	3.20 ±0.1 <sup>d</sup>	3.00 ±0.1 <sup>d</sup>	2.90 ±0.1 <sup>d</sup>
LSD			0.08	0.08	0.09	0.07	0.07	0.09	0.09	0.09
N1		3.62	3.58 <sup>b</sup>	3.53 <sup>b</sup>	5.09 <sup>b</sup>	3.14 <sup>b</sup>	3.04 <sup>b</sup>	3.00 <sup>b</sup>	2.80 <sup>b</sup>	2.70 <sup>b</sup>
N2		3.60	3.49 <sup>a</sup>	3.44 <sup>a</sup>	4.93 <sup>a</sup>	3.07 <sup>a</sup>	2.97 <sup>a</sup>	2.91 <sup>a</sup>	2.71 <sup>a</sup>	2.61 <sup>a</sup>
LSD			0.11	0.11	0.13	0.10	0.10	0.13	0.13	0.13
P1		3.62	3.48 <sup>a</sup>	3.48 <sup>ab</sup>	3.27 <sup>a</sup>	2.97 <sup>a</sup>	2.87 <sup>a</sup>	2.82 <sup>a</sup>	2.62 <sup>a</sup>	2.52 <sup>a</sup>
P2		3.60	3.50 <sup>a</sup>	3.50 <sup>ab</sup>	3.28 <sup>a</sup>	2.98 <sup>a</sup>	2.88 <sup>a</sup>	2.82 <sup>a</sup>	2.62 <sup>a</sup>	2.52 <sup>a</sup>
P3		3.60	3.50 <sup>a</sup>	3.40 <sup>a</sup>	3.30 <sup>a</sup>	3.07 <sup>a</sup>	2.97 <sup>a</sup>	2.88 <sup>a</sup>	2.68 <sup>a</sup>	2.58 <sup>a</sup>
P4		3.62	3.67 <sup>b</sup>	3.57 <sup>b</sup>	3.50 <sup>b</sup>	3.40 <sup>b</sup>	3.30 <sup>b</sup>	3.30 <sup>b</sup>	3.10 <sup>b</sup>	3.00 <sup>b</sup>

Ten days after rearing start, the P4 treatment had a significantly higher dissolved oxygen content than the P3 treatment, but it was not significantly different from the P1 and P2 treatments. The factors of commercial nitrification bacteria, microbial addition and their interaction significantly influenced the dissolved oxygen content. The dissolved oxygen test on the 15, 20, 25, 30, 35 and 40<sup>th</sup> days are presented in Table 9.

Ammonia analysis results on day 0 were not significantly influenced by the factor of commercial nitrification bacteria, addition and their interaction. The variance analysis results on days 5, 10, 15, 20, 25, 30, 35 and 40 showed that the factor of commercial nitrification bacteria, microbial addition and their interaction significantly affected ammonia content. They are presented in Table 10. The results of LSD test on the 5, 10, 15, 20, 25, 30, 35 and 40<sup>th</sup> days on the main effect of the addition of commercial

nitrification bacteria showed that the ammonia content in the rearing media with commercial nitrification bacteria was significantly lower than in the treatments without commercial nitrification bacteria. The main effect of the addition of microbes from the swamp suggested that the ammonia content in the rearing media with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. was the best for reducing ammonia concentration of rearing media compared to the other treatments. The results of LSD on the 5, 10, 15 and 20<sup>th</sup> days, in the scenario of the interaction between factors, demonstrated that the N1P4 treatment had significantly lower ammonia levels compared to the other treatments, but not significantly different from N2P2 treatment. The results of LSD on the 25, 30, 35 and 40<sup>th</sup> days in the scenario of the interaction between factors, suggested that the N1P4 treatment had significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 and N2P4 treatments.

Table 10

LSD test results for ammonia every 5 days on the rearing media

		Mean of ammonia concentration ( $\text{mg L}^{-1}$ )								
		Days after rearing								
		0	5	10	15	20	25	30	35	40
LSD			0.013	0.013	0.014	0.013	0.020	0.021	0.019	0.022
N1P1		0.290 $\pm 0.07$	0.383 $\pm 0.01^e$	0.393 $\pm 0.0^d$	0.410 $\pm 0.01^d$	0.327 $\pm 0.01^e$	0.540 $\pm 0.01^d$	0.674 $\pm 0.01^e$	0.691 $\pm 0.01^f$	0.948 $\pm 0.02^e$
N1P2		0.323 $\pm 0.03$	0.273 $\pm 0.01^d$	0.283 $\pm 0.01^c$	0.223 $\pm 0.01^c$	0.203 $\pm 0.01^c$	0.293 $\pm 0.01^b$	0.314 $\pm 0.01^b$	0.321 $\pm 0.01^c$	0.324 $\pm 0.01^b$
N1P3		0.283 $\pm 0.01$	0.257 $\pm 0.01^c$	0.267 $\pm 0.01^b$	0.207 $\pm 0.01^b$	0.197 $\pm 0.01^{bc}$	0.363 $\pm 0.02^c$	0.482 $\pm 0.02^d$	0.585 $\pm 0.01^e$	0.692 $\pm 0.02^d$
N1P4		0.267 $\pm 0.01$	0.220 $\pm 0.01^a$	0.230 $\pm 0.01^a$	0.170 $\pm 0.01^a$	0.147 $\pm 0.01^a$	0.243 $\pm 0.02^a$	0.260 $\pm 0.02^a$	0.265 $\pm 0.02^a$	0.270 $\pm 0.02^a$
N2P1		0.230 $\pm 0.01$	0.253 $\pm 0.01^c$	0.263 $\pm 0.01^b$	0.203 $\pm 0.01^b$	0.193 $\pm 0.01^{bc}$	0.282 $\pm 0.02^b$	0.363 $\pm 0.02^c$	0.385 $\pm 0.01^d$	0.392 $\pm 0.02^c$
N2P2		0.290 $\pm 0.04$	0.233 $\pm 0.01^{ab}$	0.243 $\pm 0.01^a$	0.183 $\pm 0.01^a$	0.160 $\pm 0.01^a$	0.250 $\pm 0.01^a$	0.268 $\pm 0.01^a$	0.273 $\pm 0.01^a$	0.277 $\pm 0.01^a$
N2P3		0.250 $\pm 0.02$	0.277 $\pm 0.01^d$	0.287 $\pm 0.01^c$	0.227 $\pm 0.01^c$	0.187 $\pm 0.01^b$	0.277 $\pm 0.01^b$	0.296 $\pm 0.01^b$	0.300 $\pm 0.01^b$	0.306 $\pm 0.01^b$
N2P4		0.303 $\pm 0.03$	0.237 $\pm 0.0^b$	0.247 $\pm 0.01^b$	0.187 $\pm 0.01^b$	0.163 $\pm 0.01^b$	0.250 $\pm 0.01^a$	0.268 $\pm 0.01^a$	0.273 $\pm 0.01^a$	0.277 $\pm 0.01^a$
LSD			0.006	0.006	0.007	0.006	0.010	0.011	0.010	0.011
N1		0.291	0.283 <sup>b</sup>	0.293 <sup>b</sup>	0.253 <sup>b</sup>	0.218 <sup>b</sup>	0.360 <sup>b</sup>	0.432 <sup>b</sup>	0.466 <sup>b</sup>	0.558 <sup>b</sup>
N2		0.268	0.250 <sup>a</sup>	0.260 <sup>a</sup>	0.200 <sup>a</sup>	0.176 <sup>a</sup>	0.265 <sup>a</sup>	0.299 <sup>a</sup>	0.308 <sup>a</sup>	0.313 <sup>a</sup>
LSD			0.009	0.009	0.010	0.009	0.014	0.015	0.013	0.016
P1		0.260	0.318 <sup>d</sup>	0.328 <sup>d</sup>	0.307 <sup>d</sup>	0.260 <sup>d</sup>	0.411 <sup>d</sup>	0.518 <sup>d</sup>	0.538 <sup>d</sup>	0.670 <sup>d</sup>
P2		0.307	0.253 <sup>b</sup>	0.263 <sup>b</sup>	0.203 <sup>b</sup>	0.182 <sup>b</sup>	0.272 <sup>b</sup>	0.291 <sup>b</sup>	0.297 <sup>b</sup>	0.300 <sup>b</sup>
P3		0.267	0.267 <sup>c</sup>	0.277 <sup>c</sup>	0.217 <sup>c</sup>	0.192 <sup>c</sup>	0.320 <sup>c</sup>	0.389 <sup>c</sup>	0.443 <sup>c</sup>	0.499 <sup>c</sup>
P4		0.285	0.228 <sup>a</sup>	0.238 <sup>a</sup>	0.178 <sup>a</sup>	0.155 <sup>a</sup>	0.247 <sup>a</sup>	0.264 <sup>a</sup>	0.269 <sup>a</sup>	0.274 <sup>a</sup>

Related to the factor of adding nitrifying bacteria, the lowest ammonia content was observed in the treatment with nitrifying bacteria. It is assumed that the added nitrification bacteria are able to carry out the process of nitrification on the rearing media. According to Rurangwa et al (2014), nitrification takes place through 2 reaction stages, where in the first stage the oxidation of ammonium to nitrite is carried out by ammonium oxidizing microbes (*Nitrosomonas* sp.) and in the second stage nitrite oxidation is performed by nitrite oxidizing microbes (*Nitrobacter* sp.). Among the addition of swamp microbial factors, the lowest ammonia content was observed in the treatment with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. It is suspected that the three microbes could break down ammonia. Ammonia is the main source of nitrogen in addition to nitrate, which could be used by microalgae for their metabolic processes

(Nurhayati et al 2014). *Bacillus* sp. could oxidize ammonia to nitrite through heterotrophic and chemotrophic processes (Edwards 2011).

Among the factors' interactions, N1P4 treatment had the lowest ammonia levels on the 5<sup>th</sup> day until the end of rearing. It was suspected that microbes from the swamp were able to break down the organic material, derived from feces or feed, into compounds that were not harmful to *C. striata*. The N1P4 results were not significantly different from the N2P2 and N2P4 results. In presence of the commercial nitrification bacteria in the same ecosystem as *Bacillus* sp. and *Streptomyces* sp., the nitrification process activity and the growth of nitrifying bacteria are inhibited. Nitrifying bacteria (autotrophic bacteria) produce very small biomass and slow growth. Nitrifying bacteria takes 12 hours to regenerate, while heterotrophic bacteria *Bacillus* sp. and *Streptomyces* sp. only need 30 minutes (Ebiling et al 2006). If there is a limited nitrogen to carbon (high C:N ratio), the nitrification process is inhibited and heterotrophic bacteria develop rapidly. Heterotrophic bacteria will assimilate ammonia directly into bacterial proteins, and they will develop rapidly. It will cause competition in the struggle for oxygen, space and nutrients with autotrophic bacteria (Blancheton et al 2013).

Ammonia levels in all treatments with microbes on 10<sup>th</sup> day to 20<sup>th</sup> day of rearing decreased and increased until the 40<sup>th</sup> day. On the 10<sup>th</sup> to 20<sup>th</sup> day, it was presumed that the ammonia accumulation from metabolic waste had not yet occurred, therefore the microbes given to the rearing media were able to work optimally. On the 25<sup>th</sup> to 40<sup>th</sup> day, it was supposed that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the added microbes couldn't optimally reduce their ammonia levels. Meanwhile, the treatment without the addition of microbes (N1P1) experienced an increase in ammonia along with the increase in the rearing time. Increasing ammonia levels in the N1P1 treatment was caused of the rearing without siphoning during 40 days. It resulted in a buildup of organic material derived from metabolic waste and levels of ammonia in the rearing media. The decomposing bacteria from natural swamp water added to the media have not been able to make an optimal decomposition.

**Conclusions.** The addition of swamp microbes Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. on the *C. striata* rearing media was more efficient than other treatments because they provided better water quality values and gave the best survival rate, feed efficiency and growth of *C. striata* in swamp aquaculture, although there was no nitrification bacteria used. *Bacillus* and *Streptomyces* were the best combination of microbial swamp for *C. striata* culture in swamp water aquaculture which used Chlorophyta as green water system.

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