



Enriching Probiotics of Feed Using Curcuma to Increase Growth Rates of Tilapia Seeds (*Oreochromis niloticus*)

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Abstract

Tilapia fish (*Oreochromis niloticus*) is a freshwater fish commodity widely cultivated since it is both easily cultivated and in demand by consumers. The growth of tilapia is influenced by the quality of the feed. Probiotics and herbal ingredients can optimize fish growth. One example of probiotics and herbal ingredients is PHL Pro and curcuma. This study aims to determine the effect of probiotics dose of the feed on the growth rates of tilapia seeds. This research was conducted in Pangandaran Marine and Fisheries Polytechnic Campus. The study was carried out using 4 treatments and 3 replications, namely Control or without the addition of probiotics, (P1) 150ml/kg feed, (P2) 200 ml/kg feed, (P3) 250 ml/kg feed. Fish growth measured by the researchers was the average weight and total length of fish every 7 days. Based on observations of the data, it can be concluded that the provision of probiotics in the feed affected the growth rate and total length of tilapia. The optimal dose of probiotics was 150 ml/kg of feed. Based on the study results, the best treatment was P1.

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INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the important species in the World. The total production of tilapia in Asia is 72%, 19% in Africa, and 9% in America (FAO, 2012). Tilapia is a commodity with a vital economic value. Tilapia fish has several pluses, such as being easily cultivated and favored by consumers. People's demands on tilapia lead to increasing fish production which brings about more feed demand. The price of raw material for high-quality feed is getting increased, it is, therefore, necessary to do

a feed efficiency to meet those needs to increase fish production with probiotics fermentation. Putra (2010) stated that the addition of probiotics into the feed increases the number of bacterial populations compared to other treatments, so it is assumed that enzyme activity indigestion and the feed digestibility increase.

Fermentation is the breakdown of indigestible material, such as cellulose into a simple sugar that is easily digested with the assistance of microorganisms.

Enzymes can improve nutrition value, growth and increase the digestibility of coarse fiber, protein, and other feed nutrition (Amarwati *et al.*, 2015). Probiotics is a product composed of cultured microbes or microscopic natural feed which is beneficial and increases the balance of microbes in host animal intestines. Giving probiotics in feed is expected to intensify feed fermentation in the digestive tract, so it will significantly assist the process of food absorption in fish digestion (Anggriani *et al.*, 2012).

Bacteria contained in probiotics have a mechanism to produce several enzymes for feed digestion, such as amylase, protease, lipase, and cellulose. The addition of enzyme-producing probiotics to the feed can improve the feed efficiency; hence it is easily digested and allows the enzymes to work more effectively to improve fish growth performance. Putri *et al.* (2012) stated that the provision of EM4 probiotics on tilapia fish may increase its growth rates in each treatment in which the probiotics doses are 5 ml/kg, 10 ml/kg, 15 ml/kg, and 20 ml/kg. The highest growth rate of tilapia was obtained in the treatment using a dose of 15 ml/kg. It is further described by Mulyadi (2011) stating that probiotics bacteria in the feed can perform significantly in the fish digestion; resulted in higher digestibility of the fish in absorbing food essence which leads to better growth.

Alternative to overcome the problem is using herbal ingredients, e.g., curcuma. It is because curcuma is a medicinal plant that can increase appetite due to its various content namely proteins, carbohydrates, and essential oil consisting of camphor, glucose, tumerol, and curcumin (Anand *et al.*, 2007). Curcuma is a natural antibacterial; it contains compounds controlling the process of metabolism and physiology of the body organ resulting in high-quality probiotics bacteria (Samsundari, 2006; Purwanti *et al.*, 2017). For those reasons, further study should be conducted related to the use of various doses of probiotics on tilapia fish.

This study aims to determine the application of various doses of probiotics provision into the feed to optimize tilapia growth.

METHODOLOGY

Place and Time

The study was carried out in March-May 2020 in Wet Lab of Polytechnic of Marine and Fisheries Pangandaran, Jalan Raya Babakan KM2 Babakan village, District of Pangandaran, Regency of Pangandaran, West Java.

Research Materials

The equipment used during the study was a tub, scale, ruler, spray, pails, panic, drain, basket, basin, pH meter, DO meter, refractometer, strainer, stationery, and pump. Materials are tilapia fish, feed pellets, curcuma, coconut sugar, fine bran, and yeast.

Research Design

Probiotics were applied by spraying them on 1 kg of feed following the treatment. Before being given to the fish tested, the feed was fermented with the following method: the feed was sprayed and then stirred and cooled off to allow the probiotics absorbed evenly; the feed was then let stand for 15 – 30 minutes before being given to the fish tested. It is in line with the opinion of Harianto *et al.*, (2016) stating that all homogeneous ingredients were sprayed evenly into 2500 g of feed using a spray bottle (based on the dosage on the commercial probiotics label) and was then air-dried for 15 minutes and stored in a closed container (jar) at room temperature of 25°C. This study was carried out referring to the study by Puspitasari (2017) that treatment with a dose of 200 ml resulted in good growth of the fish compared to the dose of 50 ml and 10 ml.

The use of probiotics consisted of 3 treatments with 3 replications in each treatment. The treatments are as follow: Control (without probiotics); P1 (Tilapia cultivation with probiotics addition of 150

ml/kg feed); P2 (Tilapia cultivation with probiotics addition of 200 ml/kg feed); and P3 (Tilapia cultivation with probiotics addition of 250 ml/kg feed).

Work Procedure

Probiotics Manufacturing Process

The process of making probiotics began with preparing required materials and fermentation. The materials were 1 kg of coconut sugar, 20 liters of rice water, 2 kg of grated curcuma, 2 kg of fine bran, 60 grams of yeast, and 2 liters of probiotic PHL pro. Those materials were mixed up evenly in a container to get fermented. The process of fermentation was done in sealed tight containers for 1 week and stored in a place with indirect sun rays exposure. Gas will appear during the fermentation process. The containers were opened once every two days to reduce gas. The aim was to eliminate gas fermentation i.e., CO₂ (carbon dioxide). By 1 week, the probiotics can be used to the feed (Arsyad *et al.*, 2015). Technically, probiotic was sprayed onto the feed-in dose of 5 ml, 10 ml, 15 ml solution of probiotics per kilogram of feed; the feed was then allowed to stand for a while before being given to the fish. The dose of probiotics is modified from the results of a study by Fadri *et al.* (2016) indicating that the optimum dose of probiotics EM-4 in the feed is 5 ml/kg.

Data Analysis

The data analysis used in this study is divided into two, namely: quantitative descriptive analysis and qualitative descriptive analysis. Quantitative descriptive analysis is presented in the form of tables, graphs, and pictures. Quantitative descriptive analysis using analysis of variance (ANOVA).

Mulyani *et al.* (2014) stated that survival rate is the percentage of cultivator survival which is calculated by the following formula:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Description:

SR = survival rate (%)

N_t = number of fish at the end of the rearing

N₀ = number of fish at the beginning of rearing

Absolute length growth is the total growth of the length of the final weight minus the length of the initial weight. Absolute length growth can be calculated using the formula of Effendi (1997) as follows:

$$L = L_t - L_0$$

Description:

L = absolute length growth (cm)

L_t = final fish length (cm)

L₀ = initial fish length (cm)

Specific growth rate (SGR) was calculated by collecting and measuring the fish at the beginning and the end of sampling. SGR can be calculated using a formula of Muchlisin *et al.* (2003), as follows:

$$SGR = \frac{W_t - W_0}{t} \times 100\%$$

Description:

SGR = Daily growth rate (%)

W_t = Average weight at the end of cultivation

W₀ = Average weight at the beginning of cultivation

t = period of cultivation (days)

Feed Conversion Ratio (FCR) is the ratio between the feed weight given in the period cycle and the total weight (biomass), which is generated in sampling (Kusriani *et al.*, 2012).

$$FCR = \frac{F}{(W_t + D) - W_0}$$

Description:

FCR = Feed conversion ratio

W₀ = Weight of animal tested (g)

W_t = Weight of animal-tested at the end of study (g)

D = Total Weight of Dead Fish

F = Amount of feed (g)

The value of protein efficiency ratio (PER) can be calculated using the formula of Tacon (1988) as follows:

$$PER = \frac{(W_t - W_0)}{P_i} \times 100\%$$

Description:

PER = protein efficiency ratio (%)
 W0 = weight of test animal biomass at the beginning of rearing (g)
 Wt = weight of test animal biomass at the end of rearing (g)
 Pi = protein content x amount of feed consumed by fish

F = feed amount of gift tilapia during rearing (g)

Value of feed efficiency can be calculated using the formula by Tacon (1988) as follows:

$$FE = \frac{Wt}{W0} \times 100\%$$

Description:

FE = Feed Efficiency
 W0 = biomass weight of gift tilapia at the beginning of rearing (g)
 Wt = biomass weight of gift tilapia biomass at the end of rearing (g)

Water Quality

Measurement of water quality was done *in situ* (directly on-site) and *ex-situ* (a water sample was taken previously). Measurement of *in situ* includes the parameter of temperature, DO, and pH, while measurements of *ex-situ* include the parameter of ammonia (Mukarromah, 2016).

RESULTS AND DISCUSSION

Observation of the growth rates of fish seed resulted in a different value for each treatment for 28 days of rearing. Growth rates of fish seed are presented in Table 1.

Table 1. Fish growth during rearing.

| Parameter | Treatments | | | |
|--------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| | Control | P1 (150 ml) | P2 (20 ml) | P3 (25 ml) |
| SR (%) | 64 ± 0.57 ^a | 65 ± 0.57 ^a | 60 ± 0.57 ^b | 60 ± 0.57 ^b |
| Absolute Length (cm) | 1.7 ± 0.1 ^b | 2 ± 0.1 ^a | 1.7 ± 0.2 ^{ab} | 1.5 ± 0.1 ^b |
| SGR (%) | 1.57 ± 0.03 ^b | 1.83 ± 0.04 ^a | 1.55 ± 0.06 ^{ab} | 1.42 ± 0.05 ^b |
| Feed Conversion Ratio | 2.67 ± 0.04 ^b | 2.42 ± 0.04 ^a | 2.85 ± 0.01 ^c | 3.18 ± 0.04 ^d |
| Protein Efficiency Ratio | 1.05 ± 0.01 ^b | 1.17 ± 0.02 ^a | 0.99 ± 0.02 ^c | 0.88 ± 0.01 ^d |
| Feed Efficiency | 0.37 ± 0.00 ^b | 0.41 ± 0.00 ^a | 0.35 ± 0.00 ^c | 0.31 ± 0.00 ^d |

The level of survival rate is the percentage of living fish at the end of the determined period of rearing. In the fish farming business, the value of the survival rate is a major factor determining the success of harvest and the rearing of organisms/fish. The level of fish survival rate during the study was significantly different; it was identified that the best treatment was in P1 and C treatments, this condition is assumed to increase immunity and affect survival. Based on the whole observation, fish mortality occurred on the 8th day of rearing and it began to stop on the 14th day. This is presumably since the condition of the study environment did not meet the needs of tilapia seeds on day 8 to day 14 for weather changes which thus the fish was stressed and susceptible to disease. Fish' physiological disorders (stress) lead to a drastic decrease in appetite so the fish will find it difficult to

do activities such as swimming and breathing because of the lack of nutrient intake causing less energy of the fish. Energy is needed by the fish for its activities (Subandiyono and Hastuti, 2010). Probiotics addition to Treatment P1 may presumably increase immunity and affect survival. Some researchers found that the use of probiotics increases the survival rate and immunity of fish against pathogenic infections (Iribarren *et al.*, 2012).

The observation results showed that each treatment has a significantly different absolute length, except in treatment C and P3 indicating the insignificant value of absolute length. The highest value was in P1 i.e., 2 cm and the lowest value was in P3 and C. Growth is influenced by energy sources from the available feed. The energy sources are carbohydrates, fats, and proteins, while non-protein energy

sources (carbohydrates and fats) may reduce the use of protein as an energy source. If the source of non-protein energy is sufficient, then the function of protein for growth can be carried out (Wijayanti, 2010). In treatment C, the growth of tilapia seeds was lower than that of Treatment P1 using probiotics. The difference in absolute length growth obtained from each treatment indicated that the feed with 150ml/kg probiotics effectively helps to accelerate the growth of tilapia seed.

Results of data analysis calculation showed that treatment P1 had the best result compared to treatment P2, P3, and C. It is assumed since the dose of probiotics contains bacteria *Lactobacillus*, *Actinomyces sp.*, and *Saccharomyces cerevisiae* in (P1) of 150 ml/kg of feed was efficient and increase the number of bacteria in the digestion tract. Those bacteria secrete digestive enzymes such as protease and amylase in the digestion tract (Setiawan, 2012). Probiotics in the feed can hydrolyze proteins into a simple compound to be easily absorbed through the walls of blood vessels and is used as a deposit to boost growth. Growth occurs when nutrients feed ingested in the form of amino acid absorbed by the fish body is larger than that required to maintain its body (Ramadhana *et al.*, 2012; Fitriadi *et al.*, 2020). While the feed without probiotics (C) had the lowest growth rate among the other treatments. It is due to the lack of bacteria in treatment Control resulting in the absence of enzyme digestion increase. This treatment was carried out based on Puspitasari (2017) stating that treatment using a dose of 200 ml showed the best result for the growth of catfish when compared to that of 50 ml and 100 ml. It indicates that the growth rates of each type of fish depend on the quality of feed and the cultivator to support growth.

Probiotics supplement on day 7 in the treatment C was 0.09% with an average weight of 0.63 grams, treatment P1 was 0.1% with an average weight of 0.7 grams, treatment P2 was 0.08 % with an

average weight of 0.6 grams, and treatment P3 was 0.1% with an average weight of 0.7 grams. On day 4, the highest value was 0.1% in treatment P1 and P3 and the lowest daily result was 0.08% on treatment P2. Based on the observation for 14 days, probiotics supplement on day 14 resulted in the overall weight of fish; treatment C was 0.14% with an average weight of 1.03 grams, P1 was 0.14% with an average weight of 1 gram, P2 was 0.14% with an average weight of 1.03 grams, and P3 was 0.15% with an average weight of 1.1 grams. The effect of probiotics on the process of tilapia growth on day 14 had the highest value in P3 of 0.15%, the daily growth rate was in P1, P2, Control was 0.14%.

Based on 21 days' observations, the results can be illustrated in the above image. Supplementary feeding with probiotics on day 21 resulted in the overall as follows: treatment C was 0.17 % with an average weight of 1.2 grams, P1 is 0.19% with an average weight of 1.33 grams, P2 was 0.18% with an average weight of 1.26 grams, and P3 was 0.17 % with an average weight of 1.23 grams. On day 21, the highest value was 0.19% in P1 and the lowest daily growth rate was 0.17% in treatment C. Based on 28 days observations, the results were as follows: treatment C was 0.2% with an average weight of 1.4 grams, P1 was 0.21% with an average weight of 1.5 grams, P2 was 0.20% by an average weight of 1.46 grams, and P3 was 0.20% with an average weight of 1.43 grams. On day 21, the highest value was 0.21% in P1 and the lowest daily growth rate was 0.2% in treatment C.

The correlation of curcuma supplement in the feed formula to the feed conversion ratio of tilapia seed (*Oreochromis sp.*) shows a significantly different result of each treatment. The best treatment was in P1 with the value of FCR of 2.42 ± 0.04 and the lowest result was in treatment C of 2.67 ± 0.04 and P3 of 3.18 ± 0.04 . The calculation result showed that the average FCR value of tilapia during rearing ranges from 2.42 –

3.18. The average value of FCR tended to increase along with the curcuma supplement. FCR of this treatment is much lower compared to the treatment carried out by Listiowati and Pramono (2014) conducting research using substitution of fermented cassava leaves with an FCR value of 2.25. FCR value will increase along with the supplement of cassava leaves fermentation.

Results of the study showed that the best value of protein efficiency ratio was 1.17 in treatment P1. This result is lower than the study by Amarwati *et al.* (2015) conduction a study on the supplement of fermented cassava leaves flour by 10% (1.68). It is assumed that the feed with the supplement of fermented cassava leaves

flour by 10% was able to utilize the protein in the feed efficiently. According to Marzuqi *et al.* (2004), the value of protein efficiency ratio is influenced by the ability of fish to digest feed. This ability is influenced by several factors, namely the feed composition, in which the higher protein utilized by the body indicates the more protein efficiency. Protein will be used as energy and the excess protein in the feed will be used for growth.

Good water quality is one of the keys to success in fish farming. The water parameters observed during this observation included temperature, pH, and ammonia. Water quality measurements were carried out 3 times a day in the morning and evening.

Table 2. Data analysis of water quality measurements.

| Parameter | Initial Data | Sampling 1 | Sampling 2 | Sampling 3 | Sampling 4 |
|------------------|--------------|------------|------------|------------|------------|
| Temperature (°C) | 30 | 30 | 30 | 30 | 30 |
| pH | 7 | 7 | 7 | 7 | 7 |
| Ammonia (mg/l) | 3.5 | 0 | 0 | 0.25 | 0.25 |

Table 2 shows that each treatment has the same water quality value because the maintenance system uses recirculation except for the ammonia content. Temperature value during the study was 30°C, pH 7, dan ammonia 0-0.25 mg/l indicated in P2 was 0.6 cm and the lowest value indicated in P3 was 0.3 cm. it is in line with the opinion of Monalisa and Minggawati (2010) stating that suitable water quality for tilapia is the temperature of 25-30°C, DO 5 – 7 ppm, pH of 6 – 8.5 and ammonia <1 ppm.

CONCLUSION

The difference of absolute length growth obtained from each treatment indicated that the feed with 150ml/kg probiotics supplement could effectively accelerate the growth of tilapia seed. The absolute length growth of tilapia in each treatment was different. The highest value was found in P1 which was 2 cm and the lowest value was in P3 which was 1.5 cm. The survival rate of tilapia in each treatment was different; the highest value

was at P1 which was 65%, P2 of 61%, P3 of 61%, and Control of 65%.

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