**FRONTPAGE**

This manuscript should contain the full names of the main and co-authors. However, the author's name should not include a title. Mark authors clearly for correspondence for ease of contact during the publication process.  **(CODE 20)**

 First author:

 1. Name : Rahmi

 2. Affiliation : University of Muhammadiyah Makassar

 3. E-mail : rahmiperikanan@unismuh.ac.id

 4. Orcid ID : https://orcid.org/0000-0001-7105-5277

 5. Contributions to this manuscript: Lead author/Author for correspondence

Second author:

 1. Name : Andi Ninnong Renita Relatami

 2. Affiliation : Hasanuddin University

 3. E-mail : relatamirenita11@gmail.com

 4. Orcid ID : https://orcid.org/0000-0003-4262-1334

 5. Contributions to this manuscript: Other Authors

Third author:

 1. Name : Akmal

 2. Affiliation : Takalar Brackish Water Cultivation Fisheries Center

 3. E-mail : akmal\_bbaptakalar@yahoo.com

 4. Orcid ID : https://orcid.org/0000-0003-2005-7859

 5. Contributions to this manuscript: Other Authors

Fourth author:

 1. Name : Sri Wahyuni Firman

 2. Affiliation : Muhammadiyah University of Education Sorong

 3. E-mail : sriwahyunifirman@gmail.com

4. Orcid ID : https://orcid.org/0000-0001-7090-2508

 5. Contributions to this manuscript: Other Authors

Fifth author:

 1. Name : Bunga Rante Tampangallo

 2. Affiliation : Brackish Water Cultivation Fisheries Research Institute and Fisheries Extension

 3. E-mail : bungatampangallo@yahoo.com

 4. Orcid ID : https://orcid.org/0000-0002-1999-4039

 5. Contributions to this manuscript: Other Authors

Sixth Author:

 1. Name : Andi Chadijah

 2. Affiliation : University of Muhammadiyah Makassar

 3. E-mail : andichadijah@gmail.com

4. Orcid ID : https://orcid.org/0000-0002-4596-4511

 5. Contributions to this manuscript: Other Authors

 Seventh Author:

 1. Name : Dida Ardiyana

 2. Affiliation : Pertamina DPPU Hasanuddin

 3. E-mail : ardian0104@gmail.com

4. Orcid ID : https://orcid.org/0000-0001-7000-9287

 5. Contributions to this manuscript: Other Authors

**Journal of Fisheries, Gadjah Mada University**

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

**Rahmi1\*, Andi Ninnong Renita Relatami2, Akmal3, Sri Wahyuni Firman4, Bunga Rante Tampangallo5, Andi Chadijah1, Dida Ardiyana6**

*1Aquaculture Study Program, Faculty of Agriculture, University of Muhammadiyah Makassar*

*2 Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University*

*3 Takalar Brackish Water Cultivation Fisheries Center*

*4Aquaculture Study Program, Faculty of Science and Technology, Muhammadiyah Education University Sorong*

*5Research Institute For Coastal Aquaculture and Fisheries Extension (RICAFE)*

*6Pertamina DPPU Hasanuddin Makassar*

*\*Corresponding Author: rahmiperikanan@unismuh.ac.id*

**Abstract.** This study aims to test the growth performance of tilapia (*Oreochromis niloticus*) seeds fed with different quantities of symbiont, which consists 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, which consists of 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 105 CFU / mL, 107 CFU / mL, and 109 CFU / mL, respectively, as well as 1% banana flour. The results showed that there was no significant difference associated with survival rate (SR), FCR, weight gain (WG), hepatosomatic index (HSI), and condition factor (K). Meanwhile, digestive somatic 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: Fish Seeds, Probiotics, Dosage, Growth**

**INTRODUCTION**

Tilapia (*Oreochromis niloticus*) is a 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 of tilapia has resulted in the emergence of 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 in order 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 symbiont feed, which is 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. Probiotics, when administered at the appropriate dose, can improve digestibility, growth, and the immune system of fish (M Abdeh Tawaf et al. 2008;A Bomba et al. 2008). Subsequently, Feed efficiency can be achieved through the use of probiotics and prebiotics, which produce enzymes that help in digesting the feed, thereby effectively increasing growth performance. In addition, a symbiont 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 (MH Abdelhamid et al. 2014;M Lara Flores et al. 2013), while prebiotics can provide food for the growth of probiotic bacteria (Ringgo et al. 2010).

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

Meanwhile, previous research on symbiont feed has shown a variety of 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 important to the *B. subtilis*, resulting in a synergy between the probiotics and prebiotics in the symbiont feed. 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 cm3 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 cm3. 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, which is a basic 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 then suspended in saline solution after being centrifuged at 6000 rpm for 10 minutes. 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). Faeces 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 0C, 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 7 days before the first administration of feed in the morning.

Challenge Test

On day 31, saline tilapia were tested with a suspension of the pathogenic bacteria, which was administered by intramuscular (IM) injection. Also, there was an observed hydrophilia at a concentration of 106 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 10 days, and observed daily.

Research design

The research design consisted of artificial feeds prepared as various treatments, which consists of 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 105 CFU / mL, 107 CFU / mL, and 109 CFU / mL, respectively, as well as 1% banana flour. There were 3 replica of the three treatments, as well as 3 replica 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 Effendi (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, measurement of body physical index was carried out using clove oil which was then cooled to -4 oC in order to preserve the fish. Three fish from each aquarium were taken from each treatment in order to obtain recordings of 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; Amoah et al. 2019). Also, the percentage of the hepatosomatic index (HSI) was calculated using Nikolsky's formula postulated in 1969 as follows: HSI = (Heart Weight/Fish Body Weight) × 100 %. Similarly, the percentage of the digestive somatic index (DSI) was calculated as DSI= (Digestive Tract Weight/Fish Body Weight) ×100 %. Lastly, the Condition Factor (K) given in g/cm3, was calculated using the Le Cren formula of 1951, as follows: K= (Fish Body Weight/(Fish Body Length)3) × 100.

*Data analysis*

The data was tabulated in MS.Office Excel 2013 and analysed 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 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), and body weight (WG) ranged between 2.33-3.49 g, SGR ranged from 0.61-1.14 %/day, whereas FCR ranged from 0.07-1.05, which was also 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. Survival Rate, WG, SGR, and FCR treated during the Study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | Survival Rate(%) | WG(gr) | SGR(%/day) | FCR |
| A | 95,00±4,08a | 2,33±1,65a | 0,61±0,001a | 0,09±0,02a |
| B | 96,67±2,36a | 3,49±1,04a | 1,14±0,000a | 1,05±0,13a |
| C | 95,00±4,08a | 2,80±2,06a | 1,00±0,001a | 1,17±1,29a |
| D | 96,67±2,36a | 2,93±1,31a | 1,01±0,002a | 1,10±0,42a |

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

The treatment had no effect on the Hepatosomatic Index (HSI) (0.570 -1.385 %). More so, the Digestive Somatic 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/cm3. Table 2 shows the HSI, DSi, and K levels in saline tilapia during the research treatment.

Table 2. HSI, DSi and K in saline tilapia during the study

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | HSI (%) | DSI(%) | K(g/cm3) |
| A | 0,570±0,45a | 2,360±0,28a | 1,020±2,05a |
| B | 1,385±0,19a | 3,488±0,73a | 1,097±1,93a |
| C | 1,007±0,78a | 6,231±1,45b | 1,120±2,17a |
| D | 0,680±1,36a | 5,981±1,19b | 1,167±2,36a |

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

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, SGR is the Specific Growth Rate, FCR is the Food Conversion Ratio, HIS is the Hepatosomatic Index, DSI is the Digestivesomatic Index, and lastly, K is the Condition Factor.

*Discussion*

Symbiont feed is a combination of probiotics and prebiotics that can improve feed quality, growth efficiency, survival rate, and population of lactic acid bacteria (LAB) in fish (Pangaribuan et al. 2017). The results showed that the survival rate (SR) during the study was not significantly different (P>0.05), but there was a tendency for treatment without using symbiont feed to be lower when compared to treatment using symbiont feed. This study is in line with Rusdani et al. (2016), who found that when *Bacillus* spp. were included in feed, tilapia had a higher average tendency than when no probiotics were present. According to research by Lusiastuti et al. (2017), feeding a combination of probiotics increases the survival rate, biomass, and immune response of fish. This shows that the use of symbiont feed can increase the survival rate of tilapia because it can improve the balance of microflora in the digestive tract (Nayak, 2010; Mural et al. 2017) and also improve digestive tract enzyme activity (Van Doan et al., 2019). Furthermore, symbiontz feeding can have a beneficial effect by increasing the gut microbiota through the growth and survival rate of tilapia. (Rusdani et al. 2016).

Figure 1. Survival Rates of Salted Tilapia during Research

Added WG, SGR and FCR

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

Meanwhile, the introduction of body weight (WG), specific growth rate (SGR), and feed conversion (FCR) are important parameters in the observation of tilapia. These observations showed that WG, SGR, and FCR in this research were found to be insignificant (P>0.05), as shown in Table 2. However, treatment B containing 105CFU/mL of *B. subtilis* and 1% banana flour gave a better outcome when compared to other treatments. The average weight percentage (WG) of 3.491.04a, and the SGR of 1.14 % 0.000a during the research were relatively higher, despite the fact that 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 symbiont feed, when given effectively to feed, could improve the balance between probiotics and prebiotics, which had an effect on improving digestive tract function. Widanarni et al., 2016 proposed that symbiotics refer to nutritional supplements that combine probiotics and prebiotics. The effectiveness of digestive enzymes increases as well, which leads to increased growth (TL Welker and Lim, 2011; Abareethan & Amsath, 2015), improved immune systems in fish (Kesarcodi Watson, 2008), and increased host resistance to 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 very good when compared to not feeding symbiont.

Figure 2. Increase in Body Weight (WG) of Tilapia During Research

The lower FCR value of 1.07 in the artificial feed treatment with the addition of B. subtilis 105CFU/mL and 1% banana flour (Treatment B) indicated better feed utilisation and nutrition by tilapia, resulting in a higher bodyweight value of tilapia as shown in 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 digestive somatic 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. 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 (Mary A Opiyo et al. 2019). This can be seen from the increase in WG and SGR values in the treatment-fed symbionts.

Conversely, the condition factor was influenced by probiotic supplementation; tilapia fed a symbiont diet in treatment B (artificial feed with the addition of B. subtilis 105 CFU/mL and 1% banana flour) had significantly higher condition factors than the control but were not significantly different (P > 0 0.05) from tilapia not treated in treatment A. This is most likely due to the uniformity of the age of the tilapia kept. According to Enchina and 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*

Symbiont feed, which is an artificial feed containing 105 CFU/mL of B. subtilis and 1% banana flour yielded relatively better results, with SR, FCR, and WG values of 96.67%, 1.05, and 3.49 gr.

*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 Makassar University, DPPU Hasanuddin Makassar, Head of the Research Center on Brackish Water Cultivation Fisheries and the Fisheries Extension 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 salted tilapia used in this study, as well as Universitas Hasanuddin and Sorong.

**BIBLIOGRAPHY**

1. Abareethan M. & Amsath A. (2015). Characterization and evaluation of probiotic fish feed. *International Journal of Pure and Applied Zoology*, 3(2): 148- 153.
2. Aly, S. M., Ahmed, Y. A. G., Ghareeb, A. A. A., & Mohamed, M. F. (2008). Studies on *Bacillus subtilis* and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. *Fish & shellfish immunology*. 25 (12) :128–136.doi:[10.1016/j.fsi.2008.03.013](file:///C%3A/Users/UNS-Ethanol/Downloads/10.1016/j.fsi.2008.03.013)
3. Bomba, R. Nemcová, S. Gancarcíková, R. Herich, P. Guba, D. Mudronová. (2002) Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acids, Br. J. Nutr. 88 S95. doi:[10.1079/BJN2002634](file:///C%3A/Users/UNS-Ethanol/Downloads/10.1079/BJN2002634).
4. Cerezuela R, Meseguer J, dan Esteban MA. 2011. Current Knowledge in Synbiotic Use for Fish Aquaculture: A Review. *J Aquac Res Development* S1:008.

doi:10.4172/2155-9546.S1008.

1. Effendie MI. (1979). *Metode Biologi Perikanan*. Yayasan Dewi Sri Bogor, Bogor.
2. E.R. El-Haroun, A.M.A.S. Goda, M.A. Kabir Chowdhury. (2006). Effect of dietary probiotic biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia, *Oreochromis niloticus* (L.). *Aquac. Res*. 37:1473–1480,

doi:[10.1111/j.1365-2109.2006.01584.x](file://C:/Users/UNS-Ethanol/Downloads/E.R.%20El-Haroun%2C%20A.M.A.-S.%20Goda%2C%20M.A.%20Kabir%20Chowdhury%2C%20Effect%20of%20dietary%20probiotic%20biogen%C2%AE%20supplementation%20as%20a%20growth%20promoter%20on%20growth%20perfor-%20mance%20and%20feed%20utilization%20of%20Nile%20tilapia%2C%20Oreochromis%20niloticus%20%28L.%29%2C%20Aquac.%20Res.%2037%20%282006%29%201473%E2%80%93148).

1. Encina, L., and C. Granado-Lorencio. (1997). Seasonal Changes in Condition, Nutrition, Gonad Maturation and Energy Content in Barbel, Barbus sclateri, Inhabiting A Fluctuating River. *Environmental Biology of Fishes*. 50: 75–84.
2. FAO, The State of World Fisheries and Aquaculture 2018 – Meeting the Sustainable Development Goals, FAO, Fisheries Department, Rome, Italy, 2018.
3. Gatesoupe, F.J. (1999). The use of probiotics in aquaculture. *Aquaculture*. 180 : 147–165.doi: [https://doi.org/10.1016/S0044-8486(99)00187–8](https://doi.org/10.1016/S0044-8486%2899%2900187%E2%80%938)
4. Geraylou, ZC. Souffreau ER, L.D. Meester, C.M. Courtin, J.A. Delcour, J. Buyse, And F. Ollevier. (2013). Effects Of Arabinoxylan–Oligosaccarides (A×os) and Endogenous Probiotic On The Growth Performance, Non–Specific Immunity, And Gut Microbiota On Juvenile Siberian Sturgeon (Acipencer Baerii). *J Fish Shellfish Immunol*. 35(11):766–775. doi: [10.1016/j.fsi.2013.06.014](file:///C%3A/Users/UNS-Ethanol/Downloads/10.1016/j.fsi.2013.06.014)
5. Han, B., Long, W., He, J., Liu, Y., Si, Y., Tian, L. (2015). Effects of dietary Bacillus licheniformis on growth performance, immunological parameters, intestinal morphology and resistance of juvenile Nile tilapia (*Oreochromis niloticus*) to challenge infections. *Fish Shellfish Immunol*. 46: 225–231. [https://doi.org/https://doi.org/10.1016/j.fsi.2015.06.018](https://doi.org/https%3A//doi.org/10.1016/j.fsi.2015.06.018).
6. Huisman EA. 1987. *Principles of fish production*. Department of Fish Culture and Fisheries, Wageningen Agriculture University.Wageningen. Netherland. 170p.
7. Hossain, M, Haylor GS, Beveridge MCM. (2001). Effect Of Feeding Time And Frequency On The Growth And Feed Utilization Of African Catfish Clarias Gariepinus (Burchell 1822) Fingerlings. *Aquacuture Research*. 32(10):999–1004. doi: 10.1046/j.1365-2109.2001.00635.x
8. Kurniawan, A., Suminto, S., & Haditomo, A. (2019). Pengaruh Penambahan Bakteri Kandidat Probiotik *Bacillus Methylothropicus* Pada Pakan Buatan Terhadap Profil Darah Dan Performa Pertumbuhan Ikan Nila (*Oreochromis niloticus*) Yang Diuji Tantang Dengan Bakteri *Aeromonas hydrophila*. Sains Akuakultur Tropis: *Indonesian Journal Of Tropical Aquaculture*. 3 (1): 82-92. doi:<https://doi.org/10.14710/sat.v3i1.3956>
9. Kesarcodi-Watson A., Kaspar H., Lategan J. & Gibson L. 2008. Probiotics in aquaculture : The need, principles and mechanisms of action and screening processes. *Aquaculture*. 274: 1-14.
10. Liu, H., Wang, S., Cai, Y., Guo, X., Cao, Z., Zhang, Y., Liu, S., Yuan, W., Zhu, W., Zheng, Y., Xie, Z., Guo, W., Zhou, Y. (2017). Dietary administration of Bacillus subtilis HAINUP40 enhances growth, digestive enzyme activities, innate immune responses and disease resistance of tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunol*. 60:326–333. [https://doi.org/https://doi.org/10.1016/j.fsi.201612.003](https://doi.org/https%3A//doi.org/10.1016/j.fsi.201612.003)
11. Lusiastuti AM, Andriyanto S, Samsudin R. (2017). Efektivitas kombinasi probiotik mikroenkapsulasi melalui pakan untuk pengendalian penyakit Motile *Aeromonas* *septicemia* pada ikan lele, *Clarias gariepinus*. *J. Ris. Akuakultur*. 12(2):179–186. doi: [http://dx.doi.org/10.15578/jra.12.2.2017.179–186](http://dx.doi.org/10.15578/jra.12.2.2017.179%E2%80%93186)
12. Lumpan Poolsawat, Yifeng Yu, Xiaoqin Li, Xu Zhen, Wenxiang Yao, Pu Wang, Congyan Luo, Xiangjun Leng. (2020). Efficacy of phytogenic extracts on growth performance and health of tilapia (*Oreochromis niloticus* × O. *aureus*). *Aquaculture and Fisheries*. <https://doi.org/10.1016/j.aaf.2020.08.009>.
13. Le Cren, C. P. (1951). Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in The Perch **(***Perca fluviatilis***).** *Journal of Animal Ecology* 20(2): 201-219.
14. Mary A. Opiyo, James Jumbe, Charles C. Ngugi, Harrison Charo-Karisa. (2019). Different levels of probiotics affect growth, survival and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *Scientific African*. Volume 4, e00103, ISSN 2468-2276, <https://doi.org/10.1016/j.sciaf.2019.e00103>.
15. M.Abdel-Tawwab, A.M. Abdel-Rahman, N.E.M. Ismael. (2008). Evaluation of commercial live bakers’ yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*, *Aquaculture* 280:185–189, doi:10.1016/j. aquaculture.2008.03.055.
16. M.A. Abdelhamid, A.M.M. Refaey, A.M.E. Seden, O.A. Zenhom. (2014). Effect of different sources and levels of some dietary biological additives on: IV – immunity and haematology of Nile tilapia, *Oreochromis niloticus*. Egypt, *J. Aquat. Biol. Fish*. 18:49–60, doi:[10.12816/0011096](file:///C%3A/Users/UNS-Ethanol/Downloads/10.12816/0011096).
17. M. Lara-Flores, M.A. Olvera-Novoa. (2013). The use of lactic acid bacteria isolated from intestinal tract of nile tilapia (*Oreochromis niloticus*), as growth promoters in fish fed low protein diets, Lat. Am. *J. Aquat. Res*. 41:490–497, doi:[10.3856/vol41-issue3-fulltext-12](file:///C%3A/Users/UNS-Ethanol/Downloads/10.3856/vol41-issue3-fulltext-12).
18. Murall, C.L., Abbate, J.L., Touzel, M.P., Allen-Vercoe, E., Alizon, S., Froissart, R., McCann, K. (2017). Invasions of host-associated microbiome networks, in: Advances in Ecological Research. *Elsevier*, pp. 201–281.
19. Nayak.S.K. (2010). Probiotics and immunity: a fish perspective, *Fish Shellfish Immunol*. 29 (2010) 2–14, doi:10.1016/j.fsi.2010.02.017.
20. Nikolsky G.V. (1963). *The ecology of fishes*. Academic Press. New York. 325 p.
21. Nuez-Ortín, W.G. (2013). Natural growth promoters in aquaculture practices. *Centro Tecnológico del Mar-Fundación.* 9–23.
22. Pangaribuan, E., Sasanti, AD, & Amin M. 2017. Efisiensi Pakan, Pertumbuhan, Kelangsungan Hidup dan Respon Imun Ikan Patin (*Pangasius* sp.) yang Diberi Pakan Bersinbiotik. *JKR Indo*nesia. *5*(2): 140–154.
23. Putra AN., Utomo NBP. & Widanarni. (2015). Growth Performance of Tilapia (*Oreochromis niloticus*) Fed with Probiotic, Prebiotic and Synbiotic in Diet. Pakistan, *Journal of Nutrition*. 14(5): 263- 268.
24. Ringo, E, Olsen RE, Gifstad TTO, Dalmo RA, Amlund H, Hemre GL, dan Bakke AM. (2010). Prebiotics in aquaculture: a review. *Aquaculture Nutrition* 16:117-136.
25. Rusdani, M. M., Waspodo, S. A. S., & Abidin, Z. (2016). Pengaruh Pemberian Probiotik Bacillus Spp. Melalui Pakan Terhadap Kelangsungan Hidup Dan Laju Pertumbuhan Ikan Nila (*Oreochromis niloticus*). *J. Bio. Tropis*. 16 (1): 34-40. doi:[10.29303/jbt.v16i1.103](file:///C%3A/Users/UNS-Ethanol/Downloads/10.29303/jbt.v16i1.103)
26. Tacon, AEJ. 1987. The Nutrition And Feeding Formed Fish And Shrimp. *A Training Manual Food And Agriculture Of United Nation Brazilling* , *Brazil*.
27. T.L. Welker, C. Lim, Use of probiotics in diets of tilapia, J*. Aquac. Res*. Dev. s1. 2011. 1–8, doi:[10.4172/2155-9546.S1-014.](file:///C%3A/Users/UNS-Ethanol/Downloads/10.4172/2155-9546.S1-014)
28. Van Doan, H., Hoseinifar, S.H., Khanongnuch, C., Kanpiengjai, A., Unban, K., Van Kim, V., Srichaiyo, S. (2018). Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis* *niloticus*). *Aquaculture* 491, 94–100. <https://doi.org/10.1016/j.aquaculture.2018.03.019>.
29. Van Doan, H., Hoseinifar, S.H., Ringø, E., Ángeles Esteban, M., Dadar, M., Dawood, M.A.O., Faggio, C. (2019). Host-Associated Probiotics: A Key Factor in Sustainable Aquaculture. Rev. *Fish. Sci. Aquac*. 1–27.
30. Widanarni, W., Sukenda, S., & Septiani, GR. (2016). Aplikasi Sinbiotik Untuk Pencegahan Infeksi Infectious Myonecrosis Virus Pada Udang Vaname (Litopenaeus Vannamei) (Synbiotic Application For Prevention Of Infectious Myonecrosis Virus Infection In White Shrimp (*Litopenaeus* *Vannamei*)*.  J Vet Sci*. *10*(2), 121–127. doi: [/10.21157/j.ked.hewan.v10i2.5041](https://doi.org/10.21157/j.ked.hewan.v10i2.5041)