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Effects of stocking density on growth and survival of tilapia cultured in biofloc technology system in brackish water

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Abstract
This study examined the effect of stocking density on growth and survival of tilapia cultured in biofloc technology system. Three different stocking densities cultured in biofloc technology were 6 fish/m^3, 8 fish/m^3 and 10 fish/m^3 for 86 days in triplicate for each treatment. The stocking density of the control lot was 3 fish/m^3 cultured without biofloc technology. Initial stocking weight ranged from 2–3 g/fish. The water quality parameters were monitored and regulated in the suitable ranges for biofloc technology and for the growth and development of tilapia. The results showed that specific growth rate of fish cultured at a density of 6 fish/m^3 was higher than that in the treatments of 8 fish/m^3 and 10 fish/m^3 with the average values of 5.72%; 5.62% and 5.43%, respectively, and the specific growth rate of fish in the control treatment was 5.71%. Daily growth rate of fish cultured at a density of 6 fish/m^3 was higher than that cultured at densities of 8 fish/m^3 and 10 fish/m^3 with average values of 3.19 g/day, 2.98 g/day, and 2.55 g/day, respectively; and the daily growth rate of the control treatment was 3.27 g/day. Survival rate of tilapia cultured at densities of 6 fish/m^3 and 8 fish/m^3 was 100%, whereas survival rate of tilapia cultured at a density of 10 fish/m^3 was 95.75%, and it was 88.9% for the control lot. The research results provide a scientific basis to propose tilapia culture technique in biofloc technology in brackish water, with the density of 6–8 fish/m^3.

Keywords: Stocking density, tilapia, biofloc technology (BFT), brackish water.

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INTRODUCTION

Biofloc technology (BFT) is a new biotechnology solution in sustainable development, biosafety and environmental-friendly aquaculture production [1, 2]. The feed conversion rate is reduced by applying BFT as the aquatic animals are fed with suspended biofloc particles formed by the combination of a cheap source of carbohydrate food and heterotrophic microbiota. Heterotrophic bacteria in suspended biofloc can assimilate the waste ammonium for new biomass production. Hence, ammonia can be maintained at a low and non-toxic concentration, therefore water replacement is no longer required [2–4].

The technical process of intensive culture of tilapia in brackish water is now being applied at an average stocking density of 3 fish/m^3. It does not use continuous aeration system, so it cannot be cultured at a higher density. The water is replaced regularly from the 3rd month of culture, once a week on average volume of 1/3 the amount in the pond to ensure the water quality. Aeration operates at night or on a cloudy day at the end of the second month of culture. However, BFT requires the operation of a continuous aeration system to form and maintain biofloc. It is necessary to determine the appropriate density to avoid wasting energy, reduce production costs and gain production efficiency.

The research provides the necessary information on fluctuations of environmental factors, growth rates and survival rates of tilapia cultured with BFT at different densities. Then, the most appropriate tilapia stocking density in biofloc system is determined to achieve the highest efficiency.

MATERIAL AND METHODS

Time and experimental site

Time: from May 2, 2019 to July 30, 2019.

Experimental site: The experiment was conducted at a hatchery belonging to Hoang Huong Fisheries Development Co. Ltd. that is located in Tan Thanh ward of Duong Kinh district, Hai Phong city.

Experimental design

The experiment was carried out with three different density treatments with BFT and the control without BFT (under current water exchange technology with the density of this technology). Each treatment was conducted in triplicate.

The experiments were set up completely randomly in tanks of 4 m^3. The initial salinity of cultured water was 7‰ with biofloc. Non-experimental factors such as environmental conditions (temperature, salinity, DO,…) and food of each experiment were similar. To make biofloc, we used molasses, fish feed, soybean powder mixed together with a ratio of 3:1:3 in weight, then composted with probiotics containing *Bacillus* spp. strain (CP-Bioflus 30 g/m^3). The incubation process was carried out under aeration conditions at 25–28^{o}C, stirring for 48 hours to ferment, then putting into the pond continuously for 3 days, once a day at 9–10 am. When the clarity of cultured water reached 30–40 cm, a probiotic supplement with the main ingredient of *Bacillus* spp. was conducted continuously for 3 days at 10 am, with the amount of inoculants 0.15 g/m^3/day until the biofloc appeared in the pond. The determination of biofloc in the pond was based on the floc volume index (FVI), calculated from the floc volume after 30 minutes of sedimentation in an Imhoff cone [5], with a hopper reaching 0.1–0.2 ml/l, then the creation of biofloc was stopped.

Experiment was cultured with BFT systems, three stocking densities as I: 6 fish/m^3; II: 8 fish/m^3; III: 10 fish/m^3; IV (control treatment without BFT, common cultured technique, periodical water replacement): 3 fish/m^3.

The tilapia fingerlings used in the experiment were the male unisexual tilapia (*Oreochromis* sp.*)*. Fingerlings acclimate to salinity, its length ranged from 4–6 cm and its weight ranged from 2–3 g/fish.

Biofloc was maintained in ponds weekly with the addition of carbohydrates and probiotics (CP-Bioflus) containing mainly *Bacillus* spp., with bacterial density higher than 10^7 CFU/g. The amount of CP-Bioflus was 0.15 g/m^3/time. The carbon source was from molasses containing 50% carbohydrate (C). The amount of carbohydrate was determined

according to Avnimelech, 2007 [6] and calculated quickly by the following formula:

$$X = [C/N\ (\%\ \text{protein} \times \%N_{protein}) - \%C_{feed}]/\%C_{molasses}$$

In which X was the amount of molasses added to achieve the desired *C*/*N* ratio; *C*/*N* was the ratio of *C*/*N* reached; $\%N_{protein}$ was the nitrogen content contained in 1 g of protein; $\%C_{feed}$ was the percentage of carbon in the feed component; $\%C_{molasses}$ was the carbon content in the molasses.

According to the guidance of Avnimelech, 2012 [1] and the research results of the authors (not published), the appropriate *C*/*N* ratio in the BFT system of brackish tilapia culture was 15/1. Molasses contained 50% of carbohydrates, the amount of molasses was supplemented from 30–40% of the feed for fish, calculated from the previous molasses addition, depending on the protein in the feed, supplemented once a week. During stocking, water was added flexibly due to evaporation and maintained biofloc.

Environmental factors such as temperature, pH, DO, salinity, and alkalinity were monitored daily to timely adjust in the pond.

TAN, TSS, NO_2, NO_3, were monitored once a week.

The growth of fish was checked every 15 days.

Daily feed intake was monitored in the experimental tanks.

The criteria of experimental evaluation include:

Survival rate (S - %).

Weight growth (WG).

Specific growth rate (SGR - %/day).

Daily growth rate (DGR - gr/day).

Dry feed intake (DFI) (g/fish).

Feed efficiency: feed conversion ratio (FCR); protein efficiency ratio (PER) (g/g).

Parameter analysis

Environmental factors including water temperature, pH, DO, salinity parameters were measured by a quick tester or the SERA test kit: Water temperature, DO (portable DO meter YSI 55 - USA), pH (portable DO meter pH315i/set - Germany), salinity (ATAGO - Japan).

The samples of nutrient factors including total ammonia nitrogen (TAN), nitrite ((NO_2), nitrate (NO_3^-) were collected, analyzed and processed for each parameter according to the guidance of the APHA, 1998 "Standard methods for the examination of the water and wastewater (22^{nd} ed.) [7].

Method of evaluating the growth of fish and feed coefficient:

Weight growth (*WG*) (g) = *Mean final weight* (*Wf* (g)) – *Mean initial weight* (*Wi* (g))

Specific growth rates (SGR - %/day) is calculated by the formula:

$$SGR\left(\%.day^{-1}\right) = \frac{\left(\ln W_f - \ln W_i\right)}{t} \times 100$$

Daily growth rates (DGR – g/day) is:

$$DGR\left(g.day^{-1}\right) = \frac{\left(W_f - W_i\right)}{t}$$

Where: W_i, W_f: Initial weight and final weight respectively; *t*: days of experiment.

Determination of survival rate (%) and productivity of fish after finishing the experiment.

Survival rate (%) = (*Total number of fish surviving/total number of fish stocked*) × 100

Feed conversion ratio (*FCR*):

FCR = Total weight of feed given/*Total weight of fish gain*

Dry feed intake (*DFI*):

DFI (g/fish) = *Daily feed intake* (g)/*Total fish*

Protein efficiency ratio (*PER*):

PER = Net weight gain/*Protein consumed* (g)

Data analyses

Microsoft Office Excel 2010 was used to analyze, calculate, process data and diagram.

ANOVA was used to verify the significant differences in environmental parameters and the fish growth rate.

RESULTS

Fluctuation of environmental factors during the experiment

The environmental factors

The environmental factors including temperature, pH, DO and salinity of the stocking densities were monitored and adjusted to ensure the similarity between these treatments. The ratio C:N was monitored and analyzed to suit the experiments.

Table 1. Fluctuation of the environmental factors during the experiments

Environmental factors		Stocking density treatments			
		I	II	III	IV
Temperature (^oC)	Morning	29.8 ± 0.4 (27.8–30.6)	29.8 ± 0.4 (27.8–30.6)	29.8 ± 0.4 (27.8–30.6)	29.8 ± 0.4 (27.8–30.6)
	Afternoon	30.7 ± 0.6 (28.6–31.8)	30.7 ± 0.6 (28.6–31.8)	30.7 ± 0.6 (28.6–31.8)	30.7 ± 0.6 (28.6–31.8)
pH (1-14)	Morning	7.7 ± 0.3 (7.4–8.5)	7.6 ± 0.5 (7.3–8.4)	7.5 ± 0.4 (7.3–8.2)	7.8 ± 0.5 (7.4–8.6)
	Afternoon	7.9 ± 0.4 (7.6–8.4)	7.9 ± 0.5 (7.6–8.5)	8.1 ± 0.4 (7.7–8.6)	7.9 ± 0.5 (7.6–8.5)
DO (mg/l)	Morning	6.2 ± 0.6 (5.2–6.8)	5.9 ± 0.4 (4.8–6.5)	4.8 ± 0.5 (4.6–6.2)	4.5 ± 0.6 (3.8–5.4)
	Afternoon	6.8 ± 0.7 (5.6–7.9)	6.6 ± 0.6 (5.4–7.6)	5.6 ± 0.5 (4.8–6.8)	5.5 ±0.7 (4.6–6.9)
Salinity (‰)	Morning	7 ± 1 (6–8)	7 ± 1 (6–8)	7 ± 1 (6–8)	7 ± 1 (6–8)
	Afternoon	7 ± 1 (6–8)	7 ± 1 (6–8)	7±1 (6–8)	7 ± 1 (6–8)

Notes: I, II, III with BFT included I: 6 fish/m^3; II: 8 fish/m^3; III: 10 fish/m^3; IV (control without BFT): 3 fish/m^3.

Table 1 showed that the temperature ranged from 29–30^{o}C, pH ranged from 7.5–8.1, DO ranged from 4.5–6.8 mg/l and the salinity ranged around 7‰ in each treatment. The environmental factors (T^{o}C, DO, pH, S‰**)** in experimental treatments with biofloc systems (I, II and III) show no significant difference compared to the control treatment (IV). This environmental condition was suitable for tilapia culture and biofloc growth [8–10].

Monitoring results of nutrient factors

Monitoring results of total ammonia nitrogen (TAN) in table 2 showed that the mean value of TAN in the treatment I was 0.53 mg/l, with a range from 0.16–1.55 mg/l; in the treatment II was 0.70 mg/l with a range from 0.22–1.82 mg/l; in the treatment III was 0.83 mg/l with a range from 0.14–2.28 mg/l; in the control treatment IV was 1.42 mg/l with a range from 0.12–3.22 mg/l. TAN tended to rise in the treatments, then gradually decreased, when adding carbon and biofloc it grew rapidly as heterotrophic bacteria had a large biomass to absorb nitrogen to produce biofloc particles. TAN value in the control treatment tended to be higher than that in the treatments with BFT application due to no carbon adding. The treatments with higher density had higher TAN value than the treatments with lower density, but there was no statistically significant difference ($P < 0.05$).

Figure 1 showed that, from the 7th week of culture onwards, the fish food intake was needed more along with biofloc decomposition, because fish did not used up, it caused the process of high N accumulation, resulting in increasing TAN value. TAN value was the highest in the 9th week in culture systems and biofloc sediment needed to be removed. In the control treatment, TAN value decreased due to the water replacement by 20% in the 4th and 5th weeks and by 50% in the 9th week.

These experimental results were consistent with the results of Emerenciano et al., (2017). Emerenciano et al., (2017) and Azim and Little (2008) [4, 10] also recommended that the amount of TAN is less than 1 mg/l when applying BFT. There is no TAN limit in the environmental regulation on tilapia culture.

Table 2. Monitoring results of the nutrient factors in experiments

Nutrient factors	Stocking density treatments			
	I	II	III	IV (Control)
TAN (mg/l)	0.53 ± 0.4[a] (0.16–1.55)	0.7 ± 0.49[a] (0.22–1.82)	0.83 ± 0.67[ab] (0.14–2.28)	1.42 ± 0.94[cb] (0.12–3.22)
TSS (mg/l)	247.1 ± 97.3[a] (57.3 – 409.0)	307.5 ± 84.6[a] (132.7–437.3)	330.9 ± 85.2[a] (142.9–445.7)	188.8 ± 82.4[b] (38.7–331.3)
NO_2-N (mg/l)	0.13±0.09[a] (0.01–0.36)	0.16 ± 0.11[a] (0.02–0.41)	0.20 ± 0.16[a] (0.02–0.56)	0.28 ± 0.21[b] (0.02–0.84)
NO_3-N (mg/l)	1.98 ± 1.32[a] (0.21–4.35)	2.39 ± 1.69[a] (0.24–05.66)	2.7 ± 1.91[ab] (0.22–6.27)	3.36 ± 2.35[cb] (0.25–7.79)

Notes: Values with different lowercase letters in the same row show statistically significant differences ($P < 0.05$). Values with same lowercase letters in the same row show no significant difference ($P > 0.05$); I, II, III with BFT included I: 6 fish/m^3; II: 8 fish/m^3; III: 10 fish/m^3; IV (control without BFT): 3 fish/m^3.

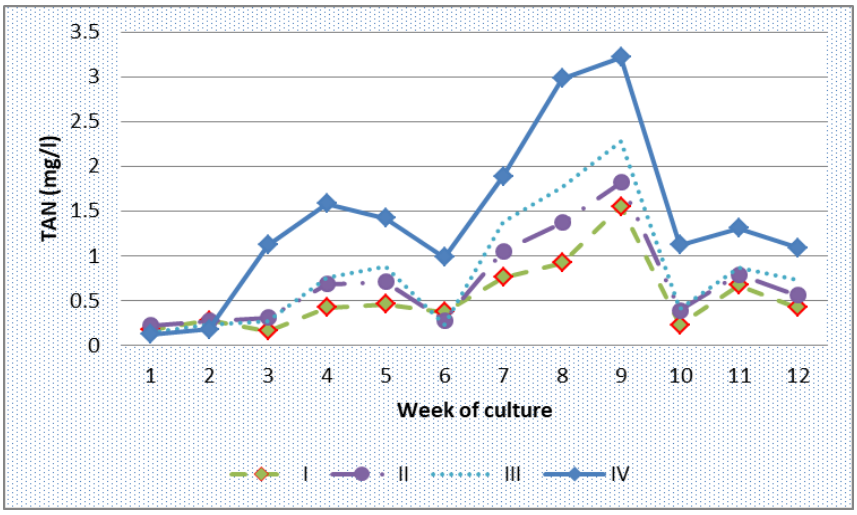

Figure 1. The variation of TAN value during the experiment

The monitoring results of total suspended solids (TSS) in table 2 showed that the mean value of TSS in the treatment I was 274.1 mg/l with a range from 57.3–409 mg/l; in the treatment II was 307.0 mg/l with a range from 132–437 mg/l; in treatment III was 330.0 mg/l with a range from 142–445 mg/l; in the control IV was 188.8 mg/l with a range from 38.7–331 mg/l.

TSS was produced right after fish stocking because the biofloc formation of TSS tended to increase during adding more feed and biofloc growth. TSS in the control was lower than in other treatments because the control did not add carbon, causing less biofloc.

In the 4th and 5th monitoring of the control treatment, the water replacement by 20% in the 4th week and the 5th week also caused the decrease of TSS. In the next monitoring, TSS increased rapidly due to the more feed intake and the biofloc decomposition, and TSS was the highest in the 9th week. In the experimental treatments, the biofloc sediment was then removed and clean water was added. In the control treatment, water was replaced by 50% to reduce TSS, then TSS continued to rise during feeding and adding carbon (figure 2).

The experiment result in table 2 and fig. 2 showed that the amount of TSS in the biofloc system ranged from 16.6–560 mg/l, which was consistent with the result of Azim and Little (2008) [10]. TSS value in the treatments was maintained less than 500 mg/l, which was within the proposed limit of Emerenciano et al., [4].

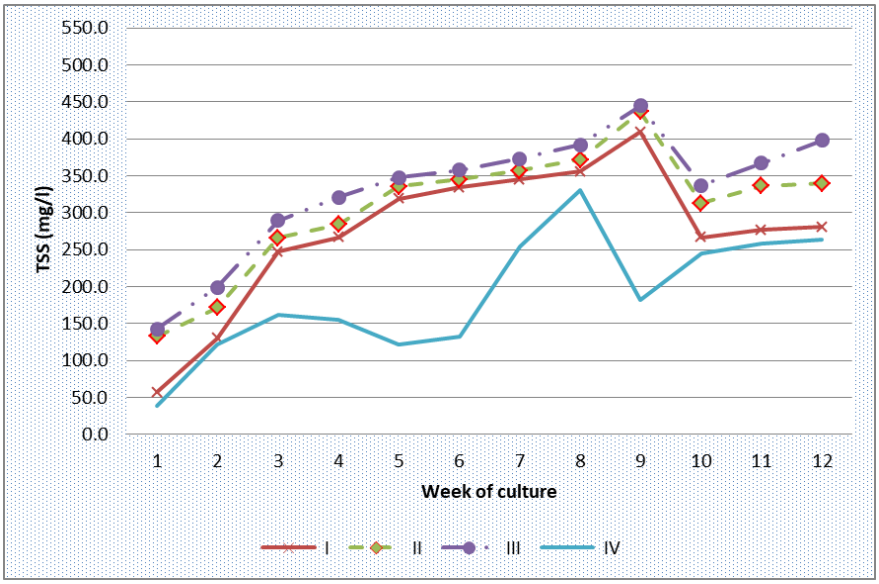

Figure 2. Variation of TSS in the experiment

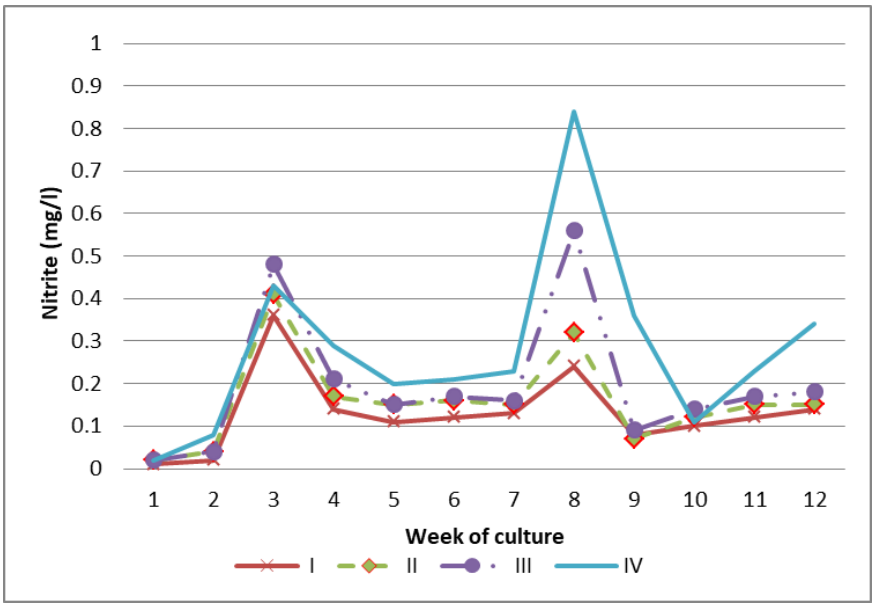

Figure 3. Variation of nitrite (mg/l) in the treatments

The monitoring result of nitrite (NO_2-N) (mg/l) in figure 3 showed that the nitrite ranged from 0.01–0.84 mg/l. Nitrite tended to increase in the very first weeks, then decreased in the 4th week and increases in the 8th week, then dropped and stabilized in the next weeks. The amount of nitrite was maintained less than 1 mg/l, within the proposed limit of Emerenciano et al., (2017) [4].

The monitoring result of nitrate (NO_3-N) (mg/l) in figure 4 indicated that the amount of nitrate in the high density treatments was higher than in the low density treatments. The control treatment had higher nitrate than the other treatments. Nitrate tended to rise in the very first weeks, then decreased and increased again in the 8th week, then dropped and stabilized in the next weeks. The nitrate in the

treatments ranged from 0.01–0.84 mg/l, which was less than 20 mg/l within the proposed limit of Emerenciano et al., (2017) [4].

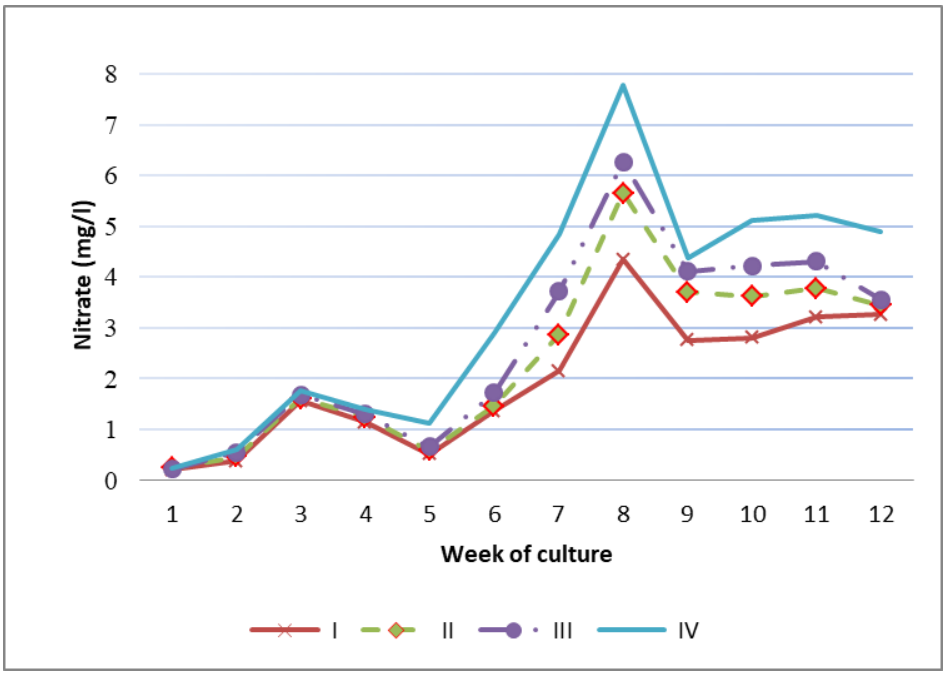

Figure 4. Variation of nitrate (mg/l) in the treatments

The growth rate and the survival rate of tilapia

The growth rate

The result in table 3 showed that, after 86 days of tilapia culture with BFT at different densities, the average weight of tilapia in the treatments I, II, III was 263.2 g/fish, 248.7 g/fish and 212.3 g/fish, respectively. The growth rate of tilapia in the control treatment with low density was higher than that in the other treatments, the average weight of tilapia was 269.4 g/fish.

The result in figure 5 and table 4 showed that in the same BFT system with the allowable environmental conditions, the growth rate of fish in the low density treatment was higher than that in the high density treatment.

Table 3. The monitoring result of the growth rate of tilapia (gram)

Date of monitoring	I	II	III	IV
Initial fish (2/5/2019)	2.22 ± 0.38^{a}	2.23 ± 0.29^{a}	2.25 ± 0.39^{a}	2.22 ± 0.29^{a}
1st (17/5/2019)	6.3 ± 0.23^{a}	6.1 ± 0.47^{a}	5.9 ± 0.60^{a}	5.5 ± 0.35^{b}
2nd (3/6/2019)	23.1 ± 2.68^{a}	22.9 ± 4.06^{a}	18.3 ± 2.68^{a}	30.6 ± 7.34^{b}
3rd (17/6/2019)	74.1 ± 4.39^{ac}	72.4 ± 3.56^{a}	65.3 ± 5.85^{b}	77.7 ± 9.05^{c}
4th (3/7/2019)	147.2 ± 5.54^{ac}	144.5 ± 6.85^{a}	121.3 ± 13.97^{b}	149.1 ± 8.07^{c}
5th (18/7/2019)	194.3 ± 5.47^{ac}	191.7 ± 4.80^{a}	160.4 ± 10.29^{b}	198.1 ± 9.03^{c}
6th (26/7/2019)	263.2 ± 4.2^{ac}	248.7 ± 9.1^{a}	212.3 ± 12.5^{b}	269.4 ± 5.1^{c}

Notes: Values with different lowercase letters in the same row show statistically significant differences ($P < 0.05$). Values with the same lowercase letters in the same row show no significant difference ($P > 0.05$); I, II, III with BFT included I: 6 fish/m^3; II: 8 fish/m^3; III: 10 fish/m^3; IV (control without BFT): 3 fish/m^3.

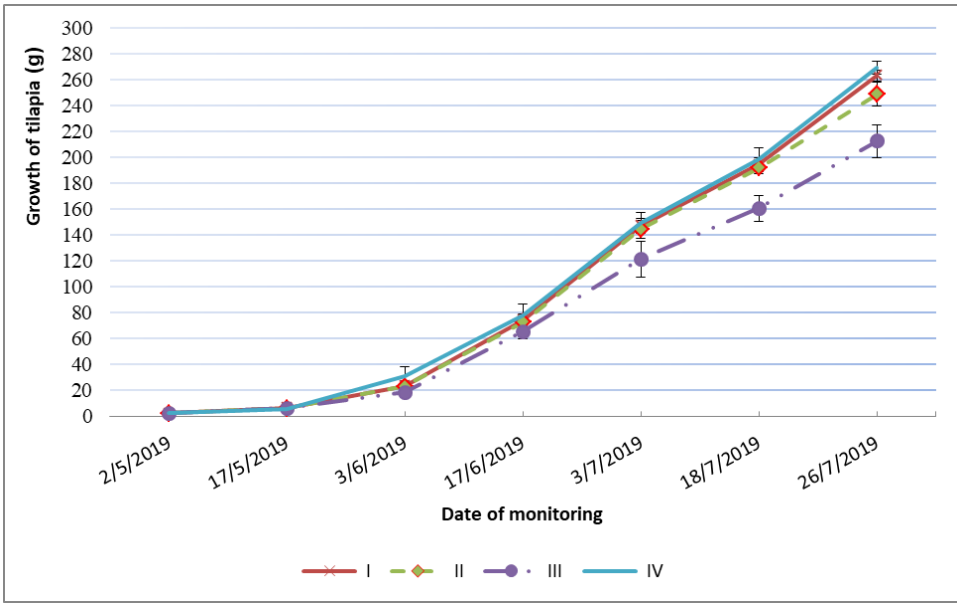

Figure 5. The growth of tilapia in the experiments

The result in table 4 showed that, after 86 days of tilapia culture with BFT at different densities, the average SGR of tilapia in the treatments I, II, III was 5.72 %.day^{-1}, 5.62 %.day^{-1} and 5.43 %.day^{-1}, respectively. The average SGR of tilapia in the control treatment was 5.71 %.day^{-1}; The average DGR of tilapia in the treatments I, II, III and IV (control treatment) was 3.13 g.day^{-1}, 2.98 g.day^{-1}, 2.55 g.day^{-1} and 3.27 g.day^{-1}, respectively.

Table 4. Specific growth rate - *SGR* (%.day^{-1}) and daily growth rate - *DGR* (g.day^{-1})

Days	I		II		III		IV	
	SGR (%.day^{-1})	*DGR* (g.day^{-1})	*SGR* (%.day^{-1})	*DGR* (g.day^{-1})	*SGR* (%.day^{-1})	*DGR* (g.day^{-1})	*SGR* (%.day^{-1})	*DGR* (g.day^{-1})
14	8.69	0.34	8.39	0.32	8.03	0.30	7.56	0.27
16	8.66	1.12	8.82	1.12	7.55	0.83	11.44	1.67
15	7.77	3.40	7.67	3.30	8.48	3.13	6.21	3.14
15	4.58	4.87	4.61	4.81	4.13	3.73	4.35	4.76
15	1.85	3.14	1.88	3.15	1.86	2.61	1.89	3.27
11	2.76	6.26	2.37	5.18	2.55	4.72	2.79	6.48
TB	5.72	3.19	5.62	2.98	5.43	2.55	5.71	3.27

Notes: I, II, III with BFT included I: 6 fish/m^3; II: 8 fish/m^3; III: 10 fish/m^3; IV (control without BFT): 3 fish/m^3.

The survival rate

The results showed that the survival rate of tilapia was 100% in the treatments I, II (6 fish/m^3 and 8 fish/m^3) and it was 95.75% and 88.9% in the treatment III and in the control, respectively. Tilapia cultured with BFT at 6 fish/m^3 and 8 fish/m^3 indicated the similar survival rate of fish, which was higher than that when cultured at 10 fish/m^3 and without BFT (figure 6).

The results in table 5 showed that after 86 days, the feed conversion ratio (FCR), daily feed intake (DFI) and protein efficiency ratio (PER) in treatments I and II were nearly equivalent. FCR in the treatments I and II was less than that in the treatment III and in the control treatment. In the treatment I, the size of fish was more uniform than that in the three remaining treatments. The dry feed intake in the treatments I, II, III, and control was 333.3 g/fish/86 days;

312 g/fish/86 days; 275 g/fish/86 days and 416.7 g/fish/86 days, respectively. The PER in the treatments I, II, III and IV control was 2.24 gram fish/gram protein; 2.25 gram fish/gram protein, 2.07 gram fish/gram protein; 1.83 gram fish/gram protein, respectively.

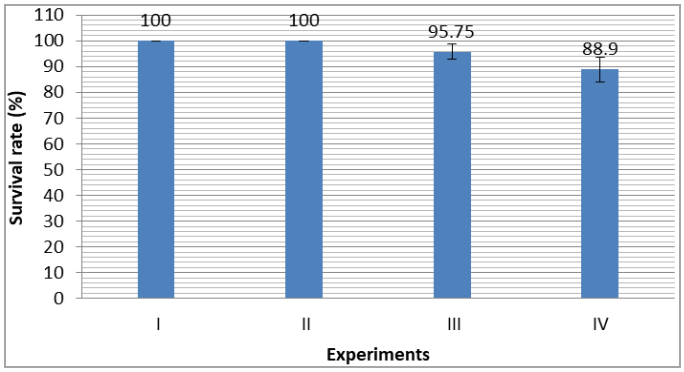

Figure 6. The survival rate of tilapia (%) in the experiments

Table 5. The criteria for evaluation of the stocking density after 86 days

Criteria	Stocking density treatments			
	I	II	III	IV
Initial weight (g/fish)	2.22 ± 0.38	2.23 ± 0.29	2.25 ± 0.39	2.22 ± 0.29
Final weight (g/fish)	263.2 ± 4.2	248.7 ± 9.1	212.3 ±12.5	269.4 ± 5.1
FCR after 86 days	1.28	1.27	1.38	1.56
DFI (g/fish/86 days)	333.3	312.5	275.0	416.7
PER (g/g)	2.24	2.25	2.07	1.83
Productivity - 86 days (g/m^3)	1579.2	1989.6	2016.9	808.2

Notes: I, II, III with BFT included I: 6 fish/m^3; II: 8 fish/m^3; III: 10 fish/m^3; IV (control without BFT): 3 fish/m^3.

CONCLUSIONS

The values of TAN, TSS, NO_2, NO_3 in the treatments with high density tended to be higher than in the treatments with low density. The control with low density and without BFT had TAN, NO_2, NO_3 higher and TSS lower than with BFT.

The tilapia cultured with BFT in the brackish water at treatment I (6 fish/m^3) had values of growth rate, survival rate, and PER higher than those in the treatments II, III (8 fish/m^3; 10 fish/m^3). FCR of the tilapia cultured with BFT was lower than that without BFT.

The study proposed that the density of tilapia culture with BFT in brackish water is 6–8 fish/m^3. However, when applying BFT in the production scale, it is necessary to find out the appropriate farming model and improve practical skills, monitoring and quick response to the problem in the culture system.

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