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EFFECT OF ADDITION EM4 PROBIOTICS IN FEED ON GROWTH RATE OF NILEM FISH (*OSTEOCHILLUS HASSELTI*) *JUVENILE*

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KeyWords
Osteochillus hasselti, probiotics EM4, survival rate, specific growth rate, feed efficiency.

ABSTRACT

This research aims to analyze the effective concentration of EM4 probiotics as an additive in commercial feed to show the best survival rate, growth rate and feed efficiency of Osteochillus hasselti juvenile. The research method used is the experimental Complete Random Design (CRD) with 4 treatments and 3 replications. The treatment A (control), B (concentration of probiotic EM4 10 ml kg-1), C (concentration of probiotic EM4 15 ml kg-1) and D (concentration of probiotic EM4 20 ml kg-1). Observed parameters are survival rate, specific growth rate, growth of length, feed efficiency and water quality. The results showed that the addition of EM4 probiotic concentration 15 ml kg-1 in feed gave the best results on the specific growth rate of Osteochillus hasselti which was 2.14% and feed efficiency is 59.26%.

INTRODUCTION

Aquaculture is the one important aspect in the fisheries sector in Indonesia. This relates to their role in the availability of national food, creating income and employment. Also, aquaculture is considered as an important sector in supporting rural economic development. One of the developed aquacultures is Osteochillus hasselti aquaculture.

Ostheochilus hasselti is a popular endemic fish in West Java and has great potential to be developed because apart from being a consumption fish, Osteochilus hasselti can act as a biological agent for cleaning the environment of water (bio cleaning agent). The presence of Osteochillus hasselti populations in public waters is known to decrease. This population decline is due to exploitation and is also a result of changes in the aquatic environment.

One of the obstacles in the aquaculture is the slow growth that causes a low amount of production. This is presumably because fish are still not perfect in utilizing commercial feed even though the protein content is quite high. Feeding is an important factor in the aquaculture business. The feed is one of the important elements that support the process of survival and the growth of fish in aquaculture (Sahwan 2004). One way that can be done to increase fish growth is by increasing feed digestibility through additional intake or supplements (Gil-Gomes et al., 2000 in Faziel et al. 2017). One of the common additions given is the addition of probiotics into a commercial feed.

The use of probiotics in aquaculture has been very popular among farmers. Probiotics are applied to improve feed efficiency and increase fish growth productivity so they can be harvested faster (Andisan 2012). Wang et al. (2008) in Arief (2014) explained that probiotic bacteria produce enzymes that can break down complex compounds into simple ones so that they are more easily absorbed by fish. Bacteria contained in probiotics have a mechanism in producing several enzymes for feed absorption such as amylase, protease, and lipase. These enzymes will help hydrolyze feed nutrients (complex molecules) such as breaking down carbohydrates, proteins and fats into simpler molecules which will facilitate the digestion and absorption process in the digestive tract of fish (Arief et al. 2014).

One of the probiotics known in the market is probiotics with the trademark Effective Microorganisms-4 (EM4) produced by PT Songgolangit Persada. EM4 is chosen by many farmers because of the microorganism content contained therein, the price is relatively cheaper and easier to obtain. The use of EM4 in the field of fisheries has provided many positive results in fish farming.

This research aims to analyze what is the optimal concentration level of the use of EM4 probiotics as additives in commercial feed to get the best survival, growth rate and feed efficiency in Osteochillus hasselti juvenile.

MATERIAL AND METHODS

The materials used in this research are 350 tails of Osteochillus hasselti size of 5-7 cm from Ciparanje Faculty of Fisheries and Marine Science Universitas Padjadjaran, EM4 brand probiotics, commercial feed brands FF-999 and molasses. This research uses an experimental method with a Complete Random Design (CRD) using four treatments and three replications.

Fish are acclimated for two weeks with a density of 20 fish/ aquarium. Feed was weighed and EM4 probiotics added with different concentrations (control, 10, 15, 20 ml kg -1) then fermented for 48 hours.

The parameters observed in this research are survival, growth rate, feed efficiency and water quality including temperature, pH and DO.

Survival Rate

Survival is observed every day by weighing and measuring the length of the fish when someone dies. Survival is the number of fish that live at the end of a certain period (Effendie 1997). To find out the survival of fish, the following formula is used:

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

Description:

SR = Survival of fish during the experiment.

Nt = Number of fish at the end of the experiment.

No = Number of fish at the beginning of the experiment

Specific Growth Rate

Growth is observed every 7 days by weighing the weight of all fish each treatment. Calculation of daily growth rates using the formula Effendie (1997):

$$\text{SGR} = \frac{LnWt - LnWo}{t} \text{ x } 100\%$$

Description:

SGR : Specific growth rate (%)

LnWt : Ln Weight of fish at the end of the study (g)

LnWo : Ln Weight of fish at the beginning of the study (g)
t : Research time (days)

Growth of Length

Observation of the length is done once every 7 days together with weight observations. The length measured is the total length. According to Effendie (1997), long growth can be expressed by the following formula:

$$L = Lt - Lo$$

Description :
L : Growth in absolute length (cm)
Lt : Average length of fish end (cm)
Lo : Average beginning length of fish (cm)

Feed Efficiency

Feed efficiency is the ratio between the body weight produced and the amount of feed given during the study. Calculation of feed efficiency using the formula Zonneveld et al. (1991) as follows:

$$FE = \frac{(Wt+D)-Wo}{F} \times 100\%$$

Description :
FE : Feed Efficiency (%)
Wt : Fish biomass at the end of the study (g)
Wo : Fish biomass at the beginning of the study (g)
D : Weight of fish that died during maintenance (g)
F : Amount of feed consumed (g)

Water Quality

The observed water quality includes temperature, pH and dissolved oxygen (Dissolved Oxygen). Water quality measurements were observed before fish sampling every 7 days.

Data Analysis

Data processing was performed using statistical calculations with the ANOVA (Analysis of Variance) method to find out the differences in each treatment given. If the results of Fcount> Ftable, then proceed with Duncan's multiple distance analysis with a confidence level of 95% (Gaspersz 1995), except for water quality analyzed descriptively by comparing with water quality standards.

RESULTS AND DISCUSSION

Survival Rate

Survival is the presentation of the number of fish that live in a certain period of time (Effendie 2002). Survival can be influenced by internal factors such as fish species and fish age. External factors also affect the survival of fish such as environmental conditions, fish health, stocking density, availability of food and water quality. The survival value of the study results for 35 days for each treatment is presented in Figure 1.

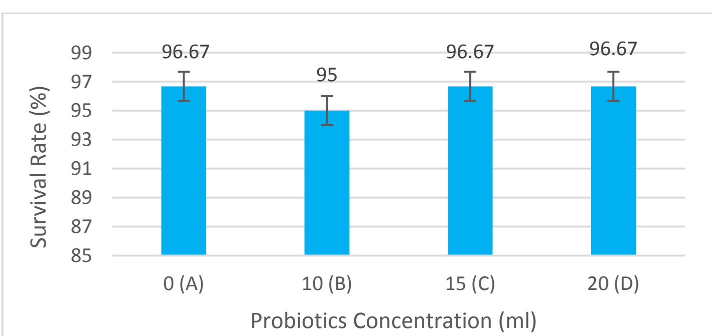

Figure 1. Survival rate *Osteochillus hasselti* juvenile Diagram

According to Husen (1985) in Mulyani et al. (2014) the survival rate (SR)> 50% is in the good category, 30-50% is moderate and <30% is classified as unfavorable. Effendi (2004) states that survival rate is influenced by biotic factors such as competition, age, density and human handling, while abiotic factors are physical and chemical properties in water. High density will cause a decrease in water quality, especially dissolved oxygen content (Noor and Pakaya 2018). A decrease in water quality can cause stress on fish, even if a decrease in water quality has exceeded the tolerance limit it will result in death. In addition, decreased water quality can also affect the appetite of fish and when appetite is reduced, the intake of feed into the body of the fish will also be reduced so that energy for maintenance and growth is not optimally fulfilled.

The results showed that all treatments resulted in values above 80%, this was due to environmental conditions in the maintenance container during the study were still within the optimal limits for the survival of *Osteochillus hasselti*. In addition, the provision of good quality feed makes the nutritional needs of fish fulfilled. The lowest yield was obtained in treatment B (10 ml kg^{-1}) in the amount of 95% (Figure 1).

The results of analysis of variance with a 95% significant showed that the treatment with probiotic EM4 in feed on the survival of the nilem fish produced no significant effect on each treatment, according to the statement of Parinduri et al. (2015) that the adition of probiotics in feed given to catfish did not have a significant effect on fish survival, where F counts <F table (0.05). The use of EM4 probiotics in feed has no significant effect, both in beneficial and detrimental processes.

Specific Growth Rate

The results of analysis of variance showed that the addition of probiotics in commercial feed had a significantly different effect on the specific growth rate *Osteochillus hasselti*. Treatment A (Control) showed the lowest growth rate compared to those treated with the addition of probiotics 10 ml kg^{-1}, 15 ml kg^{-1} and 20 ml kg^{-1} in feed. Duncan's multiple range test results at a 95% confidence level indicate that all treatments were significantly different (Table 1).

Table 1. Average Specific Growth Rate of *Osteochillus hasselti*

Treatment	Specific Growth Rate (%)
Control (A)	2,01 $\pm$ 0,06 [a]
EM4 10 ml kg^{-1} (B)	2,08 $\pm$ 0,02 [b]
EM4 15 ml kg^{-1} (C)	2,14 $\pm$ 0,01 [c]
EM4 20 ml kg^{-1}(D)	2,06 $\pm$ 0,01 [ab]

The results showed that treatment with the addition of probiotics as much as 15 ml kg^{-1} in feed (treatment C) gave the highest specific growth rate is 2.14%, this is because probiotic bacteria added to the feed provide an active role in the digestive tract of fish. Probiotic in feed that enters the digestive tract can grow and colonize the intestine (Sugih 2005). According to Gatesoupe (1999) in Juliani (2013) the activity of bacteria in digestion will change quickly when there are microbes that enter through the media feed, causing a balance between the bacteria that are already in the digestion with the bacteria that enter. Probiotic bacteria that enter the digestive tract of fish are antagonistic to pathogenic bacteria in the digestive tract of fish so that it makes the digestive tract of fish better in digesting feed.

Increased digestive activity is due to an increase in probiotic populations in the digestive tract and enzyme production due to bacteria. According to Haryanto (1977) in Juliani (2013) an increase in the number of probiotics in feed increases the number of bacterial populations in the digestive tract and increases enzyme activity. With the increase in enzyme activity, the process of food remodeling will be faster so that the process of food absorption increases and in turn will increase fish growth.

Feed that enters the digestive tract will be digested into simple particles by digestive enzymes to be absorbed through the intestinal wall. *Lactobacillus* sp. is able to produce lactic acid from simple carbohydrates that can affect the pH of the digestive tract in a more acidic direction so that *Saccharomyces cerevisiae* can grow well (Jin 1996 in Juliani 2013). *Saccharomyces cerevisiae* is able to make the condition of the digestive tract to be anaerobic which will grow *Lactobacillus* sp. well and able to maintain the balance of probiotics in the digestive tract (Fuller 1992). Haetami (2008) states that the use of probiotics directly will increase the effectiveness of intestinal microbes which in turn will increase growth. The role of *Lactobacillus* sp. in addition to producing lactic acid is able to improve the functions of digestive enzymes, breaking down complex molecules into simpler molecules so that food is easily absorbed (Meutia 2008).

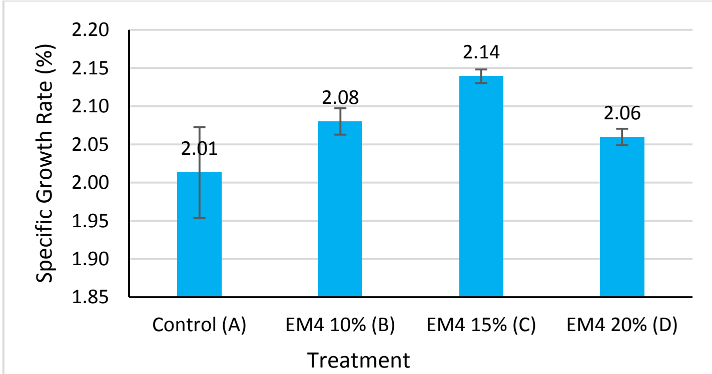

Figure 2. Chart of Specific Growth Rate of *Osteochillus hasselti*

Based on the results of the study note that the addition of probiotics in commercial feed with various treatments gives an average yield of individual weight. The average weight of *Osteochillus* during the study (35 days) increased with time of maintenance. The addition of probiotics as much as 15 ml kg^{-1} in feed (treatment C) resulted in the highest average individual weight gain of 2.14%, followed by treatment B of the addition of 10 ml kg^{-1} of probiotic feed of 2.08% and treatment D of addition of probiotics 20 ml kg^{-1} in feed that is equal to 2.06% and the lowest yield is shown in treatment A (control) without the addition of probiotics which is equal to 2.01%.

Generally, growth of *Osteochillus hasselti* is relatively slow due to the low absorption of fish. The treatment without the addition of probiotics (control) gives the lowest growth rate is 2.01%, this is due to the absence of the role of probiotic bacteria added to the feed so that the food digested by fish is less than optimal which causes a low daily growth rate.

Growth of Length

Based on the results of the F test that the growth of nilem fish seed length was not significantly different but it was seen that there was a tendency of different values in the treatment given additional probiotics compared to treatments that were not given probiotics (Table 2).

Table 2. Average Growth of *Osteochillus hasselti* Length

Treatment	Growth of Lengt (cm)
A (Control)	$1,10 \pm 0,02^a$
B (10 ml kg^{-1})	$1,15 \pm 0,07^a$
C (15 ml kg^{-1})	$1,20 \pm 0,02^a$
D (20 ml kg^{-1})	$1,15 \pm 0,01^a$

Description : Values followed by the same letter are not significantly different
Duncan's multiple range test at 95% confidence level.

The results showed that the smallest length increase was obtained in treatment A (control) is 1.10 cm (table 2), this was due to the absence of the role of probiotic bacteria like other treatments so that the nutrient content of the control treatment was lower than other treatments. The highest length growth was obtained in treatment C (15 ml kg^{-1}) is 1.20 cm. This is consistent with the results of maintenance Asma et al. (2016) that the growth of nilem length ranged from 0.98 - 1.66 cm.

Setiawati et al. (2013) explained that fish will grow if the nutrients of the feed that are digested and absorbed by the body are greater than the amount needed to maintain the body. This can occur if the supporting factors are in optimal condition and will be different as if the supporting factors such as temperature are within the limits that can be tolerated by fish, so the food eaten is more used to defend themselves for life (Akhyar et al. 2016).

Feed Efficiency

Feed is one of the important factors in a fisheries aquaculture activity. The availability of feed is very influential on the survival and growth of fish. The addition of probiotics into the *Osteochillus hasselti* feed gives significantly different results in each treatment. Feed efficiency is the ratio between the body weight produced and the amount of feed given during the study. Efficient use of feed by fish shows the results (percentage) of food that can be utilized by the fish's body (Buwono 2000). Efficiency of feed is directly proportional to body weight gain, so the higher the value of feeding means the more efficient the fish in utilizing the food consumed for growth (Djajasewaka 1985).

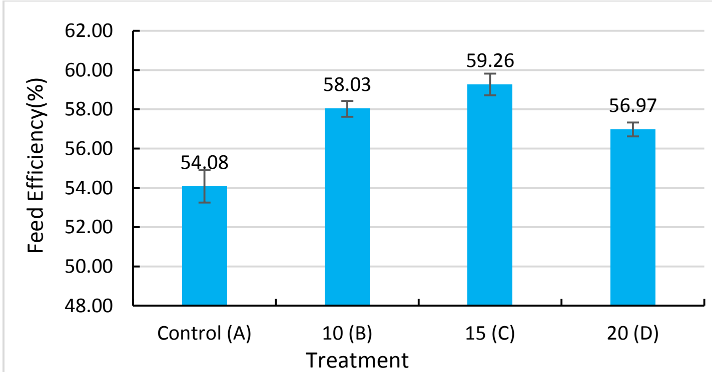

Figure 3. Feed Efficiency of *Osteochillus hasselti* Chart

The results of observations on the level of feed efficiency obtained in all treatments ranged from 54.08 - 59.26%. The best results or those that have the highest value of feed efficiency are treatment C (15 ml kg^{-1}) of 59.26% (Figure 3). The high value of feed efficiency is due to the role of bacteria that enter the digestive tract of fish in producing enzymes needed during the absorption process so that it can break down food that is difficult to digest into food that is easily digested and increases the value of feed efficiency (Mulyadi 2011). The use of feed by fish shows the percentage value of feed that can be utilized by the body of the fish. Factors that influence the high and low feed efficiency are the types of sources of nutrition and the amount of each component of the source of nutrients in the feed. The amount and quality of feed given to fish affects the growth of fish. The value of feed efficiency obtained in the results of this study can be seen in Table 3.

Table 3. Feed Efficiency of *Osteochillus hasslti*

Treatment	Feed Efficiency
A (Control)	54,08 $\pm$ 0,83 [a]
B (10 ml kg^{-1})	58,03 $\pm$ 0,40 [c]
C (15 ml kg^{-1})	59,26 $\pm$ 0,55 [d]
D (20 ml kg^{-1})	56,97 $\pm$ 0,35 [b]

The value of feed efficiency in treatment A (control) shows the lowest results compared to other treatments that is equal to 54.08%. This is consistent with the statement of Arief (2014) that low feed efficiency is caused by the lack of absorption of feed due to the absence of the addition of probiotic bacteria in treatment A (control) in digestive activity causing imperfect absorption of energy for growth. According to Putra (2011), the addition of probiotics in feed aims to increase the population of probiotic bacteria in the digestive tract of fish, so the mechanism of probiotics in producing exogenous enzymes for digestion is increasing. The existence of probiotic bacteria that produce several enzymes will increase the absorption of nutrients in the feed (Zahidah 2017). Craig and Helfrich (2002) stated that feed can be said to be good if the value of feed efficiency is more than 50%. The value treatment A shows quite significantly different results when compared with the treatment given additional probiotics (Table 3).

The value of feed efficiency increases with the increase in the concentration of probiotics given, namely in Treatment B (10 ml kg^{-1}) and C (15 ml kg^{-1}). However, there was a decrease in the results of the treatment D (20 ml kg^{-1}), it was suspected because of the increasing number of bacteria in the digestive tract so that the digestibility of the feed was not optimal. Anggriani et al. (2012) explain that the increasing number of probiotic bacteria will cause the accumulation of metabolites and competition in the use of nutrients. According to Mulyadi (2011), the number of bacteria that is too much causes the bacteria to rapidly undergo sporulation (forming spores) so that the function and activity of the bacterium *Lactobacillus* sp. not optimal. So that the more bacteria in the digestive tract of nilem fish, the digestibility of feed is not optimal and causes a decrease in the value of feed efficiency, although it is still better when compared to treatment A (control).

Water Quality

Observation of water quality can be used as supporting parameter data during the maintenance of *Osteochillus hasselti*. Water quality parameters during the study included temperature, pH and dissolved oxygen (DO). Water quality measurements were carried out once a week during the study period starting on days (0, 7, 14, 21, 28 and 35). The results of water quality measurements obtained during the study generally showed that the water quality during the study was still in a good range to support the maintenance of *Osteochillus hasselti.*

Table 4. Water Quality

Treatment	Parameter		
	Temperature (°C)	pH	DO (mg l^{-1})
A (control)	22–25,1	6,92–7,6	6,5–8,7
B (10 ml kg^{-1})	22–25,1	6,85–7,6	6,5–8,4
C (15 ml kg^{-1})	22–25	6,78–7,8	6,3–8,7
D (20 ml kg^{-1})	22–25	6,77–7,6	6,5–8,7
Optimal*	18–28*	6,7–8,6*	5–8**

Description:
*: Wicaksono 2005
**: Cholik et al. 2005

The average temperature during the study was relatively stable in the range of 22 - 25.1°C. This value is good for the growth of *Osteochillus hasselti*. The temperatures are too high or too low, can cause fish to not grow and develop properly. According to Wicaksono (2005), the ideal temperature range for the growth of *Osteochillus hasselti* is 18-28°C.

The degree of acidity (pH) during the study was still in the normal range of 6.77 - 7.8. This value is still in the optimal limit, according to Wicaksono (2005) the productive pH of waters for nilem is 6.7 - 8.6.

Dissolved oxygen content (DO) during the study ranged between 6.3 - 8.7 mg L^{-1}. This shows that water quality during the study was still a limiting factor.

CONCLUSIONS

Provision of EM4 probiotics of 15 ml kg^{-1} in feed produced 2.14% of specific growth rate and the best feed efficiency is 59.26% but had no effect on survival rate and also the length growth of *Osteochillus hasselti*. Survival rate and growth of length are 95% - 96.67% and 1.10 - 1.20 cm.

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