JURNAL PERIKANAN
UNIVERSITAS GADJAH MADA
Terakreditasi Ristekdikti No: 158/E/KPT/2021
ISSN: 2502-5066 (Online)
ISSN: 0853-6384 (Print)
Vol. 23 (2), 113-118
DOI 10.22146/jfs.68510

The Growth Performance of Tilapia (*Oreochromis niloticus*) Seeds Fed with Different Quantities of a Synbiont Feed

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Submitted: 18 August 2021; **Revised:** 19 November 2021; **Accepted :** 15 December 2021

ABSTRACT This study were aims to test the growth performance of tilapia (*Oreochromis niloticus*) seeds fed with different quantities of the synbiont, consisting of a probiotic and a prebiotic, such as *B. subtilis* and banana flour, respectively. This study employs an experimental approach with a completely randomized design (CRD). Furthermore, artificial feeds were prepared as various treatments, consisting of *B. subtilis* and banana flour concentrations. They include treatment A, which does not contain *B. subtilis* and banana flour, and also treatment B, C, and D, which consists of *B. subtilis* at concentrations of 10^5 CFU/mL, 10^7 CFU/mL, and 10^9 CFU/mL, respectively, as well as 1% banana flour. The results showed that weight gain (WG) and specific growth rate SGR were significantly different in treatment B but the survival rate (SR), food conversion rasio (FCR), hepatosomatic index (HSI), and condition factor (K) did not show significant difference. Meanwhile, somatic digestive index (DSI) showed a significant difference with treatment A, C, and D, but not with treatment B. However, treatment B showed a relatively better performance based on the SR, FCR, and WG at 96.67 %, 1.05 %, and 3.49 %, respectively.

Keywords: Dosage; fish seeds; growth; probiotics

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is fresh water and brackish water fishery commodity with significant economic value, and it is the second most widely cultivated finned fish species after carp (FAO, 2018). The high demand for tilapia has resulted in several problems, including the quality of feed composition. According to Kurniawan *et al*. (2019), the nature of the fish feed is a primary factor for growth, and this accounts for the majority of production costs at 60-70 %. This is because of the increase in the price of high-quality feed ingredients. Therefore, it is necessary to increase feed efficiency to meet these needs to increase fish production. Also, the high cost of feed promotes the efficiency of the feed given to tilapia to meet these needs, one of which is through the use of synbiont feed, a combination of a probiotic and prebiotic. According to Aly *et al*. (2008), the use of probiotics or beneficial bacteria is considered a promising alternative approach in controlling pathogenic bacteria, especially with the addition of prebiotics to fish feed. When administered at the appropriate dose, probiotics can improve digestibility, growth, and the immune system of fish (Bomba *et al*., 2002; Abdel-Tawwab *et al*., 2008) Subsequently, Feed efficiency can be achieved through probiotics and prebiotics, which produce enzymes that help digest the feed, increasing growth performance. In addition, a synbiont feed which is a balanced combination of probiotics and prebiotics, can increase the survival and growth of beneficial bacteria in the digestive tract of an organism. Probiotic bacteria are given because they can benefit cultured organisms by increasing nutritional value and host response to harmful microorganism agents, as well as improving environmental quality (Abdelhamid *et al*., 2014; Lara-Flores *et al*., 2013), while prebiotics can provide food for the growth of probiotic bacteria (Ringo *et al*., 2010).

Similarly, the use of synbiont feed can boost survival, stimulate growth, and boost the host immune system (Cerezuela *et al*., 2011). The probiotics in the synbiont feed can increase bacterial activity, which aids the fish's digestive process and increases feed digestibility, allowing the fish to grow well (Widanarni *et al*., 2016). One of such probiotic agents is *Bacillus subtilis*, which can stimulate enzyme activity, digestion, and feed absorption to promote tilapia growth (Gatesoupe, 1999; Nayak, 2010). The addition of a prebiotic to the feed also increases the digestibility of the feed. (Han *et al*., 2015; Liu *et al*., 2017).

Meanwhile, previous research on synbiont feed has shown various significant outcomes in cultured fish (Putra *et al*., 2015). Also, the bacteria used as a probiotic candidate in this study is *Bacillus subtilis*, which can improve the growth performance of tilapia (Van Doan *et al*., 2018). Conversely, the addition of prebiotics derived from banana flour is vital to the *B. subtilis*, resulting in a synergy between the probiotics and prebiotics in the synbiont feed. Prebiotics (banana flour), as a material that digestive enzymes cannot hydrolyze, can selectively support the growth of probiotic bacteria for the intestinal microflora (de Vrese & Schrezenmeir, 2008). The purpose of this study was to determine the appropriate dose of synbiotic feed on growth performance in tilapia. However, the recommendations for the feasibility of symbiont feeding in tilapia are based on possible results obtained before the end of the research period.

MATERIALS AND METHODS

Material

The materials used were tilapia seeds with an average weight of ± 5 g purchased from the Brackish Water Cultivation Fisheries Centre (BPBAP), located in Takalar. Other materials obtained from the facility include *Bacillus subtilis*, banana flour, Nutrient broth (NB), saline solution, and anticoagulants. Furthermore, an aquarium measuring 40x30x30 cm^3 was used, as well as a centrifuge, a water bath shaker, aeration equipment, a washbasin, commercial standard feed, and a 1 mL syringe.

Method

Fish preparation test

Tilapia seeds were acclimatized for one week before being used as test fry, and they were reared for 30 days at a density of 20 fish per aquarium measuring 40x30x30 cm^3. The Maros Brackish Water Cultivation Research Institute produced the artificial feed used in this study.

Maintenance

The Maros Brackish Water Cultivation Research Institute provided the fish feed for the 20 saline tilapia used in this study. Furthermore, the test feed, a primary feed supplemented with *B. subtilis*, was grown in NB (nutrient broth) and incubated for 24 hours in a shaker incubator. Subsequently, the bacterial pellet was suspended in saline solution after being centrifuged at 6.000 rpm for 10 minutes. Bacterial isolates were obtained following Rahmi *et al.* (2021). As much as 1% prebiotics (banana flour) were added to the probiotic solution and incubated for 15 to 30 minutes in a shaking water bath before being sprayed onto the feed in a ratio of 1 volume of symbiotic feed to 10 equivalent weights of regular feeding based on the various treatments was done at the station three times a day at 08.00, 13.00, and 18.00 for eight weeks (Hossain *et al.*, 2001). Feces are discarded every day after 2 hours of feeding, and water changes are performed by sucking every three days. Water quality was measured by maintaining water temperature between 28 to 32 °C, ammonia levels at 0.2 mg/L, pH levels between 7.5 to 9.5, and dissolved oxygen at 6 mg/L. The measurements were done every seven days before the first feed administration in the morning.

Challenge test

On day 31, saline tilapia were tested with a suspension of the pathogenic bacteria, administered by intramuscular (IM) injection. Also, there was an observed hydrophilia at a concentration of 10^6 CFU/ml as much as 0.1 mL/head using a sterile syringe. Furthermore, the fishes reared in the negative control pond were transferred to another pond with the same water conditions and injected with phosphate-buffered saline (PBS). Subsequently, saline tilapia was reared with standard commercial feed for ten days and observed daily.

Research design

The research design consisted of artificial feeds prepared as various treatments containing various concentrations of *B. subtilis* and banana flour. They include treatment A, which does not contain *B. subtilis* and banana flour, and also treatment B, C, and D, which consists of *B. subtilis* at concentrations of 10^5 CFU / mL, 10^7 CFU / mL, and 10^9 CFU / mL, respectively, as well as 1% banana flour. There were three replicas of the three treatments and three replicas of the control set up totaling 12 aquariums. According to Kurniawan *et al.* (2019), this research aims to determine the immune response of tilapia to symbiont feed.

Observed parameters

The survival rate of fish (survival) is calculated by comparing the percentage of fish at the end of the rearing period to the number of fish at the beginning of the rearing period. More so, the formula below was used to calculate the survival rate of fish given by Effendie (1997) as follows: Survival rate= (Nt/No) x 100%, where Nt is the population at t (tail) and No is the initial population (tail). Similarly, the Tacon (1987) formula was used to calculate the Feed Conversion Ratio (FCR): FCR = (Wt–Wo)/F, where FCR is the Feed Conversation Ratio, Wt is the total weight of test animals at the end of the study (gr), and Wo is the total weight of test animals at the beginning or end of the study (gr), F is the amount of feed consumed during the study (gr). Additionally, weight gain (WG) was also calculated using the formula Body Weight (WG) (gr) = final weight - initial weight. The following formula was postulated Huisman (1987) was used to calculate the Specific Growth Rate (SGR): Specific Growth (%/day) = 100 x (Ln (W2)-Ln (W1))/long maintenance.

Meanwhile, Clove oil was used to anesthetize fish samples at 100mg/L. The whole fish body (3 fish/aquarium) was cooled at -4 °C for physical body index. Three fish from each aquarium were taken from each treatment to record their weights and lengths. Furthermore, they were dissected to determine the factors of their liver weight, digestive tract, and condition of the fish kept (Abarike *et al.*, 2013; Mary *et al.*, 2019). Also, the hepatosomatic index (HSI) percentage was calculated using Nikolsky (1969) formula as follows: HSI = (Heart Weight/Fish Body Weight) × 100 %. Similarly, the somatic digestive index (DSI) percentage was calculated as DSI= (Digestive Tract Weight/Fish Body Weight) ×100 %. Lastly, the Condition Factor (K) given in g/cm^3 was calculated using the Le Cren (1951) formula, as follows: K= (Fish Body Weight/(Fish Body Length)^3) × 100.

Data analysis

The data was tabulated in MS.Office Excel 2013 and analyzed using ANOVA in Minitab version 16 with a 95% confidence interval. However, if there is a significant difference, the Tukey test was used.

RESULTS AND DISCUSSION

Results

The results of the survival rate, body weight (WG), specific growth rate (SGR), and food conversion rasio (FCR) were obtained as follows. Tilapia survival from each treatment ranged from 95.00 to 96.67%, which was not significantly different (P>0.05). Bodyweight (WG) ranged between 2.33-3.49 g, and SGR, which ranged from 0.61-1.14 %/day were significantly different in treatment B (P<0,05), while the FCR ranged from 0.07-1.05, there was not significantly different (P>0.05). In addition, Table 1 shows the survival rates, WG, SGR, and FCR of saline tilapia in the treatment during the study.

Table 1. The survival rate, body weight (WG), specific growth rate (SGR), and food conversion rasio (FCR) were treated during the study.

Treatment	Survival rate(%)	WG (gr)	SGR (%/day)	FCR
A	95.00±4.08[a]	2.33±1.65[a]	0.61±0.001[a]	0.09±0.02[a]
B	96.67±2.36[a]	3.49±1.04[b]	1.14±0.000[b]	1.05±0.13[a]
C	95.00±4.08[a]	2.80±2.06[a]	1.00±0.001[a]	1.17±1.29[a]
D	96.67±2.36[a]	2.93±1.31[a]	1.01±0.002[a]	1.10±0.42[a]

Note: Different superscript letters and the same parameters in tilapia showed significantly different treatment effects (Tukey's test; P<0.05).

The treatment did not affect the hepatosomatic Index (HSI) (0.570-1.385 %). More so, the somatic digestive index (DSI), which ranged between 2.360 to 6.231%, showed a significant difference in treatment A compared to treatments C and D. However, there was no significant difference in treatment B regarding the condition factor (K), which ranged between 1,020-1.167 g/cm^3. Table 2 shows the HSI, DSi, and K levels in saline tilapia during the research treatment.

Table 2. Hepatosomatic Index (HIS), somatic digestive index (DSI), and condition factor (K) in saline tilapia during the study.

Treatment	HSI (%)	DSI(%)	K(g/cm^3)
A	0.570±0.45[a]	2.360±0.28[a]	1.020±2.05[a]
B	1.385±0.19[a]	3.488±0.73[a]	1.097±1.93[a]
C	1.007±0.78[a]	6.231±1.45[b]	1.120±2.17[a]
D	0.680±1.36[a]	5.981±1.19[b]	1.167±2.36[a]

Data are shown as mean ± standard deviation (SD) (n = 3). Also, the mean with different superscripts on the same line was significantly different (P<0.05). Where WG is the weight Gain, S.G.R. is the Specific Growth Rate, FCR is the Food Conversion Ratio, HIS is the Hepatosomatic Index, DSI is the Digestivesomatic Index, and K is the Condition Factor.

Discussion

Synbiont feed combines probiotics and prebiotics to improve feed quality, growth efficiency, survival rate, and population of lactic acid bacteria (LAB) in fish (Pangaribuan *et al*., 2017). The results of the survival rate (SR) observation study showed that the values obtained tended to be the same in each treatment, statistically also showed the same thing where there was no significant difference in each treatment (P>0.05). These results indicate that the symbion combination with banana flour in tilapia does not substantially impact the survival rate of tilapia (Figure 1). The SR value was classified as good during the study because it was still above 80%. By the opinion of Mulyani & Fitriyani (2014), the survival rate of ≥50% is good, SR 30-50% is moderate, and ≤30% is not good. According to research by Lusiastuti *et al*. (2017), feeding a combination of probiotics increases fish survival rate, biomass, and immune response. This shows that the use of synbiont feed can increase the survival rate of tilapia because it can improve the balance of microflora in the digestive tract (Nayak, 2010; Murall *et al*., 2017) and improve digestive tract enzyme activity (Van Doan *et al*., 2019). Furthermore, synbionts feeding can benefit by increasing the gut microbiota through tilapia's growth and survival rate (Rusdani *et al*., 2016).

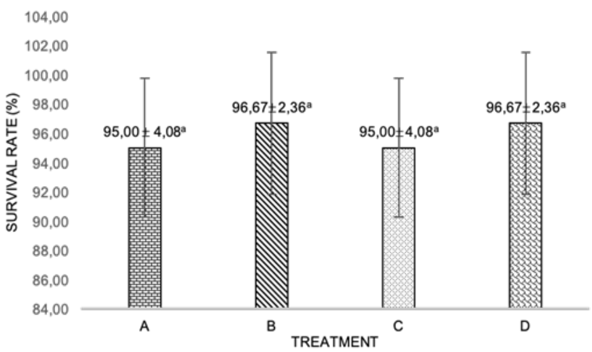

Figure 1. Survival rates of saline tilapia during research.

Synbiont feed provides certain benefits, such as increasing the utilization of nutrients, health levels, stress response, and disease resistance and optimizing the microbial balance in the farmed animal environment (Van Doan *et al*., 2018). Another benefit is producing extracellular enzymes that increase feed utilization (Nuez-Ortin, 2013).

Meanwhile, the introduction of body weight (WG), specific growth rate (SGR), and feed conversion (FCR) are essential parameters in the observation of tilapia. The results showed that WG, SGR in treatment B were significantly different from other treatments (P<0.05), but FCR was not found to be significantly different between treatments (P>0.05), as shown in Table 2. Treatment B containing 10^5 CFU/mL of *B. subtilis* and 1% banana flour gave a better outcome when compared to other treatments. The average weight percentage (WG) of 3,49±1,04, and the SGR of 1,14%±0,000 during the research were relatively higher, although the FCR value did not show a clear pattern. The higher percentage of body weight (WG) and SGR (%) during the research compared to other treatments proved that synbiont feed could improve the balance between probiotics and prebiotics, which affected the balance between probiotics and prebiotics, improving digestive tract function. Probiotic bacteria in the feed will enter the digestive tract and suppress pathogenic bacteria in the intestine so that the absorption process of fish feed will be faster (Adeline *et al*., 2020). Widanarni *et al*. (2016) proposed that synbiotics refer to nutritional supplements that combine probiotics and prebiotics. The effectiveness of digestive enzymes increases, which leads to increased growth (Welker & Lim, 2011; Abareethan & Amsath, 2015), improved immune systems in fish (Kesarcodi-Watson, 2008), and increased host resistance pathogen infections. (Geraylou *et al*., 2013). Similarly, Rusdani *et al*. (2016) discovered that the addition of probiotic *Bacillus* spp. and molasses (10v/v) increased the survival rate and daily growth rate to 96.67% and 1.01 %/ day, respectively. According to (Pangaribuan *et al*., 2017), adding symbiotics to catfish (*Pangasius* sp.) feed increased feed efficiency, protein digestibility, and SGR by 55.46 %, 82.41%, and 4.18 %, respectively, as well as the SR. As a result, these results are excellent compared to not feeding symbiont.

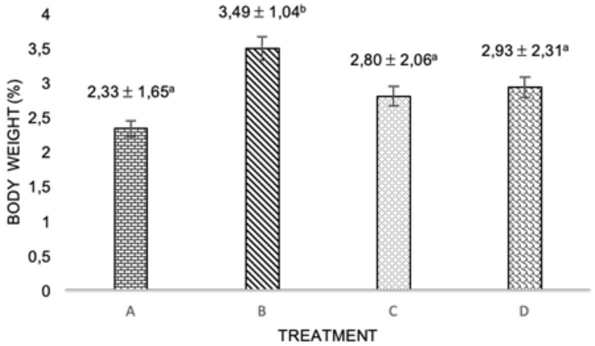

Figure 2. Increase in body weight (WG) of tilapia during research.

The results of the bodyweight (WG) observation study also showed that the values obtained tended to be the same in each treatment, statistically also showed the same thing where there was no significant difference in each treatment (P>0.05). These results indicate that the synbiont combination with banana flour in tilapia does not substantially impact the bodyweight of tilapia (Figure 2). Also, El-Haroun *et al.* (2006) reported up to 33% higher body weight gain and a 43% decrease in FCR in tilapia (*O. niloticus*) fed Biogen®, which is a commercial feed product containing *B. subtilis*.

The hepatosomatic index (HSI) and condition factor (K) yielded no significant results; however, the somatic digestive index (DSI) differed significantly between treatments A and C and D, but not between treatments B and C, as shown in Figure 3.

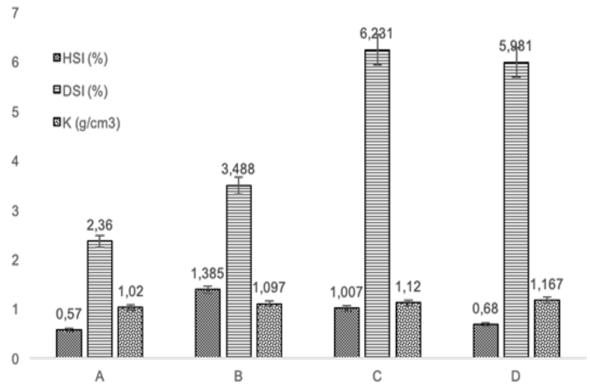

Figure 3. Value of HSI, DSI, and condition factors in tilapia during research.

The HSI and DSI values in treatment B indicate the number of energy reserves in tilapia, implying that tilapia growth will increase (Figure 3). Also, the DSI value revealed significantly different results when comparing treatment A to treatments C and D. However, these HSI and DSI values were used as parameters to assess various metabolic activities in fish (Lumpan, 2020), the greater the body weight and length of the fish, the condition of the liver and digestive tract. The HSI and DSI values of fish indicate that digestion will improve (Opiyo *et al.*, 2019). This can be seen from the increase in WG and SGR values in the treatment-fed synbionts. Tilapia-fed synbionts in the treatment had a trend that showed no effect (P>0,05) of giving synbiotics and banana flour with the condition factor of saline tilapia. This is presumably due to the uniformity of the age of the tilapia used during the research. This is most likely due to the uniformity of the age of the tilapia kept. According to Encina & Granado-Lorencio (1997), the difference in size or age of the fish is another factor that is thought to be the cause of fluctuations and changes in the value of the fish condition factor.

CONCLUSIONS AND SUGGESTIONS

Conclusion

Different quantities of synbiont do not affect fish growth and survival rate but affect the HSI and DSI values.

Suggestion

Probiotics can be used in fish feed by incorporation into feed as nutrients to increase tilapia production.

ACKNOWLEDGMENT

The authors would like to thank the LP3M Muhammadiyah University of Makassar, DPPU, Hasanuddin Makassar, Head of the Research Center on Brackish Water Cultivation Fisheries and the Fisheries Extension (RICAFE) for the laboratory facilities used in this study. The authors would also like to thank the Takalar Fisheries and Brackish Water Cultivation Center (BPBAP) for the contribution of saline tilapia used in this study and Hasanuddin University Muhammadiyah Education University Sorong.

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How to cite this article Rahmi, R., A.N.R. Relatami, A. Akmal, S.W. Firman. B.R. Tampangallo, A. Chadijah & D. Ardiyanana. 2021.The growth performance of tilapia (*Oreochromis niloticus*) seeds fed with different quantities of a symbiont feed. Jurnal Perikanan Universitas Gadjah Mada. 23 (2): 113-118. DOI https://doi.org/10.22146/jfs.68510