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Impact of Probiotic Swamp Bacteria on the Prevention of *Aeromonas hydrophila* Infection in Striped Snakehead (*Channa striata*)

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ABSTRACT

Probiotics are microbes that can modify the bacterial structure in fish digestive systems. Probiotics are used as feed additives and can improve the host's health. As a result, it can help to prevent Aeromonas infection. This study aims to determine the effectiveness of bacteria from the swamp (*Bacillus* sp. and *Streptomyces* sp.) on feed in preventing *Aeromonas hydrophila* infection in striped snakeheads. The study used P0 (control), P1 (*Bacillus* sp.), P2 (*Streptomyces* sp.), P3 (*Bacillus* sp. and *Streptomyces* sp. combination), each bacterium 10 ml kg^{-1} feed. Total of bacteria, prevalence, fish growth, survival rate, and water quality are among the variables assessed. The fish survival, fish growth, and feed efficiency in Channa treated with a combination of Bacillus and Streptomyces (P3) were significantly highest among other, resulting of best prevalence 2.38%.

INTRODUCTION

Snakehead fish (*Channa striata*) is a swamp fish that lives in most Indonesian swampy waters and is highly commercially valuable (Setyaningrum *et al*., 2024). One of the diseases that often attacks freshwater fish, including Channa fish, is Motile Aeromonas Septicaemia (MAS), which is caused by the bacteria *Aeromonas hydrophila* (Dao *et al*., 2022). The histological results of the intestines of snakehead fish showed inflammation, which was suspected to be caused by *Aeromonas hydrophila* (Wu *et al*., 2024). One alternative that can be done to prevent this disease is by applying probiotics (Sayyed *et al*., 2014). Based on Setiyaningsih *et al*. (2018) statement that probiotic bacteria in feed can inhibit the growth of *A. hydrophila* bacteria and increase fish survival by 93% in catfish after a challenge test. The LD_{50} challenge test with an injected dose of *A. hydrophila* 10^5 cfu. mL^{-1} was able to attack snakehead fish (Olga, 2012).

Probiotic bacteria can help increase nutrient absorption so that it can improve host performance of growth (Zhang *et al*., 2012; Wijayanti *et al*., 2020), improve microbial balance and reduce the presence of pathogens in the digestive tract (Dawood, 2021), and increase the microbial population and produce helpful enzymes that aid in digestion and protect the gastrointestinal tract of fish (Assan *et al*., 2022). Based on the results of (Wijayanti *et al*., 2020), *Streptomyces* sp. bacteria are cellulolytic and proteolytic bacteria isolated from swamp and pond water sediments where *Bacillus* sp. and *Streptomyces* sp. can help the digestion process of snakehead fish, which can increase the value of feed efficiency and feed digestibility. The types of probiotic bacteria that are usually used in aquaculture activities are *Lactococcus* sp., *Staphylococcus* sp., *Micrococcus, Rhodococcus, Bacillus* sp., *Paenibacillus, Lactobacillus* sp., *Aeromonas, Alteromonas, Arthrobacter, Microbacterium, Carnobacterium* sp., *Bifidobacterium, Clostridium, Rhodosporidium, Vibrio, Enterovibrio, Aliivibrio, Phaeobacter, Roseobacter, Pseudoalteromonas, Pseudomonas, Rhodopseudomonas, Shewanella* and *Kocuria,* as probiotics in finfish aquaculture (Ringø *et al*., 2022). The results of the study by Setiawati *et al*. (2013), the addition of commercial probiotics containing *Bacillus* sp. 10^6 cfu.mL^{-1} with a dose of 10 mL.kg^{-1} feed produced a feed efficiency value of 65.32% and protein retention of 36.15% in catfish. Based on research by Bernal *et al.*, (2017), the combination of *Bacillus* sp. and *Streptomyces* sp. given through spraying on feed to shrimp are both bacteria and probiotic candidates that can increase growth and survival and increase disease resistance. In this study, a combination of probiotic bacteria from swamps was added in the form of *Bacillus* sp. and *Streptomyces* sp., which are expected to affect preventing *Aeromonas hydrophila* bacterial attacks on snakehead fish, which are typical swamp fish.

This study aims to know the impact of giving probiotic candidate from bacteria *Bacillus* and *Streptomyces* in feed to decrease *A. hydrophila* bacterial infection. The usefulness of this study is as an alternative in preventing *A. hydrophila* infection in snakehead fish cultivated in swamp areas.

METHODS

Time and Place

The study was conducted in 2019. This research was conducted in the Laboratory of Aquaculture and Experimental Pond Study Program, Laboratory of Microbiology and Biotechnology, Faculty of Agriculture, Universitas Sriwijaya.

Tools and Materials

This study used the following tools: Erlenmeyer flask, measuring cup, spatula, hot plate, laminar air flow, spreader rod, colony counter, vortex, syringe, micropipette, petri dish, test tube, tube, shaker, pH meter, DO meter, aquarium, thermometer, magnetic stirrer, aerator, aeration stone, micropippet, autoclave, ose needle, digital scale, analytical scale, spray bottle, and digital caliper. The materials used in this study were swamp water, snakehead fish (5 ± 1 cm), nutrient agar, nutrient broth, yeast extract, malt extract, bactopeptone, glucose, agar, Tryptone Soya Broth, Tryptone Soya Agar, Glutamate Starch Phenole, alcohol, distilled water, isolates of *Bacillus* sp. and *Streptomyces* sp., physiological NaCl solution, molasses, commercial feed, and rifampicin antibiotic.

Research Design

The research design used was a Completely Randomized Design, with four treatments and three replications. The treatments given were different combinations of swamp bacteria in the feed, such as a combination of bacteria *Bacillus* and *Streptomyces* in shrimp research (Bernal *et al*., 2017). The research design is as follows:

P0: Control, feeding without the addition of bacteria
P1: Feeding with the addition of *Bacillus* sp. (10^6 CFU mL^{-1}) 10 ml.kg^{-1} feed
P2: Feeding with the addition of *Streptomyces* sp. (10^6 CFU mL^{-1}) 10 ml.kg^{-1} feed
P3: Feeding with the addition of *Bacillus* sp. (10^6 CFU mL^{-1}) 5 ml.kg^{-1} feed and *Streptomyces* sp. (10^6 CFU mL^{-1}) 5 ml.kg^{-1} feed.

Research Procedures

Cultivation and Propagation of Bacteria

The swamp bacteria tested were isolates from previous research (Wijayanti *et al*, 2018) collected at the Aquaculture and Experimental Pond Laboratory. Pure *Bacillus* sp bacteria were streaked in a petri dish containing NA (Nutrient Agar) media that had been given a rifampicin resistance marker (Rif^R) at a 25 µg/mL dose. Then the petri dish was wrapped using wrapping paper and incubated for 2 days at a temperature of (28-30) ^oC and observed until the bacteria grew (Setyati & Subagiyo, 2012). Pure *Streptomyces* sp bacteria were transferred into a petri dish containing YM (Yeast Malt Agar) media that had been given a rifampicin resistance marker (Rif^R) at a 25 µg/mL dose. Then, the culture was stored for 5 days at 28-30°C.

A single colony of *Bacillus* sp was streaked into an agar slant containing NA (Nutrient Agar) media that had been given a rifampicin resistance (Rif^R) marker at a dose of 25 µg. mL^{-1} and then incubated at room temperature (28-30^{o}C) for 2 days. A single colony of *Streptomyces* sp was streaked into an agar slant containing YM (Yeast Malt Agar) media that had been given a rifampicin resistance (Rif^R) marker at a dose of 25 µg. mL^{-1}, then incubated at room temperature (28-30^{o}C) for 5 days. The pure cultures of *Bacillus* sp. and *Streptomyces* sp were multiplied. *Bacillus* sp that grew in NA media were multiplied in liquid NB media, the suspension was taken as much as 1 ose to be cultured in NB media in an Erlenmeyer flask as much as 50 ml. *Streptomyces* sp bacteria were multiplied in liquid YM media in an Erlenmeyer flask to a volume of 50 ml. Next, each media that has been filled with bacteria is stirred for 2 days for *Bacillus* sp. and for 5 days for *Streptomyces* sp.

Fish Maintenance and Feeding Test

16 aquariums measuring 40x40x40 cm^3 were prepared as maintenance media. The aquariums were first washed using potassium permanganate for 24 hours, then rinsed using clean water and dried. The aquariums were filled with 24 L of water with a stocking density of 15 fish per aquarium. The snakehead fish used were 5±1 cm long. The fish were adapted for 7 days and given commercial feed (40% protein) at satiation with a frequency of three times a day. After the adaptation period, the snakehead fish were given commercial feed (40% protein) that had been mixed with swamp bacteria, namely *Bacillus* sp. Rif^R and *Streptomyces* sp. Rif^R by spraying the feed with a dose at the beginning of spraying 10^6 $cfu.ml^{-1}$. Bacteria on the feed was carried out once a week. After spraying, the feed was left to dry and stored in the refrigerator. After the feeding containing the swamp bacteria was completed, it was continued on the 15^{th} day until the end of the observation by giving commercial feed without treatment. Feeding was done 3 times a day at satiation.

After the adaptation period, the snakehead fish were given commercial feed (40% protein) mixed with swamp bacteria, namely *Bacillus* sp. Rif^R and *Streptomyces* sp. Rif^R by spraying the feed with a dose at the beginning of spraying 10^6 $cfu.ml^{-1}$. Bacteria were sprayed on the feed once a week. After spraying, the feed was left to dry and stored in the refrigerator. After the feeding containing swamp bacteria was completed, it was continued on the 15^{th} day until the end of the observation by giving commercial feed without treatment. Feeding was done 3 times a day at satiation.

Challenge Test and Bacterial Population Counting

A. hydrophila bacterial challenge test was conducted after the fish had been fed with the treatment. Snakehead fish were injected with as much as 0.1 ml per individu of *A. hydrophila* bacteria from a liquid medium containing 10^5 $cfu.mL^{-1}$ bacterial culture (Olga, 2012) on the 15th day. Fish were dissected before and after the challenge test for bacterial counts in the snakehead fish intestines. Then, prevalence observations were made 24 hours after fish injection. During maintenance, bacterial counts were conducted on days 0, 1, 14, 16, and 23, starting with taking a 1-gram sample with 9 ml of physiological solution. The results of the 10^{-3} to 10^{-5} dilutions were taken with a pipette one by one as much as 0.1 ml and put into each petri dish then spread in a petri dish as much as 50 µl of NA media for *Bacillus* sp. Rif^R, YM for Streptomyces sp. Rif^R, and GSP for *A. hydrophila*. Colony counts were performed using a colony counter.

Observed Parameters

Number of Bacteria in the Intestines

The population of *A. hydrophila* bacteria in the intestines of snakehead fish is calculated using the following formula (Madigan *et al*, 2019):

Bacterial population (CFU/ml) = total number of colonies at agar media x 1/dilution fold

Percentage of Fish Affected by Disease (Prevalence)

Prevalence observation was conducted to determine the percentage of fish infected with *A. hydrophila* bacteria. The percentage of fish affected by the disease was calculated using the following formula (Dewi, 2018):

Prevalences (%) = (Number of fish affected by disease/total number of examined fish) x 100

Fish Survival

The survival of snakehead fish was calculated using the following formula (Do et al., 2021):

$$Survival = Nt/No \times 100\%$$

Description:

Nt : Number of fish at final of rearing (ind)

No : Number of fish at initial of rearing (ind)

Feed Efficiency

Feed utilization efficiency was calculated using the formula according to *de Verdal et al*. (2018), as follows:

$$EP = ((Wt+D)-Wo))/F$$

Description:

EP : Feed Efficiency (%)

Wt : final fish weight (g)

Wo : initial fish weight (g)

F : intake feed (g)

D : weight of dead fish during maintenance (g)

Absolute Length Growth

The absolute length growth of fish was calculated using the following formula (Lugert *et al*., 2016):

$$L = Lt - Lo$$

Description:

L : Growth of Fish Length (cm)

Lt : Average length of fish at the final rearing (cm)

Lo : Average length of fish at the initial rearing (cm)

Absolute Weight Growth

Fish weight gain during maintenance was calculated using the following formula (Lugert *et al*., 2016):

$$W = Wt - Wo$$

Description:

W : growth of fish weight (g)
Wt : Fish weight at the final rearing (g)
Wo : Fish weight at the initial rearing (g)

Water Quality

Measurement of water quality parameters includes temperature, dissolved oxygen, pH, and ammonia. The pH and temperature values were measured daily during the study. Dissolved oxygen and ammonia measurements were measured at the beginning and end of rearing.

Data Analysis

Survival, growth, feed efficiency, and prevalence data were analyzed using variance analysis with a 95% confidence level. If the results of the variance analysis show a significant response, then it is continued with the Least Significant Difference test. Data on the number of bacteria in the intestine and water quality were analyzed descriptively.

RESULTS

The results of the research that has been conducted during the maintenance of snakehead fish using swamp bacteria as probiotic candidates in feed are as follows:

Prevalence of Snakehead Fish

Prevalence is the percentage of fish that are attacked by disease. Data from calculating the prevalence of Channa fish during maintenance are presented in Table 1.

Table 1. Prevalence Data of Snakehead Fish During Maintenance

Treatment	Prevalence 24 hours (%) $LSD_{0.05}$ = 9.8383	Prevalence 48 hours (%) $LSD_{0.05}$ = 9.2286	Prevalence 72 hours (%) $LSD_{0.05}$ =10.2412
P0	62.2222^c±7.6980	59.0909^d±7.8730	58.3333^c±7.2169
P1	37.7778^b±3.8490	26.9231^c±3.3309	24.0741^b±5.2509
P2	35.5555^b±3.8490	17.5824^b±4.7900	14.2191^b±4.6562
P3	24.4445^a±3.8490	6.9842^a±0.2749	2.3810^a±4.1238

Number of Bacteria in the Gut

The results of measuring the density of bacteria isolated in the guts are in Table 2. The density of *Bacillus* sp. and *Streptomyces* sp. bacteria is in Table 3. The density of *A. hydrophila* bacteria is in Table 4.

Table 2. Data on the Density of Bacteria Isolated in the Guts of Snakehead Fish

Treatment	Total Density of Bacteria($\times 10^7$ cfu.mL^{-1})				
	Day - 0	Day - 1	Day - 14	Day - 16	Day - 23
P0	0.0100±0.0000	0.1273±0.0242	2.5587±0.3433	3.4847±0.2901	2.6730±0.1090
P1	0.0100±0.0000	0.1113±0.0006	2.6353±0.6980	3.8003±0.3286	1.5063±0.0268
P2	0.0100±0.0000	0.1143±0.0043	3.3350±0.4533	3.9253±0.3021	1.6643±0.0810
P3	0.0100±0.0000	0.2997±0.0510	6.8477±0.9849	8.6077±0.6202	4.0873±0.6601

Note: Bacterial density was calculated using the Total Plate Count method wit Colony forming unit per milliliters (cfu mL^{-1})

The density of bacteria on day 0 was calculated before being given the treatment feed. In contrast, the density of bacteria on day 1 was calculated after 24 hours of being given the treatment feed. Based on the calculation of bacteria in the intestines on days 1, 14, and 16, it showed that the total bacteria increased in each treatment. On day 23, it showed that the total density of bacteria decreased in each treatment.

Table 3. Data on the Density of *Bacillus* sp. and *Streptomyces* sp. Bacteria in the Gut of Snakehead Fish

Treatment	Total Density of Bacteria ($\times 10^7$ cfu.mL^{-1})			
	Day - 1	Day - 14	Day - 16	Day - 23
P1 B	0.0183 ± 0.0020	0.2780 ± 0.0622	0.1260 ± 0.0170	0.1343 ± 0.0061
P2 S	0.0155 ± 0.0049	0.2562 ± 0.0730	0.2091 ± 0.0511	0.2414 ± 0.0613
P3 B	0.0200 ± 0.0020	0.3654 ± 0.0912	0.2503 ± 0.0770	0.3523 ± 0.0040
P3 S	0.0214 ± 0.0015	0.4000 ± 0.0450	0.2243 ± 0.0222	0.2931 ± 0.0522

Note: Bacterial density is calculated using the Total Plate Count (TPC) method with Colony Forming Unit per Milliliter (CFU.mL^{-1}) units. B (*Bacillus* sp.) and S (*Streptomyces* sp.)

The density of *Bacillus* sp. and *Streptomyces* sp. bacteria on day 1 was calculated after 24 hours of feeding the treatment. Based on the calculation of bacteria on day 14, it showed that the population of *Bacillus* sp. and *Streptomyces* sp. bacteria increased in each treatment. On day 16, the calculation of bacteria showed that the density of bacteria decreased for each treatment. On day 23, the density of bacteria increased in each treatment.

Table 4. Data on the Density of *Aeromonas hydrophila* Bacteria in the Gut of Snakehead Fish

Treatment	Total Density of Bacteria ($\times 10^7$ cfu.mL^{-1})	
	Day - 16	Day - 23
P0	0.6080 ± 0.2260	0.5670 ± 0.2650
P1	0.1293 ± 0.0161	0.0997 ± 0.0521
P2	0.1173 ± 0.0060	0.0740 ± 0.0142
P3	0.0910 ± 0.0042	0.0347 ± 0.0250

Note: Bacterial density is calculated using the Total Plate Count (TPC) method with Colony Forming Unit per Milliliter (CFU.mL^{-1}) units.

A. hydrophila challenge test was conducted on day 15. The density of *A. hydrophila* bacteria began to be calculated on day 16, 24 hours after the challenge test. Based on the calculation of bacteria on day 23, the population of *A. hydrophila* bacteria decreased in each treatment.

Fish Performances

Data of survival, feed efficiency, weight growth, and length of snakehead fish are showed at Table 5.

Table 5. Survival, Feed Efficiency, Weight Growth and Length Growth of Snakehead Fish

Treatment	Survival Rate (%) $LSD_{0.05}$ = 7.25	Feed Efficiency (%) $LSD_{0.05}$ = 13.13	Weight Growth(g) $LSD_{0.05}$ = 0.15	Length Growth (cm)
P0	28.8889^a±3.8490	30.0560^a±4.7410	0.9367^a±0.0289	0.7933±0.1210
P1	55.5556^b±3.8490	51.8185^b±7.8821	1.6667^b±0.1498	0.8959±0.1158
P2	62.2222^b±3.8490	56.5978^b±8.8254	1.7233^b±0.0231	0.6200±0.1498
P3	71.1111^c±3.8490	67.6545^b±5.6679	1.9167^c±0.0231	1.0900±0.0200

Note: Numbers followed by different superscript letters indicate significant differences between treatments in the 5% smallest significant difference test.

Table 5 shows that feeding with the addition of swamp bacteria as probiotic significantly differs in terms of survival, feed efficiency, and weight growth of snakehead fish; however, it was not significantly different in terms of length growth of snakehead fish.

Water Quality

Water quality during the maintenance of snakehead fish can still support the activities of snakehead fish (Table 6).

Table 6. Water Quality Range During Maintenance

Treatment	Temperature (°C)	pH	Dissolved Oxygene ($mg.L^{-1}$)	Ammonia ($mg.L^{-1}$)
P0	27.1-28.7	5.1-6.1	3.87-4.10	0.09-0.08
P1	27.1-28.8	5.1-6.2	3.87-4.12	0.09-0.09
P2	27.1-28.7	5.1-6.2	3.87-4.12	0.09-0.13
P3	27.1-28.8	5.1-6.3	3.87-4.14	0.09-0.15

Note: the temperature range was 24°C-36°C (Do *et al.*, 2021), pH was 5 - 8 (Ly *et al.*, 2024), DO was 0.2 – 8.6 $mg.L^{-1}$ (Puspaningsih *et al.*, 2018), ammonia <1.65 $mg.L^{-1}$ (Ha *et al.*, 2021).

DISCUSSION

Prevalence in Snakehead Fish

The prevalence of snakehead fish was calculated at 24 hours, 48 hours, and 72 hours after *A. hydrophila* bacteria injection. Fish fed with the addition of candidate probiotic swamp bacteria (P3) had a lower prevalence value compared to control fish (feed without treatment). From the ANOVA results, it is known that the provision of candidate probiotic bacteria in the feed has a significant effect on prevalence, so it is suspected that there is one treatment that produces a different prevalence response. From the results of the $LSD_{0.05}$ test, it is known that the prevalence of snakehead fish 24 hours, 48 hours, and 72 hours after injection at P3 was significantly higher than P0, P1, and P2. The candidate probiotic swamp bacteria can colonize the gut and inhibit the growth of *A. hydrophila* colonies. Probiotics affect the community of gut environmental microorganisms, provide benefits to their hosts (Balcázar *et al*., 2006), and can be used as a feed supplement that can improve the fish's immune system (Lara-flores, 2011). So when *A. hydrophila* bacteria enter, the fish's immune system is more ready to phagocytize pathogenic bacteria. So that *A. hydrophila* bacteria did not reach the quorum sensing level to cause fish to become sick. From the results of the study, it can be proven that 24 hours after the challenge test produced a prevalence value of 24.4445%, 48 hours after the challenge test 6.9842%, and 72 hours after the challenge test 2.3810%. Each treatment given the addition of bacteria from the swamp as a probiotic candidate resulted in a lower prevalence than the control.

The research results show that during the *A. hydrophila* challenge test through injection, *A. hydrophila* bacteria were found in the intestines of snakehead fish. The highest density of *A. hydrophila* bacteria in the intestines (0.6080 x 10^7 $cfu.mL^{-1}$) was found in P0, which was given without treatment and tested for challenge. This treatment resulted in the highest prevalence (62.2222%). In this case, the density of *A. hydrophila* bacteria in the intestines affected the prevalence of snakehead fish. The greater the bacterial population in the intestines, the higher the prevalence of snakehead fish. The lowest density of *A. hydrophila* bacteria in the intestines (0.0347 x 10^7 $cfu.mL^{-1}$) was found in P3, which was given feed with the addition of candidate probiotic swamp bacteria and tested for challenge. It is suspected that in the P3 treatment, fewer *A. hydrophila* bacteria attached to the intestines because of the presence of swamp bacteria that had previously colonized the intestines of snakehead fish, which caused the *A. hydrophila* bacteria to have no room to grow. Ahmadi *et al*. (2012) stated that probiotic activity contained in feed, such as forming colonies and attaching to the intestines, will suppress pathogenic bacteria from growing. In addition, when probiotics attach to the intestinal lymphoid tissue, it will accelerate the stimulation of macrophages to detect the presence of antigens (Nayak, 2010).

Total Bacteria in the Gut

Providing swamp-based bacteria, the probiotic candidate *Bacillus* sp. and *Streptomyces* sp. in feed for 14 days increased bacterial population, both total and rifampicin-resistant bacteria. The highest bacterial density results in the intestines of snakehead fish were obtained in treatment P3, namely Streptomyces sp. (0.4000 x 10^7 cfu.mL^{-1}) and Bacillus sp. (0.3654 x 10^7 cfu.mL^{-1}) before being challenged. After the *A. hydrophila* challenge test was carried out on the 15th day, there was an increase in the total bacterial population on the 16th day and a decrease in the bacterial population on the 23rd day in each treatment (Table 2). The results of (Syaifullah & Satyantini, 2024) study showed that the administration of probiotics in feed using EM-4 microorganisms consisting of *Lactobacillus* sp., *Streptomyces* sp, *Actinomycetes* sp, and yeast could increase the population of *Lactobacillus* sp. in the guts of snakehead fish. Bernal *et al.* (2017) stated that the combination of *Streptomyces* sp. and *Bacillus* sp. given through feed causes synergistic activity with each other, which can provide benefits to produce several extracellular enzymes (*Bacillus* sp.) and antibiotic compounds (*Streptomyces* sp.). According to Ringø *et al*. (2022), *Bacillus* sp. and LAB can produce antimicrobial compounds in the form of bacteriocins and antibiotics so that it can suppress the presence of pathogenic bacteria. Salamoni *et al*. (2010) stated that *Streptomyces* sp. has the potential to control pathogenic bacteria by competing, parasitizing, or producing secondary metabolite compounds and various biologically active compounds, such as antibacterial, antifungal, antiparasitic, and antiviral.

Survival, Feed Efficiency, and Growth of Snakehead Fish

The addition of swamp bacteria as probiotic candidates significantly differed on the survival of snakehead fish. Based on the $LSD_{0.05}$ test, the survival of snakehead fish treated with feed with the addition of a combination of *Bacillus* sp. (10^6 cfu.mL^{-1}) 5ml/kg feed and *Streptomyces* sp. (10^6 cfu.mL^{-1}) 5ml.kg^{-1} feed (P3) resulted in the highest survival and was significantly different from other treatments. This is thought to be due to using a combination of swamp bacteria as probiotic candidates that enter the digestive tract first and live in it. Furthermore, these bacteria in the fish's digestive tract will secrete digestive enzymes such as protease and amylase (Champasri *et al*., 2021). In addition, these bacteria can dominate the fish's digestive tract, and the presence of pathogenic bacteria will decrease so that the fish will utilize these good bacteria to grow and the fish will be healthy (Setiawati *et al*., 2013). Based on research results (de Moraes *et al*., 2022), the application of probiotics through feed and testing against *A. hydrophila* in Nile tilapia resulted in a survival rate of 98.10%.

Based on the $LSD_{0.05}$ test, feed treatment with the addition of a combination of Bacillus sp. and Streptomyces sp. (P3) produced significantly higher feed efficiency than P0, but not significantly different from treatments P1 and P2. The feed efficiency value is related to the amount of feed consumed by fish and the growth produced. Treatment P3, with the highest feed efficiency value, also produced the highest survival value and absolute weight growth during maintenance. Treatment P0, without the provision of candidate probiotic swamp bacteria in the feed, produced the lowest feed efficiency, survival, and absolute weight growth values compared to other treatments. This is thought to be due to the presence of *A. hydrophila* bacterial infection, which causes fish stress and fish begin to respond slowly to the feed given and the amount of feed consumed until the end of maintenance is also less than other treatments. This is by the statement (Muslim *et al*., 2009) that the response to fish appetite stimulation decreases due to infection with *A. hydrophila*, which is caused by metabolic disorders in the body, causing internal organ abnormalities in the form of swelling or inflammation of internal organs after injection of *A. hydrophila* bacteria.

Based on the $LSD_{0.05}$ test, the absolute weight growth of snakehead fish fed with a combination of candidate probiotic swamp bacteria *Bacillus* sp. and *Streptomyces* sp. (P3) was significantly different compared to other treatments. Meanwhile, the feed treatment without candidate probiotic swamp bacteria (P0) produced the lowest absolute weight growth compared to treatments P1, P2, and P3. (Irianto & Austin, 2002) stated that the provision of probiotics in aquaculture can be given through water or feed. The provision of probiotic bacteria in feed affects the speed of feed fermentation in the digestive tract, which can help the process of food absorption in fish digestion. So, in this study, the provision of a combination of *Bacillus* sp. and *Streptomyces* sp. bacteria had a significant effect on the absolute weight growth of snakehead fish. This is by the statement Setiyaningsih *et al*. (2018) that probiotics through feed can produce a more efficient level of feed utilization and can contribute to increasing digestive enzyme activity, thereby improving growth performance.

Bacillus ND2 has immunostimulant properties and can increase feed digestibility because it has lipolytic, proteolytic, and amylolytic enzymes (Lusiastuti *et al*., 2016). *Streptomyces* sp. is a cellulolytic and proteolytic bacteria isolated from swamp and pond water sediments. *Streptomyces* sp. is also able to produce bioactive compounds such as antibiotics, namely tetracycline, streptomycin, nystatin, neomycin, kanamycin, cycloheximide, cycloserine, lincomycin, aminoglycosides, aureomycin, chloramphenicol, nystatin, amphotericin, etc (de Lima Procópio *et al*., 2012; Madigan *et al*, 2019). According to Tan *et al*. (2016), *Streptomyces* sp. is a bacterium that has the potential as a probiotic that can improve growth performance because it can produce antagonistic compounds in the form of antibiotics (streptomycin) and antimicrobials. *Streptomyces* sp. bacteria are bacteria that can produce two antibiotic pigments, namely, undecylprodigiosin (red) and actinorhodin (blue) (Naeimpoor & Mavituna, 2000). The production of undecylprodigiosin in S*treptomyces* sp. increased after *Bacillus* sp. was added to the culture medium. This shows that *Bacillus* sp. bacteria can increase antibiotic production in *Streptomyces* sp.

Based on Table 2, the absolute length growth ranges from 0.7933 - 1.0900 cm. Based on the results of the analysis of variance, it is known that the provision of *Bacillus* sp. and *Streptomyces* sp. bacteria does not affect the absolute length growth of snakehead fish. The highest absolute length growth of snakehead fish was found in the feed treatment added with a combination of *Bacillus* sp. and *Streptomyces* sp. (P3), which was 1.09 cm. The treatment without the addition of swamp bacteria produced the lowest absolute length, which was 0.7933 cm.

Water Quality

The water temperature of snakehead fish maintenance during maintenance was in the range of 27.1-28.8°C. The water temperature value of maintenance during the study was still in the optimum range for snakehead fish maintenance media. The ideal temperature for striped snakehead feedings is 30°C, which promotes higher growth and survival. Temperature fluctuations between 24°C and 36°C had a substantial impact on snakehead fry growth *(Do et al.*, 2021*)*. The pH value of the maintenance media during the study ranged from 5.1 to 6.3. The pH value is still within the tolerance range to support the life of snakehead fish. Ly *et al.*

(2024) stated that the pH range of 5.5-8.0 was appropriate for the early stages of *Channa*, with pH 5.5-6.0 being preferable for incubation and larval growth, and pH 5.5-6.5 for fry growth.

The dissolved oxygen content during maintenance was in the range of 3.87-4.14 mg. L^{-1}. The dissolved oxygen content value during maintenance was still within the tolerance range for the survival of snakehead fish. Purnamawati *et al.* (2017) stated that snakehead fish can survive with dissolved oxygen level less than 5 mg. L^{-1}. Snakehead fish are fish that can survive in low oxygen conditions, they can tolerance dissolved oxygen from 0.2 until 8 mg.L^{-1} (Puspaningsih *et al*., 2018). This is because snakehead fish have additional respiratory organs on the top of their gills, labyrinths so that they can utilize oxygen directly from the free air but snakehead fish can still maintain their survival. The ammonia content in the maintenance media ranges from 0.04 to 0.15 mg. L^{-1}. The ammonia content is still in the optimal range for maintaining snakehead fish. (Ha *et al*., 2021) stated that snakehead fish can still live at an ammonia content of $<1.65\pm0.43$ mg. L^{-1}.

CONCLUSION

The provision of swamp bacteria as probiotic candidates significantly affected the prevalence of MAS and survival, growth, and feed efficiency of snakehead fish. Treatment with *Bacillus* and *Streptomyces* produced the lowest prevalence of 2.3810% and the lowest density of *A. hydrophila*, 3.47×10^5 cfu. mL^{-1} in the guts of snakehead fish.

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