# Growth Performance of Tilapia Fed Commercial Feed with Cellulolytic Bacteria from Ruminants

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**Abstract.** Bacteria have the ability to decompose cellulose into glucose monomers and make it a carbon source and energy source. Cellulolytic bacteria are found in the digestive tract of ruminants. Utilization of cellulolytic bacteria as a producer of cellulase enzymes can be applied to fish to increase feed digestibility so that it can support growth. Cellulolytic bacteria isolated from ruminant were introduced to tilapia through feed. The study used a completely randomized design with four treatments and three replications. A total of 25 tilapia with a length of 10 cm were kept in each aquarium with a capacity of 250 L for 2 mo. During feed maintenance, cellulolytic bacteria were added at a dose of 1 %, 2 %, 3 % and without bacteria as a control. The specific growth rate of tilapia treated with 2 % and 3 % bacteria significantly increased. The feed was more efficiently utilized by tilapia when compared to that without the addition of cellulolytic bacteria or at a dose of 1 %. The amount of feed consumption did not differ between treatments, while the survival rate of tilapia treated with bacteria was significantly higher than without bacteria. Cellulolytic bacteria isolated from the digestive tract of ruminants added to commercial feed significantly improved the growth performance of tilapia.

**Keywords:** Feed efficiency, fish nutrition, nila, *Oreochromis niloticus* (Linnaeus, 1758), probiotic.

## 1 Introduction

In aquaculture, feed is one of the important elements that support the growth and survival of cultured fish. About 60 % to 70 % of the total production costs incurred are feed costs [1–3]. Quality feed raw materials and having sufficient nutritional content will affect the quality of the feed so that it has an impact on the growth performance of fish [4–6]. Poor quality feed will not be able to meet the nutritional needs of fish. In addition, undigested materials will pollute the waters and have the potential to have other negative impacts

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[7–9]. Probiotic bacteria can optimize the absorption of nutrients in feed through an enzymatic process to digest complex nutrients in feed so that nutrition is easily absorbed in the fish intestines [10–12].

To support growth, fish generally depend on nutrients in the form of protein. The protein sources used are usually fish meal and soybean meal which are relatively expensive [13]. Another form of nutrition that is abundantly available, inexpensive but cannot be utilized optimally by fish is carbohydrates. This is due to the ability of fish to digest carbohydrates is low when compared to protein or fat [14]. To be able to increase the ability to digest carbohydrate sources in feed, it can be done by adding cellulolytic bacteria.

Cellulolytic bacteria are bacteria that produce cellulase enzymes that can degrade cellulose into simpler sugars. This bacterium has the opportunity to be used as a predigest to hydrolyze cellulose contained in feed. Studies on the use of cellulolytic bacteria to reduce cellulose content have been carried out on several types of vegetable raw materials such as lamtoro (*Leucaena leucocephala* (Lam.) de Wit) leaf meal [15], tapioca by-products [16] and cassava peel (*Manihot esculenta* Crantz) [17].

In previous studies, bacterial exploration from the rumen was carried out and bacterial isolates were obtained that have cellulolytic activity [18]. The bacterial isolates have been tested and have the potential to be used as probiotics. In this study, isolates of cellulolytic bacteria were added to feed and given to tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] to determine its impact on growth.

## 2 Material and methods

### 2.1 Research procedure

Research used a completely randomized design with four treatments and three replications. Bacterial isolates commercial feed was added at a density of $8 \times 10^6$ CFU $mL^{-1}$ at doses of 0 % as control (P0), a dose of 1 % (P1), 1.5 % (P2) and 2 % (P3). The materials used in the study were tilapia with a size of 10 cm, commercial pellet feed (Bravion 330 with 30 % protein). The equipment used is a refractometer, thermometer, pH meter for water, ammonia, DO meter, stationery and camera.

This research begins by preparing a research container in the form of a rectangular aquarium measuring 90 cm × 60 cm × 60 cm (total number of aquariums is 12) which has been cleaned as well as other equipment such as aeration, and replenishment of deposited water for 24 h. Each aquarium was filled with 25 ind with a body length of 8 cm. Tilapia were obtained from Freshwater Aquaculture Management Unit (*Unit Pengelola Budidaya Air Tawar* – UPBAT) Punten, East Java, Indonesia. Each aquarium was filled with 250 L of water with a stocking density of 25 fish each. Cellulolytic bacteria at a density of 108 CFU $mL^{-1}$ diluted appropriate treatment dose. The bacteria are sprayed on the feed and given to the tilapia. Fish body weight was measured during maintenance with a measurement interval of 1 wk. Water quality is recorded every day including data on temperature, pH, ammonia, dissolved oxigen.

### 2.2 Data analysis

Growth and water quality data were analyzed using Analysis of Variance (ANOVA) at a significant level (α) 0.05 and (α) 0.01. If there is a difference in the effect on the treatment, then the Least Significant Difference is further tested.

# 3 Results and discussion

## 3.1 Specific Growth Rate (SGR)

Results of specific growth rate of tilapia within 30 d of observation can be seen in Figure 1.

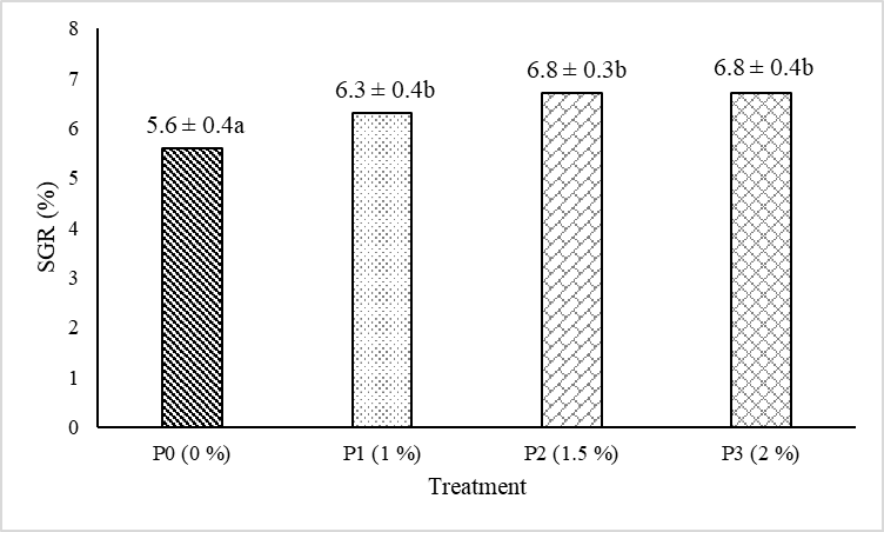


**Fig. 1.** Specific growth rate tilapia.

Based on Figure 1 specific growth rate of tilapia showed that the treatment with a commercial feed the addition of a dose of 1.5 % (P2) and a dose of 2 % (P3) cellulolytic bacteria had the same growth rate of 6.8 %. The results of the ANOVA analysis showed that the growth of the control and P1 treatments was significantly different from that of P2 and P3. So, it can be concluded that the administration of cellulolytic bacteria with a dose of 1 % has not shown an increase in growth performance. The dose that can show an increase in growth performance is more than 2 %. This is in accordance with the opinion of De *et al.* [19] that the use of cellulolytic bacteria causes an increase in optimal growth in Asian seabass, *Lates calcarifer* (Bloch, 1790) juveniles fed diets supplemented with cellulolytic and amylolytic gut bacteria isolated from brackishwater fish.

According to Dawood *et al.* [20], probiotics have a very important role in being able to break down nutrients in feed into simpler ones so that they can assist in the process of optimal nutrient absorption. Increased nutrition due to the addition of probiotics that produce enzymes that are able to hydrolyze nutrients so that they are easily absorbed by the fish body. The increase in the growth of tilapia given multi-species probiotics is a cumulative interaction in aspects of nutrition, water quality and health [21]. The addition of cellulolytic bacteria resulted in an increase in the specific weight of tilapia reared for 30 d, this is in accordance with Rodde *et al.* [22], that the addition of fermentative bacteria to feed can increase the growth rate and feed conversion ratio in tilapia.

## 3.2 Feed efficiency

Efficiency of tilapia feed consumption during 30 d of rearing is presented in Figure 2.

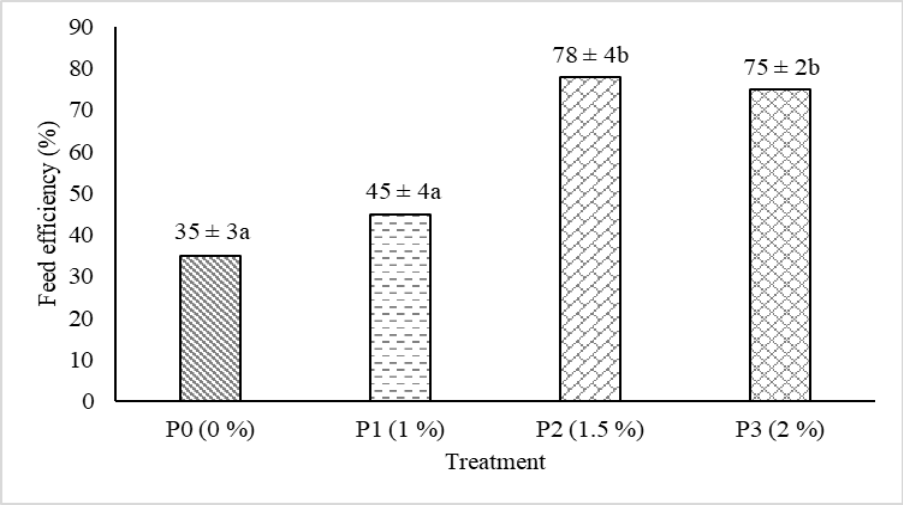


**Fig. 2.** Efficiency of tilapia feed consumption.

Based on the results of Figure 2, it shows the efficiency value of tilapia feed consumption, which is reared for 30 d, the highest value was obtained from the feed treatment with an additional dose of 1.5 % by 78 %, and the lowest value was the control treatment by 35 %.

The results of the analysis of variance in the analysis of the efficiency of feed consumption showed that the addition of cellulolytic bacteria to commercial feed had a significant effect, as seen from F 12.18 > $F_{table}$. Then proceed with BNT testing.

In the treatment in the study, it was seen that the P0 and P2 treatments had no significant effect on the efficiency of tilapia feed, but other the case with P1 and P3 treatments which had a very significant effect on the efficiency of tilapia feed consumption during the study, so the feed consumed by the treated fish was more efficient than fish feed without the presence of cellulolytic bacteria. This is in accordance with the opinion of Prasetyo *et al.* [23] that the use of cellulolytic bacteria from the rumen of sheep causes an increase in optimal growth of catfish juveniles reared for 28 d in tarpaulin pond media with a specific growth value of 27 %. The addition of growth if the feed can be digested optimally, so that it becomes energy that can be utilized optimally by fish.

The addition of probiotics can improve the digestive system of fish so that it can increase the growth rate optimally and can streamline the use of commercial feed so that it can reduce expenditure costs [24]. Feed efficiency is also directly proportional to the amount of feed consumption because it can be seen that the amount of feed that is optimally absorbed by fish and which is not optimally absorbed by cultured fish [25]. Feed efficiency is also useful for reducing feed residue caused by fish without decreasing growth in weight and length, so that the feed consumed efficiently becomes fish meat [26]. Kankainen *et al.* [27] said that the value of fish feed efficiency is a major factor in aquaculture activities so that it becomes important in handling before aquaculture activities are carried out, one of which is the provision of probiotics in feed which is useful for optimizing feed absorption even though the feed given is relatively small.

### 3.3 Absolute growth (length)

Absolute growth and relative growth rate of tilapia obtained results in each treatment for 30 d of selection, can be seen in Figure 3.

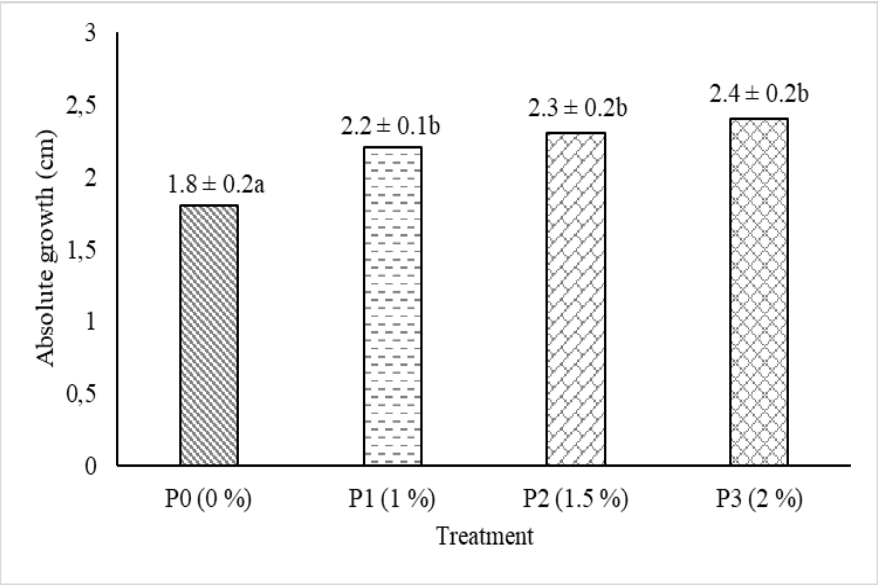


**Fig. 3.** Absolute length growth.

Based on the results Figure 3 shows the highest value for absolute growth and relative growth rate of tilapia in feed treatment with an additional dose of 1.5 % (P2) of 2.3 cm every individual, followed by treatment using a dose of 2 % (P3) of 2.4 cm every individual, then with the addition of a 1 % dose of 2.2 cm every individual and the control treatment of 1.8 cm every individual.

The value of the relative growth rate obtained is directly proportional to the absolute growth rate. Based on the results of the analysis of diversity (ANOVA) showed that the effect of treatment did not have a very significant effect on absolute growth, because the provision of cellulolytic bacteria in commercial feed was uneven, so that the feed absorbed by fish was not absorbed optimally in the fish's body and could also be affected by high fat in the fish commercial feed that makes it difficult for the fish body in the process of digesting feed in tilapia. This is in accordance with the opinion of Lall and Tibbetts [28] that the value of high fat content can affect the low consumption of feed in fish. Prabu *et al.* [29] stated that high fat feed will cause low fish feed consumption and can also be influenced by poor maintenance water quality, causing fish appetite to decrease which results in fish being stressed. The absolute weight growth rate is influenced by the presence of cellulolytic bacteria which have a role to facilitate the process of absorption of nutrients in commercial feed so that fish growth becomes more rapid [23]. The increase in absolute growth and relative growth experienced by tilapia cannot be separated from the role of the environment and probiotic (cellulolytic) bacteria that work in optimally absorbing nutrients from feed, the best occurred in the treatment with a dose of 2 % cellulolytic bacteria. This is in accordance with Abarike *et al.* [30], that the addition of good bacteria or probiotics with a certain dose can affect the growth rate of tilapia to be more optimal and can help in maintaining the condition of the fish body so that it is not susceptible to disease and probiotics can increase the growth rate of tilapia ranging from between 7 % to 9 % [31].

### 3.4 Total feed consumption

The amount of tilapia feed consumption during the study is presented in Figure 4.

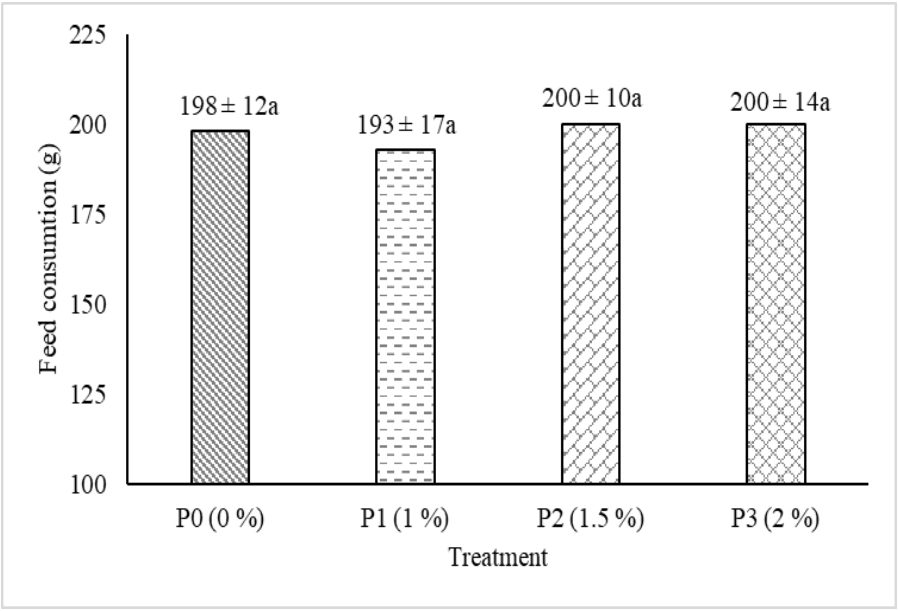


**Fig. 4.** The results of the amount of feed consumption during the study.

Based on the results of Figure 4 show that the best amount of tilapia feed consumption in the P2 and P3 treatments with the addition of 1.5 % and 2 % cellulolytic bacteria doses was more optimal and efficient in consuming the given feed, this is it can be seen that the role of cellulolytic bacteria which can optimize the digestibility of tilapia and appetite becomes good so that the fish can absorb feed nutrients properly. However, from the value of variance in the amount of feed consumption in this study, there was no significant difference in the treatment because tilapia reared for 30 d did not receive an evenly distributed feed and it was also due to the condition of commercial feed which had high fat content so that it was difficult to be absorbed by the fish's body. This is in accordance with Siagian *et al.* [32] who stated that commercial feeds that have rations with high fat content values will result in a decrease in the amount of fish feed consumption, or high fat will be inversely proportional to the amount of feed consumption will be low.

In the opinion of Tachibana *et al.* [31] that the growth of tilapia can be influenced by the consumption of feed absorbed by the body of tilapia, it can also the efficiency of feed use during maintenance, the value of the minimum amount of feed consumption ranges from 50 % to 80 % of the amount of feed given at the beginning and end of maintenance. Feed quality and the addition of probiotics to feed are factors that can affect the amount of fish feed consumption during the cultivation process [25]. The addition of weight and length of fish is influenced by the percentage of the amount of feed consumed by fish during rearing activities. The amount of feed consumption is very important for aquaculture actors because 75 % of the cost is spent on buying commercial feed, therefore the use of probiotics serves to reduce costs so that the fish produced will still have optimal and efficient growth in length and weight [33].

### 3.5 Survival rate

Survival of tilapia during 30 d of rearing is presented in Figure 5.

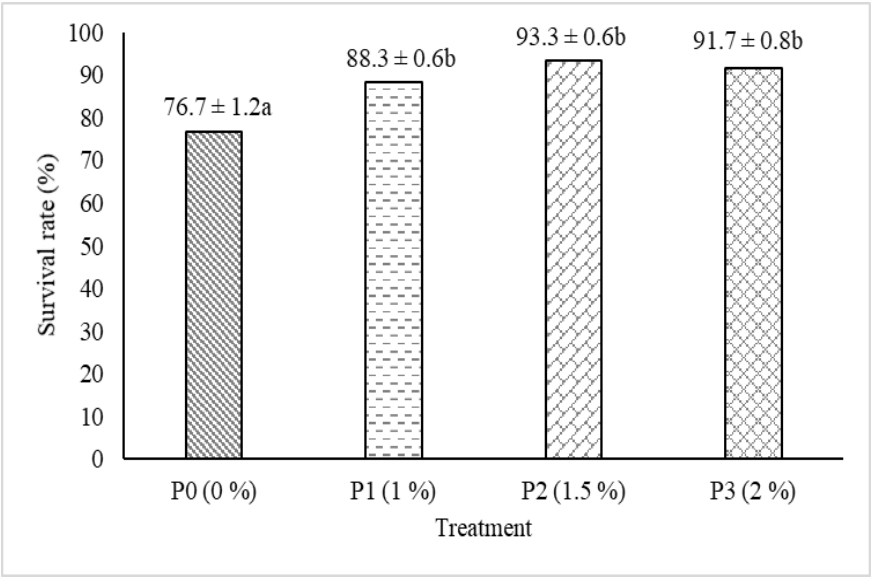


**Fig. 5.** The survival of tilapia during the study.

Based on Figure 5 on the survival of tilapia it can be seen that the highest survival value of tilapia was found in the treatment with a dose of 1.5 % cellulolytic bacteria in commercial feed of 93.3 % and the lowest value found in the control treatment (without cellulolytic bacteria) of 76.7 %. This shows that the addition of cellulolytic bacteria to commercial feed is very influential on the survival of tilapia. Where cellulolytic bacteria are good bacteria (probiotics) which are useful for the digestibility of fish so that they can absorb nutrients in the feed optimally and also survival is influenced by good water quality so that the fish's appetite is in good condition.

The results of the analysis of variance also showed a significant effect between each treatment ($P > 0.05$). The high survival rate at a dose of 1.5 % is related to the appetite response and water quality that supports well tests were then continued with the BNT test against the survival of tilapia, namely P2 treatment got the best value of 93.3 % (significantly different), followed by P3 91.7 %. This is in accordance with Lall and Tibbetts [28] statement that factors affecting feed consumption include nutrient content, temperature, palatability, age, body weight, and stomach capacity. Feed palatability is determined by the form of feed, size, taste, aroma, and color which are physical and chemical factors of feed. In addition, the palatability of the feed is also closely related to the attractiveness of the given amino acids which will affect the response. Probiotic bacteria can also improve the fish's immune system so that it can increase the survival rate of fish that are attacked by bacteria [34].

According to Buyukdeveci *et al.* [35], that the ability of cellulolytic bacteria to produce extracellular enzymes has an effect on increasing the digestibility of feed in fish. The high cellulolytic activity indicates that these bacteria have benefits for degrading cellulose. The survival value of good tilapia ranges from 73.5 % to 86.5 % which can affect several factors, namely nutrient intake, and water quality conditions [28]. The survival of tilapia in 30 d rearing experienced a significant difference from the control treatment with the addition of cellulolytic bacteria to commercial feed, this agrees with Buyukdeveci *et al.* [35] that the survival rate or fish survival can be optimal because it is influenced by conditions of feed intake that have high nutrients or feed added with probiotics and environmental conditions of live fish. The death that occurred in the control treatment was due to the fish not being able to consume the feed given properly. So, it affected the water

quality which was the main mortality factor in the control treatment. This is in accordance with the opinion of Prasetyo *et al.* [23] where the survival value of tilapia fry can be influenced by the condition of the feed ingredients provided which affect the body's metabolism so that it can make water quality dangerous for the fish.

### 3.6 Water quality

Results of water quality measurements during the study included measurements of temperature, ammonia, pH meter, and dissolved oxygen meter are presented in Table 1.

**Table 1.** Water quality measurement water.

| Quality | Dose 1 % | Dose 1.5 % | Dose 2 % |
|---|---|---|---|
| Temperature | 26 C | 26 C | 26 C |
| pH | 7 | 7 | 7 |
| Dissolved oxygen | 5.98 | 6.5 | 5.8 |
| Ammonia | 0 | 0 | 0 |

Based on these data during the study the water quality in each aquarium showed good water quality to be able to support the growth of tilapia during the study. This is reinforced by the statement, the National Standardization Agency (SNI 6141:2009) that the optimum temperature range for the maintenance and growth of tilapia ranges from 25 ℃ to 30 ℃, then fish can adapt well to waters that have a pH ranging from 6.5 to 8.5. The value of the degree of acidity in the waters can affect the metabolism in the fish body so it is very important, the value of a good degree of acidity is between 6.5 to 7 [36]. A good range of dissolved oxygen levels for tilapia rearing media is > 5 mg $L^{-1}$, so that survival can be well maintained and the level of ammonia value in the waters can be tolerated or under normal conditions and is good for rearing tilapia is < 0.002 mg $L^{-1}$. The appetite and growth rate of tilapia will be inhibited or decreased if the concentration value of ammonia levels is > 0.008 mg $L^{-1}$ resulting in a decrease in the body's resistance of the fish at that concentration.

## 4 Conclusion

The results of this study indicate that the dosage of 1 %, 1.5 % and 2 % cellulolytic bacteria to commercial feeds can affect the growth performance of the tilapia in research, in terms of good survival rate, SGR (Specific Growth Rate), EP (efficiency feed), JKP (amount of feed consumption) which is also optimal for the growth performance of the tilapia, but this is inseparable from the good water quality. So, that the fish can optimally absorb the nutrients in the feed that has been added with cellulolytic bacteria and the best provision in this study occurred in the treatment of P2 (1.5 % dose) and P3 (2 %). It can be concluded that the higher the dose of cellulolytic bacteria can affect the rate of growth performance of tilapia.

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## References

1. J.E. Setiawati, T. Tarsim, Y.T. Adiputra, S. Hudibah, J. Rekayasa Teknologi Budidaya Perairan., **1**,2: 151–162 (2013) [in Bahasa Indonesia]

https://jurnal.fp.unila.ac.id/index.php/bdpi/article/view/119
2. F.A. Ansari, A. Guldhe, S.K. Gupta, I. Rawat, F. Bux, Environ. Sci. Pollut. Res., **28**: 43234–43257 (2021) https://doi.org/10.1007/s11356-021-14989-x
3. P.C. Oostlander, J. van Houcke, R.H. Wijffels, M.J. Barbosa, Aquac., **525**,735310: 1–10 (2020) https://doi.org/10.1016/j.aquaculture.2020.735310
4. O.J. Eriegha, P.A. Ekotutu, Anim. Res. Int., **14,**2: 2697–2709 (2017) https://www.ajol.info/index.php/ari/article/view/186884/176157
5. R. Teodósio, S. Engrola, R. Colen, K. Masagounder, C. Aragão, Aquac. Nutr., **26**,2: 422–431 (2020) https://doi.org/10.1111/anu.13004
6. D. Rachmawati, R.H. Setyobudi, J. Burlakovs, T. Elfitasari, A.H. Purnomo, Jordan J. Biol. Sci., **14**,2: 297–302 (2021) https://doi.org/10.54319/jjbs/140215
7. J.R. Bogard, G.C. Marks, S. Wood, S.H. Thilsted, Glob. Food Sec., **16**: 54–64 (2018) https://doi.org/10.1016/j.gfs.2017.09.004
8. F. Kokou, E. Fountoulaki, Aquac., **495**: 295–310 (2018) https://doi.org/10.1016/j.aquaculture.2018.06.003
9. D. Rachmawati, A.A. Prihanto, R.H. Setyobudi, O. Anne, Proc. Pak. Acad. Sci.: B. Life Environ Sci., **55**,3: 31–39 (2018) https://www.ppaspk.org/index.php/PPAS-B/article/view/120
10. J. Olmos, M. Acosta, G. Mendoza, V. Pitones, Arch. Microbiol., **202**: 427–435 (2020) https://doi.org/10.1007/s00203-019-01757-2
11. M.B. Munir, R. Hashim, Y.H. Chai, T.L. Marsh, S.A.M. Nor, Aquac., **460**: 59–68 (2016) https://doi.org/10.1016/j.aquaculture.2016.03.041
12. D. Rachmawati, J. Hutabarat, O. Anne, R.H. Setyobudi, T. Efitasari, Sarhad J. Agric., **37**,Special issue 1: 55–63 (2021) https://dx.doi.org/10.17582/journal.sja/2021/37.s1.55.63
13. A.G.J. Tacon, M. Metian, Aquac., **285**,1–4: 146–158 (2008) https://doi.org/10.1016/j.aquaculture.2008.08.015
14. E.M. Ferreira, A.V. Pires, I. Susin, M.V. Biehl, R.S. Gentil, M.O.M. Parente, et al., Anim. Feed Sci. Technol., **216**: 30–39 (2016) https://doi.org/10.1016/j.anifeedsci.2015.09.007
15. A. De Angelis, L. Gasco, G. Parisi, P.P. Danielli, Animals, **11**,8: 1–16 (2021) https://doi.org/10.3390/ani11082230
16. W. Wizna, H. Abbas, Y. Rizal, A. Dharma, I.P. Kompiang, Pak. J. Nutr., **8**,10: 1636–1640 (2009) https://doi.org/10.3923/pjn.2009.1636.1640
17. F. Nasiu, W.L. Salido, A.M. Tasse, S. Syamsuddin, H.A. Hadini, A. Indi, J. Ilmu Teknologi Peternakan Tropis, **7**,2: 127–132 (2020) [in Bahasa Indonesia] http://dx.doi.org/10.33772/jitro.v7i2.11482
18. A. Zubaidah, D. Prasetyo, H. Handajani, S.P. Rohmah, D.A. Puspita, J. Riset Akuakultur, **14**,4: 261–271 (2019) [in Bahasa Indonesia] http://ejournal-balitbang.kkp.go.id/index.php/jra/article/view/7998/6509
19. D. De, T.K. Ghoshal, R.A. Raja, S. Kumar, Aquac. Res., **46**,7: 1688–1698 (2015) https://doi.org/10.1111/are.12325
20. M.A.O. Dawood, S. Koshio, M.M. Abdel-Daim, H.V. Doan, Rev. Aquac., **11**,3: 907–924 (2019) https://doi.org/10.1111/raq.12272
21. B.T. Standen, A. Rodiles, D.L. Peggs, S.J. Davies, G.A. Santos, D.L. Merrifield, Appl. Microbiol. Biotechnol., **99**: 8403–8417 (2015) https://doi.org/10.1007/s00253-015-6702-2
22. C. Rodde, B. Chatain, M. Vandeputte, T.Q. Trinh, J.A.H. Benzie, H. de Verdal, Aquac. Rep., **17**,100349: 1–8 (2020) https://doi.org/10.1016/j.aqrep.2020.100349
23. D. Prasetyo, A. Zubaidah, H. Handajani, E. Hudin, Indones. J. Trop. Aquatic, **4,**2: 51–61 (2022) https://doi.org/10.22219/ijota.v4i2.20360

24. N.V. Hai, Fish Shellfish Immunol., **45**,2: 592–597 (2015) https://doi.org/10.1016/j.fsi.2015.05.026
25. S. Craig, L. Halfrich, Virginia Cooperative Extension, 1–6 (2017) https://vtechworks.lib.vt.edu/handle/10919/80712
26. H. de Verdal, H. Komen, E. Quillet, B. Chatain, F. Allal, J.A.H. Benzie, et al., Rev. Aquac., **10**,4: 833–851 (2018) https://doi.org/10.1111/raq.12202
27. M. Kankainen, J. Setala, I.K. Berrill, K. Ruohonen, C. Noble, O. Schneider, Aquac. Econ. Manag., **16**,4: 341–364 (2012) https://doi.org/10.1080/13657305.2012.729247
28. S.P. Lall, S.M. Tibbetts, Vet. Clin. North Am. Exot. Anim. Pract., **12**,2: 361–372 (2009) https://doi.org/10.1016/j.cvex.2009.01.005
29. E. Prabu, S. Felix, N. Felix, B. Ahilan, P. Ruby, Int. J. Fish. Aquat. Sci., **5**,6: 349–355 (2017) https://www.fisheriesjournal.com/archives/?year=2017&vol=5&issue=6&part=E&ArticleId=1443
30. E.D. Abarike, J. Cai, Y. Lu, H. Yu, L. Chen, J. Jian, et al., Fish Shellfish Immunol., **82**: 229–238 (2018) https://doi.org/10.1016/j.fsi.2018.08.037
31. L. Tachibana, G.S. Telli, D.C. Dias, G.S. Goncalves, C.M. Ishikawa, R.B. Cavalcantle, et al., Aquac. Rep., **16**,100277: 1–7 (2020) https://doi.org/10.1016/j.aqrep.2020.100277
32. D.R. Siagian, D. Jusadi, J. Ekasari, M. Setiawati, Aquac. Int., **29**: 1935–1947 (2021) https://doi.org/10.1007/s10499-021-00726-1
33. M.A.O. Dawood, S. Koshio, Rev. Aquac., **12**,2: 987–1002 (2019) https://doi.org/10.1111/raq.12368
34. S. Andriawan, S. Hadi, S. Andayani, E. Sanusi, M. Maftuch, AACL Bioflux, **12**,6: 2167–2176 (2019) http://www.bioflux.com.ro/docs/2019.2167-2176.pdf
35. M.E. Buyukdeveci, I. Cengizler, J.L. Balcazar, I. Demirkale, Dev. Comp. Immunol., **138**: 104553 (2023) https://doi.org/10.1016/j.dci.2022.104553
36. A.D. Robert, M.C. Barnhart, J. North Am. Benthol. Soc., **18**,4: 477–487 (1999) https://doi.org/10.2307/1468380