Review Article

Beneficial Implications of Probiotics in Fresh Water Fish Aquaculture: A Review

ABSTRACT

Listed as one of the highest producer of aquaculture fish, Indonesia's fisheries sector holds a promising potential to be developed as source of global food security. The total production value of both inland and marine farming from both fish and non-fish species were reported to be increased each year. The current government targeted to excel the production up to 25% by 2024. To support this, many innovation and research have been done to accelerate the production of cultured fish, especially fresh water fish which demand is always high in the market. Several fresh water fish commodities have a poor growing performances, such as growth rate, feed conversion ratio (FCR) and survival rate. Thus, a probiotics were used to boost their performance. In this review, a comprehensive analysis of how probiotics help to increase ~~aquaculture~~aquaculture efficiency of fresh water fish will be thoroughly discussed. The discussion will be focus on three fresh water fish species, which are Gouramy fingerlings (*Oshpronemus gouramy*) Siamese catfish (*Pangasius hypopthalmus*) And Nilem fish (*Osteochillus hasselti*)

Keywords: Probiotics, Fresh Water Fish, Aquaculture, Growth rate, Survival Rate, Feed Conversion Ratio

1. INTRODUCTION

Indonesia is one of the top producers of aquaculture fish in the world, listed the third in 2018 after China and India [1]. According to Directorate General of Aquaculture, Ministry of Marine Affairs and fisheries Indonesia, the aquaculture fisheries practices in 2020 yielded 18, 44 million tons for both fish and non-fish species and targeted to reach 22,65 million tons by 2024 [2]. The global demand of fish forces the stakeholders to upscale its production which implicated to greater needs of better and efficient aquaculture practice, in terms of its system and growth performances.

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One of the obstacles in the aquaculture is the slow growth that causes a low amount of production. This is presumably because fish are still not perfect in utilizing commercial feed even though the protein content is quite high. Feeding is an important factor in the aquaculture business. The feed is one of the important elements that support the process of survival and the growth of fish in aquaculture [3]. One way that can be done to increase fish growth is by increasing feed digestibility through additional intake or supplements [4]. One of the common additions given is the addition of probiotics into a commercial feed.

The use of probiotics in aquaculture has been very popular among farmers. Probiotics are applied to improve feed efficiency and increase fish growth productivity so they can be harvested faster. Wang et. al [5] explained that probiotic bacteria produce enzymes that can break down complex compounds into simple ones so that they are more easily absorbed by fish. Bacteria contained in probiotics have a mechanism in producing several enzymes for feed absorption such as amylase, protease, and lipase. These enzymes will help hydrolyze feed nutrients (complex molecules) such as breaking down carbohydrates, proteins and fats into simpler molecules which will facilitate the digestion and absorption process in the digestive tract of fish [6].

Fresh water fish has a remarkably high demand throughout the year, both in local or international market. Indonesia has approximately 2,8 million hectares of potentially used area for inland aquaculture ~~spreadout~~spread-out from the west to the east, but only 2,28% of that were already used [2]. Thus, there is a high opportunity to increase the number of production of this aquaculture. One of the main commodities that have a good potential to be cultured and produced are Gouramy fingerlings (*Oshpronemus gouramy*) Siamese catfish (*Pangasius hypopthalmus*) ~~A~~and Nilem fish (*Osteochillus hasselti*). However, ~~The~~the three fish~~es~~ species have a similar major problems to be efficiently cultured, their slow growth performance hindered its economic benefits.

The use of probiotics to those fresh water fish species have been studied and resulted to some improve growing performances. Here we will have discussed how the probiotics increase the metabolism of the fish and how it affected to several parameters of aquaculture such as growth rate, Feed Conversion ratio and survival rate.

2. PROBIOTICS

Probiotics are defined as single or mixed bacterial cultures which, when consumed by livestock or humans, have a beneficial effect on the health of the host by enhancing the properties of the microflora in the digestive tract. Microorganisms are said to be probiotics if they meet several requirements, including: a) are non-pathogenic, b) viability in high populations of around 10^6 - 10^8 cfu/ml c) produce microbial substances that will inhibit pathogenic bacteria in the digestive tract, d) be able to compete with pathogenic bacteria e) resistant to enzymes and bile salts, f) can be prepared as a living cell product on an industrial scale and g) can maintain stability and survival for a long time in storage and in the field. Species of probiotic bacteria should be part of the normal gut microflora so that these bacteria more easily adapt to the intestinal environment and can reach the location where the required effect takes place [7].

According to Irianto [8], there are basically 3 working models of probiotics, namely: (1) suppressing the microbial population through competition by producing anti-microbial compounds or through competition for nutrients and attachment sites on the intestinal wall, (2) changing microbial metabolism by increasing or decreasing activity. enzymes, and (3) stimulate immunity through increased antibody levels or macrophage activity. According to Wang [5] the mechanism of ~~action of~~ probiotics action is to improve water quality by eliminating harmful compounds in the waters. The next mechanism ~~of action of probiotics~~ is to increase growth performance by increasing the nutritional value of feed through increasing the activity of digestive enzymes in the digestive tract of fish and shrimp [9].

Bacillus are g~~G~~ram positive bacteria, form spores, and produce high amounts of antagonistic components. *Bacillus subtilis* and *Bacillus licheniformis* are bacteria that generally act as probiotics in shrimp culture. These bacterial species are found in freshwater and seawater environments and are also found in the intestines of shrimp [10]. Several studies that show

the ability of Bacillus as a probiotic include spores of *Lactobacillus plantarum* and *Bacillus* spp. can reduce the number of occurrences of vibriosis in turbot fish larvae through encapsulation in rotifers and increase the weight and survival of turbot fish larvae. The addition of B. subtilis to the feed was reported to be able to increase the growth of tilapia (*Oreochromis niloticus*), and increase the immune response, the addition of *Bacillus* spp. rearing water can increase the survival and production of catfish by improving water quality and stimulating the immune response (Add references).

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3. EFFECTIVE MICROORGANISM 4 (EM4)

In 1980, Prof. Dr. Teruo Higa developed a fermentation technology called EM4 (Effective Microorganisms 4). EM4 is a mixed culture of several non-pathogenic microorganisms. The natural microorganisms contained in EM4 consist of five groups of microorganisms, namely lactic acid bacteria (*Lactobacillus* sp), photosynthetic bacteria (*Rhodopseudomonas* sp), *Actinomycetes* sp, *Streptomyces* sp, and yeast (yeast) [11].

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Photosynthetic bacteria are bacteria that can synthesize nitrogen compounds and sugars. Photosynthetic bacteria can form substances that produce amino acids, nucleic acids and bioactive substances [12]. Fermentation ~~mushrooms~~ fungi function to ferment organic matter into organic compounds (in the form of alcohol, sugar and amino acids) that are ready to be absorbed.

Fermentation is the process of changing a complex compound into simpler compounds. Fermentation involves oxidation and reduction reactions. Complex compounds in the form of carbohydrates, proteins and fats will be converted into glucose compounds, amino acids, fatty acids and glycerol. The fermentation process can also remove unwanted odors, improve digestibility and remove toxins contained in raw materials. Fermented feed contains simple compounds that will be fed to fish. Feed that already contains simple compounds will be easier for fish to absorb. The fermented carbohydrates will be used by fish as an energy source.

Lactobacillus bacteria is one type of probiotic bacteria. According to Arief et al. [13] the role of Lactobacillus sp. is to maintain the balance of microbes in the digestive tract so as to increase the digestibility of fish. The work of Lactobacillus sp. convert carbohydrates into lactic acid, thereby producing endogenous enzymes to increase nutrient absorption, feed consumption, growth, and inhibit the growth of pathogenic organisms.

Actinomycetes are mycelium-shaped microbes (filaments in the form of threads). Actinomycetes will take amino acids and substances produced by fermenting fungi which will become antibiotics. EM4 when added to feed works in two ways. First, the fermentation process will be carried out by fermenting fungi which convert complex molecules into simpler molecules. The fermentation process makes food easier to absorb. Second, the probiotic bacteria in EM4 were able to inhibit the growth of pathogenic bacteria in the digestive tract of fish. Probiotic bacteria are antagonistic with pathogenic bacteria. When the growth of pathogenic bacteria is inhibited, it will make the food essence that is absorbed in the fish intestine more optimally.

4. EFFECT OF PROBIOTIC UTILIZATION TO FRESH WATER FISH

4.1 Gouramy Fingerlings (*OSHPRONEMUS GOURAMY*).

Gourami has a fairly high economic value and is very popular with the public as a consumption fish, because this gourami has dense flesh, large spines and tastes delicious

and savory. Unfortunately, gouramy is known to the public as a slow-growing fish compared to other freshwater fish. The growth of gouramy tends to be slow, this is because the gouramy undergoes changes in eating habits in each phase of its growth, namely carnivores in the one month phase of their life, omnivores in the juvenile phase and herbivores in the adult phase [14].

Gouramy is spread throughout the archipelago in Indonesia and neighboring countries as a cultured fish originating from Java. In Java, the cultivation of gouramy to produce seeds and consumption fish has been widespread in West Java (Tasikmalaya, Ciamis, Garut, Parung, Bogor, Cipanas, Indramayu), Central Java (Purwokerto, Magelang, Banjarnegara, Purbalingga, Banyumas), East Java (Kediri, Tulung Agung, Blitar), Bali (Karang Asem). In Sumatra the cultivation of gourami developed in Mungo near Payakumbuh (West Sumatra). In Sulawesi it develops in Airmadidi near Manado (North Sulawesi) [15].

Sitanggang and Sarwono [15] stated that in nature, gouramy inhabits calm and stagnant waters such as swamps, lakes and lakes. In a fast-flowing river, it is rare to find gouramis. His its life of liking free-flowing waters is evident, when gouramis are very easy to maintain in stagnant ponds. Gouramy can live in rivers, swamps, lakes and freshwater ponds. Gouramis can adapt to slightly brackish and slightly salty water. However, according to Saparinto [16], although it has a high adaptability to environmental conditions, gouramy is more suitable to live in fresh water. The most optimal waters for cultivation are located at an altitude of 50-400 meters above sea level. This fish is still tolerant up to an altitude of 600 meters above sea level. The ideal temperature for carp is 240 -280 C. The ideal water depth is between 70-100 cm so that sunlight can touch the bottom of the pond so that a fertile layer can develop. For a productive aquaculture pond, the best pH is between 6.5-8 [15]. Gouramy has a low sensitivity to toxic compounds in water. Most freshwater fish will die at a dissolved carbon dioxide (CO2) level of 15 ppm but gouramy can still survive at a dissolved carbon dioxide level of 100 ppm [16].

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According to Susanto [17] gouramis are fish which are carnivorous when they are small, while as adults they are herbivores. Because this type of food is an obstacle to the growth of gourami. Susanto [17], also said that the foods that are often eaten by small and parent carp are taro leaves, papaya tree cassava, cucumber, genjer, sweet potato, pumpkin. The growth of gouramy is very slow when compared to other types of cultured fish such as tombro (carp), catfish, and tilapia. Gouramy growth is strongly influenced by heredity (strain), health, feed, living space and age (time) [15]. Meanwhile, according to Saparinto [16] the growth of gouramy is influenced by several factors, namely internal factors and external factors. Internal factors include heredity (genetic), sex, age, and parasites and disease. Meanwhile, external factors that dominantly influence are feed, water temperature and water chemical factors. These things are a major concern in the maintenance of gouramy in order to get gouramy that has good quality.

A study conducted previously by our group using liquid and powder probiotic to gourami fish showed a promising results. The results obtained during 40 days of maintenance showed that administration of probiotic in growth, feed conversion ratio, and survival had a good effect. Probiotic treatment with combination 25% (3,75 ml/kg) : Powder 75% (7,5 g/kg) has the best results with a value of 1.63 ± 0.09% specific growth rate, absolute weight growth 25.27 ± 2.62 g, feed conversion ratio 1.59 ± 0.02 and a survival rate of 93.3 ± 5.77% (Figure 1). There are various factors that affect the amount of feed consumption in fish are feeding habits, physiological status, fish weight, temperature, dissolved oxygen, feed composition and the level of preference so that each treatment has different feed conversion values. According to Verschuere et al. [18] when probiotic had successfully entered and inhabited

the digestive tract of their host, they can secrete exoenzymes that can help degrade feed ingredients into smaller molecules (monomers) there by increasing the effectiveness and efficiency of their absorption process.

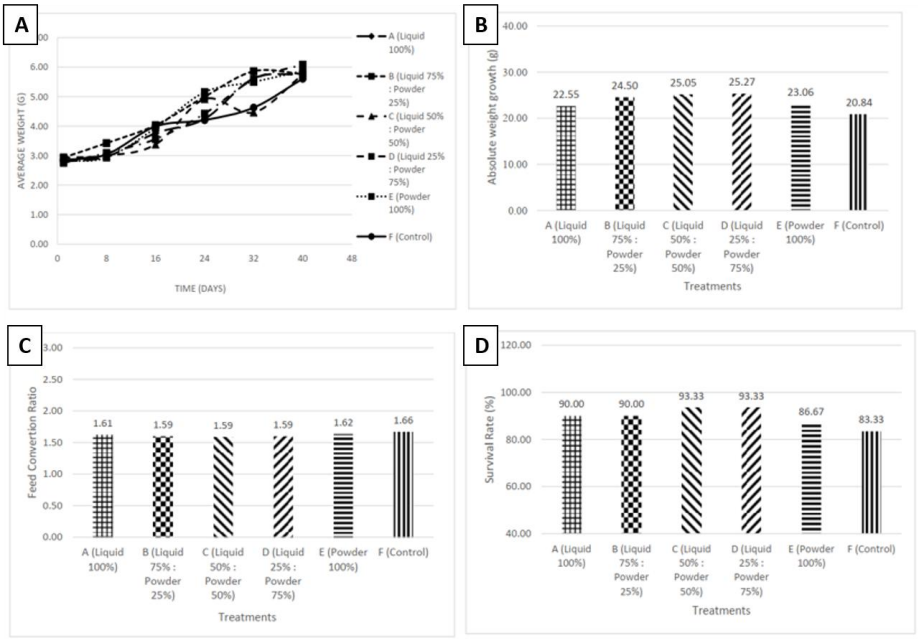

Fig 1. Aquaculture Performance of Gourami fish after administered by probiotics for 40 days. Average weight growth (A), Absolut weight growth (B), Feed Conversion Ratio (FCR) (C), Survival Rate (D).

4.2 Siamese Catfish (*PANGASIUS HYPOPTHALMUS*)

Siamese catfish (*Pangasius hypopthalmus*) is a type of freshwater consumption fish native to Indonesia which is spread in parts of Sumatra and Kalimantan. Catfish meat has a fairly high calorie and protein content, the taste of the meat is distinctive, delicious~~, delicious~~ and savory so that it is favored by the public. Catfish is considered safer for health because it has low cholesterol levels compared to livestock meat. In addition, catfish have several other advantages, namely the size per individual is large and in nature the length can reach 120 cm [19].

Catfish (*Pangasius* sp.) is one of the economically important freshwater fish commodities. The catfish rearing place does not require running water. This fish has many advantages compared to other freshwater fish, including being a voracious fish for food, at the age of 6 months, catfish can reach a length of 35-40 cm [20].

Catfish do not have scales, the head is relatively small with the mouth located at the end of the head. The catfish has an elongated body that is white like silver with a bluish back. Body length can reach 120 cm. In cultivation at the age of 6 months, catfish can reach a size of 35-40 cm [19]. The caudal fin is fork-shaped and symmetrical in shape. The long anal fin

consists of 30-33 soft rays. The pelvic fin has 8-9 soft rays. The dorsal (dorsal) fin has hard rays that turn into serrated patels on the back. The soft rays of the dorsal fin are 7-8 pieces.

According to Djariah [21], catfish need an energy source that comes from food for growth and survival. Catfish are omnivorous fish, but tend to be carnivorous. Susanto and Amri [19] explained that in nature, the main food of catfish is small shrimp (crustaceans), insects and mollusks. While the complementary food of catfish in the form of rotifers, small fish and leaves in the waters. In accordance with Cholik et al. [22] which states that catfish are very responsive to artificial feed.

In nature, the geographical distribution of catfish is quite wide, almost all over Indonesia. Naturally, this fish is found in large rivers and calm waters in Sumatra, such as the Way Rarem, Musi, Batanghari and Indragiri rivers. Other major rivers in Java, such as the Brantas and Bengawan rivers. Even the catfish's close family is also found in major rivers in Kalimantan, such as the Kayan, Berau, Mahakam, Barito, Kahayan and Kapuas rivers. Generally, these fish are found in certain locations in the river, such as deep lubuk (river valleys). Susanto and Amri [19] said that catfish are nocturnal or carry out activities at night like other catfish. The catfish likes to hide in burrows on the banks of the river where it lives and includes bottom fish, this can be seen from the shape of its mouth which is slightly downwards.

Our group previously performed research of probiotic to boost growth performance of tilapia catfish using liquid and dry probiotics available commercially in the market. From the results it can be concluded that the addition of a combination of liquid and dry probiotics with the best results is the concentration of liquid administration of 3.75 ml / kg of feed and dry 7.5 g / kg of feed with an average specific growth rate (Weight) of 2.66 %, the average specific growth rate (length) is 1.95 cm, and the feed conversion ratio is 1.01 and the survival rate is 100% for all treatments (Figure 2).

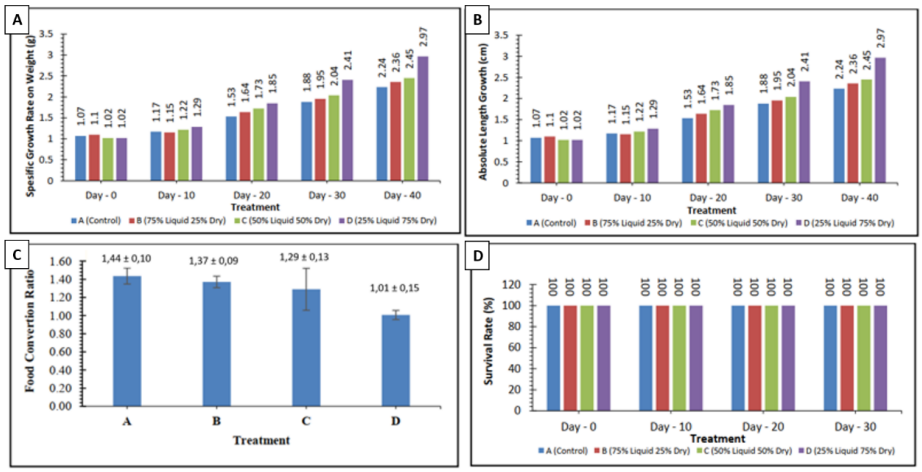

Fig 2. Aquaculture Performance of Tilapia Catfish after administered by probiotics. Weight growth rate (A), Length growth rate (B), Feed Conversion Ratio (FCR) (C), Survival Rate (D).

The nutritional content of the feed will affect the growth of fish, feed is given to determine the effect of nutrients contained in the feed provided by observing the growth of fish for some

time. The activity of bacteria in digestion will change quickly if there are microbes that enter through feed or water which causes a change in the balance of bacteria that already exists in the digestive tract with bacteria that enter [23]. According to Effendie [24], growth is influenced by internal and external factors. Internal factors largely depend on the condition of the fish's body, for example the ability of fish to utilize the remaining energy and protein after metabolism for growth. Meanwhile, external factors such as environmental factors and feed are very influential on fish plants. Both of these factors will balance the state of the body of the fish while in the media maintenance and support the growth of the body of the fish.

We used the tilapia catfish in the seed stage to see how the addition of probiotics help the young fish hone his survival as it's a critical stage in nutrional fulfillment. The digestive tract of fish in the seed stage is still not perfect, so it is difficult for fish to utilize fiber where fish have limitations in terms of the availability of cellulotic enzymes in the digestive tract. Fish also need a protein high enough to support growth and survival [25]. Basically, probiotics added to the feed are used to smooth the digestive system in fish so that the food eaten is more efficient. The value of the feed conversion ratio obtained shows that the performance of the digestive system of each treatment is different or not all are in maximum condition, so it looks after the addition of probiotic treatment affects the digestion and growth of fish.

4.3 3. Nilem Fish (*OSTEOCHILLUS HASSELTI*)

In Indonesia, nilem fish are known as nilem, lehat, magut, regis, milem, muntu, trough, palau, pawas, puyau, asang, penopa, and carp. Areas of distribution include: Malaysia, Thailand, Vietnam, Cambodia, Indonesia (Java, Sumatra, Kalimantan and Sulawesi). Nilem fish have a flat body shape, the mouth can be poked out. The position of the mouth is located at the tip of the nose (terminal). The position of the pelvic fins is located behind the pectoral (abdominal) fins. Nilem fish are classified as circular scales (cycloid). The upper jaw is equal to or longer than the eye diameter, while the muzzle barbels are shorter than the head length. Beginning of dorsal fin opposite the 8th to 10th lateral line scales. The shape of the anal fin is slightly erect, the beginning of the anal fin opposite the 22nd or 23rd lateral line scales behind the last dorsal fin rays. The pelvic and pectoral fins are almost the same length. The beginning of the pelvic fins is separated by 4 – 4 1/2 scales from the 10th to 12th lateral line scales. The pelvic fins do not reach the anus. Forked tail fin. The height of the caudal peduncle is almost the same as the caudal peduncle and is surrounded by 16 scales [26]. According to Hardjamulia [27] based on the color of the scales, Nilem fish can be divided into 2, namely blackish brown nilem fish (nilem fish which is brown green on the back and bright on the belly) and red nilem fish (nilem fish which is red or reddish in color). red on the back and on the belly a bit light).

Nilem fish are classified as omnivores (eating everything). The diet consists of detritus, attached bodies, periphyton and epiphyton so that the Cyprinidae fish species live more often at the bottom of the waters [20]. In addition, Cyprinidae fish include plankton eaters and aquatic plants. 8 At the larval and seed stages, Cyprinidae fish eat phytoplankton and zooplankton or single-celled algae species such as diatoms and algae belonging to the Cyanophyceae and Chlorophyceae classes. As stated by Hardjamulia [27] that the seeds of Cyprinidae fish such as nilem and carp eat phytoplankton and zooplankton belonging to the Bacillariophyceae, Chlorophyceae, Desmidiaceae and Cyanophyceae classes.

We used EM4 probiotics to enhance the growth performance of nilem fish with 4 treatments and 3 replications. The treatment A (control), B (concentration of probiotic EM4 10 ml kg-1), C (concentration of probiotic EM4 15 ml kg-1) and D (concentration of probiotic EM4 20 ml kg-1). The results showed a good relation between the used of probiotics to nilem fish growth. Provision of EM4 probiotics of 15 ml kg-1 in feed produced 2.14% of specific growth

rate and the best feed efficiency is 59.26% but had no effect on survival rate and also the length growth of *Osteochillus hasselti*. Survival rate and growth of length are 95% - 96.67% and 1.10 - 1.20 cm, respectively (Figure 3).

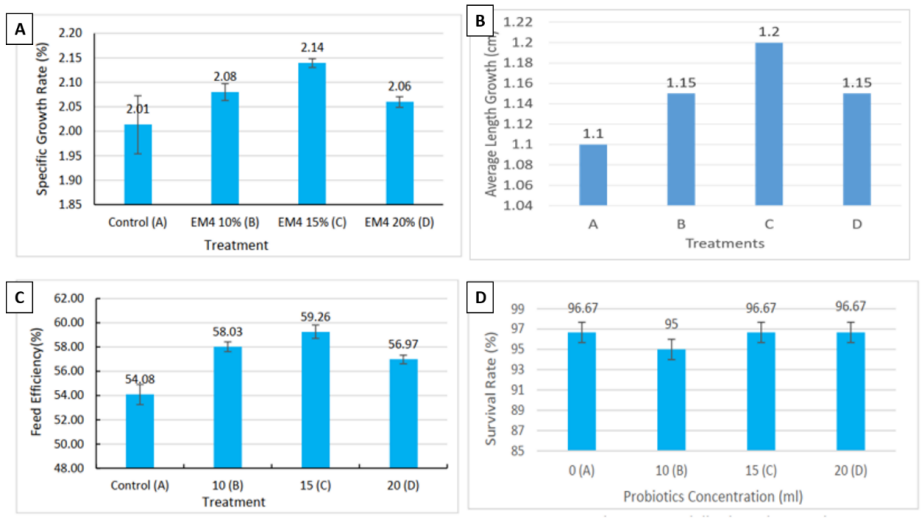

Fig 3. Aquaculture Performance of Nilem Fish after administered by probiotics. Specific growth rate (A), Length growth rate (B), Feed Conversion Ratio (FCR) (C), Survival Rate (D).

According to Mulyani et al. [28] the survival rate (SR)> 50% is in the good category, 30-50% is moderate and <30% is classified as unfavorable. Effendi [29] states that survival rate is influenced by biotic factors such as competition, age, density and human handling, while abiotic factors are physical and chemical properties in water. High density will cause a decrease in water quality, especially dissolved oxygen content [30]. A decrease in water quality can cause stress on fish, even if a decrease in water quality has exceeded the tolerance limit it will result in death. In addition, decreased water quality can also affect the appetite of fish and when appetite is reduced, the intake of feed into the body of the fish will also be reduced so that energy for maintenance and growth is not optimally fulfilled.

Increased digestive activity is due to an increase in probiotic populations in the digestive tract and enzyme production due to bacteria. According to Juliani [31] an increase in the number of probiotics in feed increases the number of bacterial populations in the digestive tract and increases enzyme activity. With the increase in enzyme activity, the process of food remodeling will be faster so that the process of food absorption increases and in turn will increase fish growth. Feed that enters the digestive tract will be digested into simple particles by digestive enzymes to be absorbed through the intestinal wall. *Lactobacillus* sp. is able to produce lactic acid from simple carbohydrates that can affect the pH of the digestive tract in a more acidic direction so that Saccharomyces cerevisiae can grow well [31]. Saccharomyces cerevisiae is able to make the condition of the digestive tract to be anaerobic which will grow Lactobacillus sp. well and able to maintain the balance of probiotics in the digestive tract [32]. Haetami [33] states that the use of probiotics directly will increase the effectiveness of intestinal microbes which in turn will increase growth. The role of *Lactobacillus* sp. in addition to producing lactic acid is able to improve the functions of

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digestive enzymes, breaking down complex molecules into simpler molecules so that food is easily absorbed [34].

5. CONCLUSION

The addition of probiotic to improve the quality of fresh water fish growth performances have been studied and proved to be effective. Both liquid and dry probiotic were reported to help fish to digest food better, especially to those which still in the younger stage, and improve water quality of the aquaculture systems. From the three species of fish discussed here, administration of probiotic to the feed of Gouramy fingerlings (*Oshpronemus gouramy*) Siamese catfish (*Pangasius hypopthalmus*) And Nilem fish (*Osteochillus hasselti*) successfully improved the growth rate performance, feed conversion ratio (FCR) and survival rate of fish. Further study of The use of probiotic in different species of fish and different type of aquaculture system will be needed to enrich the full potential of probiotic in fisheries sector.

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