Aqua Simba Probiotic Application On The Sustainability And Growth Of Nirwana Tilapia (*Oreochromis niloticus*) Nursery II

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ABSTRACT

This study aimed to analyze the appropriate concentration of Aqua Simba probiotics in nurseries II nirwana tilapia (Oreochromis niloticus) feed so that it can produce the highest growth and survival. This research was carried out for 35 days from February 2021 to March 2021 at the Laboratory of Building 4, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The research method used is experimentally using a completely randomized design (CRD) with four treatments and five replications. The research data were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple-distance test with a 95% confidence level if there was a difference in treatment. The treatment given was the addition of probiotic Aqua Simba with different concentrations into the nirwana tilapia nursery II feed which consisted of treatment without the addition of probiotics as a control, the addition of probiotics 3 mL/kg of feed, the addition of probiotics 5 mL/kg of feed and the addition of probiotics 7 mL/kg of feed. The results showed that the addition of probiotic Aqua Simba to nirwana tilapia nurseries II feed had an effect on survival, growth, growth rate, feed conversion ratio and water quality of nirwana tilapia nurseries II seeds. The addition of probiotic Aqua Simba with a concentration of 5 mL/kg of feed resulted in a survival rate of 89.09%, length growth of 0.79%, weight growth of 0.91%, daily growth rate of 1.64%, feed conversion ratio of 3.77 and with good water quality and still within the threshold for nirwana tilapia nurseries II.

Keyword : - *Nirwana tilapia, aqua simba, survival rate, growth, daily growth rate, food conversion ratio, water quality*

1. INTRODUCTION

Tilapia (Oreochromis niloticus) is a freshwater fish consumed in tropical waters which contains 17.7% protein and 1.3% fat [1]. Nirwana tilapia is one of the leading fishery commodities in Indonesia, where the development of cultivation is quite good. Efforts that can be made to increase feed utilization are by adding probiotics to artificial feed which aims to increase feed digestibility in fish. Probiotics are live microorganisms that benefit the host by modifying the microbial community associated with the host, improving nutritional value and feed utilization, optimizing feed use, increasing host response to disease, and improving the environmental quality of microorganisms [2]. Probiotics optimize the performance of enzymes in the digestive tract of fish that are able to break down complex compounds into simple ones so that the feed absorption process will occur more optimally than before [3].

Lactobacillus sp. are bacteria that produce lactic acid which can suppress harmful microorganisms and can decompose organic matter quickly. *Bacillus* sp. is one of the gram-positive bacteria, is a proteolytic bacteria that can decompose protein into amino acids, disaccharides or polysaccharides into simple sugars and is able to produce pectin, which is a complex carbohydrate with pectinolytic properties [4]. *Saccharomyces* sp. can produce amylase and has the potential as an immunostimulant that can improve the fish's immune system by interacting directly with cells that activate the immune system. Saccharomyces sp. is also the main source of nitrogen in water [5, 6].

2. MATERIALS AND METHODS

2.1 Time and Place of Research

The research was carried out in the Laboratory of Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University for 35 days from February 2021 to March 2021.

2.2 Materials and Tools

The tools used in the research are an aquarium measuring 20 × 30 × 40 cm^3, aeration installation, thermometer, DO meter, pH meter, ammonia test kit, nitrate test kit, nitrite test kit, digital scale, tray, spray, zipper plastic, millimeter block and measuring cup. Meanwhile, the materials used were nirwana tilapia seeds measuring 3-5 cm with a weight of 2.5-4.5 g from Balai Benih Ikan (BBI) Cibiru, Aqua Simba probiotics containing *Lactobacillus* sp., *Bacillus* sp. and *Saccharomyces* sp. with a microbial population density of 10^8 CFU/mL, commercial feed PF 1000 with 39-41% protein and molasses.

2.3 Research Methods

Complete randomized design (CRD) was used for the study, consisting of 4 treatments with 5 replications. The data from the research on survival (SR), growth, daily growth rate, feed convention ratio (FCR) of water were analyzed using analysis of variance (ANOVA). Test the real difference between treatments using Duncan's multiple distance test with a 95% confidence level [7]. Water quality data including temperature, dissolved oxygen (DO), pH, ammonia, nitrate and nitrite were analyzed descriptively comparatively.

- Treatment A : Control or without probiotics
- Treatment B : Provision of probiotics with a concentration of 3 mL/kg of feed
- Treatment C : Provision of probiotics with a concentration of 5 mL/kg of feed
- Treatment D : Provision of probiotics with a concentration of 7 mL/kg of feed

The research was carried out through several stages, including preparation of rearing containers, acclimatization of test animals, giving probiotics to feed with a fermentation process and rearing fish. The study was conducted for 35 days with ad libitum feeding twice a day and measuring body weight and water quality every 7 days.

2.4 Procedure for Preparation of Fish Raising Containers

The stages in the preparation of fish rearing containers include :

1. Washing the aquarium and immersing the aquarium using chlorine at a dose of 10 ppt for 24 hours for sterilization, then rinsing the aquarium with clean water.
2. Fill the aquarium with water as much as 20 L or of the volume of the aquarium and aerate for 24 hours.
3. Putting Nirwana tilapia seeds into the aquarium with a density of 1 fish/L as many as 11 tails for the acclimatization process.
4. Perform the acclimatization process for seven days.
5. Give treatment to fish according to each treatment.

2.5 Probiotic Administration Procedure

The stages in the process of giving probiotics include :

1. Activating probiotic microorganisms according to each treatment using 2 mL of molasses.
2. Giving probiotics as much as each treatment carried out on the feed by spraying it on the surface of the feed to be given to the fish evenly.
3. Ferment the feed that has been sprayed with probiotics in a zipper plastic for 24 hours at room temperature, after which the feed can be used.

2.6 Fish Maintenance Procedure

The stages carried out in fish maintenance include :

1. Weigh the test fish the day before the research activity to determine the amount of feed to be given each day.
2. Keeping fish for 35 days in an aquarium that has been equipped with oxygen supply.

3. Feeding ad libitum, namely feeding techniques according to the consumption ability or fish needs with the frequency of feeding twice a day, which is around 10.00 and 17.00 WIB.
4. Sponge and replace the maintenance water as much as 25% of the water volume every two days during the maintenance period.
5. Sampling the weight and length of five fish fry every seven days.
6. Conduct water quality measurements including temperature, dissolved oxygen and pH once a day and ammonia, nitrite and nitrate every seven days.

3. RESULT AND DISCUSSION

3.1 Survival Rate

The survival of fish is very dependent on the adaptability of fish to food and the environment, stocking density and sufficient water quality to support fish growth [8]. The viability of Nirwana tilapia seeds ranged from 83.64-89.09% (Table -1). The range of values for the survival rate of Nirwana tilapia nursery II has exceeded the value set by Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia NO. KEP.23/MEN/2012 with a minimum score of 70% [9]. The use of probiotics in fish farming can reduce fish mortality so that it has an impact on increasing the survival rate of fish and fish resistance to pathogenic infections as well as reducing the environmental burden of fish due to accumulation of waste in the waters [10]. The highest survival rate was found in treatments B and C, namely with the provision of probiotics 3 ml/kg of feed and 5 ml/kg of feed with a percentage value of 89.09% which indicates that the survival needs of Nirwana tilapia nursery II during rearing are met. Factors that affect the necessities of life include stocking density, quality maintenance media, feeding and disease. Probiotics play a role in optimizing the performance of enzymes in the digestive tract of fish and maintaining water quality. The amount of probiotic use also determines the effect of probiotics in increasing the survival rate of Nirwana tilapia seeds, because the use of high doses of probiotics in fish culture does not guarantee good protection for the host animal [11].

Table -1: Survival Rate

Treatment	Survival Rate (%)
A (Control)	87,27
B (3 mL/kg of feed)	89,09
C (5 mL/kg of feed)	89,09
D (7 mL/kg of feed)	83,64

3.2 Growth

Growth is a change in the size of either weight or length in a certain period of time [12]. Each treatment showed different absolute growth. The treatment that added probiotics to the feed had a significant effect on the growth in length and weight growth of Nirwana tilapia nursery II, namely treatment C (Table -2). Treatment A as a control has a lower value than treatment C due to the low activity of bacteria in the digestive tract of fish to convert complex nutrients into simpler molecules so that nutrient absorption is not optimal [13]. The addition of probiotics to fish feed with insufficient or excessive amounts is also not effective in increasing fish growth so that fish cannot use feed optimally. Excess number of probiotic bacteria can lead to increased accumulation of metabolites and competition for the use of nutrients. The number of enzymes as metabolite products will cause some bacteria to die, causing a decrease in digestibility [14, 4].

Table -2: Growth

Treatment	Length Growth (%)	Weight Growth (%)
A (Control)	0,55 ± 0,05 b	0,67 ± 0,12 a
B (3 mL/kg of feed)	0,58 ± 0,13 b	0,55 ± 0,05 a
C (5 mL/kg of feed)	0,79 ± 0,02 c	0,91 ± 0,08 b
D (7 mL/kg of feed)	0,43 ± 0,04 a	0,68 ± 0,25 a

3.3 Daily Growth Rate

The growth rate is related to the increase in body weight that comes from the use of protein in the feed. Probiotics are the addition of microorganisms that have a beneficial effect on the host through modifying the form of association with the host or the microorganism community in its environment, optimizing the use of feed or increasing its nutritional value, competing with pathogenic microorganisms, improving the host response to disease and improving water quality [2]. The best value of daily growth rate was in treatment C with the addition of 5 mL/kg Aqua Simba probiotic feed into the feed (Table -3). The presence of probiotic bacteria in the digestive tract of fish fry will be very beneficial because probiotic bacteria produce exogenous enzymes such as amylase, lipase and protease in the digestive system of fish where these enzymes can reduce energy expenditure (expenditure energy) for the digestive process so that the available energy can be used for growth [15]. The daily growth rates in treatments A, B and D were not significantly different because the energy contained in the feed was first used by fish to meet the energy needs of body maintenance. If there is any remaining energy, it will then be used to meet its growth needs. If the energy in the feed is limited, the energy is only used for metabolism and not for fish growth. If the quality of the feed is poor, the amount of feed is insufficient and the environmental conditions are not supportive, the growth rate of fish will be hampered [16].

Lactobacillus is able to balance digestive tract microbes that can increase digestibility by converting carbohydrates into lactic acid to lower pH thereby stimulating the production of endogenous enzymes to increase nutrient absorption, feed intake and growth and inhibit the growth of pathogenic microorganisms [13]. Lactobacillus also produces the enzyme lactase which breaks down lactose into glucose and galactose. Glucose is used in the lactic acid fermentation process through the glycolysis process to produce lactic acid and energy [17]. Bacillus bacteria are able to remodel feed in a relatively shorter time and play a role in protein degradation reactions that are able to produce proteases so that protein is more easily absorbed by Nirwana tilapia. In addition, Saccharomyces is a biological catalyst that helps biochemical processes in simplifying compounds in feed and plays a role in binding various kinds of pathogenic bacteria that enter the fish body with feed and dispose of them through feces so that fish can grow better [18, 19].

Table -3: Daily Growth Rate

Treatment	Daily Growth Rate
A (Kontrol)	0,90 ± 0,13 a
B (3 mL/kg of feed)	0,95 ± 0,21 a
C (5 mL/kg of feed)	1,64 ± 0,15 b
D (7 mL/kg of feed)	0,84 ± 0,13 a

3.4 Food Conversion Rate (FCR)

The results of the feed conversion ratio between treatments were significantly different and treatment C was the best treatment (Table -4). The value of the feed conversion ratio is closely related to the quality of the feed, so the lower the value, the better the quality of the feed and the fish utilize the feed consumed efficiently, so that the body weight of the fish can increase because the feed is digested optimally. The presence of probiotic bacteria in the feed then enters the digestive tract and suppresses pathogenic bacteria in the intestines so that it can help the process of absorption of food faster. Probiotics optimize the performance of enzymes in the digestive tract of fish so that the provision of probiotics in feed affects the speed of feed fermentation and has an impact on the process of absorption of food in fish digestion which occurs faster and optimally than before [20]. Direct feeding of microbes such as Lactobacillus sp. benefits the host animal by increasing appetite, increasing gut microbes, synthesizing vitamins and stimulating the immune system. The addition of nirwana tilapia seed weight is also related to the efficiency of fish seeds in utilizing and digesting the feed provided.

Probiotics given to feed must be in optimal quantities because the number of bacteria is not right, especially if too many will cause the bacteria to rapidly sporulate so that the function and activity of bacteria in helping the digestive process is not optimal. The FCR value obtained is still high because nirwana tilapia is still in the seed phase where the meat formation process has not been maximized. This shows that Nirwana tilapia seeds have not been able to utilize the feed given optimally so that the feed is not absorbed properly to be converted into meat.

Table -4: Food Conversion Ratio

Treatment	FCR
A (Kontrol)	6,30 ± 0,91 b
B (3 mL/kg of feed)	7,10 ± 1,16 b
C (5 mL/kg of feed)	3,77 ± 0,42 a
D (7 mL/kg of feed)	5,93 ± 2,27 b

3.5 Water Quality

Water quality is one of the main environmental factors in fish farming systems. Water quality is a limiting factor that can affect the environment of the rearing media and support the life and growth of fish which indirectly affects the metabolic processes of the test fish. Poor water quality can make fish susceptible to disease [21]. The results of water quality measurements obtained during the study were still within the optimal threshold (Table -5). The water temperature during the study was in the range of 23.5-28.5°C where the average temperature value was in the optimal temperature range for rearing media for nirwana tilapia nursery II, which was 22-32°C [9]. The metabolic rate of fish is determined by the amount of oxygen consumed, the metabolic rate of full fish will be higher so that the rate of oxygen consumption will increase after the fish eats because fish need oxygen to carry out food intake. If the amount of oxygen needed is not appropriate then the food intake will be suppressed which results in disrupted growth. pH and DO during the study were still in the normal range which is in good conditions for the growth of tilapia nirwana seeds based on the Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia No. KEEP. 23/MEN/2012 [9].

Table -5: Water Quality

Water Quality	Treatment			
	A (Control)	B (3 mL/kg of feed)	C (5 mL/kg of feed)	D (7 mL/kg of feed)
Temperature (°C)				
	24,2 - 28,1	24,3 - 28,5	24,3 - 27,8	23,5 - 27,2
Average	26,12	26,50	26,22	25,28
pH				
	7,4 - 7,8	7,2 – 7,7	7,3 - 7,9	7,1 - 7,6
Average	7,58	7,44	7,59	7,32
DO (mg/L)				
	6,1 - 7,7	5,3 - 7,5	6,2 - 7,7	5,0 - 7,5
Average	6,92	6,52	7,02	6,26
Ammonia (mg/L)				
	0,13 - 0,32	0,18- 0,34	0,17 - 0,33	0,17 - 0,35
Average	0,24	0,28	0,26	0,29
Nitrate (mg/L)				
	0,30 - 0,53	0,28 - 0,43	0,27 - 0,43	0,30 - 0,43
Average	0,38	0,35	0,34	0,36
Nitrite (mg/L)				
	0,08 - 0,31	0,05 - 0,23	0,04 - 0,23	0,05 - 0,29
Average	0,16	0,13	0,12	0,14

Ammonia content during the study ranged from 0.13 to 0.35 mg L, which was still within the normal range of < 1 mg/L [22]. *Bacillus* sp. and *Saccharomyces* sp. contained in probiotics can indirectly suppress the ammonia content contained in the maintenance media. Ammonia accumulation in culture media is one of the causes of a decrease in the quality of maintenance water which can result in production failure in fish farming [23]. Nitrate levels ranged from 0.27-0.53 mg/L, which was still within the tolerance limit for Nirwana tilapia growth, which was <20 mg/L in waters [24]. Nitrite levels ranged from 0.04-0.31 mg/L which was still within reasonable limits of the nitrite content in the waters for nirwana tilapia cultivation 0.5-5.0 mg/L [25]. The decrease in nitrite levels is caused by the nature of nitrite whose concentration is very easy to change because it is influenced by the presence of dissolved oxygen (DO). The presence of oxygen in the water can oxidize nitrite to nitrate, causing unstable nitrite concentrations in

waters. Good probiotics besides being able to be used to improve feed quality can also be used to improve the quality of rearing water so as to increase the survival and growth of fish [26, 27].

4. CONCLUSIONS

Based on the results of the research that has been carried out, it can be concluded that the appropriate amount of Aqua Simba probiotics to be given to Nirwana tilapia (Oreochromis niloticus) nurseries II to increase survival and seed growth is 5 mL/kg of feed. Through the treatment of adding probiotics in the amount of 5 mL/kg of feed, the SR value was 89.09%, the length and weight gain of fish were 0.79 cm and 0.91 g, the growth rate was 1.64%, the feed conversion ratio (FCR) with a value of 3.77 and with good water quality and still within the threshold for nirwana tilapia seeds.

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