Efficiency of intensive tilapia culture in earthen ponds applied biofloc technology (BFT), probiotics and off-flavor control methods

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ABSTRACT

The aim of this study was to assess the efficiency of Nile and red tilapia cultured in intensive systems applied a combination of biofloc technology (BFT) and probiotics in earthen ponds with different methods to control the off-flavor problem. A growing-out experiment was conducted for 150 days in which both Nile tilapia (5.88 ± 0.59 g) and red tilapia (9.72 ± 0.72 g) fingerlings were randomly allotted into three replicates of earthen ponds which were subdivided into four treatment groups, including: off-flavor controlled by algae management in Nile tilapia ponds (NT - AM) and red tilapia ponds (RT - AM), and off-flavor controlled by active water exchange in Nile tilapia ponds (NT - WE) and red tilapia ponds (RT - WE). The results showed that water quality was maintained in suitable ranges for fish growth. There were no significant differences in growth performance (final weight, daily weight gain and specific growth rate) among all treatments ($P < 0.05$). Feed conversion ratio of Nile tilapia (1.28 - 1.31) was significantly lower than that of red tilapia (1.35 - 1.37) ($P < 0.05$). The survival ratio (about 80%) and extrapolated yield (21.50 tons/ha in 150 days) of fish was similar and high for both Nile and red tilapia. The quality of the harvested fish in term of condition factor, size even and off-flavor intensity was also excellent. The economic efficiency of red tilapia farming in this system was higher as compared to cage systems. This study clearly demonstrated that the technique of combined BFT and probiotic application in earthen ponds could contribute to the sustainable development of tilapia production in Vietnam by reducing production cost, saving water resource and avoiding environmental pollution.

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1. Introduction

Tilapias originate from Africa and the Middle East but now are introduced into many territories and cultivated worldwide, particularly in Asia and the Pacific (De Silva et al., 2004). Tilapias are herbivorous and fed mainly on planktonic organisms, aquatic macrophytes and detritus, and they are able to tolerate low oxygen tension and a wide range of salinity and temperature, and resist to diseases (Ng & Romano, 2013). All tilapia species with important commercial values farming outside Africa belong to *Oreochromis* genus and more than 90% is Nile tilapia (*Oreochromis niloticus*) (Watanabe et al., 2002). In 2018, tilapias were the second species of cultured food-fish, after carps, with a production of 4,525.5 thousand tons and accounted for 8.3% of the major aquaculture finfish (FAO, 2020).

Early introduced to Vietnam since 1950s, tilapias have commonly raised in mono - and polyculture systems in earthen ponds and cages in both freshwater and brackish water environments with different intensification levels. So far, Nile tilapia strains have been commonly cultured in ponds (Nguyen et al., 2004; Nguyen et al., 2006) and red tilapia (*O. niloticus x O. mossambicus*) strains in cages (Phan, 2015; Tran, 2016). In 2015, total tilapia production was 187,000 tons with a total 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 (GSO, 2021). Tilapia production was also planned to increase and oriented toward export in the future (MARD, 2016).

According to Yue et al. (2016), land-based recirculating aquaculture, and cage and offshore aquaculture can help reduce the negative impacts of tilapia culture on the environment and global biodiversity. One of problems of tilapia cultured in earthen ponds is the development of off-flavor (Fitzsimmons, 2008). Tran (2016) noted that water quality in areas of red tilapia culture in cages in the Mekong River Delta (MRD) has declined, leading to increased disease incidence, mortality and feed utilization. Recently, several modern technologies, new approaches and alternative methods, including probiotics, have been applied to improve the production as well as quality of Nile tilapia (Aly et al., 2008). The biofloc technology (BFT) combines the removal of nutrients from the water with the production of microbial biomass, which can *in situ* be used by the cultured species as additional food source (De Schryver et al., 2008). Aquaculture using these technologies offers aquaculture a sustainable tool to simultaneously address its environmental, social and economic issues concurrent with its growth (Crab et al., 2012). In Vietnam, farming tilapia with BFT has been implemented in tanks (Nguyen, 2012; Le et al., 2016) and in concrete ponds (Nguyen et al., 2013). Phan et al. (2021) was successful in combined application of BFT and probiotics in Nile tilapia culture in earthen ponds. This experiment aimed to assess the ability of combination of BFT, probiotic and off-flavor control application in intensive tilapia culture in earthen ponds, as an alternative solution for red tilapia culture in cages.

2. Materials and Methods

Study site

The study was carried out in Long Xuyen city of An Giang province in the MRD of Vietnam from May to December, 2020.

Experimental design

The experiment was set up as a completely randomized design with four treatments to assess the efficiency of tilapia strains cultured intensively in earthen ponds. The combination of BFT and probiotic use but different off-flavor control methods was applied for treatments as following:

- NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds;

- NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds;

Each treatment was replicated in three 200 m^2 earthen ponds with a water depth of 1.5 m. The ponds were drained, completely removed bottom mud, applied $CaCO_3$ 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 then fertilized with urea at a dose of 1 kg/100 m^2 to develop natural feed. Pond water was aerated with air-tubes fixed on round aluminum dishes and convectively moved with air-lift systems (Phan et al., 2021).

All-male fingerlings of GIFT strain of Nile tilapia with initial weights of 5.88 ± 0.59 g were supplied from the Tilapia Selection Center of the Research Institute for Aquaculture No. 1. The fingerlings of selected red tilapia strain with initial weights of 9.72 ± 0.72 g were supplied from the National Breeding Center for Southern Freshwater Aquaculture of Research Institute for Aquaculture No. 2. When pond water became green, the fingerlings were immersed in salt water of 2 - 3% for 5 - 10 min to eliminate parasites and stocked at a density of 5 ind/m^2 in the morning. The air-tube and air-lift systems were operated for 24/24 h after fish stocking.

Biofloc, feeding and algae management

Biofloc booter was prepared by mixing 30 g of feed and 30 g of probiotic Pond Plus of Bayer Company with a mixture of *Bacillus* spp. (*B. subtilis, B. megaterium, B. amyloliquefaciens, B. licheniformis and B. pumilus* $\geq 1.0 \times 10^9$ CFU/g) in 3,000 mL clean water. The mixture was aerated and steered for 24 - 48 h at pH of 6.0 - 7.2. The ponds were supplied the biofloc booter at a dose of 5 ppm/day for the first month. In following months, the ponds were supplied the mixed *Bacillus* spp. probiotics at a dose of 10 g/100 m^2 and at an interval of 10 days. Moreover, 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/L and stimulate the flocs formation (Nguyen et al., 2013; Phan et al., 2021).

Pelleted feed for tilapia of Green Feed Company was supplied to the fish followed a feeding regime as presented Table 1.

The fish was fed twice a day at 08:00 - 09:00 and 15:00 - 16:00. The feeding was stopped one day per week to stimulate the fish consuming flocs (Nguyen et al., 2013). At thirty-day intervals, a sample of 30 fish individuals of each replicate was randomly collected for size (total length and weight) measurement.

In the NT-AM and RT-AM ponds, no water exchanged was applied but copper sulfate ($CuSO_4.5H_20$) was used at a dose of 2 kg/ha pond to kill algae when transparency dropped below 30 cm then *Bacillus* spp. probiotics was applied to improve water quality. In the NT-WE and RT-WE ponds, active water exchange was applied during the last month of the cultivation to maintain the transparency ≥ 30 cm.

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 of HANNA Company at a 3 days interval. Transparency and ammonia were measured weekly using secchi dish and indophenol blue method (APHA, 1995). At the end of the experiment, a pooled water sample of the ponds was primarily tested for quality parameters (pH, biological oxygen demand, chemical oxygen demand, total suspended solids and *Coliform*) (APHA, 1995). These parameters were assessed for waste water quality followed the National Technical Regulation No. 02-26:2017/MARD (MARD, 2017).

Production efficiency analysis

The fish growth, feed utilization, product quality and economic efficiency were assessed as follows:

- The specific growth rate (SGR) (Mehrara et al., 2009):

Table 1. Feeding regime and pelleted feed (Green Feed company) given to tilapia in the experiment

Crude protein (%)		35		30		25	
Feeding rate (% BW)	7	5	4	3	3	2.5	2
Weeks	1 - 2	3 - 4	5 - 8	9 - 10	11 - 12	13 - 16	17 - 20

SGR (%/day) = 100*(LnW_f – LnW_i)/T, where W_i = initial fish weight (g), W_f = final fish weight (g), T = experiment period (day) and Ln = natural logarithm.

- The feed conversion ratio (FCR) (De Silva & Anderson, 1994)

FCR = $F_c/(M_f - M_i)$, where F_c = total feed consumed by fish (kg), M_i = total fish weight at beginning (kg) and M_f = total fish weight (kg) at the end of the experiment.

- Assessment of off-flavor of the harvested fish followed Fitzsimmons (2008): fillets of three randomly sampled fish of each replicate were wrapped in aluminum foil and steamed in a microwave oven for 60 sec. Off-flavor intensity of the fillets was sensuously assessed by a judge of nine untrained persons based on a five-level scale with 1 = very strong and 5 = no off-flavor. Flavor intensity was expressed by the average of level scores of the judge.

- Economic efficiency was estimated followed Do (2010) based on total cost and revenue for 1 ha/crop as following: benefit = total revenue – total cost, and capital efficiency (benefit-cost ratio, BCR (%)) = 100*benefit/total cost.

Statistical analysis

All experiment data were statistically analyzed by one-way analysis of variance (ANOVA), using Tukey's post hoc ANOVA test for individual comparisons ($P < 0.05$, level of significance). All statistical analyses were carried out by using the Minnitab software version 16.0 program.

3. Results and Discussion

3.1. Water quality

There were no significant differences in the water parameters among the ponds (Table 2). The mean transparency of the ponds was low during the experiment, hence implying high density of algae. The temperature, pH and DO average values in the afternoon were higher than in the morning related to the photosynthesis and respiration of the algae. Ammonia was maintained at low concentrations.

3.2. Growth performance

The growth performance in terms of total length and weight of the fish in all treatments during the cultivation was quite consistent and

Table 2. Average values of water quality parameters of the experimental ponds

Water parameters	Time	Treatments[1]			
		NT-AM	NT-WE	RT-AM	RT-WE
Temperature (°C)	Morning	25.50 ± 2.19	25.41 ± 2.15	25.72 ± 2.16	24.61 ± 2.15
	Afternoon	28.32 ± 2.18	28.59 ± 2.12	28.62 ± 2.19	28.45 ± 2.18
pH	Morning	7.20 ± 0.10	7.16 ± 0.08	7.18 ± 0.10	7.18 ± 0.08
	Afternoon	7.82 ± 0.10	8.78 ± 0.09	7.80 ± 0.09	7.82 ± 0.09
Dissolved oxygen (mg/L)	Morning	5.22 ± 0.33	5.18 ± 0.35	5.20 ± 0.36	5.18 ± 0.35
	Afternoon	6.25 ± 0.34	6.24 ± 0.39	6.26 ± 0.37	6.27 ± 0.39
Ammonia (NH_3-N) (mg/L)		0.08 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Transparency (cm)		26.70±1.70	26.70 ± 1.70	26.70 ± 1.70	26.70 ± 1.70

[1]NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds; NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds.

in a same trend (Figures 1 and 2). In general, the length of the fish showed a high increase in the first ninety days but slow in the last sixty days. Different from length growth, the increase in body weight was low in the first sixty days but high thereafter.

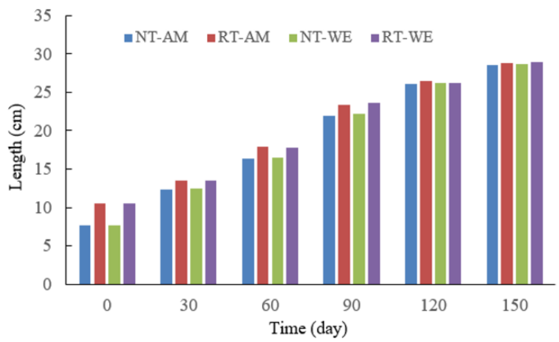

Figure 1. Growth in total length of the fish during culture period. NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds; NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds.

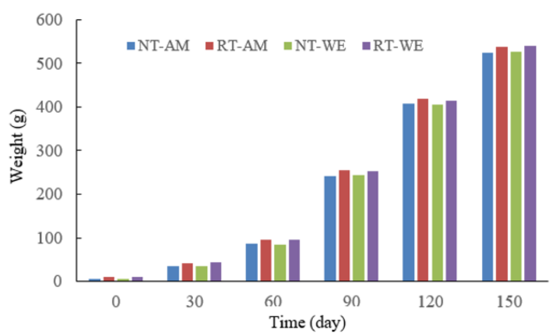

Figure 2. Growth in weight of the fish during culture period. NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds; NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds.

Although the initial weight of the stocked fingerlings of red tilapia was higher than that of Nile tilapia, there were no significant differences in the means of total harvested biomass among the treatments ($P > 0.05$). The total consumed feed, average feed intake (AFI) and feed conversion ratio (FCR) of red tilapia were significantly

Table 3. Growth performance of the experiment fish

Parameters	Treatments[1]			
	NT-AM	NT-WE	RT-AM	RT-WE
Final weight (g)	523.50 ± 2.09	526.72 ± 3.50	538.39 ± 7.66	539.78 ± 8.34
Daily weight gain (g/day)	3.45^a ± 0.01	3.47^a ± 0.02	3.52^a ± 0.05	3.53^a ± 0.06
Specific growth rate (%/day)	2.99^a ± 0.00	3.00^a ± 0.00	2.68^a ± 0.01	2.68^a ± 0.01

[1]Means within the same row with different superscript letters are significantly different at $P < 0.05$ where a < b; NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds; NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds.

higher than those of Nile tilapia ($P < 0.05$), but no significant difference was found between any these values of the same fish strains ($P > 0.05$) (Table 4).

Survival ratio (SR) of the fish were high and not significantly different ($P > 0.05$), which resulted in high extrapolated yields. Significantly lower coefficient of variation (CV) expressed an even weight size of the harvested fish in the NT-AM ponds compared to the others ($P < 0.05$). High scores pointed out the good quality in terms of off-flavor intensity of all treatments (Table 5).

The quality in terms of pH, BOD, COD, TSS and *Coliform* of the effluent after fish harvesting of the ponds responded the Vietnam's criteria to be directly discharged into the environment (Table 6).

Economic efficiency which was estimated for red tilapia of the RT-AM treatment extrapolated to 1 ha for five-month cultivation as a demonstration is presented in Table 7. Feed cost occupied the highest ratio of the production cost, followed by energy, probiotics and labor costs. The production cost was estimated about 27,900 VND. With a farm price of 32,000 VND/kg, The net benefit was 88,136,4000 VND with a ratio of benefit/cost was 14,69%.

4. Discussion

Cruz et al. (2013) proposed that the use of probiotics is recommended in culture system with high concentrations of produced nitrogen compounds, especially the highly toxic total ammonia, to improve water quality. The ammonia (NH_3-N) concentrations in this experiment (0.04 - 0.05 mg/L) were lower than those of tilapia intensively cultured in earthen ponds without BFT (0.36 - 0.70 mg/L) (Nguyen et al., 2006) and in concrete ponds with BFT and no probiotic application (0.21 - 0.27 mg/L) (Nguyen et al., 2013). According to Dauda et al. (2013), improved water quality was particularly associated with *Bacillus* spp. in aquaculture. Zhou et al. (2010) found that the separate application of probiotics of *Bacillus subtilis, B. coagulans* and *Rhodopseudomonas palustris* in 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 further improved compared to that of the tilapia BFT ponds (Nguyen et al., 2013)

Table 4. Feed utilization of the experiment fish

Parameters	Treatments[1]			
	NT-AM	NT-WE	RT-AM	RT-WE
Total stocked fish biomass (kg)	5.88 ± 0.59	5.88 ± 0.59	9.72 ± 0.72	9.72 ± 0.72
Total harvested fish biomass (kg)	422.99^a ± 3.08	430.14^a ± 0.23	430.52^a ± 6.18	433.23^a ± 3.90
Total consumed feed (kg)	544.69^a ± 2.94	544.84^a ± 3.67	576.23^b ± 5.06	573.24^b ± 5.02
Average feed intake (g)	674.13^a ± 3.65	667.17^a ±4.50	720.62^b ± 6.32	714.21^b ± 6.24
Feed conversion ratio	1.31^a ± 0.01	1.28^a ± 0.01	1.37^b ± 0.02	1.35^b ± 0.02

[1]Means within the same row with different superscript letters are significantly different at P <0.05 where a < b; NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds; NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds.

Table 5. Culture efficiency, biometric indice and quality of the harvested fish

Parameters	Treatments[1]			
	NT-AM	NT-WE	RT-AM	RT-WE
Extrapolated yield (ton/ha)	21.15	21.51	21.53	21.66
Survival ratio (%)	$80.80^a \pm 0.44$	$81.67^a \pm 0.55$	$79.97^a \pm 0.70$	$80.30^a \pm 0.99$
Coefficient of variation of final BW (%)	$5.65^a \pm 0.49$	$7.82^b \pm 3.41$	$6.77^b \pm 0.25$	$7.35^b \pm 0.86$
Score of off-flavor intensity	$4.70^a \pm 1.06$	$4.92^a \pm 0.19$	$4.85^a \pm 0.77$	$4.96^a \pm 0.27$

[1]Means within the same row with different superscript letters are significantly different at $P < 0.05$ where a < b; NT - AM and RT - AM: off-flavor controlled by algae management in Nile and red tilapia ponds; NT - WE and RT - WE: off-flavor controlled by active water exchange in Nile and red tilapia ponds.

and probiotic applied tanks (Zhou et al., 2010) coming from the combined application of BFT and mixed Bacillus spp. probiotics (Loan et al., 2021). In general, the water parameters in this experiment were within suitable ranges for tilapia growth (Balarin & Haller, 1982).

Many studies have suggestedthat probiotics can serve asa growth promoter for important species in aquaculture (Dawood & Koshio, 2016). In this study, with the same stocking density of 5 fish/m^2, the DWG of Nile tilapia (3.45 - 3.47 g/day) and red tilapia (3.52 - 3.53 g/day) was higher than that of tilapia intensively cultured in the concrete tanks with BFT (2.3 - 2.5 g/day) (Nguyen, 2012) and similar to that of tilapia intensively cultured in concrete ponds with BFT (3.46 - 3.60 g/day) (Nguyen et al., 2013). The growth in terms of SGR of Nile tilapia (2.99 - 3.00%/day) and red tilapia (2.68%/day) was also higher that of tilapia (2.50 - 2.58%/day) cultured in tanks with BFT and different salinities (Le et al., 2016). In the present study, FCR of Nile tilapia (1.28 - 1.31) and red tilapia (1.35 - 1.37) was similar to those of tilapia cultured in concrete ponds (1.33 - 1.37) (Nguyen et al., 2013) and in tanks (1.29 - 1.41) (Le et al., 2016). The lower FCR of Nile tilapia compared to red tilapia indicates the better use of flocs of the former.

There were no significant differences in the SR and extrapolated yield between Nile and red tilapia strains. The SR of the fish in this study (about 80%) was higher than that of red tilapia cultured in cages (69.2%) (Phan, 2015). The extrapolated yield of all treatments (21.15 - 21.66 tons/ha in 150 days) was similar to that of tilapia cultured in concrete ponds with BFT (23.00 - 24.80 tons/ha in 172 days) (Nguyen et al., 2013). In addition to the combination of BFT and probiotic supplement, the methods of algae management and active water exchange further improved the quality of harvested fish in terms of off-flavor intensity (Table 4).

Coldebella et al. (2018) found that the concentrations of TSS, COD and other of organic matters in the pond water of intensive tilapia culture increased significantly at the end of the farming cycle, which caused a progressive deterioration of the environment. The primary analysis of discharged water quality included parameters of pH, BOD, COD, TSS and *Coliform* at the end of the cultivation showed that the combination of BFT and probiotic application resulted in the ful-

Table 6. Quality parameters of pooled pond water after harvesting

Parameters	Unit	Analyzed values	National Technical Regulation No. 02-26:2017/MARD
pH		7.2	5.5 - 9
Biological oxygen demand (BOD_{5-20}°C)	mg/L	39.72	≤ 50
Chemical oxygen demand (COD)	mg/L	85.95	≤ 150
Total suspended solids (TSS)	mg/L	94.7	$\leq$100
Coliform	MPN/100 mL	4,289	$\leq$ 5,000

Table 7. Economic efficiency analysis for red tilapia cultured in the experiment

Parameters	Amount	Value (VND)	Ratio of total cost (%)
Extrapolated yield (ton/ha in 5 months)	21.5		
Total cost		599,863,600	100
Fixed cost (Depreciation of aeration system)		15,000,000	2.50
Variable cost		584,863,600	97.50
Feed (ton)	29,025	440,193,600	73.38
Electricity for aeration, water supply (kW)	17,000	37,400,000	6.23
Probiotics (kg)	21.5	25,800,000	4.30
Permenant labor (1 labor × month)	5	25,000,000	4.17
Others (molasses, Vitamin C, $CuSO_4.5H_20$,...)		56,470,000	9.41
Production cost for 1 kg of red tilapia		27,900	
Total revenue		688,000,000	
Net benefit		88,136,400	
Benefit-cost ratio (%)		14.69	

fillment of the effluent quality after fish harvesting to Vietnam's criteria to be directly discharged into receiving water bodies (Table 5).

Crab et al. (2012) emphasized that in transferring BFT to farmers, the economic benefits of the implementation of this technique was also a very important aspect. The feed and seed cost ratios (73.38 and 0.83, respectively) of red tilapia culture in this study was lower than those (80.04% and 14.67%, respectively) of red tilapia cultured in cages. The high ratio of seed cost in cage systems due to larger fingerlings required (Phan, 2015). The BCR of red tilapia of the experiment (14.69%) was higher than that of red tilapia cultured in cages (11.4%) (Phan, 2015) but lower than that of tilapia cultured in different systems such as in earthen ponds without BFT (18.97%) (Nguyen et al., 2006) and in concrete ponds with BFT (19.17%) (Nguyen et al., 2013). In the case of an increased shortage and degradation of running water, this method could avoid the risk of diseases, thereby save the cost of medicines, increasing the quality of product and contributing to sustainable development of tilapias in general and red tilapia farming in particular.

5. Conclusions

The major goal of the present study was to develop and assess the efficiency of applying a combination of BFT and probiotics with different off-flavor control methods in intensively cultured tilapia in earthen ponds. The results showed that the water quality of this system was maintained within suitable ranges for the growth of fish. The growth performance (DWG and SGR), feed consumption, production efficiency (FCR, SR, yield and quality of the fish) were also improved compared to the separate use of BFT and probiotics. The quality of the harvested fish in term of size even and off-flavor intensity was also excellent. This system brought higher economic efficiency for red tilapia farming compared to cage system. This study clearly demonstrated that the combined application of BFT, probiotic and off-flavor control is an advanced approach for production of high-quality tilapias to support the plan of government for the development of tilapia aquaculture for export.

Conflict of interest

The authors have no conflicts of interest to declare.

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