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THE EFFECT OF PROBIOTICS ON GROWTH AND SURVIVAL RATES OF MILKFISH (CHANOS-CHANOS FORSKAL) LARVAE IN FRESHWATER

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ABSTRACT
Milkfish is a commodity of high economic value because of its essential nutritional status for the people. This study examines the effect of giving different doses of probiotics mixed in feed on the growth of milkfish (*Chanos chanos* Forskal) larvae. The study used a completely randomized design with 4 treatments and 3 replications. The treatments given were 3 cc/kg feed, 6 cc/kg feed, 9 cc/kg feed, and 12 cc/kg feed. This research was conducted from July to August 2021 at the freshwater fish farming ponds of SUPM Tegal. The results showed that the highest average biomass growth was obtained at a dose of 12 cc/Kg of feed, which was 3,189.43 grams. The lowest average biomass growth was obtained at a dose of 3 cc/Kg of feed which was 606.93 grams. The growth response to the different doses of probiotics was linear, and the survival rate of larvae reached 93%. The water quality range during the research was still suitable to support growth, where the temperature ranged from 23 to 31ºC, pH ranged from 6.5 to 7.5, dissolved oxygen content ranged from 5.5 to 5.6 ppm, and salinity ranged from 8 to 12 ppt.

KEY WORDS
Probiotics, growth, milkfish.

Milkfish have relatively diverse food preferences, including zooplankton, diatoms, small benthos, filamentous algae, mat algae, and detritus (Bagarinao, 1994). The long and dense gill filter of milkfish functions as a filter for aquatic microorganisms (such as plankton), completed with an epibranchial organ as a tool to condense food before being swallowed (Huisman, 1987).

Research shows that milkfish reared in biofloc systems without artificial feeding appear to have relatively slow growth; it is suspected that milkfish cannot grow optimally only by relying on biofloc as a single food source (Usman et al., 2011). In connection with this, research has been carried out on the growth and use of biofloc as a food source in milkfish cultivation by regulating the amount of stocking density to increase protein efficiency in feed and water quality.

Strategies to increase production, including regulating density levels, good environmental conditions, adequate feeding, and appropriate densities, will increase fish growth. The main differences between modular (traditional) and semi-intensive systems are feeding, fish density, pond construction, and milkfish growth.

High-density fish farming increases waste in the waters from cultivation activities, such as leftover feed, feces, and other metabolic waste. Waste containing high nutrients can be detrimental because it may lead to diseases caused by viruses, bacteria, and other organisms that can kill fish and potentially damage the aquaculture environment (Husain, 2014). One technology that can overcome the problem of aquaculture waste is the biofloc system.

Biofloc technology is an alternative to solve the problem of intensive aquaculture waste. This technology has been considered the most profitable because, in addition to reducing inorganic nitrogen waste from leftover feed and manure, it can provide additional protein feed for farm animals to increase growth and feed efficiency for fish (Rangka and Gunarto, 2012), overcome aquaculture waste (Riani, 2012), and reduce inorganic nitrogen (ammonia, nitrite, and nitrate) to improve water quality (Ekasari, 2009). Therefore, biofloc systems have the potential as an additional feed with high protein nutrients to increase growth and feed efficiency.

Milkfish rearing in freshwater is no different from brackish because milkfish are euryhaline (can adapt to a wide range of salinity). Therefore, milkfish can live well in freshwater. However, it takes longer for *nener* (fries or juvenile milkfish) to adapt to freshwater than brackish water. Generally, nener is produced in seawater systems from parent to nursery with water salinity ranging from 30 to 32 ppt (eggs and larvae) and 10 to 20 ppt (*nener*). Thus, milkfish need an adaptation process before being reared in freshwater with salinity ranging from 0 to 5 ppt because the lower the salinity level, the more fries will die. It is actually easy for *nener* to adapt to freshwater as long as the water salinity decreases are done gradually.

Therefore, we conducted a study to examine the effect of probiotics on the survival and growth of milkfish larvae (*Chanos chanos* Forskal) in freshwater.

METHODS OF RESEARCH

The study occurred at the freshwater fish farming ponds of SUPM Tegal, Indonesia, from July 13 to August 3, 2021. The study used a completely randomized design with 4 treatments and 3 replications. We used *nener* (fries or juvenile milkfish). The treatments given were 3 cc/kg feed (Treatment A), 6 cc/kg feed (Treatment B), 9 cc/kg feed (Treatment C), and 12 cc/kg feed (Treatment D). Samples were collected once a week for a month.

Observations were made on Absolute Weight Gain, Specific Growth Rate (SGR), survival rate, and water physicochemical factors. We used 4 pools measuring 5 x 9 meters, each with a *happa* measuring 3 x 1 meter.

Absolute Weight Gain is calculated using the formula by Effendie (1997):

$$Wm = Wt – Wo$$

Where: Wm = Growth absolute weight (grams), Wt = Biomass weight of at the end of the study (grams), Wo = Biomass weight of at the beginning of the study (grams).

Specific Growth Rate (SGR) is the percentage difference between the final and initial weight, divided by the cultivation time. According to Zonneveld et al. (1991), the formula for calculating the Specific Growth Rate is:

$$SGR = (\ln Wt – \ln Wo)/T*100 \text{ (\%/day)}$$

Where: Wo = Average weight at the beginning of the study (g), Wt = Average weight on day t (g), T = Cultivation time (days).

Survival Rate (SR) is the ratio of the number of fish that live from the beginning to the end of the study. Survival can be calculated by the formula (Muchlisin et al., 2016):

$$SR = \text{Survival (\%)} = (Nt – No)/No*100$$

Where: Nt = Number of fish at the end of the study, No = Number of fish at the beginning of the study.

Water Physicochemical Factors determine water quality as a cultivation medium during the study. The physicochemical parameters of the water were observed once a week, including temperature, pH, DO, salinity, and nitrate. Dissolved Oxygen (DO) concentration was measured using a DO meter. Acidity (pH) was measured using a pH meter. The temperature was measured using a thermometer, and nitrate was measured using a spectrophotometer. The analyzed water samples were taken from fish-rearing containers.

RESULTS AND DISCUSSION

Weekly measurements on the weight of milkfish confirmed that the fish experienced weight gain, as presented in Table 1.

Table 1 – Biomass Weight of Juvenile Milkfish (grams) during the Study

Treatment	A	B	C	D
1	584.70	1,469.20	2,289.00	3,240.60
2	602.60	1,469.50	2,286.60	3,206.50
3	633.50	1,377.20	2,112.80	3,121.20
Total	1,820.80	4,315.90	6,688.40	9,568.30
Average	606.93	1,438.63	2,229.47	3,189.43

Source: Research Data, 2021.

Table 1 shows that the highest biomass growth at 3,189.43 grams happens in Treatment D with a probiotics dose of 12cc/kg feed, followed by 2,229.47 grams in Treatment C with a probiotics dose of 9 cc/kg feed, and 1,438.63 grams in Treatment B with a probiotics dose of 6 cc/kg feed. Meanwhile, the lowest biomass growth at 606.93 grams happens in Treatment A with a probiotics dose of 3 cc/kg feed.

Figure 1 presents the increasing biomass growth along with increasing probiotics doses.

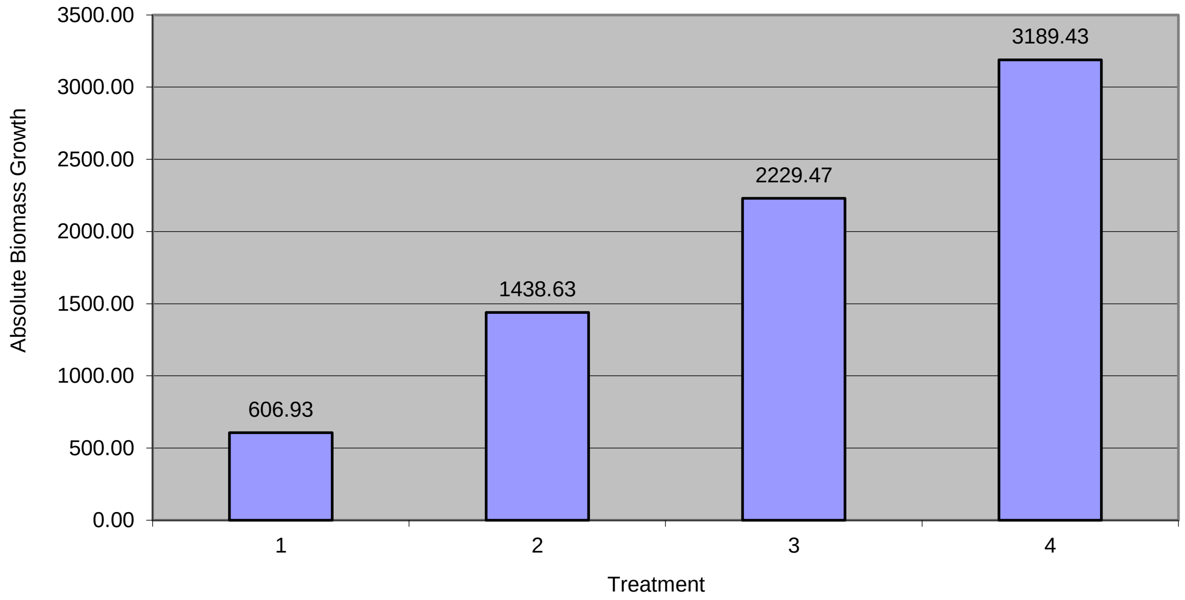

Figure 1 – Biomass Growth of Juvenile Milkfish (Source: Research Data, 2021)

The analysis of variance with the RAL test confirmed that the difference in probiotic doses had a very significant effect on the absolute individual weight gain (grams) of milkfish in nurseries (F count = 837.86 > F table 0.05 (3;8) = 4.07). In other words, the higher the dose of probiotics given in feed, the higher the weight gain of milkfish in nurseries, and vice versa. This might have happened due to competition in space and feed intake.

The growth represents changes in the size of either weight or length in a certain period (Huet, 1971). Growth will only occur if the energy content in the feed exceeds the energy required for body maintenance and replacing damaged cells (Zonneveld et al., 1991). External factors that affect growth include water and environmental conditions, while internal factors are species, sex, genetics, and the physiological status of fish.

Physical growth occurs with changes in the number or size of cells that make up body tissues, while morphological growth can be seen from changes in body shape. Growth will occur when the energy needs for metabolism and maintenance of body tissues have been met following the needs of fish (Hepher, 1988) and if the amount of feed consumed is greater than the amount needed for body maintenance and energy (Huet, 1971).

Feed affects fish performance, which includes growth and reproduction. Thus, quality feed is expected to increase feed efficiency, which is reflected in increased growth. Based on food types, milkfish are herbivores that tend to be omnivorous with a toothless mouth and a very long intestine (the intestine can be several times the length of the body). At the larval stage, milkfish are carnivores that eat zooplankton. At the postlarval stage, they become omnivores that eat zooplankton, diatoms, and benthos. At the juvenile stage, they are herbivores that eat filamentous algae, detritus, and small benthos and can consume the pelleted feed. During the study, milkfish were given artificial feed. The accumulated leftover feed and other metabolic waste affected plankton abundance as the study proceeded. The plankton was used optimally by the milkfish for its growth. The plankton observed during the study is presented in Table 3.

Table 3 – Plankton Observations during the Study

Plankton Amount (Total) cell/ml	Treatment A	Treatment B	Treatment C	Treatment D
Chlorophyta	905,000	1,877,500	2,582,500	1,030,000
Cyanophyta	150,000	190,000	100,000	100,000
Chryptophyta	10,000	-	22,500	-
Crysophyta	25,000	-	25,000	-

Chlorophyta and Cyanophyta had the highest amount. According to Lukstadt (2002), filamentous algae (Cyanophyta) is one of the stomach contents of juvenile milkfish. Amores (2003) confirms that juvenile and adult milkfish eat cyanobacteria, algae, and small invertebrates on the bottom of the water. Likewise, Garcia (1990) states that the food preferred by all ages of milkfish is blue-green algae.

The results of the Specific Growth Rate analysis are presented in Figure 2.

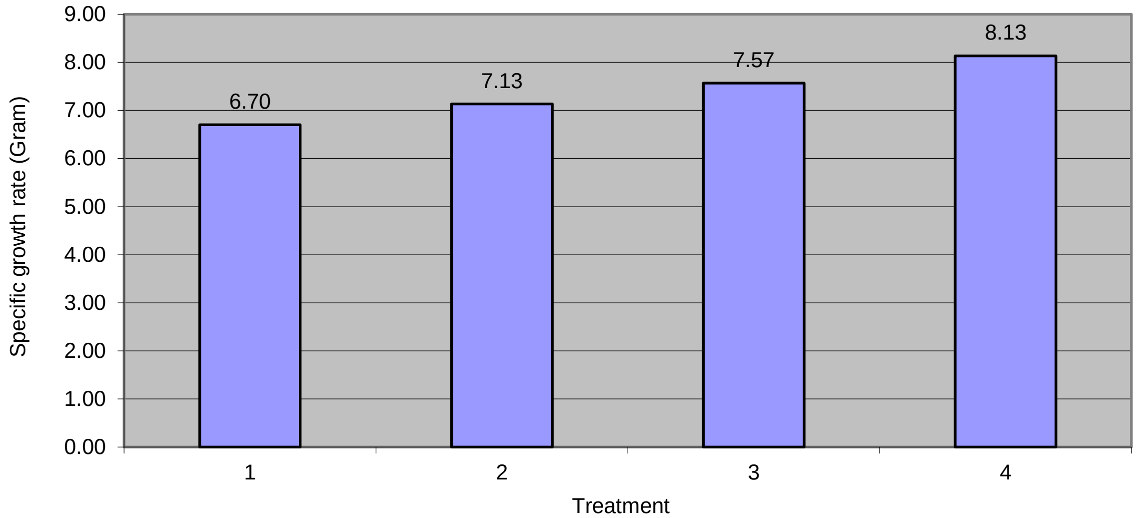

Figure 2 – Differences in Specific Growth Rates of Milkfish during the Study

The analysis of variance with the RAL test confirmed that the difference in probiotic doses did not cause a very significant effect on the specific growth rate of milkfish in nurseries (F count = 3.4887 > F table 0.05 (3;8) = 4.07).

The specific growth rate in Treatment A was 6.70% body weight per day, which showed the lowest growth; this was possible because the low dose of probiotics caused milkfish to grow slower.

The specific growth rate of each individual will slow down when the dose of probiotics is low. A high dose enables fish to utilize food well compared to a low dose because food is an external factor affecting growth (Syahid et al., 2006). Fish growth is also influenced by the ability of fish to utilize feed. Fish cannot make most of the feed if probiotics are given in a dose too small. Sabariah and Sunarto (2009) add that the speed of growth depends on the amount of feed consumed, the amount of protein in the feed, water quality, and other factors such as heredity, age, endurance, and the ability of the fish to utilize the feed.

The high level of feed digestibility is suspected to be caused by *Lactobacillus* sp. bacteria in the probiotics given. Lactobacillus sp. balances the digestive tract microbes to increase digestibility by converting carbohydrates into lactic acid, stimulating the production of enzymes for nutrient absorption, feed consumption, and growth, and inhibiting pathogenic organisms (Samadi, 2002 in Arief et al., 2008).

The survival rate of milkfish ranged from 85.33% to 93%; this was possible due to environmental adaptation, suitability of feed types, ability to utilize feed, availability of natural feed, and the role of probiotics. During environmental adaptation, milkfish adjusted to the salt content where the initial environment had a salt content of 15%. At a dose of 12 cc/kg feed, the average survival rate was 92.33%, which was higher than at a dose of 3 cc/kg feed. The density factor will affect the survival of the population. Effendie (2002) adds that internal and external factors influence fish survival. Internal factors include resistance to disease, feed, and age. External factors include stocking density, disease, and water quality.

Dissolved oxygen levels during the study ranged from 3.11 to 3.87 ppm—these levels were still within the acceptable range for milkfish growth. According to SNI 8005 (2014), the ideal dissolved oxygen content in water for the life and growth of milkfish is > 3.50 ppm. The temperature during the study was around 27.8 to 29.3°C and was still tolerable for the growth of milkfish. Milkfish can live at temperatures between 30 to 35°C (Ghufron and Kordi, 2007). During the study, the degree of acidity (pH) was in the optimal pH range of 7.8. According to Ahmad et al. (1993), the pH of water that can provide normal growth in milkfish is from 6.5 to 8.5, while according to SNI 8005 (2014), the optimal pH for milkfish is from 7.5 to 8.5. Milkfish is a euryhaline fish that can live in a fairly high salinity range (0 – 140 promil). Therefore milkfish can live in freshwater (ponds and rice fields), brackish water, and salt water (sea) (Purnowati et al., 2007). The nitrite levels during the study varied in the range of 0.001 ppm to 0.017 ppm and were still safe for milkfish life. The safe level of nitrite for milkfish growth is less than 0.30 ppm (Ahmad et al., 1993) in the nitrite assimilation process.

Results showed that probiotics from banana hump mixed with fish feed did not affect the mortality of the fish in our study because the average survival rate from the beginning to the end of the study was between 86.67%. Data on the survival of milkfish during the study are presented in Table 3.

Table 3 – Milkfish Survival of (*Chanos chanos* Forskal)

Treatment	Replication	Initial	Sampling							
			I		II		III		IV	
			Total	%	Total	%	Total	%	Total	%
A	1	100	98	98	96	96	95	95	93	93
	2	100	95	95	94	94	93	93	93	93
	3	100	98	98	95	95	94	94	91	91
Average		100	97.00	97.00	95.00	95.00	94.00	94.00	92.33	92.33
B	1	200	190	95	187	93.5	185	92.5	184	92
	2	200	193	96.5	188	94	185	92.5	185	92.5
	3	200	189	94.5	187	93.5	184	92	184	92
Average		200	190.67	95.33	187.33	93.67	184.67	92.33	184.33	92.17
C	1	300	288	96	280	93.33	274	91.33	271	90.33
	2	300	29	93	271	90.33	268	89.33	262	87.33
	3	300	272	90.67	267	89	260	86.67	256	85.33
Average		300	279.67	93.22	272.67	90.89	267.33	89.11	263.00	87.67
D	1	400	374	93.5	362	90.5	353	88.25	347	86.75
	2	400	367	91.75	358	89.5	350	87.5	345	86.25
	3	400	364	91	356	89	352	88	348	87
Average		400	368.33	92.08	358.67	89.67	351.67	87.92	346.67	86.67

According to Yusriman and Heltonika (2010), the factors that influence the survival rate of an organism are biotic factors and abiotic factors. Biotic factors include competitors, population density, age, and the ability of organisms to adapt to the environment. Abiotic factors include temperature, dissolved oxygen and pH. Thus, the addition of probiotics can better affect the survival of milkfish.

In general, water quality, including temperature, pH, and dissolved oxygen content, was still in the normal range during the cultivation period and supported growth. Water quality, food, and fish biology are some of the most determining factors in fish life and growth. The temperature range during the study was 27 to 31°C. According to Achmad (2004), the optimal water temperature for milkfish is between 26 and 33°C. The pH value during the study was still in the optimum range, around 6.5 and 7.5. Kordi (2011) states that pH indicates whether the water environment is good or bad; the pH range for milkfish cultivation is between 6-8. Dissolved oxygen in water is one of the water quality parameters that affect hatchery. Oxygen greatly determines the life of fish and organisms in these waters, especially in the biological function of growth. DO measurement results during the study ranged from 3.5 to 5.6 ppm. According to Achmad (2004), the optimum range of dissolved oxygen for milkfish is between 3.0-8.5 ppm. The salinity range during the study was 8 to 12 ppt. Kordi (2011) mentions that milkfish can adapt to water salinity so that they can live in freshwater (salinity between 0-5 ppt) and salt water (salinity > 30 ppt).

CONCLUSION

The effect of stocking density on the absolute individual weight gain (grams) of milkfish ranged from 6.70 cm to 8.13 cm, and the highest weight gain happened in Treatment 1 (stocking density of 25 fish/m^2).

The RAL test showed a significant effect of probiotics on the absolute individual weight gain (grams) of milkfish in the nursery (F count = 837.86 > F table 0.05 (3;8) = 4.07). The absolute biomass weight gain ranged from 889.63 grams to 2.842.77 grams, and the highest was in Treatment 4 (stocking density of 100 fish/m^2). The RAL test showed that the difference in stocking density significantly affected the absolute biomass weight gains (grams) of milkfish (F count = 126.5352 > F table 0.05 (3;8) = 4.07). The specific growth rate ranged from 6.20% to 5.67%, and the highest was 6.20% in Treatment 1 (stocking density 25 fish/m^2). The RAL test showed that the difference in stocking density had no significant effect on the specific growth rate of milkfish in the nursery (F count = 3.4887 < F table 0.05 (3;8) = 4.07). The survival rate of milkfish until the end of the study was around 85.33% to 93%.

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