Can the combination of biofloc technology and probiotic application improve feed utilization and production of Nile tilapia (*Oreochromis niloticus*)?

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Abstract. Biofloc technology (BFT) and probiotic application are widely used in aquaculture. This study aimed to assess the effects of combined BFT and probiotic application on intensive tilapia culture in modified earthen ponds, namely, high-density polyethylene (HDPE) dyke and bottom lined pond (DBL); HDPE bottom lined pond (OBL); and no HDPE lined pond (NoL). A trial was conducted for 150 days in the three mentioned pond types in triplicate. The pond had 200 m^2 and 1.5 m in depth. Mono-sex tilapia fingerlings (sizing 3.20 ± 0.89 g each) were stocked with a density of 5 fish m^{-2}. Biofloc booster was applied into the ponds at the rate of 5 mg L^{-1} per day for the first month. Thereafter, the ponds were supplied the mixed *Bacillus* spp. probiotic at a dose of 10 g per 100 m^2 every 10 days, and molasses (C = 37.5%) at a dose of 3.5 g m^{-3} weekly. The growth performance of tilapia in the DBL ponds was significantly higher ($p < 0.05$) than those in the OBL treatment, but not significantly different from those in the NoL treatment. The lowest survival rate (66.6%) of the fish in the OBL treatment compared to those in the other treatments. Feed conversion ratio was low (1.27) for all ponds. The quality of the harvested fish was evaluated as excellent. This study proves that the combination of BFT and probiotic application could improve the efficiency of tilapia farming in earthen ponds.

Key Words: biofloc, earthern pond, feed utilization, growth, probiotic.

Introduction. The aquatic animals in general and fish in particularly are the highly nutritious and offer cheap protein sources compared to meat to fulfill food demand and alleviate malnutrition problem to the poor people (Pradeepkiran 2019; Yuvarajan et al 2019). Tilapias originate from Africa and the Middle East (Lim and Webster 2006), but it is now worldwide cultured, particular in Asia and the Pacific (De Silva et al 2004). Tilapias can survive at low oxygen tension, tolerate a wide range of salinity and temperature, and resist to diseases. Moreover, they are low on the food chain, herbivorous, and feeding mainly on planktonic organisms, aquatic macrophytes and detritus (Pandian 1988; Ng and Romano 2013). Tilapias encompass 51.1 ± 0.46% of high-quality protein (whole body dry weight basis) and essential fatty acids; and the nutrient profile of tilapias indicated the low protein feed makes it more economical (Yuvarajan et al 2019). All tilapia species with important commercial values farming outside Africa belong to *Oreochromis* genus and more than 90% is Nile tilapia (*Oreochromis niloticus*) (Popma & Masser 1999; Watanabe et al 2002). In 2018, tilapias were the second species of cultured food-fish, after carps, with a total production of 4.526 million metric tons, occupying 8.3% of major aquaculture finfish (FAO 2020).

In Viet Nam, aquaculture has significantly contributed to the development of the country's economy in terms of food security, income generation, restructuring strategy in agriculture, export earning as well as the implementation of hunger alleviation and poverty reduction programs. In 2019, aquaculture production accounted for 54.3% of the total fisheries outputs (8.27 million metric tons); in which farmed fish accounted for 69.9% (GSO 2021). Early introduced to Viet Nam in 1950s, Tilapia was commonly cultured in mono- and polyculture systems in earthen ponds and cages in both freshwater and brackish water environments with different intensification levels. In 2015, the total production of Tilapia was 187,000 MT with a value of 200 million USD. In 2017, tilapia product was exported to 68 international markets with a revenue of 45 million USD, an increase of 32% compared to 2016 (MARD 2019). Tilapia production is also planned to increase and oriented to export in the future (MARD 2016).

Aquaculture is playing a vital role in the developing countries in national economic development, and global food supply (Pradeepkiran 2019). However, the expansion of the aquaculture production can be restricted due to environmental problems caused by the discharge of its waste products in the water bodies. Accumulation of toxic inorganic nitrogen (NH_4, NO_2) is prevented in bio-flocs system by maintaining a high C/N ratio and inducing the uptake of ammonium by the microbial community (Avnimelech et al 1994). These bacteria become inhibited due to the limited carbon source in the water. Therefore, the addition of organic carbon in the pond is able to overcome the problem of increasing ammonia in the water due to a number of bacteria in the water able to utilize the nitrogen element derived from the rest of the feed but the performance depends on the C/N ratio and the microbial community. Aquaculture using bio-flocs technology (BFT) offers aquaculture a sustainable tool to simultaneously address its environmental, social and economic issues concurrent with its growth (Crab et al 2012). The BFT combines the removal of nutrients from the water with the production of microbial biomass, which can *in situ* be used by the culture species as an additional food source (De Schryver et al 2008). The most widely used definition of probiotics is given by Fuller (1989) as 'a live microbial feed supplement which beneficially affects the host animal by improving intestinal balance'. A lot of studies on probiotic use in fish and shellfish aquaculture worldwide have been published, there are fewer publications on probiotic use in tilapia (Welker and Lim 2011; Hai 2015). Among all the routes of probiotic administration in aquaculture, supplementation of rearing water is the only method which is applicable for all ages of fish (Jahangiri and Esteban 2018). Among probiotic species employed in aquaculture, *Bacillus* spp. is likely the most widely used as a probiotic to enhance growth performance, innate immune responses, and disease resistance (Hai 2015). In Viet Nam, farming tilapia with BFT was implemented in tanks (Hoa 2012; Viet et al 2016) and in concrete ponds (Tien et al 2013). Researchers are challenged to further develop this technique and farmers to implement it in their future aquaculture systems (Crab et al 2012). Recently, several modern technologies such as closed-circulation systems, new approaches such as low impact, zero waste aquaculture and alternative methods such as using immunostimulants instead of antibiotics, including probiotics, have been applied to improve the production as well as the quality of Nile tilapia (Aly et al 2008). This study aimed to assess the ability of a combination of BFT and probiotic application as an alternative solution for improving feed use efficiency, food safety and effluent quality in intensive tilapia culture in earthen ponds.

Material and Method

Experimental design. The experiment was conducted on a production farm in Long Xuyen city of An Giang province in the Mekong Delta, Viet Nam. A completely randomized design with three treatments was used including (i) treatment 1: high-density polyethylene (HDPE) dyke and bottom lined pond (DBL); (ii) treatment 2: HDPE bottom lined pond (OBL); and (iii) treatment 3: with no HDPE lined pond (NoL). Each treatment was replicated in three 200 m^2 ponds with a water depth of 1.5 m.

Pond water was aerated with air-tube and convectively moved with air-lift systems. The air-tubes were distributed evenly with a distance of 4 m on pond bottom and fixed on round aluminum dishes to avoid bottom disturbance and water turbidity. The ponds were prepared by draining water, removing bottom mud, and treating with $CaCO_3$ at a dose of 7–10 kg 100 m^{-2} and dried for 2–3 days. Water was supplied into the ponds using filter bags to eliminate unwanted animals. The ponds were then fertilized with urea at a dose of 1 kg 100 m^{-2} to develop natural feed.

Sex-reversal fingerlings of GIFT tilapia (*Oreochromis niloticus*) using methyl testosterone treatment method were supplied from the Research Institute for Aquaculture No. 1, Vietnam. The fingerlings with an average initial weight of 3,20 ± 0,89 g were stocked into experimental ponds at a density of 5 fish m^{-2}. Prior to stocking, the fish was immersed in salt water of 2-3% for 5-10 minutes to eliminate parasites. The air-tube and air-lift systems were daily operated for 24 hrs for the whole culture period. The ponds were only supplied new water to compensate for evaporation.

***Biofloc booter, feeding, and management*.**

Biofloc booter: Biofloc booster was prepared by mixing 30 g of feed and 30 g of probiotic PondPlus of Bayer Company with a mixture of *Bacillus* spp. (*B. subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *B. licheniformis* and *B. pumilus*, ≥ 1.0 × 10^{9} CFU g^{-1}) in 3,000 mL clean water. This mixture was aerated and steered for 24 - 48 hours at pH 6.0-7.2. The biofloc booter was then added into the ponds at a dose of 5 mg L^{-1} per day during the first month. Since the second month onward, the ponds were supplied with the *Bacillus* spp. probiotic at a dose of 10 g 100 m^{-2} every 10 days. In addition, molasses (C = 37,5%) as a supplementary carbon source was weekly added to the ponds at a dose of 3.5 g m^{-3} of pond water to maintain the C : N ratio of about 11.5 : 1 and to stimulate the formation of flocs (Tien et al 2013).

Feed and feeding: Fish were fed commercial pellet for tilapia (GreenFeed Company) twice a day at 08:00–09:00 and 15:00-16:00. Crude protein contents of the feed of 30 - 35% were adjusted according to fish growth. Feeding rates were 2 - 5% body weight and adjusted at every 15 days based on fish biomass. To stimulate the fish to consume more flocs, the daily feeding rate was reduced to 90% of the requirements. Moreover, the feeding was cancelled one day per week to stimulate the fish to use flocs (Hoa 2012; Tien et al 2013). At fifteen-day intervals, 30 fish of each replicate were randomly sampled for growth measurement including total length and weight. The experiment was carried out for 150 days.

Water quality monitoring: Water temperature, dissolved oxygen (DO), and pH were measured in the morning (06:00) and afternoon (15:00) using portable DO and pH meters (HANNA Company) at a 3-day interval. Transparency and ammonia were measured weekly using secchi dish and indophenol blue method (APHA 1995). Biological oxygen demand (BOD$_{5\text{-}20^{o}C}$), chemical oxygen demand (COD), total suspended solids (TSS) and *Coliform*) were measured at the end of the culture period using methods described by APHA (1995). These parameters were compared with the standard guidelines of the national technical regulation for Tilapia farm: A technical requirement for veterinary hygiene, environmental protection and food safety issued by the Ministry of Agriculture and Rural Development dated 7/6/2017 (QCVN 02-26: 2017/BNNPTNT).

Growth and survival measurement. The survival of fish was determined at the end of the culture period, while the growth was measured every 15 days.

Survival rate (SR):

$$SR(\%) = 100 \times (No.of\,harvested\;\;fish / No.of\;\;stocked\;\;fish)$$

Specific growth rate (SGR):

$$SGR\left(\%/\mathrm{day}\right) = 100 \times \left(LnW_f - LnW_i\right)/t$$

Daily weight gain (DWG):

$$DWG\left(\mathrm{g/\,day}\right) = \left(W_f - W_i\right)/t$$

Where: Wi is initial weight (g); Wf is final weight (g), and t is experimental period (day)

Feed conversion ratio (FCR):

$$FCR = F_c / \left(M_f - M_i\right)$$

$$FI = F_c \,/\, \text{Number of fish at harvest}$$

Where: Fc is total feed consumed by fish (kg), Mi is total fish weight at beginning (kg) and Mf is total fish weight at the end of the experiment (kg).

Condition factor (K) (Froese 2006)

$$K = 100 \times W / L^3$$

Where: W is fish weight (g) and L is total length (cm)

Extrapolated yield (EY):

$$\text{MT/ ha} = M_f \times 10.000 / \text{Area of experiment ponds}$$

Fish quality. The harvested fish was assessed on non-indigenous microbial food safety namely *Escherichia coli*, *Staphylococcus* spp. and *Salmonella* spp. Three harvested fish were sampled and preserved following the national standards of TCVN 6404:2016 and TCVN 6507-3:2019. The national standards of TCVN4884-1:2015 and TCVN4884-2:2015 were applied for quantifying *Escherichia coli* and *Staphylococcus* spp., and TCVN4829:2005 for *Salmonella* spp. Food safety of the harvested fish was assessed following Decision No. 46/2007/QD-BYT on the regulation of the maximum level of biological and chemical pollution in food issued by the Viet Nam Ministry of Health on 19/12/2007 and the Commission Regulation (EC) No 2073/2005 dated on 15 November 2005 on microbiological criteria for foodstuffs.

The assessment of off-flavor of the harvested fish was followed by a method modified from van der Ploeg (1991) and Fitzsimmons (2008). Fillets of three randomly sampled fish of each replicate were wrapped in aluminum foil and steamed in a microwave oven for 60 seconds. Off-flavor intensity of the fillets was assessed by a judge of nine untrained persons based on a scale of five levels including 1 = very strong, 2 = strong, 3 = slight to distinct, 4 = very slight and 5 = no off-flavors. The flavor intensity was expressed by the average of level scores given by the judges.

Statistical analysis. All data were subjected to statistical analysis using a one-way ANOVA followed by Duncan's multiple range test using Minnitab software version 16.0. The significant difference between treatment means was set at the probability of 0.05. Data of survival rate were transformed to arsin before analyzing. Results were presented as mean ± standard deviation.

Results

Water quality. The water quality parameters of the experimental ponds are shown in Table 1. No significant differences ($p > 0.05$) in the water parameters were found among the ponds. The water transparency means from 26.7 to 27.4 cm were low implying a high density of algae. The means of temperature (27.4 to 30.6^{0}C), pH (7.66 to 8.42) and DO (5.08 to 6.28 mg/L) were slightly increased in the afternoon. Ammonia concentration was low (0.04 - 0.05 mg/L).

Table 1

Effect of different pond construction on water quality of tilapia ponds

Water parameters	*Time*	*Treatment*		
		DBL	*OBL*	*NoL*
Temperature (^{0}C)	Morning	27.4 (25.0-31.6)	27.5 (25.0-31.8)	27.4 (25.0-31.8)
	Afternoon	30.6 (28.0-34.4)	30.6 (28.0-34.3)	30.5 (28.0-34.2)
pH	Morning	7.70 (7.50-7.91)	7.68 (7.51-7.91)	7.66 (7.51-7.76)
	Afternoon	8.42 (7.96-8.71)	8.40 (7.91-8.71)	8.38 (7.91-8.56)
DO (mg/L)	Morning	5.09 (4.40-5.70)	5.10 (4.40-5.80)	5.08 (4.40-6.20)
	Afternoon	6.24 (5.50-6.80)	6.28 (5.50-6.80)	6.2 (5.50-7.00)
Ammonia (NH_3-N) (mg/L)		0.04 (0.00-0.09)	0.04 (0.00-0.10)	0.0 (0.00-0.10)
Transparency (cm)		27.4 (22.0-35.0)	26.7 (21.5-34.0)	26.7 (21.0-34.0)

Note: values are presented as mean (min-max)

Growth performance. The fish growth in total length and weight of the fish at sampling points are presented in Figures 1 and 2; and the growth parameters at the end of the culture period are in Table 2. In terms of absolute growth in length and weight of fish in the treatment OBL were lower in the other treatments at the sampling point from 75 days to the harvest. The final weight (g), DWG (g/day) and SGR (%/day) of the DBL treatment were significantly higher if compared to those of the OBL ($p < 0.05$), but no significant difference of these parameters were found between OBL and NoL and DBL and NoL ($p > 0.05$).

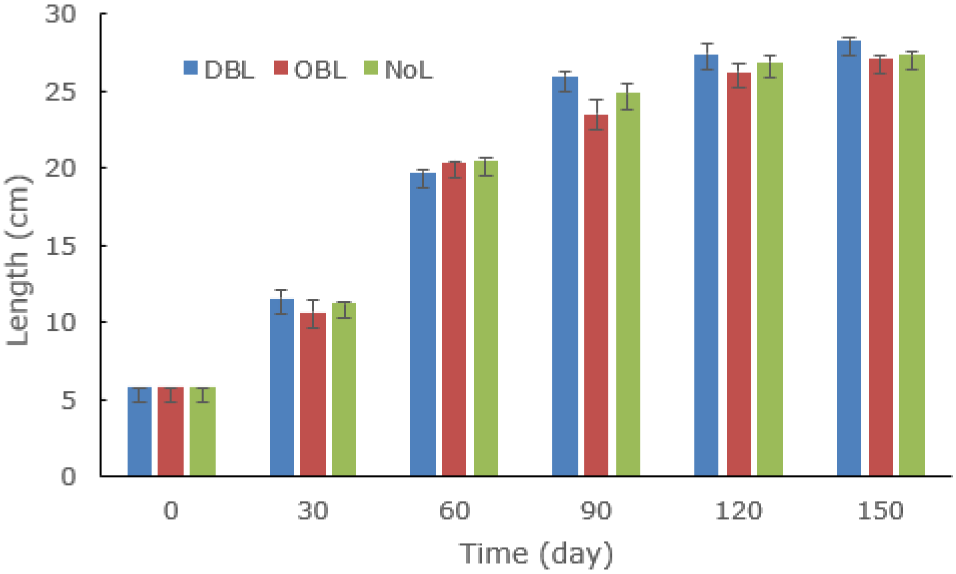

Figure 1. Growth in total length (± std) of the fish during the culture period.

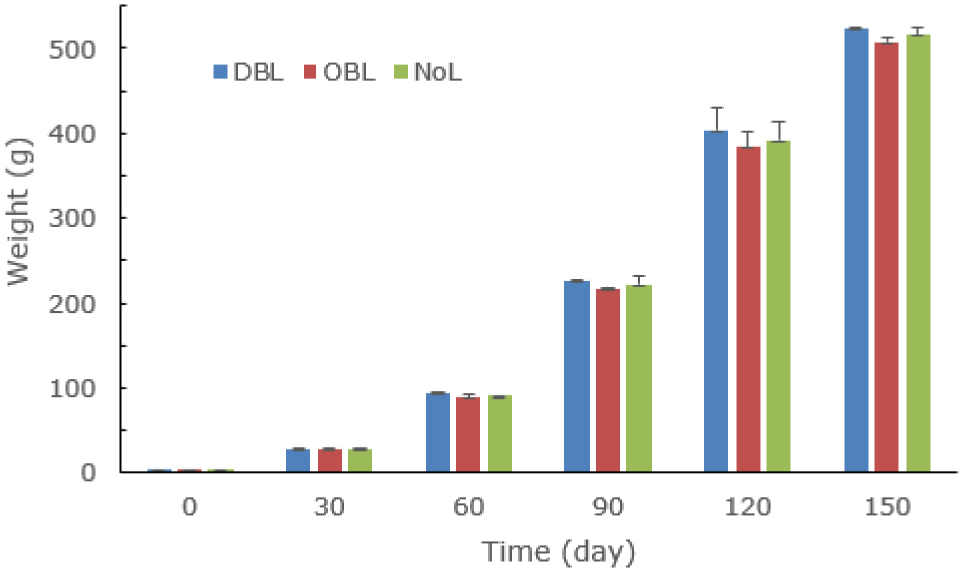

Figure 2. Growth in weight (± std) of the fish during the culture period.

Table 2

Growth performance of the fish of different pond construction

Parameters	*Treatment*		
	DBL	*OBL*	*NoL*
Final weight (g)	524.01^{b}±0.68	507.01^{a}±6.85	515.92^{ab}±8.25
Daily weight gain - DWG (g/day)	3.47^{b}±0.01	3.36^{a}±0.05	3.42^{ab}±0.05
Specific growth rate - SGR (%/day)	3.40^{b}±0.01	3.38^{a}±0.05	3.39^{ab}±0.05

Note: Different superscripts in the same row indicate significant differences ($p < 0.05$) by LSD ($n = 180$). The values shown are the average value and standard deviation.

Harvest biomass, survival, and feed use efficiency. Means of total harvested biomass and feed intake (AFI) of the DBL treatment were highest, followed by those of the NoL and OBL treatments (Table 3). There was a significant difference of means of the total harvested biomass and FI between the DBL and OBL treatments ($p<0.05$), but no significant difference in any of these variables between the NLP treatment and the other treatments ($p>0.05$). The extrapolated yields of the DBL and NoL were higher than that of the OBL. No significant difference in means of feed conversion ratio (FCR) was noted among the treatments (Table 3). The survival rate of the fish in the DBL and NoL treatments was significantly higher than that of the OBL treatment ($p<0.05$).

Table 3

Biomass, yield, survival and feed utilization of the fish in different treatments

Parameters	*Treatment*		
	DBL	*OBL*	*NoL*
Total stocked fish biomass (kg)	3.19±0.90	3.19±0.90	3.19±0.90
Total harvested fish biomass (kg)	420^{b}±5.55	337^{a}±1.24	412^{ab}±2.97
Extrapolated yield (MT/ha)	21.01	16.87	20.59
Survival rate – SR (%)	80.2^{b}±0.95	66.6^{a}±1.03	79.8^{b}±1.63
Feed intake – FI (g)	662^{b}±7.86	639^{a}±9.80	649^{ab}±13.35
Feed conversion ratio – FCR	1.27±0.00	1.27±0.02	1.27±0.02

Note: Different superscripts in the same row indicate significant differences ($P<0.05$) by LSD ($n=9$). The values shown are the average value and standard deviation.

Fish quality. The condition factor (K) of fish in the DBL treatment was significantly lower than that of the OBL and NLP ($p<0.05$) (Table 4). Significantly lower means of coefficient of variation (CV) expressed an even length size of the harvested fish of the DBL and NoL compared to the OBL ($p<0.05$). High scores pointed out the good quality in terms of off-flavor intensity of all treatments (Table 4).

Infected bacteria found in the harvested fish is presented in Table 5. The ratio and density of *E. coli* were highest in the fish of the OBL treatment and followed by those of the NoL and DBL treatments. The trend was similar to *Staphylococcus* spp. However, *Salmonella* spp. Was not presented in the fish of any treatments. The results showed that the harvested fish met the food safety standard criteria for human consumption of Vietnam and European Commission (EC).

Table 4

Biometric indices and quality of harvested fish of different treatments

Parameters	*Treatment*		
	DBL	*OBL*	*NoL*
Condition factor – K	$2.33^a \pm 0.03$	$2.59^b \pm 0.12$	$2.54^b \pm 0.04$
Coefficient of variation of final length - CV (%)	$3.76^a \pm 0.91$	$5.61^b \pm 1.35$	$3.58^a \pm 0.83$
Score of off-flavor intensity (SOI)	$4.56^a \pm 1.28$	$4.11^a \pm 1.69$	$4.41^a \pm 1.45$

Note: Different superscripts in the same row indicate significant differences ($P<0.05$) by LSD (n=180 for K & CV and n=27 for SOI). The values shown are the average value and standard deviation.

Table 5

Bacterial analysis on harvested fish of different treatments

Treatments & criteria	*Escherichia coli*		*Staphylococcus spp.*		*Salmonella spp.*	
	Ratio (%)	*Density (10^2 MPN g^{-1})*	*Ratio (%)*	*Density (10^2 CFU g^{-1})*	*Ratio (%)*	*Density (10^2 CFU g^{-1})*
DBL	50	0.08 ± 0.09	67	0.10 ± 0.08	0	Negative
OBL	100	0.37 ± 0.14	100	0.33 ± 0.20	0	Negative
NoL	67	0.11 ± 0.09	67	0.13 ± 0.11	0	Negative
Vietnamese & EC criteria	$<10^2$		$<10^2$			not present

Pond water at harvest. The quality in terms of pH, BOD, COD, TSS and *Coliform* of the water or effluent at harvest met the Viet Nam's standard criteria to directly discharge into the water environment (QCVN 02-26:2017/BNNPTNT) (Table 6).

Table 6

Quality parameters of pooled pond water after harvesting

Parameters	*Unit*	*Values*	*QCVN 02-26:2017/BNNPTNT*
pH		7.6	5.5-9
Biological oxygen demand ($BOD_{5\text{-}20^oC}$)	mg/L	43.2	≤ 50
Chemical oxygen demand (COD)	mg/L	80.6	≤ 150
Total suspended solids (TSS)	mg/L	84.4	≤ 100
Coliform	MPN/100 mL	3,989	≤ 5,000

Discussion. Viet et al (2016) found that BFT application in tilapia culture in concrete tanks had no effects on temperature and pH but declining total ammonia nitrogen (TAN) concentrations at studied salinities. The ammonia (NH_3-N) concentration of the treatments in this study (0.04 - 0.05 mg L^{-1}) was lower than that of tilapia intensively cultured in earthen ponds without BFT (0.36 - 0.70 mg L^{-1}) (Dan et al 2006), and also lower than that of tilapia intensively cultured in concrete ponds with only BFT (0.21 - 0.27 mg L^{-1}) (Tien et al 2013). According to Dauda et al (2013) improved water quality was especially associated with *Bacillus* sp. in aquaculture. Zhou et al (2010) found that the separate application of probiotics of *Bacillus subtilis*, *B. coagulans* and *Rhodopseudomonas palustris* in the water had no improvement of water quality in Nile tilapia culture in recycled water tanks. The water quality of the ponds in this study were improved if compared to that of the tilapia BFT ponds (Tien et al 2013) and probiotic applied tanks (Zhou et al 2010) which contributed from the application of a mixed

Bacillus spp. probiotic. In general, the water parameters in this study were within the suitable ranges for tilapia growth (Balarin and Haller 1982).

Dan et al (2006) found that the daily weight gain of the tilapia intensively cultured in the earthen ponds without BFT was 2.4-3.1 g/day. This parameter of the tilapia intensively cultured in the concrete tanks and ponds with BFT was 2.3 - 2.5 and 3.46 - 3.60 g/day, respectively (Hoa 2012; Tien et al 2013). Many studies pointed out that probiotics could be a growth promoter for important species in aquaculture (Hai 2015; Dawood and Koshio 2016). In this study, with the same cultured species and stocking density of 5 fish m^{-2}, the DWG of the fish (3.36 - 3.47 g day^{-1}) was higher than that (2.4 - 3.1 g day^{-1}) of the study of Dan et al (2006) and somewhat lower to that (3.46 - 3.60 g day^{-1}) of the study of Tien et al (2013). The growth in terms of SGR of the fish (3.38 - 3.40% day^{-1}) was also higher than that of tilapia (2.50 - 2.58% day^{-1}) cultured in tanks with BFT and different salinities (Viet et al 2016). The high growth performance of the fish in this study showed positive effects of the BFT and probiotic combination on tilapia. Probiotics can regulate the water environment, improve the digestibility of nutrients, and increase tolerance to stress in fish so that higher ratios of feed energy that enters the fish's body are used for growth. Moreover, the administration of multispecies probiotics shows the size of the harvested fish to be uniform.

Bossier and Ekasari (2017) reported that the nutrient waste in an aquaculture system is mostly generated from unconsumed feed and the digestion and metabolic processes of feed. This nutrient waste can be indirectly converted into microbial biomass that may eventually be consumed by the cultured animals as their food source. Taoka et al (2006) found that the probiotics treatment, both oral administration and supply to rearing water, enhanced non-specific immune parameters, and resulted in the improvement of resistance of Nile tilapia to *Edwardsiella tarda* infection. Cruz et al (2013) proposed the use of probiotics in culture systems with high concentrations of produced nitrogen compounds, especially the highly toxic total ammonia for improving water quality. The feed conversion ratio (FCR) of the fish in the present study (1.27) was better than that of tilapia cultured in different systems such as in earthen ponds without BFT (1.7 - 1.8) (Dan et al 2006), in concrete ponds with BFT (1.33 - 1.37) (Tien et al 2013) and in tanks with BFT (1.29 - 1.41) (Viet et al 2016). This result proves that the application of BFT combined with regular probiotic use improves feed efficiency of the fish directly by providing a food source of microbial biomass and indirectly by improving water quality and fish health. Growth increases if the feed can be digested properly, 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 optimal growth rate and reduce FCR. Moreover, the bio-floc and the microbial community served as an addition of high value feed and microbial protein can contribute to reduce the FCR of the fish in this study.

De Schryver et al (2008) pointed up factors such as dissolved oxygen concentration, choice of organic carbon source and organic loading rate having an influence on the growth of flocs. These are all strongly interrelated. The good growth performance of the fish in this study compared to tilapia cultured with BFT of the above authors also implied the efficiency of carbon source, and supplementary dose and frequency to stimulate floc development in the ponds.

It was found that the pond construction had clear impacts on feed consumption and survival rate of the fish. These parameters of the OBL treatment were lowest compared to the DBL and NoL treatments (Table 3 and 4). In the OBL treatment, the HDPE canvas could not cling firmly into bottom soil. Therefore, the motion of the loosened canvas made noise resulting in hesitance to take feed of the fish. Moreover, many fish were found to be trapped and burrowed in holes created by the loosen canvas. Based on water quality, growth performance and production efficiency (FCR, SR, yield, and quality of the fish), it could be emphasized that the BFT could be well implemented for tilapia intensive culture in earthen ponds without HDPE lining

The condition factor of the fish in the present study (2.33 - 2.59) was higher than that of tilapia fed with *Bacillus* strains mixture probiotic (1.67 - 1.68) (Elsabagh et al 2018) and cultured with BFT (2.1 - 2.3) (Crab et al 2012). Moreover, the combination of

BFT and probiotic supplementation was also improving the quality of harvested fish in terms of off-flavor intensity. By-products of the ponds applied BFT and supplied probiotics are the growth of the microbial community and the production of bio-flocs, which results in the reduction nutrients from waste materials available for odor-producing cyanobacteria development. According to Boari et al (2008), *Staphylococcus* spp. was not isolated in the cultivation water but in tilapia tegument and gut, and fresh fillets; *Salmonella* spp. was not detected. Huong et al (2014) found that there were no harmful bacteria of *E. coli*, *Staphylococcus* spp. and *Salmonella* spp. in bottom muds of tilapia intensive culture ponds. The presence of *E. coli*, *Staphylococcus* spp. bacteria in the fish of this study may be infected by unknown sources after harvesting. However, the harvested fish fully responded to the standard criteria of Viet Nam and EC to be safe food for consumers in national as well as international markets. This finding is similar to the study of Thuy et al (2013) on tilapia cultured in intensive ponds with BFT.

Coldebella et al (2018) carried out a study on the quality of effluent from ground-excavated ponds in an intensive tilapia culture area (in the Western region of the State of Paraná in Brazil) and found that the concentrations of suspended solids, total solids, chemical oxygen demand, and total phosphorus increased significantly at harvesting, which caused a progressive deterioration in the effluent released into the environment. The authors recommended the application of good management practices during culture period and/or the use of decantation ponds to reduce the nutrient load that could cause eutrophication in recipient bodies. The primary analysis of wastewater included parameters of pH, BOD, COD, TSS and *Coliform* at the end of the culture period showed that the combination of BFT and probiotic application resulted in the fulfillment of the effluent quality after fish harvesting to the Viet Nam's criteria to be directly discharged into receiving water bodies.

Crab et al (2012) emphasized that in transferring BFT to farmers, in addition to technical knowledge, the economic benefits of the implementation of this technique were also a very important aspect. De Schryver et al (2008) estimated a gain of a tilapia culture system with BFT about 10% in terms of feed costs per kilogram of produced fish compared to a system without BFT. Feeding represented over 65% of the operational costs of tilapia intensive culture (Dan et al 2006). Based on the FCR, the combination of BFT and probiotic application in this study could save about 6.3% and 37.8% in terms of feed costs per kilogram of tilapia fish produced in only with BFT (Tien et al 2013) and without BFT ponds, respectively (Dan et al 2006). Since ammonium/ammonia can be maintained at a low and non-toxic concentration with BFT application so that water replacement is no longer required (Bossier and Ekasari 2017). In the case of increased shortage and degradation of running water situation (MONRE 2019), the application of BFT and probiotics in the trial ponds could be an appropriate solution for water quality management in intensive culture of tilapia. Therefore, the added value that combined BFT and probiotics brought to tilapia culture in earthen ponds was represented by the reduced costs for water exchange and treatment as well as effluent treatment.

Conclusions. The combination of BFT and probiotics maintains good water quality for tilapia growth. The quality of the harvested fish was also excellent and met the standard criteria for human food consumption. In addition, the combined BFT and probiotic contributes to the improvement of water effluent and capital saving from reducing costs of feed, water exchange and treatment, and effluent treatment. With the increasing concerns of consumers on water resource use, environmental pollution problems, and food saftey, the combination of BFT and probiotics in the intensive system in earthen ponds can contribute to sustainable development of tilapia culture in the country.

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