Addition of EM4 Probiotics in Commercial Feed For The Growth of Milkfish Juvenils (*Chanos chanos*)

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	ABSTRACT		
Keywords:	Milkfish production can be increased in aquaculture if feeding and the		
EM4; milk fish; use of artificial high protein feed can be optimized. Optimal f			
juvenile; feed;	be done by adding probiotics to the feed, one of which is by using EM4.		
growth;	This study aims to compare the concentration of the addition of		
probiotic	probiotic EM4 in commercial feed so that optimal growth of juvenile		
	milkfish (Chanos chanos) is obtained. This research was conducted in		
	the community ponds of Toiba Village, Bualemo District, Banggai		
	Regency from May to July 2022. This research uses an experimental		
	method which is carried out in one pond plot. There were nine fixed		
	cages with a width of 30 cm and a height of 30 cm used for maintenance.		
	The experimental design used in this study was a completely		
	randomized design (CRD) with 3 treatments and 3 replications.		
	Parameters observed in this study were Specific Growth Rate (SGR),		
	Feed Conversion Ratio (FCR), Survival (SR) and water quality. The		
	results of the research showed that treatment B provided the best		
	Specific Growth Rate and Feed Conversion Ratio for juvenile milkfish		
	because the difference in the number of bacteria contained in EM4		
	problotics can affect the absorption of fish nutrients because the more		
	bacterial population in the digestive tract will increase the availability		
	of nutrients that are ready to be absorbed in the digestive tract. This		
	increase in digestibility is the same as the higher nutrients available to		
	be absorbed by the body which will affect growth and Feed Conversion		
	Ratio.		

INTRODUCTION

Milkfish (*Chanos chanos*) is one of the results of fish farming that lives in brackish water or fish that comes from ponds that have quite good prospects for development. This is because the market demand is quite high because the taste of the meat is delicious, the price is relatively stable, and it is easy to maintain. Milkfish is a food ingredient that contains sufficient nutrition and is beneficial for the body. In 100 grams of milkfish contains 123 kcal of energy, 20 grams of protein, 4.80 grams of fat (Agustina & Anugrah, 2023).

The traditional cultivation system still uses natural feed, so to improve cultivation technology, it needs to be changed to use an intensive cultivation system (Linayati et al., 2021). The intensive cultivation system uses more advanced technology, one of which is using artificial feed. Artificial feed is one of the important production factors to support the success of milkfish cultivation. The costs that must be incurred for the procurement of artificial feed are very large when compared to other production costs. Yuniar (2017) in (Ningsih et al., 2024), feed is a source of material and energy to support the survival and growth of fish, but on the other hand, feed absorbs quite high costs, it can reach 50-70% of the total production costs. Milkfish production can be increased in cultivation activities if the provision of feed and the use of high-protein artificial feed can be optimized. Optimal feeding can be done by adding probiotics to the feed. The addition of probiotics to the feed is useful for increasing the availability of amylase enzymes so that the use of protein as an energy source can be replaced by utilizing carbohydrates as an energy source for fish (Anis & Hariani, 2019).

Probiotics are additional food in the form of live microbes that have a beneficial effect on the host by improving the balance of microbes in the digestive tract. The direct use of probiotics can increase the effectiveness of microbes which can ultimately increase growth. The use of probiotics is an internal solution to produce optimal growth and feed efficiency, reduce production costs and ultimately reduce the environmental burden due to the accumulation of waste in the waters (feces and leftover feed). One of the probiotics used is EM4. EM4 is a mixed culture of beneficial microorganisms, useful for improving water quality and increasing fish growth. Most EM4 contains 90% *Lactobacillus* sp. microorganisms, namely lactic acid-producing bacteria, and in small amounts contains photosynthetic bacteria, *Streptomyces* sp. and yeast (Telaumbanua et al., 2023).

Providing probiotics in feed affects the speed of the digestive tract, so it will greatly help the process of food absorption in the digestion of milkfish. (Ramadhana et al., 2012) in their research, feeding with the addition of probiotic *Lactobacillus* sp. using doses of 3%, 5% and 7% can increase the digestibility value in tilapia. Feed containing 7% probiotics showed a digestibility value of 68.09%, 5% probiotics of 64.99, and 3% probiotics of 63.26%. Probiotic administration with a percentage of 3%, 5% and 7% can increase the number of intestinal mucosa bacteria in tilapia. Furthermore, in the study (Harmilia et al., 2019) feeding with the treatment of additional doses of EM4 probiotics in feed of 7%, 9% and 11%, showed that the addition of a dose of EM-4 probiotics in feed of 11% had a significant effect on the growth of red tilapia (*Oreochromis niloticus*) weight by 6.98 grams and the highest survival rate with a value of 88%. EM4 predominantly contains Lactobacillus bacteria. This bacteria is one of the fermentation microorganisms, so that if it is present in food or feed ingredients, it will be able to improve the quality of the feed so that it can increase digestibility which can increase growth. The purpose of this

study was to compare the concentration of additional EM4 probiotics in commercial feed so that optimal growth of juvenile milkfish (*Chanos chanos*) was obtained.

METHOD

This research was conducted in the community ponds of Toiba Village, Bualemo District, Banggai Regency from May to July 2022 with environmental conditions suitable for the life of juvenile milkfish. The tools used in this study include DO meters, pH meters, hand refractometers, thermometers, and digital scales. While the materials used are young milkfish (juveniles) and EM4 probiotics. Juvenile milkfish were 45 fish with a length of 4 cm and a weight of 1.65 g/fish. The test feed used was from PT. Pangan Tengah Pertiwi Karawang with a protein content of 20%. There are nine tanjap cages with a width of 30 cm and a height of 30 cm used for maintenance.

The obtained milkfish juveniles were placed in a maintenance container for three days for acclimatization. The maintenance container (cage) was prepared according to the treatment. After adapting to its environment, the milkfish juveniles were spread in each maintenance container with a density of 5 fish/container. Feed that had been mixed with EM4 probiotics was given as much as 5% of the weight of the biomass of the test organism with feeding carried out three times a day (morning, afternoon, and evening).

Completely Randomized Design (CRD) with three trials and three replications was the experimental strategy used in this study. The procedure tried involved the addition of various amounts of EM4 to commercial feed namely Treatment A (11% EM4 probiotic concentration in 100 grams of commercial feed), Treatment B (13% EM4 probiotic concentration in 100 grams of commercial feed) and Treatment C (Commercial feed without probiotics or control).

In this study, daily specific growth rate, survival rate, and water quality will all be monitored. At the beginning, middle, and end of the study, observations were made on water quality including temperature, pH, salinity, and dissolved oxygen. To determine the effect of treatment on the weight gain of the test fish, weighing was carried out using an electric scale with an accuracy of (1g) calculated at the time of feeding the test feed.

Parameter measurements include:

1. Specific growth rate using the formula (Zonneveld et al., 1991):

$$SGR = \frac{LnWt - LnWo}{t} \times 100\% \dots (1)$$

Information:

SGR	=	Specific Growth Rate (%);
Wt	=	Average weight of fish (g/tail) at the end of maintenance;
Wo	=	Average weight of fish (g/tail) at the beginning of maintenance;
t	=	maintenance duration (days)

2. Feed Conversion Ratio based on the formula Effendi (2002) in (Chilmawati et al., 2014):

$$FCR = \frac{F}{(Wt+D)-Wo}$$
....(2)

Information:

FCR = Feed Conversion Ratio;

F = Weight of feed given (g);

Wt = Weight of fish biomass at the end of maintenance (g);

D = Weight of dead fish (g);

Wo = Weight of fish biomass at the beginning of maintenance (g)

3. Survival based on formula Effendi (1997) in (Islamiyah et al., 2018):

SR= $\frac{Nt}{No} \times 100\%$(3)

Information:

SR = Number of fish that survive (%);
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No = Total number of fish present at birth (tail);

Nt = Total number of fish that are still alive (tail)

To determine whether the provision of EM4 probiotics in commercial feed has an effect on the growth and Feed Conversion Ratio of juvenile milkfish (*Chanos chanos*), then analysis of variance (ANOVA) was carried out at the 5% and 1% levels. If there is a difference between treatments, the BNT test is continued (Gaspersz, 1995).

RESULT AND DISCUSSION

Specific Growth Rate

Examination of the specific growth rate by administering EM4 probiotics in each commercial feed with treatment A (11% dose of EM4 probiotics; treatment B (13% dose of probiotics); and treatment C (without the addition of probiotics) obtained the averagevalue of weight gain of juvenile milkfish as follows (Figure 1).



Figure 1. Specific growth rate of milkfish juveniles (Chanos chanos) (%)

The results of statistical analysis of specific growth rates with the provision of EM4 probiotics in commercial feed, namely treatment (A) EM4 probiotic dose of 11%, treatment (B) probiotic dose of 13% and treatment (C) without the addition of probiotics on the growth of juvenile milkfish (*Chanos chanos*) showed that there was a very significant difference between treatments (p<0.01). In (Figure 1), treatment B (6.443%) showed the highest average growth value, followed by treatment A (4.827%), while treatment C (2.997%) showed the lowest average value. The provision of Lactobacillus sp. probiotics through feed is also thought to be able to maintain the balance of microbes in the digestive tract by suppressing the growth of harmful bacteria. The addition of EM4 probiotics contains Lactobacillus bacteria, multivitamins, which are beneficial and can help the growth and quantity of feed aroma which causes a high appeal to feed consumption so that it affects the growth of juvenile milkfish.

Microorganisms in probiotics work to break down metabolic waste and stimulate the immune system to improve the health of farmed fish and affect their growth. Probiotics are able to increase the absorption process of nutrients in feed because they are assisted by probiotic bacteria in the digestive tract, thereby increasing growth. The addition of probiotics to commercial feed also increases the number of microflora in the intestines, thereby increasing the activity of digestive enzymes (Telaumbanua et al., 2023).

While the lowest average growth value was shown in treatment C (control), it is suspected that the feed given could not be digested properly by the digestive tract of the test organism. This is in accordance with the opinion of (Setiawati et al., 2013) that *Lactobacillus* sp. bacteria are able to balance digestive tract microbes so that they can increase fish digestibility by converting carbohydrates into lactic acid which can lower pH, stimulate endogenous production to increase nutrient absorption, feed consumption, growth and suppress the growth of pathogenic organisms.

Growth is a change in shape due to an increase in length, weight and volume in a certain period individually. Growth can also be interpreted as an increase in the number of cells through mitosis which ultimately causes changes in tissue size. Growth for a population is an increase in the number of individuals, where the factors that influence it can be internal and external factors. Internal factors include age, heredity and gender, while external factors include temperature, food, disease, cultivation media, and so on (Andriani et al., 2019).

Feed Conversion Ratio

Based on the results of research on the addition of EM4 Probiotics to commercial feed on the feed conversion ratio of milkfish juveniles, the average value of the Feed Conversion Ratio (FCR) was obtained for each treatment (Figure 2).



Figure 2. Feed conversion ratio (FCR) of milkfish juveniles (Chanos chanos)

The results of data analysis (Figure 2) show that the difference in the provision of EM4 probiotic concentration in feed on the average value of the feed conversion ratio shows a very significant difference between treatments (p<0.01). Based on the results of the BNT test, it shows significant results between each treatment, where treatment B (using 13% EM4 probiotics), treatment A (using 11% EM4 probiotics), and treatment C (control) affect the feed digestion process in the fish intestines will be slow so that the utilization of the feed given is not optimal in the growth process. This explains that there is a tendency that treatment A and treatment B with the addition of probiotics to the feed will be able to make fish more optimal in utilizing the feed given. The statement (Setiawati et al., 2013) that the amount of secreted enzymes increases according to the amount of probiotics in the digestive tract, will ultimately increase the amount of feed digested.

Based on the average value of the Feed Conversion Ratio during the study, it showed that treatment B which was given EM4 probiotics had the lowest average Feed Conversion Ratio result (2.043) followed by treatment A (2.244). The Feed Conversion Ratio value in treatment B is the best, because the best feed conversion shows that if the calculation result of the Feed Conversion Ratio has the lowest value, then the amount of feed used to increase weight in a certain time is small, so it is efficient in feed utilization. This statement is in accordance with the opinion of (Anis & Hariani, 2019) that the feed conversion ratio is a comparison between the amount of feed given and the weight of the fish seeds produced. Providing feed in minimal amounts but being able to provide a maximum response to fish seed growth is an indication that the feed has good quality.

Differences in the number of bacteria contained in probiotics can affect the absorption of fish nutrients because the greater the population of bacteria in the digestive tract will increase the availability of nutrients that are ready to be absorbed in the digestive tract through protein hydrolysis into simpler compounds, namely amino acids, so that metabolism becomes easier because protein absorption is assisted by the presence of protease enzymes. This is in accordance with the statement (Setiawati et al., 2013) that the amount of secreted enzymes increases according to the number of probiotics in the digestive tract, which will ultimately increase the amount of feed digested. This increase in digestibility is the same as the increasing number of nutrients available to be absorbed by the body.

Survival Rate

The results of the statistical analysis of survival rate by administering EM4 probiotics in commercial feed, namely treatment A (EM4 probiotic dose 11%), treatment B (probiotic dose 13%) and treatment C (without adding probiotics) on the growth of juvenile milkfish (*Chanos chanos*) obtained an average survival rate value (Figure 3).



Figure 3. Survival rate of milkfish juveniles (Chanos chanos) (%)

Based on the results of the analysis of the survival of milkfish juveniles (*Chanos chanos*), it shows that there is no significant difference between treatments (p>0.05). The results of survival observations at the beginning and at the end of the study did not find any deaths for 42 days, it is suspected that the addition of EM4 probiotics containing Lactobacillus sp. to the feed has a good impact on the survival of milkfish juveniles. The survival of milkfish juveniles is influenced by several factors, one of which is the availability of feed that suits the needs of the fish. Survival can be used to determine the tolerance and ability of fish to survive (Andriani et al., 2019). The addition of probiotics to the treatment is thought to increase immunity and affect survival. Several researchers have found that the use of probiotics can increase the survival and resistance of fish to pathogen infections (Noviana et al., 2014), thus the use of feed supplemented with probiotics can increase growth and reduce mortality rates caused by pathogens.

Water Quality

According to the results of water quality measurements during the study, namely temperature, acidity level (pH), dissolved oxygen (DO), the range of values for several of these parameters was obtained as shown in (Table 1). Table 1. Range of water quality parameters during the study

	Measurement Time			
Parameters	Moorning	Afternoon	Evening	
	(06.00)	(13.00)	(17.00)	
Temperature (°C)	23-24	26-32	26-30	
Dissolved Oxygen (mg/L)	4,6	4,5	4,4	
рН	7,0	7,1	6,8	
Salinity (ppt)	27-29	28-30	28-30	
Source: Processed primary data (202)	2)			

Source: Processed primary data (2022)

The results of the analysis of Table 1 above, the media temperature during maintenance ranged from 23-30°C. The temperature during the study during maintenance was still tolerable and suitable for the life of milkfish. Purnamawati, (2002) in (Irawan & Handayani, 2021), temperature is a very important physical measure for fish life. Each type of fish has a different temperature range, because temperature can affect the growth and appetite of fish. Sudden changes in temperature can cause fish death, even though other environmental conditions are optimal.

This is in accordance with the statement (Putriani et al., 2024) that the optimum temperature for milkfish growth is around 28-32°C. The results of pH measurements during the study ranged from 6.8-7.0, still within the tolerance limits of milkfish life or in good condition. This is in accordance with the statement (Irawan & Handayani, 2021) The right pH value for milkfish cultivation ranges from 6.5 to 9. Death can occur outside this range, and fish growth is also poor. In fact, at pH 4 or 11, milkfish death can occur. Seawater is usually alkaline, so water changes can be done to increase the pH.

The data from the results of dissolved oxygen measurements during the study ranged from 4.4-4.6 mg/L, still within the feasible range. This is in accordance with the statement (Zonneveld et al., 1991) the range of dissolved oxygen for fish cultivation is around 3-5 mg/L. The salinity range obtained during the study was 27-30 ppt, this range still supports the life of milkfish. The appropriate range for the life and growth of milkfish is between 15-35 ppt (Arfan et al., 2022)

CONCLUSION

Based on the results of research and discussion on the addition of EM4 probiotics in commercial feed for the growth of milkfish juveniles (*Chanos chanos*), it can be concluded that the Specific Growth Rate and Feed Conversion Ratio in each treatment have a very significant effect, where the highest Specific Growth Rate and the highest Feed Conversion Ratio in treatment B (13% EM4 probiotic concentration in 100 grams of commercial feed), while the survival of milkfish juveniles (*Chanos chanos*) in each treatment was not significantly different with the average survival value of milkfish juveniles in each treatment reaching 100%. The addition of EM4 probiotics contains Lactobacillus bacteria, multivitamins, which are beneficial and can help the growth and quantity of feed aroma which causes high appeal to feed consumption so that it affects the growth of test fish. It is recommended to obtain optimal growth and survival of milkfish juveniles to provide a concentration of EM4 probiotics of 13% in 100 grams of commercial feed.

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