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Influence Of Probiotics Em-4 (*Effective Microorganism-4*) At Different Doses On The Sustenance And Growth Of Large Payau Nila Fish (*Oreochromis Niloticus*)

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ABSTRACT

Effect of Giving Probiotics EM-4 (Effective Microorganism-4) with Different Doses on the Survival and Growth of Brackish Tilapia Larvae (Oreochromis niloticus). The purpose of this study was to determine the effect of the dose of probiotic EM-4 (Effective Microorganism-4) in fish feed on the growth of survival and growth rate of brackish tilapia larvae. To determine the best and highest dose of probiotic EM-4 (Effective Microorganism-4) in fish feed that produces growth and survival of tilapia (Oreochromis niloticus). This research was conducted in January - February 2025 in Sicanang Pond, Medan Belawan Village. The method used was an experimental method using a non-factorial completely randomised design consisting of 4 treatments and 3 replicates. The highest percentage of survival of tilapia larvae in this study was found in treatment C and treatment D at 100% while treatment A (control) was 99.63% and the lowest was in treatment B at 98.89% The results of the analysis of variance showed that there was an effect of giving EM-4 very significantly different (P>0.01) on the absolute weight growth of tilapia larvae (Oreochromis niloticus). The average weight growth increased in treatment C with the addition of EM-4 dose of 20 ml/kg feed by 0.70 grams and decreased in treatment D which is the addition of EM-4 dose of 25 ml/kg feed by 0.61 grams followed by treatment B 15 ml/kg feed by 0.35 grams while the lowest treatment was in treatment A (control) without EM-4 with an average weight of 0.25 grams. The highest absolute length growth of tilapia larvae was obtained in treatment C at 35.57 mm, followed by treatment D at 29.37 mm, treatment B at 17.77 mm and the lowest was in treatment A at 15.87 mm. The results of the Sisdik analysis of variance showed that there was a highly significant effect (highly significant) of giving doses of EM-4 probiotics (P < 0.01) on the growth of brackish tilapia larvae length. The results of water quality measurements Temperature ranged from 26ºC - 29ºC, water pH ranged from 7 - 8, salinity 10 ppt and dissolved oxygen 4.0 - 4.8 mg/l.

Keywords: Survival, Tilapia larvae (Oreochromis niloticus), Growth, Probiotic EM-4, Water quality.

INTRODUCTION

Indonesia is known to have a lot of fishery resources, especially about fish species. According to Amri (2007), it is estimated that around 167 thousand species of fish in the world live in Indonesian waters. These fish species are not only marine fish, but also freshwater fish. Freshwater fish is a type of fish that lives in inland waters. In Indonesia, there are several types of freshwater fish that have been cultivated by the community. Besides having high nutrition, freshwater fish also has high economic value for trade, one of which is tilapia (Oreochromis niloticus).

Brackish tilapia (Oreochromis niloticus) is the result of hybridisation of two superior strains (sultana and gift) with a local strain of tilapia (jabir) that is tolerant of saline waters with salinities ranging from 4 ppt to 20 ppt. Compared to other fish species, this fish has many advantages to be developed because of its favourable biological characteristics such as omnivorous, wide adaptability and tolerance to high

environmental conditions (Hasbullah et al., 2013). The wide spread of tilapia farming around the world results from its adaptability to the environment and intensive farming systems, as well as in Asian countries that are the largest tilapia producers (Ath-thar and Rudy., 2010).

One indicator of successful aquaculture is reflected in high growth and production and low mortality rates. This can be realised if the environmental conditions of aquaculture are favourable as well as the high resistance of the fish body to disease during the maintenance period. Probiotics are one of the alternatives that are considered capable of improving aquatic conditions and cultured organisms from disease attacks. Probiotics in the field of aquaculture are live microorganisms that have beneficial properties for the host animal, so that the population of harmful microorganisms does not increase and maintain microbial balance and control of pathogens in the digestive tract, water, and aquatic environment (Andriyanto et al., 2010).

Probiotics in aquaculture have several roles including: 1). suppressing the population of harmful microbes in the digestive tract by competing for space (place of attachment) and opportunities to get nutrients. 2). produce anti-microbial compounds that will directly suppress microbial growth and prevent the formation of harmful microbial colonisation in the digestive system of the host animal. 3). produce compounds that are immunostimulants, namely increasing the immune system of fish (host animals) in the face of disease attacks by increasing antibody levels and macrophage activity, such as lipo polysaccharides, glycans and peptidoglycans. 4). produce vitamin compounds that are beneficial to the host animal given probiotics and will indirectly increase the nutritional value of the feed. (Atmomarsono et al., 2009).

Several studies have proven that the use of probiotics can provide benefits in tilapia aquaculture and other types of fish, including improving the immune system, growth and survival rate of fish and resistance to disease, including the provision of probiotics with different doses can increase the survival and growth of Pangasius djambal catfish seeds (Andriyanto., 2010).

In improving feed nutrition, bacteria contained in probiotics have a mechanism in producing several enzymes for feed digestion such as amylase, protease, lipase and cellulose. These enzymes will help hydrolyse feed nutrients (complex molecules), such as breaking down carbohydrates, proteins and fats into simpler molecules by facilitating the process of digestion and absorption in the fish digestive tract. giving probiotics with different doses in commercial feed can produce good growth rates and feed efficiency in tilapia, so it can be used in feed with the hope of reducing costs.
feed in tilapia aquaculture (Kamil et al., 2016)

Research on the effect of EM-4 (Effective Microorganism-4) probiotics with different doses on the survival and growth of brackish tilapia larvae has never been done, therefore it is deemed necessary to study this probiotic on the growth and survival of tilapia cultured in brackish water.

LITERATURE REVIEW

A. Classification

Khairuman & Khairul (2013), stated that initially tilapia was included in the Tilapia nilotica species, but in its development fisheries experts have decided to change the name to Oreochromis niloticus or Oreochromis sp. The name niloticus indicates the place where tilapia comes from, namely the Nile river on the African continent.

B. Morphology

Susanto (2009) states that in general the body shape of tilapia is flat to the side and elongated. Vertical lines on the body are 9-11 pieces, while the lines on the red tail fin are 6-12 pieces. On the dorsal fin there are also oblique lines, the eyes look prominent and relatively large with the edges of the eyes coloured white. The body is relatively thicker and stockier than that of tilapia. The lateral line (lateral line in the middle of the body) is interrupted followed by a line located below.

While the morphology of tilapia according to Amri and Khairuman (2007) is that the width of the tilapia body is generally one third of its body length. The body shape is elongated and slender, the fish scales are relatively large, the eyes are prominent and large with white edges. Tilapia have five fins located on the back, chest, abdomen, anus, and tail. The *anal fin* has 3 hard spokes and 9-11 weak spokes. The *caudal* fin has 2 hardened weak spokes and 16-18 weak fin spokes. The *dorsal* fin has 17 hard fin spokes and 13 weak fin spokes. The *pectoral* fin has 1 hard radius and 5 weak fin radii. The *ventral* fin has 1 hard fin radius and 5 weak fin radii. Tilapia has *cycloid* scales that cover its entire body.

C. Ecology

Tilapia is a commonly consumed fish that lives in freshwater, sometimes tilapia is also found living in slightly saline (brackish) waters. Tilapia is known as a euryhaline fish (can live in a high salinity range), tilapia inhabits a variety of freshwater habitats, including shallow waterways, ponds, rivers and lakes.

Tilapia has a habitat in fresh waters, such as rivers, lakes, reservoirs and swamps. But because of its high tolerance to salinity, the fish can live and breed in brackish waters. The preferred salinity is between 0-35 ppt. Tilapia that are still small are more resistant to environmental changes than fish that are already large (Suyanto, 2003).

D. Eating Habits of Goldfish

In general, after the age of 5 days, goldfish eat microorganisms in the form of plankton. Goldfish larvae eat vegetable plankton measuring 100-300 microns. At the age of 5 days, the size of the larvae reaches 6 mm-7 mm. At the age of 1 month, the normal size of larvae reaches 25 mm-30 mm and the size of organisms that can be swallowed ranges from 0.5 mm-2.0 mm. Even though goldfish like natural food in the form of plankton, this habit changes gradually in line with their development and growth. Goldfish are known as omnivorous aquatic animals. Adult goldfish are relatively greedy in swallowing all types of natural or artificial food (Santoso, 1993).

E. Eating and Eating Habits

Tilapia is classified as an omnivorous fish that can consume animal or plant food. Therefore, this fish is very easy to cultivate. Tilapia larvae love zooplankton (animal plankton), such as rotifers, moina, or daphnia. In addition, it also preys on algae or moss attached to objects in its habitat. Tilapia also eat aquatic plants that grow in the culture pond (Khairuman and Amri., 2013). Other types of additional food commonly given to tilapia such as fine bran, peanut meal, coconut pulp and so on (Andrianto., 2007).

F. Probiotics

According to Arief et al, (2008) probiotics are defined as live microorganisms consumed by humans or animals in sufficient quantities, able to live and pass through stomach and digestive tract conditions and benefit their host cells by improving health for the host. Probiotic bacteria must also belong to the safe group or GRAS (Generally Recognized As Safe).

Probiotics are live microorganisms that have beneficial properties for the host animal, so that the population of harmful pathogenic microorganisms does not increase and further change the balance of microflora in the digestive tract. Probiotics, in other words, are supplementary food for host animals in the form of microorganism cells (microbes) or as microscopic feed that aims to win the competition in the digestive tract system of fish (host animals) with harmful bacteria (pathogens). The competition takes place in terms of the utilisation of nutrients derived from the metabolic products of feed and efforts to place space in the digestive tract to form colonies (Supriyanto, 2009).

G. Probiotic EM-4 (Effective Microorganism-4)

The provision of probiotics containing *Lactobacillius* bacteria, *Actinomycetes sp, and Saccharmyces cerevisiae* in feed is intended to increase fish digestibility of feed by increasing digestive enzymes that can hydrolyse proteins into simpler compounds so that they are easily absorbed and used as deposits for growth. Probiotics are photosynthetic bacteria, such as *Lactobacillus sp, Actinomycetes sp, Streptomycetes sp*, and yeast. EM4 probiotics containing lignocellulotic microbes will help break lignocellulotic bonds so that lignin and cellulose will be released and proteolytic microbes produce protease enzymes that function to break down proteins into amino acids. The use of probiotics in fish feed can improve digestibility and growth of tilapia. Therefore, it is necessary to conduct research that examines the effect of probiotics in feed on the level of feed consumption and growth of tilapia (Noviana *et al*., 2014).

EM-4 product is a culture in a yellowish-brown liquid medium that is beneficial for livestock growth and production with a sweet and sour smell. EM-4 farms are able to improve microorganisms in the digestive tract of livestock so that the health of livestock will increase, not easily stressed and the smell of faeces will decrease. Giving EM4 to livestock feed and drinking water will increase livestock appetite due to the sweet and sour aroma caused (Kukuh, 2010).

H. Trajectory

To maintain fish survival, food that fulfils the nutritional needs of fish is required. The food eaten by the fish is used for survival and the rest will be utilised for growth. The quality of the feed must meet the nutritional needs of the fish and the quantity must be adjusted to the number of fish stocked. An increase in stocking density that is not appropriate will result in competition for space and food, which in turn can reduce health and physiological conditions so that fish will experience stress. While diseases that attack are usually related to poor water quality, so good water quality will reduce the risk of fish disease and fish can survive. The use of probiotics can increase fish survival as done in several previous studies.

Noviana et al, (2014) raised tilapia by feeding with probiotics added to Lactobacillus sp. producing a survival rate of 90%, while for the treatment without probiotics the survival rate obtained was only 50.8%. Similarly, Anggriani et al, (2012) in their research obtained a tilapia survival rate of 70% which was fed with the addition of probiotic Bacillus sp, while without probiotics, the survival rate was only 43%. Furthermore, Andriyanto et al, (2010) also obtained the highest survival rate of 86.67% and without the provision of probiotics produced the lowest survival rate of 76.67% with the use of probiotics Bacillus sp. added to fish feed in the maintenance of jambal catfish (Pangasius jambal).

I. Growth

Male tilapia have a faster growth rate than female tilapia. The growth rate of male tilapia averages 2.1 grams/day, while the growth rate of female tilapia averages 1.8 grams/day (Ghufran, 2009). In 3-4 months of rearing, tilapia can be obtained at an average size of 250 grams from an initial weight of 30-50 grams (Cholik, 2005). In addition to its rapid growth, tilapia also has a high survival rate during the rearing period. Wiryanta et al (2010) explained that the survival rate of tilapia in enlargement activities is 65-75%.

The use of probiotics on the growth of fish or other cultured organisms has been proven in several previous studies: Noviana et al, (2014) obtained a relative growth rate of tilapia of 3.20% fed with probiotic Lactobacillus sp. compared to the growth rate of fish without probiotics which was only 1.2%. Furthermore, Agustin et al, (2014) who raised cork fish with the addition of probiotics obtained the highest growth rate of 3.17% and the lowest growth rate of 2.14% without probiotics. The highest growth of gourami fish seeds was also obtained (1.67%) with the addition of probiotic Lactobacillus sp. compared to the growth rate of seeds fed without probiotics (0.41%) (Suminto., 2015).

J. *Life Graduation*

Survival is a success rate of a number of fish tested within a certain period of time. This success rate is influenced by factors such as feed. survival (SR) is the percentage of the number of fish that are still alive after being fed and then calculated at the end of the study (Mudjiman, 2004).

However, if poor water quality is not immediately corrected to normal, these fish will be susceptible to bacterial, fungal and protozoan infections. These diseases can result in the death of the fish, and can be transmitted from one to another quickly, resulting in mass mortality. The size of the fish itself determines its survival. A relatively small size or larvae, its survival will be small compared to fish that are already large or have become seeds.

K. Water Quality

Water quality is one of the factors that play an important role in aquaculture activities. Biota grow optimally in water quality that suits their needs (Ghufran, 2009). Water quality includes physical, chemical and biological properties of water. Physical properties include temperature, water brightness, turbidity, and water colour. Chemical properties of water include acidity (pH), dissolved oxygen (DO), carbohydrates, ammonia, and alkalinity, while biological properties of water include plankton, benthos, and aquatic plants. Some important water quality parameters in tilapia aquaculture are temperature, pH, dissolved oxygen, and ammonia. In order for the growth and development of tilapia to run well, these water quality parameters must be maintained so that the growth of tilapia seeds can take place optimally (Popma and Masser, 1999).

Water quality management includes salinity, acidity (pH), temperature, dissolved oxygen, and ammonia. Tilapia is a fish that is euryhaline so that its habitat is very wide, including fresh waters, river estuaries and brackish waters. The Agency for the Assessment and Application of Technology (2011) states that brackish water tilapia is tolerant of brackish and sea water with salinities reaching 20 ppt. Khairuman and Amri, (2002) stated that the optimum temperature for tilapia is 25-33°C. The ideal dissolved oxygen value for tilapia rearing ranges from 3.0-8.0 ppm (Djarijah, 2002). The acidity level of water (pH) where tilapia live ranges from 6-8.5. The optimal pH is 6.5-9 (Setyo, 2006).

RESEARCH METHODOLOGY

This research was conducted in January - February 2025 in Sicanang Pond, Medan Belawan Village.

The research method used is the experimental method. Silalahi (2003) states that experimental research basically wants to test the relationship between a cause and an effect. This research generally aims to determine the cause and effect relationship by giving a dose treatment of EM-4 probiotics in fish feed on the survival and growth rate of brackish tilapia larvae.

RESULTS AND DISCUSSION

A. Live Graduation of Tilapia Larvae

The percentage of survival of brackish tilapia larvae sprayed with EM-4 probiotic after 30 days of research can be seen in Table 3.

Table 3. Survival rate of Tilapia larvae (*Oreochromis niloticus*) during the study.

Repeat	Treatment			
	A	B	C	D
1	98.89	97.78	100	100
2	100	100	100	100
3	100	98.89	100	100
Total	**298.89**	**296.67**	**300**	**300**
Average	**99.63**	**98.89**	**100.00**	**100.00**

In Table 3, it can be seen that the percentage of brackish tilapia larvae survival in each treatment did not differ significantly. The highest percentage of tilapia larvae survival in this study was found in treatment C and treatment D at 100% while treatment A (control) was 99.63% and the lowest was found in treatment B at 98.89%.

Haris in Sulastri (2006) states that factors that affect survival are internal and external factors. Internal factors are the fish itself, its heredity, physiology, while external factors are water quality, temperature, pH, DO, NH3 and food. Alikhunti et al in Suhardianti (2006) distinguish 3 categories of larval survival, namely: 1) larval survival of more than 50% is classified as good, 2) 30-50% is classified as moderate and 3) Less than 30% is classified as poor.

According to Ardita et al. (2015) the ability of tilapia has a high value and the addition of probiotics does not have a significant effect on fish survival.

B. Absolute Weight Growth of Tilapia Larvae

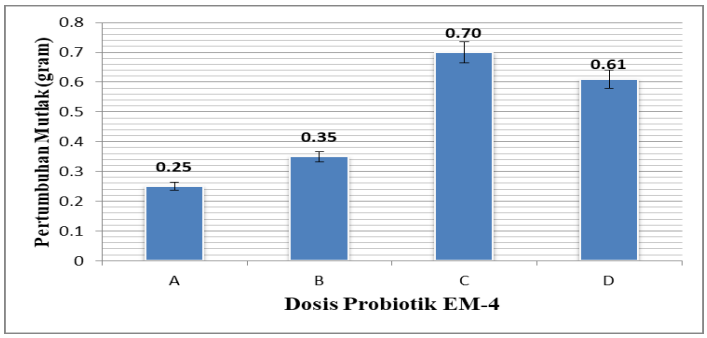
After 30 days of observation in the research container, the absolute weight growth data of tilapia larvae (*Oreochromis niloticus*) can be seen in Table 4.

Repeat	Treatment (gram)			
	A	B	C	D
1	0.21	0.29	0.72	0.63
2	0.28	0.39	0.68	0.58
3	0.25	0.38	0.71	0.62
Total	**0.74**	**1.06**	**2.11**	**1.83**
Average	**0.25**	**0.35**	**0.70**	**0.61**

Based on the analysis of variance, there is an effect of EM-4 giving very significantly different (P>0.01) on the absolute weight growth of tilapia larvae (*Oreochromis niloticus*). The average weight growth increased in treatment C with the addition of EM-4 dose of 20 ml/kg feed by 0.70 grams and decreased in treatment D, namely the addition of EM-4 dose of 25 ml/kg feed by 0.61 grams followed by treatment B 15 ml/kg feed by 0.35 grams while the lowest treatment was in treatment

A (control) without EM-4 with an average weight of 0.25 grams. It is suspected that the higher addition of probiotic concentration does not always have a positive effect on the population of bacteria in the digestive tract of tilapia larvae (Irianto, 2003).

Furthermore, the research data can be shown in the form of a diagram (histogram) in Figure 3.



This is supported by the research of Midhun *et al*. (2019); Mulyasari *et al*. (2016); Ling *et al*. (2018); Rahmawan *et al.* (2014); Hemaiswarya *el al.* (2013), the use of probiotics in aquaculture can improve growth, digestibility and feed efficiency, appetite, fish immune system, stress tolerance and resistance to pathogens. Probiotics enter the fish gut and then help the digestive process so that feed will be more efficiently utilised by fish because feed nutrients will be easily absorbed by the fish body (Setiawati *et al.* 2013).

C. Absolute Length Growth of Tilapia Larvae

From the experiments that have been carried out on the absolute length growth of brackish tilapia larvae by giving EM-4 probiotics, the data obtained from the observation of the average absolute length of brackish tilapia larvae are presented in Table 5.

Repeat	Treatment (mm)			
	A	B	C	D
1	15.10	18.8	36.00	29.70
2	17.00	17.1	34.90	28.90
3	15.500	17.4	35.80	29.50
Total	**47.60**	**53.30**	**106.70**	**88.10**
Average	**15.87**	**17.77**	**35.57**	**29.37**

Based on data table 4. The highest absolute length growth of tilapia larvae was obtained in treatment C at 35.57 mm, followed by treatment D at 29.37 mm, treatment B at 17.77 mm and the lowest was in treatment A at 15.87 mm. The analysis of variance

showed a *highly significant* effect of EM-4 probiotic dosing ($P < 0.01$) on the length growth of brackish tilapia larvae.

The provision of EM-4 probiotics containing Lactobacillus bacteria, Actinomycetes sp, and yeast in feed aims to increase fish digestibility of feed by increasing digestive enzymes so that they are easily absorbed and used as fish growth. This is in accordance with Noviana et al (2014) which states that probiotics are photosynthetic bacteria, such as Lactobacillus sp, Actinomycetes sp, Streptomycetes sp, and yeast. EM4 probiotics containing lignocellulotic microbes will help break lignocellulotic bonds so that lignin and cellulose will be released and proteolytic microbes produce protease enzymes that function to break down proteins into amino acids. The use of probiotics in fish feed can increase digestibility.

and growth of tilapia.

According to Weatherley in Hartanto (1996), growth is a change in the size of fish both in weight, length and volume over a period of time caused by changes in tissue due to cell division of muscle and bone which is the largest part of the fish body so as to cause additional fish weight.

D. Water Quality

The table below shows the value of water quality measurements including temperature, dissolved oxygen, and acidity/pH during the 30-day maintenance period. For more details can be seen in table 6

Table 6: Water Quality Range During the Study

Parameters	Unit	Treatment			
		A	B	C	D
Salinity	ppt	10	10	10	10
Temperature	^oC	26 - 28	26 -28	26-29	27-28
pH		7 - 8	7 - 8	7 - 8	7 - 8

The temperature value in this study ranged almost the same temperature range (26-29 oC. The temperature at the time of the study was in the optimal range for tilapia cultivation, which is around 26-29^{o}C. Khairuman and Amri (2011) stated that the optimal temperature for tilapia growth is around 24-32^{o}C. According to Effendi (2014), an increase in temperature causes an increase in the speed of metabolism and respiration of aquatic organisms, and results in increased oxygen consumption. An increase in water temperature of 10^{o}C causes an increase in oxygen consumption by aquatic organisms about 2-3 times, but the increase in temperature is accompanied by a decrease in dissolved oxygen levels so that the presence of oxygen is often unable to meet the oxygen needs of aquatic organisms to metabolise and respire.

The pH measurements in this study showed pH values ranging from 7-8 and pH. The pH range is a good condition for habitat and growth of tilapia. According to Kordi and Sancung (2007), the pH range for optimal growth occurs at pH 7-8, while

the pH for tilapia habitat is between 6-8.5. The effect of water pH can occur on the survival and growth of fish.

Oxygen is required in the oxidation of organic matter from food to provide energy for biological activity. Poor or below optimum oxygen concentration leads to mortality (Boyd, 2012). . Amri and Khairuman (2003) stated that tilapia requires more than 4 ppm of dissolved oxygen to grow optimally.

The salinity value obtained during the study of brackish tilapia larvae fed with EM-4 probiotic addition was 10 ppt. Brackish tilapia is tolerant of brackish and marine water with salinities up to 20 ppt (BPPT, 2011).

CONCLUSION

Based on the research that has been done, conclusions and suggestions can be drawn, among others:

1. The highest percentage of tilapia larvae survival in this study was found in treatment C and treatment D at 100%, while treatment A (control) was 99.63% and the lowest was found in treatment B at 98.89%.
2. Based on the analysis of variance, there is an effect of EM-4 giving very significantly different (P>0.01) on the absolute weight growth of tilapia larvae (Oreochromis niloticus). The average weight growth increased in treatment C by giving a dose of EM-4 20 ml/kg feed of 0.70 grams and decreased in treatment D, namely the addition of a dose of EM-4 25 ml/kg feed of 0.61 grams followed by treatment B 15 ml/kg feed of 0.35 grams while the lowest treatment was in treatment A (control) without giving EM-4 with an average weight of 0.25 grams.
3. The highest absolute length growth of tilapia larvae was obtained in treatment C at 35.57 mm, followed by treatment D at 29.37 mm, treatment B at 17.77 mm and the lowest was in treatment A at 15.87 mm. The analysis of variance showed a highly significant effect of EM-4 probiotic dosing (P < 0.01) on the length growth of brackish tilapia larvae.
4. Water quality measurement results Temperature ranged from 26^{o}C - 29^{o}C, water pH ranged from 7 - 8, salinity 10 ppt and dissolved oxygen 4.0 - 4.8 mg/l.

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