Effect of dietary probiotics SEAL on growth performance of red tilapia (Oreochromis sp.) nilasa strain

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Abstract. Aims of this study were to determine the effect of probiotic application on the growth performance and feed utilization of red tilapia nilasa strain juveniles. Yeast and bacterial strains of different origin (Saccharomyces sp., Enterobacter sp. JC10, Aeromonas sp. JC33, and Lactococcus sp. JAL12) were administered to fish in daily and three day intervals at dose of 5x10⁴ CFU/gram feed. The control groups were only administered with fish pellet with saline buffer. The fish were stocked in 95 × 72 × 55 cm containers (50 each) and fed approximately 3% of their body weight thrice a day for 62 days. Absolute, relative and specific growths of weight were examined at one and two months of probiotic application. Fish survival rate, total production, feed conversion ratio (FCR) were analyzed at the end of the experiment. The results showed that probiotics application in fish affected absolute weight, relative weight, specific weight, and total production (P<0.05). No significant effects on fish feed utilization parameters were obtained. It is concluded that probiotics SEAL application is efficacious in the growth performance of tilapia nisala strain. The application with three-day interval improves the growth and total production of red tilapia similar to the daily probiotics application.

1. Introduction
Red tilapia (Oreochromis sp.) is an economically important freshwater species cultured worldwide. Tilapia is selected by fish farmer due to the ability to eat on various feeds, rapid growth, tolerance to a wide range of culture conditions [1]. Tilapia is responsive to manipulation. Many efforts have been done to enhance the growth performance of tilapia, including genetic modification, water quality management, and feed improvement. Tilapia is also responsive to probiotics applications by using various strains and administration methods [2].

Probiotics is live microorganisms that provide beneficial effects to fish [3]. The benefit of probiotic application in aquaculture could be in the term of growth performance, survival rate, immunity, digestion system, disease resistance, water quality improvements, and stress management [4][5][6]. Probiotic should be applied in the optimum amount to obtain the best effects on the host. Incorrect application of probiotics will destroy microbiome balance of the fish intestine, and disrupt the balance of enzymatic process in the gastrointestinal tract [3].
The use of probiotics with local origin is suggested to support sustainable aquaculture. In the present study, we evaluate the efficacy of probiotics SEAL in promoting growth performance of red tilapia (*Oreochromis* sp.) nilasa strain. The probiotics was composed of yeast bakers (*Saccharomyces* sp.), and three locally isolated bacteria strains, the member of the genus *Enterococcus*, *Aeromonas*, and *Lactococcus*. These probiotics bacteria were originated from the intestine of Indonesia local fishes with strong enzymatic activity [7]. The four microbe genera have been previously reported by previous researchers on the efficacy as probiotics in various fish species worldwide [4]. This study investigated the effect of application frequencies of the probiotics SEAL on growth performance, survival rate, feed conversion ratio and total production of red tilapia (*Oreochromis* sp.) nilasa strain.

2. **Material and methods**

2.1. **Experimental design and fish**

Research was conducted in fish hatchery facility of the Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Indonesia in the period of April – December 2019. A completely randomized design was used with three treatments in triplicate, namely control (no probiotics), daily, and three day intervals of probiotics application in $5 \times 10^4$ cfu/g of feed. Red tilapia (*Oreochromis* sp.) nilasa strain juveniles with average weight of 8 g were used with the density of 50 fish/tanks. The research was conducted for 60 days. Feeding was conducted four times a day with the daily dose of 3% of fish biomass. Fish biomass was evaluated every two weeks to determine the amount of feed required in each tanks.

2.2. **Microbial strain and culture condition**

Inoculum of Yeast baker (*Saccharomyces* sp.) and three bacterial strain, namely *Aeromonas* sp. JC33, *Lactococcus* sp. JAL12, and *Enterobacter* sp. JC10 of various fish intestine origin, as the collection of fish health and environment laboratory, Gadjah Mada University were stored in $-80^\circ$C for daily culture on a sterile molase medium, containing 3g/L of molase, 3 g/L of fish flour, 1g/L of monosodium glutamate, and water. The microbial stains were cultured separately each other every day in a 10 ml medium for 24 h in room temperature prior to the application in fish feed.

2.3. **Probiotics application**

Microbial density in each culture was determined prior to mixing in feed of the day by using a mixing container. A commercial fish feed “Surya 78I-2” (contained of 15% of protein) was used in the present study. Being mixed with probiotics, the fish feed was air dried for about 1 hour prior to the application as feed for the experimental fish. The probiotic application was conducted according to the application frequency or intervals applied on the experimental fish for up to 60 days of treatments.

2.4. **Analysis of fish growth**

Fish were measured on the individual weight and length at day 30 and 60 of probiotics treatments, dan day-7 of *A. hydrophila* infection. Growth performance was evaluated base on the absolute growth rate, relative growth rate, and specific growth rate of fish weight.

2.5. **Analysis of fish survival rate, total production, and feed conversion ratio (FCR)**

Fish survival rate in each tanks was determined based on the number of fish in initial stage (50 fish) and the remaining fish at the end of the day-60. Fish total production was calculated based on the procedure mentioned [8]. Feed conversion ratio was calculated based on the amount of feed given during the treatment, compared to the fish biomass increase during the cultivation period [8].

2.6. **Data analysis**
All data obtained in the present study were analyzed by using an *Analysis of Variance* and *Duncan Multiple Range Test* (DMRT) with the IBM SPSS software. Significant differences were considered at $P<0.05$.

### 3. Results and discussion

#### 3.1 Absolute growth of weight

Absolute growth of weight data is presented in the figure 1. The absolute growth of weight of probiotic treated groups was higher than the control groups. Growth obtained by probiotics application in the three-day interval was similar to the daily application.

![Figure 1. Absolute growth of weight of the experimental fish.](image1)

#### 3.2 Relative growth of weight

Data of the fish relative growth of weight is presented in the figure 2. Probiotic treatments obtained relative growth of weight of higher than the control groups. However, there is no significant differences on the probiotics n effects on the growth parameters in daily and three-day intervals of application.

![Figure 2. Relative growth of weight of the experimental fish.](image2)

#### 3.3 Specific growth rate

Data of the fish weight specific growth rate is presented in the figure 3. All probiotic treated groups obtained better growth performance compared to the control groups with no probiotics application.
3.4. Fish survival rate
Data of survival rate of the experimental fish is shown in the figure 4. There are no significant differences on the fish survival rate between treatments. Survival rate of the experimental fish ranged between 91.33% and 94%.

3.5. Total production of fish
Data of fish total production in the present study is presented in figure 5. Probiotics application in three-day interval obtained significant higher total fish biomass than the non-probiotic groups (negative control). Meanwhile, the total fish production of the daily probiotic treated groups were similar to the non-probiotic groups.

3.6. Feed conversion ratio (FCR)
Feed conversion ratio obtained in each experimental groups is demonstrated in the figure 6. There are no significant different in the FCR value between treatment. The FCR value were in the range of 3.72 – 4.14.

![Figure 6. Feed conversion ratio obtained in each experimental group.](image)

### 3.7. Discussion
The present study confirmed that probiotics SEAL, containing a yeast bakers *Saccharomyces* sp., and three bacterial strains *Aeromonas* sp., *Lactococccus* sp. and *Enterobacter* sp., is efficacious to the fish growth performance of red tilapia nilasa strain. Probiotics application with three-day interval obtained the growth similar to the daily application. This result is better than the previously reported fish probiotics that required the daily application for significant effect in the fish growth [9]. The result of the present study is similar to previously reported bacterial probiotics *Micrococcus* sp and *Pseudomonas* sp. in enhancing the growth of tilapia [10], but different to previously reported non-eficacious *Bacillus* spp. and *Pediococcus* sp. in red hybrid tilapia [11]. Accordingly, we suggest that the result of the present study provides the valuable information for further large scale application of probiotic SEAL in fish farms. The present probiotics strains have been confirmed with strong enzymatic activities and have ability to stay in fish intestine [7]. We suspect that the growth performance improvement here is due to the enhancement of the enzymatic activity in the intestine that induce fish appetite to eat. To confirm the phenomenon, investigation on the degree of digestion process in the fish intestine and colonization of the intestine by the probiotic SEAL is required.

Probiotic SEAL application with three-day interval enhance 10% of total fish biomass production. The price for probiotic application in the present study is much cheaper that the additional 10% fish weight gained due to the the probiotics application. The probiotic SEAL amount is only $10^4$ cells/g of feed/day. This number is lower than previously recommended amount of other probiotics strain for tilapia in, which is required $10^{10}$ cfu/g of feed [12]. Hence, we suggest that the result of the present study supports the development of efficient, productive, and environmental friendly aquaculture system in Indonesia.

Probiotic application in the present study did not influence the feed conversion ratio (FCR). Many factors are influencing FCR in aquaculture system. The FCR mainly correlated with the feed quality. The better feed quality, the more efficient digestion process in fish intestine to support the fish growth. Probiotic effect in FCR will be clearly seen in application in longer period [13]. Accordingly, we suspect that the similar FCR value is due to the low feed quality used, and the medium term (two moths) of probiotic application.

### 4. Conclusion
Probiotics SEAL application in red tilapia nilasa strain improved the growth performance. The probiotics application with three-day interval improves the fish growth and total similar to the daily probiotics application.
References

[1] Opiyo M A, James J, Charles C N, Harrison C K 2019 Different levels of probiotics affect growth, survival and body composition of Nile tilapia (Oreochromis niloticus) cultured in low input ponds. Scientific African 4 e00103 https://doi.org/10.1016/j.sciaf.2019.e00103

[2] Hai N V 2015 The use of probiotics in aquaculture. Journal of Applied Microbiology 119(4) 917-935 https://doi.org/10.1111/jam.12886

[3] Gatesoupe F J 1999 Review the use of probiotics in aquaculture. Aquaculture 180(1-2) 147-165 https://doi.org/10.1016/S0044-8486(99)00187-8

[4] Hoseinifar S H, Sun Y-Z, Wang A, Zhou Z 2018 Probiotics as Means of Diseases Control in Aquaculture, a Review of Current Knowledge and Future Perspectives. Front. Microbiol. 10.3389/fmicb.2018.02429 https://doi.org/10.3389/fmicb.2018.02429

[5] Tarnecki A M, Wafapoor M, Phillips R N 2019 Benefits of a Bacillus probiotic to larval fish survival and transport stress resistance. Sci Rep 9 4892. https://doi.org/10.1038/s41598-019-39316-w

[6] Istiqomah I, Sukardi, Murwantoko, Isnansetyo A 2020 Review Vibriosis Management in Indonesian Marine Fish Farming. E3S Web of Conferences 147 01001 https://doi.org/10.1051/e3scconf/202014701001

[7] Istiqomah I, Isnansetyo A, Atitus I N, Rohman A F 2019 Isolation of Cellulolytic Bacterium Staphylococcus sp. JC20 from the Intestine of Octopus (Octopus sp.) for Fish Probiotic Candidate. Jurnal Perikanan Universitas Gadjah Mada 21(2) 93-98 https://doi.org/10.22146/jfs.39525

[8] Hariyadi D R, Isnansetyo A, Istiqomah I, Hardaningsih I, Wahyudi, Kim SS 2018 Growth, total production and feed efficiency of catfish (Clarias sp.) orally administered with shrimp waste hydrolysate. Aquacultura Indonesiana 19(1) 15-20

[9] Munir M B, Hashim R, Manaf M S A, Nor S A M 2016 Dietary Prebiotics and Probiotics Influence the Growth Performance, Feed Utilisation, and Body Indices of Snakehead (Channa striata) Fingerlings. Trop Life Sci Res. 27(2) 111-125. https://doi:10.21315/tlsr2016.27.2.9

[10] El-Rhman A M A, Khattab Y A E, Shalaby A M E 2009 Micrococcus luteus and Pseudomonas species as probiotics for promoting the growth performance and health of Nile tilapia, Oreochromis niloticus. Fish & Shellfish Immunology 27(2) 175-180 https://doi.org/10.1016/j.fsi.2009.03.020

[11] Wing-Keong N G, Kim Y-C, Romano N, Koh C-B, Yang S-Y 2014 Effects of Dietary Probiotics on the Growth and Feeding Efficiency of Red Hybrid Tilapia, Oreochromis sp., and Subsequent Resistance to Streptococcus agalactiae. Journal of Applied Aquaculture 26 (1) 22-31 DOI: 10.1080/10454438.2013.874961

[12] Dias D C, Fernanda P B F, Fábio R S, Leonardo T, Giovanni S G, Carlos M I, Mariene M N, Maria J T R P 2020 Economic feasibility of probiotic use in the diet of Nile tilapia, Oreochromis niloticus, during the reproductive period. Acta Sci., Anim. Sci. 42 . https://doi.org/10.4025/actascianimsci.v42i1.47960

[13] Toledo A, Frizzo L, Signorini M, Bossier P, Arenal A 2019 Impact of probiotics on growth performance and shrimp survival: A meta-analysis. Aquaculture 500 196-205.

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