Fermentation of probiotic-enriched commercial feed to improve tilapia (*Oreochromis niloticus*) growout performance

IOP Conf. Series: Earth and Environmental Science **674** (2021) 012056 doi:10.1088/1755-1315/674/1/012056

Fermentation of probiotic-enriched commercial feed to improve tilapia (*Oreochromis niloticus*) growout performance

S Ndobe[1], E Rosyida[1] and Z R Palallo[2]

[1]Faculty of Animal Husbandry and Fisheries, Universitas Tadulako, Palu 94118, Indonesia
[2]Postgraduate School, Universitas Tadulako, Palu 94118, Indonesia
Email: samliok@untad.ac.id

Abstract. The Nile tilapia (*Oreochromis niloticus*) is a freshwater food fish farmed in many regions around the world, including Indonesia. Feed is often the main input in aquaculture systems; however, feed quality is not always optimal for fish health and growth. Probiotics are microorganisms which can help break down complex compounds and make feed more digestible through fermentation. Benefits can include improved health, survival and faster growth. This research studied the effect of feed enrichment through fermentation with a probiotic (BOSTER^{R}) on *O. niloticus* grow out performance. The probiotic was added to commercial feed (HI-PRO-VITE 782) with eight fermentation time treatments (A = 0 days = control; B=1 day; C=2 days; D=3 days; E=4 days; F=5 days; G=6 days; H=7days). Experimental fish were 120 *O. niloticus* fingerlings (total length 4-5 cm; weight 0.90-1.25 g) from Kalawara Hatchery, Sigi District, Central Sulawesi, Indonesia. Five fingerlings were placed in each experimental unit (aerated aquaria, 20 L water) and fed 3 times/day at 5% of bodyweight. Variables measured were growth (length and weight), survival rate (100% under all treatments) and feed conversion ratio (FCR). Water quality was monitored. The best results in terms of growth and FCR were obtained with 2 days of probiotic fermentation treatment.

1. Introduction

The aquaculture of freshwater fish is making an increasingly important contribution to global food security [1, 2]. Feed is often the most costly input in freshwater aquaculture systems [3, 4]. Sourcing safe, efficient and affordable feed for the cultured fish one of the main challenges in aquaculture development [1, 5]. One the one hand it is vital that the feed provides nutrition conducive to the health and growth of the fish commodity being cultured, while on the other hand the feed market is highly competitive as fish farmers seek products which will enable them to make a profit [5]. In addition, there is a growing recognition of the importance of sustainability and minimising environmental footprint of aquaculture [1].

These multiple and sometimes conflicting concerns are putting pressure on fish farmers and feed suppliers and driving innovation [1, 5], including the growing use of probiotics in aquaculture, in particular in feed formulations [6–9]. Evidence that probiotics can improve the health and/or growout performance of cultured fish species is growing [2, 10, 11]. For example improvements in growth [2,11–17] and feed conversion ratio (FCR) [12, 13] as well as survival [14], including through improved immune response and disease resistance [2, 10, 11, 13, 16–18].

One challenge is that readily available and affordable feed ingredients with suitable proximate composition are not always well digested and thus not optimal for fish growth and health [19]. One potential solution to this challenge is fermentation using probiotics, specifically microorganisms which

can help to break down complex compounds and make feed more digestible [19, 20]. Benefits from feed fermentation using probiotics can include improved health and survival [21] as well as faster growth [19, 21, 22] and even improved flesh quality [21].

The Nile tilapia (*Oreochromis niloticus*) is a freshwater food fish originating from Africa where it has been fished since prehistoric times [23], In recent decades this species has become a mainstay of aquaculture development in many regions around the world, accounting for 8.3% of global aquaculture production in 2018 [1]. In Indonesia *O. niloticus*, known as *ikan nila*, is one of the most commonly farmed freshwater fishes, and one of the priority aquaculture commodities slated for further expansion in the 2020-2024 Strategic Plan of the Directorate General for Aquaculture of the Ministry of Marine Affairs and Fisheries [3]. Nile tilapia culture has even expanded beyond freshwater into brackishwater habitat, due to the development of increasingly euryhaline strains [24]. Enrichment of commercial feed with the probiotic (BOSTER^{R}) has been shown to improve *O. niloticus* grow out performance. The aim of this research was to study the effect on *O. niloticus* growout performance of fermentation for different time periods (numbers of days) of a readily available and popular commercial feed (HI-PRO-VITE 782) after enrichment of the feed with this probiotic.

2. Materials and Methods

2.1. Study site, experimental fish and husbandry

This study was conducted in the Faculty of Animal Husbandry and Fisheries Aquaculture Laboratory at Universitas Tadulako in Palu, Central Sulawesi, Indonesia from the last week in March to the first week in May 2020 (6 weeks). Experimental fish were Nile tilapia (*Oreochromis niloticus*) fingerlings obtained from the Kalawara Hatchery in Sigi District, Central Sulawesi Province, Indonesia. The fingerlings (N = 480) ranged from 4-5 cm in total length (TL) and 0.90-1.25 g in weight. The experimental units were aerated aquaria (30 cm x 30 cm x 40 cm) filled with 20 L of fresh water. The fingerlings were placed in the aquaria at a stocking density of 1 fish/L (20 fish per experimental unit). The fingerlings were fed 3 times/day (08:00, 12:00 and 17:00 WITA = GMT+8) at a rate of 5% of bodyweight. Water quality parameters monitored with a calibrated multi-meter probe (Lutron YK-2001PHA) were temperature (ºC), pH and dissolved oxygen (DO, mg/L). The parameters were measured 3 times/day (07:00, 13:00 and 19:00 WITA). The water was changed weekly while the fish were being weighed and measured.

2.2. Experimental design and parameters

The commercial feed used as a base feed was HI-PRO-VITE 782. The control was the method of probiotic enrichment currently used by fish farmers based on previous research, i.e. daily preparation and use on the same day. The probiotic BOSTER^{R} was added to this commercial feed with eight fermentation time treatments: A = 0 days = control; B=1 day; C=2 days; D=3 days; E=4 days; F=5 days; G=6 days; H=7days). There were three replicates of each treatment giving 24 experimental units with 5 fish per unit. A Completely Randomized Design (CRD) experimental layout was used.

The fish were measured and weighed weekly. Total length (cm) was measured using digital callipers (Vernier 150mm/6", precision 0.01 cm) and body weight was measured using digital scales (Diamond 20g scales, precision 0.001g) at the beginning of the experiment (day 0) and weekly over the five week study period. Growth in total length (TL) and in weight (W) were defined as: $GL = L_t - L_0$, $GW = W_t - W_0$, Where, L_t = total length (TL) in cm and W_t = weight at time t in g; L_0 = total length (TL) in cm and W_0 = weight at day 0 in g

Mortality was recorded and survival rate SR was defined as: $SR = n_t \cdot n_0^{-1}$, where, n_t = number of live fish at time t; n_0 = number of live fish at day 0.

The feed conversion ratio (FCR) was calculated as: $FCR = F/\{(W_t+D)-W_0\}$,where: F = Total amount of feed given (g) to the fish in the experimental unit; W_0 = total weight (g) of all fished stocked in the unit at day 0; W_t = total weight (g) of all fish alive at time t (at the end of the 5 week study period); D = weight of all fish in the unit that died during the experimental period.

2.3. Fermentation method

The commercial feed was fermented after enrichment with the BOSTER^{R} probiotic. The method followed the procedures in [25], modified to suit the probiotic used in this research (i.e. sugar was not added to the probiotic mixture). Firstly 5 ml of probiotic was added to 50 ml of distilled water. This mixture was sprayed onto the feed which was then dried in the open air. The treated feed was placed in plastic containers and sealed to allow fermentation to occur. The feed was kept in these sealed containers for a set length of time depending on the treatment (0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days and 7 days) before being fed to the fish. New batches were prepared daily, and used after the appropriate storage (fermentation) time for each treatment.

2.4. Data analysis

Data were tabulated, data analysis was conducted and graphics were produced in Microsoft Excel 2010. One-way ANOVA was applied to evaluate the significance of between treatment differences at the 95% confidence limit ($p < 0.05$). If the ANOVA indicated statistically significant between-treatment differences, a post-hoc Least Significant Difference (LSD) test was applied. If the ANOVA did not indicate significant differences ($p > 0.05$) the LSD test was not applied. The results were analysed descriptively and with reference to the scientific literature.

3. Results and Discussion

3.1. Water quality and survival rate (SR)

Water quality can significantly affect fish survival and growth. Overall, the water quality can be considered to have met the requirements for Nile tilapia culture as evidenced by the survival rate (SR) which was 100% under all treatments. The water quality monitoring data (Table 1) indicate that in general the parameters measured remained within the ranges acceptable for Nile tilapia aquaculture according to the Indonesian National Standard for Nile tilapia farming (SNI-7550-2009).

Table 1. Water quality data in the experimental units over the 5 week study period

No.	Parameter	Unit	Range measured	Recommended range[a]
1.	Temperature	ºC	26.3 – 29.1	25 – 32
2.	pH		6.30 – 8.41	6.5 – 8.5
3.	DO	mg/L	3.10 – 3.80	> 3.0

[a] Source: Indonesian National Standard for tilapia culture SNI-7550-2009

Nile tilapia can tolerate and have been reported to grow quite well over a temperature range of 14 to 38ºC [26]. The temperature during the study remained well within these extremes and indeed well within the optimal or recommended range according to SNI-7550-2009 (Table 1). According to [27], Nile tilapia can be cultured in water with a pH range of 6-8.5, although growth tends to be faster in the range pH 7-8. Therefore, it can be considered that the pH range remained within the range tolerated by Nile tilapia, although occasionally it dropped below the optimum range recommended in SNI-7550-2009. The dissolved oxygen (DO) was relatively low compared to the general recommendation of 5 mg/L or higher for aquaculture in general, but within the recommended range for Nile tilapia farming based on SNI-7550-2009 and [27]. Abdel-Tawwab et al [28] report that Nile tilapia reared with a DO range of 2.5–3.0 mg/L grew more slowly and suffered higher mortality than those reared with a DO range of 6-6.5 mg/L. Although the DO in this study never fell below 3 mg/L, it is possible that growth could be increased with improved aeration to raise the DO concentration.

3.2. Growth in length and weight gain

Growth of the Nile tilapia (*Oreochromis niloticus*) fingerlings was steady and showed a similar overall pattern for all treatments. Net growth in length (Fig.1) and weight gain (Fig. 2) of the *O. niloticus* fingerlings was highest for treatment C (2 days fermentation), followed by treatment D (3 days fermentation), after which mean growth decreased with fermentation time.

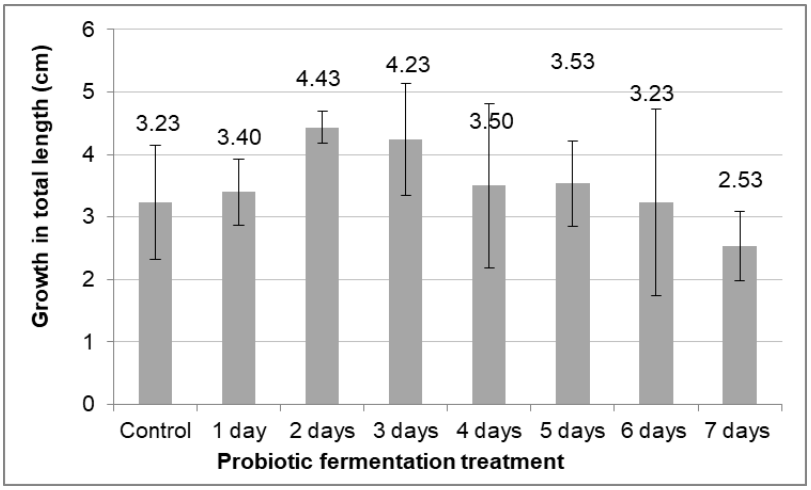

Figure 1. Growth in total length (cm) over 5 weeks of Nile tilapia (*Oreochromis niloticus*) seedlings fed commercial feed (HI-PRO-VITE 782) enriched with probiotic (BOSTER^{R}) and fermented for 0-7 days. Error bars show standard deviation

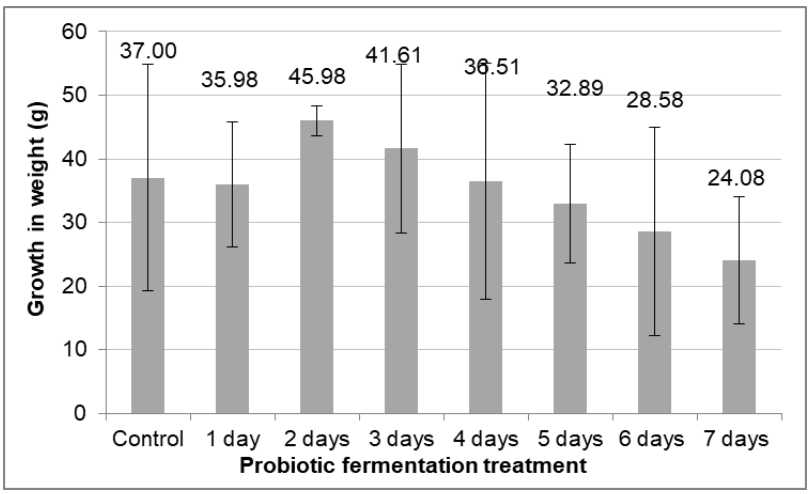

Figure 2. Growth in weight (g) over 5 weeks of Nile tilapia (*Oreochromis niloticus*) seedlings fed commercial feed (HI-PRO-VITE 782) enriched with probiotic (BOSTER^{R}) and fermented for 0-7 days. Error bars show standard deviation

In addition to the highest mean net growth, treatment C also appeared to result in more homogenous growth patterns. This is evident in the noticeably lower variability between replicates, as indicated by the smallest standard deviation values for growth in both length and weight. Despite the visually striking trend, there was no statistically significant difference between treatments in the aggregate or when paired ($p > 0.05$). This lack of statistical significance was most likely due to the small number of replicates (3) coupled with high variability between replicates in all treatments except the 2 day fermentation treatment (treatment C). This variability is reflected in the length of the standard deviation whiskers in Fig. 1 and Fig. 2.

3.3. *Feed conversion ratio (FCR)*

The feed conversion ratio (FCR) exhibited the same pattern as the growth in length and weight gain, with the highest mean FCR obtained for treatment C, with a 2 day fermentation period (Table 2). In theory, the more digestible the feed, the better fish will digest it, so that the nutrients in the feed will be more efficiently converted into fish biomass. However, as for growth, the between-treatment differences were not statistically significant ($p > 0.05$), indicating the effect on feed digestibility and

fish digestion is small and there is a greater than 5% probability that it could have occurred due chance in the absence of any real difference between the treatments.

Table 2. Feed conversion ratio (FCR) for Nile tilapia (*Oreochromis niloticus*) given feed enriched through probiotic fermentation for different fermentation times

No.	Treatment	Fermentation days	Mean FCR
1	A	0	4.261
2	B	1	4.122
3	C	2	5.857
4	D	3	4.414
5	E	4	4.153
6	F	5	4.034
7	G	6	3.971
8	H	7	3.401

3.4. Evaluation of the fermentation of probiotic enriched feed

The fact that survival rate was 100% for all treatments indicates that all feed treatments provided acceptable nutrition for Nile tilapia, as could be expected based on previous trials with different dosages of probiotic but without fermentation. That research has already been implemented at the farmer level, and fish farmers are currently preparing feed sprayed with probiotic. The farmers are using the enrichment protocol applied in this study as a control on a daily basis, i.e. preparing each day sufficient for use that same day (control treatment A in this study).

While the fermentation did not produce a large and statistically significant change in any one parameter, the consistent pattern for all parameters (growth in length, weight gain and FCR) as well as the greater consistency between replicates indicates that a 2 day fermentation is worth further investigation, in particular on-farm trials in collaboration with the fish farmers. At the very least the results show that such fermentation of the enriched feed would do no harm, while if even small improvements (as suggested by our results) could translate into noticeable gains in productivity at a farm or pond scale.

Another consideration is that, although 2 days fermentation appears to be optimal, there were also apparent improvements in mean growth and FCR compared to the control for 1 day and 3 days of fermentation. These results indicate that fish farmers might not need to prepare the probiotic-enriched feed every day, giving them potentially more flexibility in their activities and potentially resulting in increased labour efficiency. However the consistent declines in all parameters, albeit not statistically significant, after 4 days or more of fermentation indicates that it would not be advisable to allow the enriched feed to ferment (as it will if it is stored after spraying and drying) for more than 3 days.

4. Conclusion

The results of this study indicate that fermentation of a commercial feed (HI-PRO-VITE 782) for up to a week after enrichment with the probiotic BOSTER^{R} does not appear to have a large effect on the growth of Nile tilapia (*Oreochromis niloticus*) fingerlings, and survival rate was 100% under all treatments. Despite the lack of statistical significance, all parameters followed the same pattern, indicating maximum potential for improvement in terms of growth and feed conversion rate (FCR) after 2 days of fermentation. Extending the period of fermentation beyond 3 days appeared to be counterproductive. We recommend further research including scaling up and diversification. In particular, fish pond scale trials of feed enriched through 2-day probiotic fermentation over a complete stocking to harvest cycle, and trials of the fermented probiotic enrichment approach with other commercial or bespoke feeds and for other aquaculture commodities.

References

[1] FAO 2020 *The State of World Fisheries and Aquaculture 2020: Sustainability in action* (Rome: Food and Agriculture Organization of the United Nations)
[2] Welker T L and Lim C 2011 Use of probiotics in diets of tilapia *Aquac. Res. Dev.* **S1** 014
[3] Directorate General for Aquaculture 2020 *Rencana Strategis tahun 2020-2024 Direktorat Jenderal Perikanan Budidaya* [*Strategic plan for the period 2020-2024 of the Directorate General for Aquaculture*] (Jakarta: Kementerian Kelautan dan Perikanan Republik Indonesia) 104 pp
[4] Iliyasu A, Mohamed Z A and Terano R 2016 Comparative analysis of technical efficiency for different production culture systems and species of freshwater aquaculture in Peninsular Malaysia *Aquac. Reports* **3** 51–7
[5] Jennings S, Stentiford G D, Leocadio A M, Jeffery K R, Metcalfe J D, Katsiadaki I, Auchterlonie N A, Mangi S C, Pinnegar J K, Ellis T, Peeler E J, Luisetti T, Baker-Austin C, Brown M, Catchpole T L, Clyne F J, Dye S R, Edmonds N J, Hyder K, Lee J, Lees D N, Morgan O C, O'Brien C M, Oidtmann B, Posen P E, Santos A R, Taylor N G H, Turner A D, Townhill B L and Verner-Jeffreys D W 2016 Aquatic food security: insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment *Fish Fish.* **17** 893–938
[6] Sihag R C and Sharma P 2012 Probiotics: The new eco friendly alternative measures of disease control for sustainable aquaculture *J. Fish. Aquat. Sci.* **7** 72–103
[7] Pérez-Sánchez T, Ruiz-Zarzuela I, de Blas I and Balcázar J L 2014 Probiotics in aquaculture: A current assessment *Rev. Aquac.* **6** 133–46
[8] Dawood M A O and Koshio S 2016 Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review *Aquaculture* **454** 243–51
[9] Martínez Cruz P, Ibáñez A L, Monroy Hermosillo O A and Ramírez Saad H C 2012 Use of probiotics in aquaculture *ISRN Microbiol.* **2012** 1–13
[10] Standen B T, Rawling M D, Davies S J, Castex M, Foey A, Gioacchini G, Carnevali O and Merrifield D L 2013 Probiotic *Pediococcus acidilactici* modulates both localised intestinal- and peripheral-immunity in tilapia (*Oreochromis niloticus*) *Fish Shellfish Immunol.* **35** 1097–104
[11] Hai N Van 2015 Research findings from the use of probiotics in tilapia aquaculture: A review *Fish Shellfish Immunol.* **45** 592–7
[12] Suarsito S and Purbomartono C 2018 The effect of use of tofu dregs fermented on feed efficiency and growth of African cat fish (*Clarias gariepinus*) *IOP Conf. Ser. Mater. Sci. Eng.* **434** 1–8
[13] Ayyat M S, Labib H M and Mahmoud H K 2014 A probiotic cocktail as a growth promoter in Nile tilapia (*Oreochromis niloticus*) *J. Appl. Aquac.* **26** 208–15
[14] Mesalhy S, Ahmed Y A-G, Gareeb A A-A and 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 Immunol.* **25** 128–36
[15] El-Haroun E R, Goda A M A and Chowdhury M A K 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** 1473–80
[16] Nwanna L C and H T-J 2017 Effects of dietary *Lactobicillus plantarum* on the growth, carcass quality, and immune response of African catfish *Clarias Gariepinus*

challenged with *Salmonella Typhi J. Appl. Aquac.* **29** 62–80
[17] El-Rhman A M A, Khattab Y A E and 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 Immunol.* **27** 175–80
[18] Shelby R A, Lim C, Yildirim-Aksoy M and Delaney M A 2006 Effects of probiotic diet supplements on disease resistance and immune response of young Nile tilapia, *Oreochromis niloticus J. Appl. Aquac.* **18** 23–34
[19] Dawood M A O and Koshio S 2020 Application of fermentation strategy in aquafeed for sustainable aquaculture *Rev. Aquac.* **12** 987–1002
[20] Taufik D, Arief M and Kenconojati H 2019 The effect of different level of probiotic addition on commercial feed against digestibility and efficiency of Nile tilapia feed (*Oreochromis Niloticus*) *IOP Conf. Ser. Earth Environ. Sci.* **236** 012074
[21] Chilmawati D, Swastawati F, Wijayanti I, Ambaryanto and Cahyono B 2018 Probiotic use for growth improvement, feed efficiency, survival rate and nutrition value of milkfish (*Chanos chanos*) *Saintek Perikan.* **13** 119–25
[22] Anugraheni R 2016 *Pengaruh penambahan probiotik EM4 pada pakan ikan terhadap pertumbuhan ikan nila merah (Oreochromis niloticus)* [*Effect of adding probiotic EM4 to feed on growth of red Nile tilapia*] (Yogyakarta: Universitas Sanata) 130 pp.
[23] Van Neer W 2004 Evolution of prehistoric fishing in the Nile Valley *J. African Archaeol.* **2** 251–69
[24] Putra N S S U, Lapong I, Rimmer M A, Raharjo S and Dhand N K 2013 Comparative performance of four strains of Nile tilapia (*Oreochromis niloticus*) in brackish water ponds in Indonesia *J. Appl. Aquac.* **25** 293–307
[25] Harianto D K, Sasanti A D and Fitriani M 2016 Pengaruh perbedaan lama waktu penyimpanan pakan berprebiotik terhadap kualitas pakan [The effect of different storage times on quality of probiotic feed] *J. Akuakultur Rawa Indones.* **4** 117–27
[26] Khairuman H and Amri K 2013 *Budidaya ikan nila* [*Culture of Nile tilapia*] (Jakarta: Agromedia)
[27] Kordi K M G H 2010 *Budidaya ikan nila di kolam terpal* [*Nile tilapia culture in tarpaulin ponds*] (Yogyakarta: Lily Publisher)
[28] Abdel-Tawwab M, Hagras A E, Elbaghdady H A M and Monier M N 2015 Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity *Aquac. Int.* **23** 1261–74