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Effect of probiotic em-4 in feeds on the growth and survival rate of goldfish (*Carrasius auratus*)

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Abstract

EM-4 is one kind of probiotic which contains Effective Microorganisms Generation Four that could promote the growth of fish, water quality improvement and inhibit the patogen bacteria in waters. The objective of the research were to analyze the effect of probiotic EM-4 in feed on the growth and survival rate of Goldfish, and to determine the best dose of probiotic to gain the highest growth and survival rate of Goldfish (*Carrasius auratus*). The research had been conducted by using completely randomized experiment method which four treatments and three replications. The initial weight of fish was 3 to 4 grams and length 3.5 to 3.6 cm. Based on the research it was found that the probiotic doses in feeds had significant effect on the growth and survival rate of Goldfish. The best dose of probiotic was 30 ml/kg feed with weight growth was 5.6 gram, length growth was 1.2 cm, and survival rate was 100%.

Keywords: Probiotic, growth, survival rate, goldfish

1. Introduction

As increase of social welfare, the variety of amenities needed by peoples also increase. Therefore, the trade of ornamental fishes for amenities is more attention by peoples of Indonesia. This situation become a chance for ornamental fishes farmer to gain the maximum profite. The condition push the fish trader to make the ornamental fish business as main livelihood in several regions in Indonesia.

The ornamental fish business has a good prospect for fish farmer because the fish has highly price, and short period of spawning reproduction cycle (1 to 2 months). In one year period, the ornamental fishes can spawn 10 times [1, 7].

The variety of species, colour, size and body former of ornamental fishes is very important to increase the fish beautiful apperance and harmony to environment. So much peoples assume that ornamental fish culture is good for health, because the swimming movement of fish in a beautiful aquarium can appease our heart, mollifying the blood hypertention and reduce our body stress.

Goldfish (*Carassius auratus*) is one of the ornamental fishes which has good attractives, so, the fish often cultured in aquarium in many countries. The fish also can gives spritual satisfaction, especially for aging peoples [4]. Therefore, the marketing of Goldfish has a good prospect and stimulate the peoples to culture it fish.

Many efforts has been carried out to increase the survival rate of Goldfish cultures, include use of antibiotics. Application of antibiotics in fish culture without strict controlled for prevention and curation of fish disease is dangerous for fish and environment because its can increase the resistance of patogen bacteria in waters [19]. Therefore, use of probiotic is one alternative to increase the survival rate of Goldfish culture.

Several years later, the probiotic has been used for human health, livestock production, soil managenent, and fish cultures [10, 11, 13, 21]. According to Mulyana (2011) [16], the use of probiotic for fish culture is more safety compared to antibiotics, not danger for fish, and allowable by goverment regulation. Probiotics are supplement foods which contains live bacteria that affect on the hospes healthy through balancing of intestinal microbes in digestive system [9].

Probiotic could be given to fish culture through feeds, water and natural feeds such as Rotifer or Artemia. The probiotics has effect on feeds fermentation rate in digestive system, so, its very useful to improve the absorptions of nutrition in fish intestine.

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Fermentation of feeds could degrade the complex compounds to simple compounds like amino acids and forming the vitamins that could promote the growth of fish [15, 3].

Use of probiotic in feeds is ordered to balance the intestine function. Its condition makes the digestive system can produce several enzymes which increase the digestion rate, and immune system of fish [16]. Therefore, the amount of probiotics in feeding must be controlled in order to gain the optimum growth and survival rate of fish.

At present, probiotic EM-4 (Effective Microorganism Generation Four) is much used in fish culture in Indonesia. Probiotic EM-4 contains several species of microorganism involves *Lactobacillus sp*, *Azotobacter sp*, *Clostridia sp*, *Enterbacter sp*, *Agrobacterium sp*, *Erwinia sp*, *Pseudomonas sp*. and lactate formed bacteria [11, 19]. This bacteria is known has several functions, viz. biological control in water environment and in digestive system of fish to inhibit the pathogen bacteria, increase the fermentation rate in fish intestine, and improve the water qualities [21]. Based on this facts, the probiotic EM-4 would be used for ornamental fish especially for Goldfish.

2. Materials and Methods

2.1 Materials

Goldfish fries as many 240 fishes with average weight 4 grams and average length about 3.5 cm were used. The experiment had been carried out in 28 days. The pellet (Trade Mark Takari) as much 100 grams, and 250 ml of probiotic EM-4 was used as treatment in experiment. As many 12 units of glass aquarium with volume capacity 10 litres, 12 units of aerator, thermometer, DO meter, pH meter, digital weigher, ruler, volumetric flask, and volumetric pipette were used as experiment equipments.

2.2 Research Method

The research used experiment method with completely randomized design. The experiment had four treatments of probiotic dose with three replications. The probiotic doses were 0 ml (control), 10 ml, 20 ml and 30 ml per kg feed. Feeding was given three times daily as much 3% of fish body weight [17].

2.2.1 Research Procedures

- The glass aquariums for experiment were cleaned by detergent and dried by air flow of fan. Sterilization of glass aquariums used alcohol 96%.
- All of aquariums were filled by water of 10 litres, and set the aerator in the bottom.
- The fries of Goldfish as 20 fishes were entered to each aquarium, and let it acclimatization for 2 days. During acclimatization, feeding must be given three times daily.
- After 2 days, the treatment of probiotic EM-4 was begun with four doses of treatment.
- The water quality was measured every days in the morning, while the measurement of growth and survival rate of fishes were carried out in once a week until termination of experiment (28 days).

2.2.2 Data Collection

During the experiment, the data collection were: 1) absolute weight growth of Goldfish which was measured once a week,

2) absolute weight growth of Goldfishes, 3) survival rate of Goldfishes, and 4) water quality parameters (temperatures, pH and dissolved oxygen).

The absolute weight growth of fishes was calculated with formula : $W_m = W_t - W_o$, where, W_t is last weight of fish (grams), and W_o was initial weight of fish (grams). While the absolute length growth of fishes was calculated with formula : $L_t = L_t - L_o$, where L_t was last length of fish (cm), and L_o was initial length of fish (cm) [8, 18].

The survival rate of fishes in the research was calculated with formula from Effendi (1997) [8] viz. $SR = N_t/N_o \times 100\%$, where, N_t was total life fishes in the termination of experiment, and N_o was the total fishes in initial of experiment.

2.3 Analysis of Data

2.3.1 Validation of Data

Validation of data had been taken by using Error Variances Homogeneity Test (Barlett Test) in order to verify the homogeneity of data, and allow the requirements for analysis of variance. The Barlett test used Chi Square distributions with formula from Steel and Torries (2003) and Bangun (1997) [20, 5] as follow:

$$\chi^2_{\text{pure}} = \left(\frac{1}{c}\right) \cdot \chi^2_{\text{empiric}}$$

$$\chi^2_{\text{empiric}} = 2,3026 \{ \sum (r_i - 1) \times \log S^2 - \sum (r_i - 1) \times \log S_i^2 \}$$

If the value of $\chi^2_{\text{pure}} < \chi^2_{\text{Table}}$ with significant level 0.05, it means that the data was valid and variance analysis could be carried out.

2.3.2 Analysis of Variance (ANOVA)

Analysis of variance was based on the completely randomized design by using F significant test in order to know the effect of probiotic doses in feeds on the growth and survival rate of Goldfish. The best treatment of probiotic dose was determined by Least Significant Difference (LSD) test with significant level 0.05 and 0.01 and formula from Steel and Torries (2003) [20] as follow:

$$LSD_{\alpha} = t_{\alpha} (\text{Df E}) \sqrt{\frac{2MSE}{r}}$$

Where, MSE is mean square error, r is replication, df E is degree of freedom of error, and t_{α} is value of t distribution on α significant level.

3. Results and Discussions

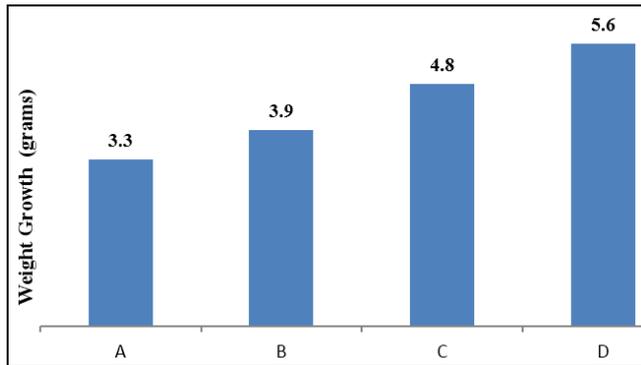
3.1 Effect of Probiotic EM-4 on the Weight Growth of Goldfish

The absolute weight growth of Goldfish during 28 days cultured with used probiotic EM-4 in feeds is presented in Table 1. The average weight growth of fishes range from 3.3 grams to 5.6 grams. The highest weight growth was recorded in D treatment, and the lowest growth was recorded in A treatment (control). The effect of all treatment (B, C and D) was higher than A (control). It means the use of probiotic EM-4 in feeds could increase the growth rate of Goldfishes.

Table 1: Weight Growth of Gold Fish for Each Treatment

Treatments	Initial Weight (g)	Final Weight (g)	Weight Growth	
			Absolute (g)	Relative (%)
A (0 ml/kg)	4.1	7.4	3.3	89.20
B (10 ml/kg)	4.1	8.0	3.9	95.10
C (20 ml/kg)	4.2	9.0	4.8	114.3
D (30 ml/kg)	3.7	9.3	5.6	151.3

The data also showed the increase of probiotic dose in feeds tend to increase the weight growth of Goldfish (Fig. 1). This fact conform to Seharsono (2010) and Mulyana (2011) [19, 16] who stated the use of probiotic in feeds could improve the fish digestive system, and increase the nutrients absorption in small intestine of fish and promotes the fish growth.

**Fig 1:** Weight Growth of Goldfish with Use Probiotic EM-4 in Feeds

Based on the analysis of variance, the different of probiotic dose in feeds caused significant effect ($P < 0.05$) on the absolute weight growth of Goldfish. This result due to

probiotic EM-4 could increase the fermentation rate of feeds in fish intestine, and increase the absorption rate of nutrition in digestive system of fish [6, 14, 15].

Through LSD test was found that average weight growth of B treatment (10 ml probiotic EM-4/kg feed), C treatment (20 ml probiotic EM-4/kg feed) and D treatment (30 ml probiotic EM-4/kg feed) was significant different compared to A (control) treatment (0 ml probiotic EM-4/kg feed). The highest weight growth of Goldfish (5.6 grams) was obtained to probiotic dose of 30 ml/kg feed, and the lowest weight growth (3.3 grams) was obtained to A treatment (control). Therefore, it was concluded that the use of 30 ml probiotic EM-4/kg feed as the best dose treatment to gain the highest weight growth of Goldfishes.

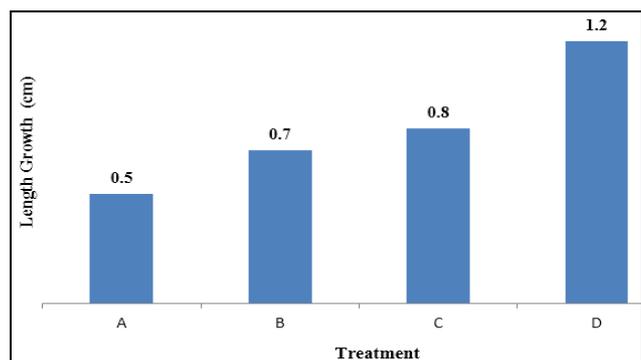
3.2 Effect of Probiotic EM-4 on the Length Growth of Goldfish

The average of absolute length growth of Goldfish for each treatment is between 0.5 cm to 1.2 cm after 28 days cultured. The data of Table 2 shows that average length growth of Goldfish on D treatment (30 ml probiotic EM-4/kg feed) twice more than A treatment (control or 0 ml probiotic EM-4/kg feed).

Table 2: Length Growth of Goldfish for Each Treatment

Treatments	Initial Length (cm)	Final Length (cm)	Length Growth	
			Absolute (cm)	Relative (%)
A (0 ml/kg)	3.5	4.0	0.5	15.14
B (10 ml/kg)	3.6	4.3	0.7	21.21
C (20 ml/kg)	3.6	4.4	0.8	23.05
D (30 ml/kg)	3.5	4.7	1.2	32.01

Beside that, the increase of probiotic doses in feeds tend to increase the length growth of Goldfish (Fig. 2). This fact is in accordance to Mulyana (2011) and Adryanto *et al.* (2010) [17, 3] who stated that used of probiotic in feeds could balance the intestine functions by producing several enzymes which increase the digestion rate, and the growth of fish. Several enzymes produced by probiotic bacteria were lactose, protease, keratinase, amylase, xylanase, cellulase, and lipase [10]. Therefore, use of probiotic EM-4 in feeds could increase the feeds efficiency and reduce feed conversion ratio of fish.

**Fig 2:** Length Growth of Goldfish with Use Probiotic EM-4 in Feeds

Analysis of variance showed that the different of probiotic dose in feeds caused the highly significant effect ($P < 0.01$) on the length growth of Goldfish. From LSD test found the average length growth of B treatment (10 ml probiotic EM-4/kg feed), C treatment (20 ml probiotic EM-4/kg feed, and D treatment (30 ml probiotic EM-4/kg feed) was significant different compared to A treatment (0 ml probiotic EM-4/kg feed). It means that D treatment is the best dose of probiotic EM-4 to gain the highest length growth of Goldfish.

3.3 Effect of Probiotic EM-4 on the Survival Rate of Goldfish

The average survival rate of Goldfish during the experiment was between 75% to 100% (see Table 2). The use of probiotic EM-4 in feeds gives positive effect on the survival rate of Goldfish. The increase of probiotic dose in feeds tend to increase the survival rate of Goldfish. This fact is in accordance to Sorharsono (2010) and Mulyana (2011) [19, 16] that probiotic could increase the immune system of body fish, so, its increase the survival rate of fish. Beside that, probiotic could improve the water quality through degradation of organic compound in waters, and inhibit the pathogen bacteria both in the waters and in the body of fishes.

Based on the analysis of variance, the different of probiotic dose in feeds caused significant effect ($P < 0.05$) on the survival rate of Goldfish. From LSD test was found that average survival rate of Goldfish for B, C and D treatment was significant different compared to A treatment (control).

The highest survival rate (100%) was recorded in C and D treatment, while the lowest survival rate was recorded in A treatment (without use probiotic EM-4). It means the use of 30 ml probiotic EM-4 in feed is the best dose to obtain the highest survival rate of Goldfish.

Table 3: Survival Rate of Goldfish for Each Treatment

Treatments	Initial Goldfish Population (No)	Final Goldfish Population (Nt)	Mortality (%)	Survival Rate (%)
A (0 ml/kg)	20	16	25.00	75.00
B (10 ml/kg)	20	18	10.00	90.00
C (20 ml/kg)	20	20	0.00	100.00
D (30 ml/kg)	20	20	0.00	100.00

3.4 Water Quality

The water quality is very important in aquaculture, therefore the physical, chemical and biological parameters of water must be controlled during fish culture period. Physically, water is the space for fishes movement, and chemically, water contains the nutrient elements and dissolved biogenic gasses, while biologically, water contains natural feeds and microbes which degrades the organic compounds in water.

During the experiment, the water quality was measured every day in the morning at 8.00 am for parameters temperature, pH and dissolved oxygen. The parameter of temperatures has range between 29 °C to 30 °C, pH has interval from 6.8 to 7.1, and dissolved oxygen has range between 5.8 mg/l to 6.4 mg/l. All of parameters still safety for Goldfish culture and suitable for fish life. According to Afrianto dan Liviawaty (1990) ^[1], the water temperature for Goldfish culture was 26 to 30 °C, pH range was 6.5 to 7.2, and dissolved oxygen was 5.4 to 6.8 mg/l.

The stability of water quality during the experiment was related to the water aeration by aerator, and the experiment conducted in the laboratory. In other words, the environment condition during the experiment was homogen. Therefore, the treatment of probiotic EM-4 in feeds give pure effect on the growth and survival rate of Goldfish.

4. Conclusions

1. Use of Probiotic EM-4 in feeds had significant effect on the weight growth, length growth and survival rate of Goldfish.
2. The best treatment of probiotic dose was 30 ml of probiotic EM-4/kg feed with absolute weight growth was 5.6 grams, absolute length growth was 1.2 cm, and the survival rate was 100%.
3. Water quality parameters of temperature, pH and dissolved oxygen during the experiment still suitable for Goldfish culture.

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