

Effect of biofloc technology at varies biological factors on the growth performance and water quality in cultivation of red hybrid tilapia

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Abstract. Aquaculture is one of the important food-producing sectors. However, the effluent of the aquaculture system consists of pollutants such as nitrogenous compounds and the ample usage of water for traditional aquaculture systems. Therefore, biofloc technology was developed in the aquaculture industry in the 1980s to improve the water quality of the system and increase the fish production. The purpose of this experimental work is to investigate the effect of biological factors on the growth performance of fish and the water quality of the biofloc technology (BFT) aquaculture system for red hybrid tilapia cultivation. In this study, 3 biological factors such as the carbon-to-nitrogen ratio, stocking density and the feeding rate were studied under BFT. Parallel experiments in thirteen suspended growth tanks were carried out within (10:1-20:1) C/N ratio, (1%-5%) feeding rate and (20-80 fish m⁻³) stocking density for 10 weeks. Growth rate of fish and water quality data such as ammonia, nitrite and nitrate concentrations were collected weekly during the study. The results revealed that the 20:1 C/N ratio gave the best water quality. Feeding rate at 4% of body weight produced good growth performance of the tilapia species and the higher stocking densities lead to lower water quality and survival rate.

1 Introduction

Aquaculture is a food-producing industry that is growing at a quicker rate than other main food-producing sectors, and it may assist in driving economic growth, eradicating hunger, reducing poverty, and making better use of natural resources [1]. The aquaculture food-producing industry should concentrate on establishing a technology that is both productive and profitable, particularly in terms of maintaining the same level of output while using fewer resources such as water and feed, as well as reducing the negative environmental effects.

Aquaculture has encountered several major concerns and challenges across the world. The pollutant nitrogenous compound in aquaculture system effluent produces an increase in the amount of chemical and biological oxygen demand in the water [2]. Furthermore, the high cost of water resources restricts aquaculture development due to the extensive use of water in the aquaculture system [3]. Furthermore, the growing price of the feed ingredient raises the expense of fish production [4]. The growing global population will exacerbate the problem of food scarcity, resulting in higher food costs [2].

Nowadays, the majority of research and development in the sustainable aquaculture business is focused on

Biofloc Technology (BFT), which boosts aquaculture productivity while using fewer resources. The use of BFT in aquaculture systems has many benefits, including high production and environmental friendliness. BFT is a technology that converts waste nutrients like faeces and nitrogen compounds into microbial biomass, which may then be processed into food and fed to fish [5]. The growth of heterotrophic bacteria in the water will be fostered by adding adequate carbon sources to attain the optimal carbon-to-nitrogen C/N ratio in the water [6]. This community may reduce the nitrogenous component in the water to a non-toxic level, and no water exchange is necessary [2].

A few studies on the influence of varying C/N ratios, feeding rate, and stocking densities on water quality and tilapia production have been published in recent years. With molasses as the carbon source, the tanks with a 10:1 C/N ratio demonstrated the best growth performance of tilapia [7] while for the wheat flour as the carbon source, a 15:1 C/N ratio spurred high tilapia production [8]. The previous study shows that while the stocking density was growing, the tilapia growth performance showed a negative trend line [9]. Their study shows that the stocking density of 33 fish m⁻³ in the BFT system is the most profitable [9] while the ideal stocking density for excellent growth performance and feed utilization is 40

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fish m⁻³ [10]. For the feeding rate, 2 studies suggest that a feeding rate of 5% body weight per day is the most appropriate level for tilapia and feeding twice a day in a BFT system [11, 12]. However, another study suggests that the feeding rate can be at 4.3% and 6.1% for lower feed costs and better growth performance respectively [13].

The objective of the study is to evaluate the growth of red hybrid tilapia and the water quality in the outdoor BFT system (open environment without greenhouse) within several parameters which are the carbon-to-nitrogen ratio, the stocking density of fish and the feeding rate. Besides, the result will be produced may improve the biofloc technology (BFT) system and make it more reliable in aquaculture systems to lower the operation cost and increase fish production in Malaysia.

2 Methodology

2.1 Fish for experiments

Red hybrid tilapia (*Oreochromis spp.*) juveniles were used in our experiment. At the beginning of the experiment, the fish were fasted for 24 hours to weigh their initial average weight. All juveniles (n= 1000; 2.205 ± 0.001g) were weighed individually and distributed in 9 big experimental tanks (2000 L) and 4 small experimental tanks (500L) according to the table below.

Table 1. Experimental Tank with Fish Number.

Experimental Tanks	Tank Volume (L)	Number of Fish
C (Control)	2000	100
A1, A2, A3, A4	2000	100
B1, B2, B3, B4	2000	100
C1	500	10
C2	500	20
C3	500	30
C4	500	40

2.2 Experimental condition

These experiments used 13 outdoor suspended growth tanks (SGTs) with the biofloc prepared inside. Air pumps were used to provide enough oxygen to the BFT system and maintain the dissolved oxygen (DO) of the water since there is the consumption of oxygen by the microbial metabolism and operating at a rate of 5 L/min. There was no water exchange during the experiment and the dechlorinated tap water was added to the tank for replacing the evaporation losses and water sampling. The fish were fed twice a day (8.00 am and 4.00 pm). The study was lasted 10 weeks and followed by 13 treatments and 3 different parameters. Table 2 shows the parameters of every experimental tank.

Table 2. Experimental Tank with Studied Parameters.

Parameters	C/N ratio	Feed Rate, %	Stock Density, fish/m ³
C (Control)	10:1	5.00%	50
A1	12.5:1	5.00%	50
A2	15:1	5.00%	50
A3	17.5:1	5.00%	50
A4	20:1	5.00%	50
B1	10:1	1.00%	50
B2	10:1	2.50%	50
B3	10:1	4.00%	50
B4	10:1	7.00%	50
C1	10:1	5.00%	20
C2	10:1	5.00%	40
C3	10:1	5.00%	60
C4	10:1	5.00%	80

2.2.1 Control set

A control set was set up based on the best parameters from previous research to compare with the other 12 treatments. Parameters of C/N ratio at 10:1, stocking rate at 50 fish/m³, and feeding rate at 5% of the body weight of fish were used in the control set.

2.2.2 Different ratio of carbon-to-nitrogen (C/N)

Four outdoor suspended growth tanks (SGTs) were used for the experiment with 4 different C/N ratios (12.5:1, 15:1, 17.5:1, 20:1). Based on Pérez-Fuentes, et al. [7] the demand carbon sources used to produce respective C/N ratio can be calculated by equation (1). Assuming the protein contains 16% of nitrogen and the tilapia only digest 35% of protein nitrogen and excrete 65% of protein nitrogen. Molasses contains 40% of carbon and the commercial feed contains 35% of protein.

$$\text{Demand carbon source} = \frac{\text{amount of diet needed (g)} \times (\text{amount of protein (g) in 100g diet} / (100 \text{g diet}) \times (16 \text{g diet nitrogen} / (100 \text{g diet protein}) \times (65 \text{ waste nitrogen} / (100 \text{g diet protein}) \times \text{C:N ratio} ((\text{carbon (g)} / (\text{waste nitrogen (g)})) \times (100 \text{g carbon sources} / (40 \text{g carbon}))} \quad (1)$$

2.2.3 Different stocking densities of tilapia

Four levels of stocking rates (20, 40, 60, and 80 fish m⁻³) were designed for this experiment by using 4 suspended growth tanks (SGTs). The C/N ratio of these 4 tanks was maintained at 10:1 by feeding the calculated amount of carbon sources and the commercial feed at 5% of the body weight of tilapia per day.

2.2.4 Different feeding rates

The 4 tanks were allocated to 4 treatments with 1%, 2.50%, 4%, and 7% of body weight per day in two equal feedings (8.00 am and 4.00 pm). In this study, the stocking density was fixed with 50 fish m⁻³ and the C/N ratio of the tanks was maintained at 10:1 by feeding the calculated amount of carbon sources and the commercial feed.

2.2.5 Biofloc preparation and maintenance

The bioflocs in the experimental tanks were prepared 1 week before the experiment started. Before biofloc preparation, the water in the tank must be sanitized and neutralized to an optimum pH value. Salt was added to every tank with an amount of 1kg of salt /m³ of water to control the parasite in the water. After that, followed by dolomite with an amount of 50 grams of dolomite per m³ of water for controlling the pH value of the water. For biofloc preparation, 100 mL of molasses and 10 mL of probiotics are added to every m³ of water. The molasses was added again to each tank on 2 days before stocking in the amount needed for the respective C/N ratio for each treatment.

At the earlier stage of the experiment (weeks 1-2), the molasses was added to the tanks every four days, after diluting in 8.0L of de-chlorinated tap water to prevent low dissolved oxygen (DO) content and maintain a low concentration of ammonia [7]. Starting from 3rd week of the experiment, the molasses was added once a week.

2.3 Water quality determination and water sample collection

During the experiment, several parameters such as the concentration of NO₂⁻ and NO₃⁻, dissolved oxygen (DO), Total ammonium nitrogen (TAN) and pH were measured weekly to analyse the quality of the water. An optical dissolve oxygen probe (Vernier) was used for measuring DO level while a freshwater master test kit (API) was used for determining the concentration of NO₂⁻ and NO₃⁻, total

ammonium nitrogen (TAN) and pH value of the water sample.

2.4 Growth performance and fish sample collection

After 10 weeks, the fish fasted for a day. Then the final weight was measured individually and there are several growth performance parameters were calculated to determine the growth performance. The growth performance parameters were calculated based on the following equations:

$$WG = [(BW_f - BW_i) / (BW_i)] \times 100 \quad (2)$$

$$SGR = [(\ln(BW_f) - \ln(BW_i)) / T] \times 100 \quad (3)$$

$$FI = (FC) / (F_i) \quad (4)$$

$$(FCR) = FI / WG \quad (5)$$

$$(PER) = WG / PI \quad (6)$$

$$S = F_r / F_i \times 100 \quad (7)$$

WG (weight gain, %), BW_f (final body weight), BW_i (initial body weight), SGR (specific growth rate, %day⁻¹), T (time, day), FI (feed intake, g fish⁻¹), FC (feed consumption per tank, g), F_i (initial fish number per tank), FCR (feed conversion ratio), PER (protein efficiency ratio), S (survival), F_r (number of fish remaining in each tank on week 10).

3 Result and discussion

3.1 Ammonia level

Table 3 Ammonia Level (ppm)

Week		1	2	3	4	5	6	7	8	9	10
Ammonia Level (ppm)	C (control set)	0	0	0	0	0	0.25	0.25	0.25	0	0
	Different Carbon: Nitrogen ratio										
	A1 : C/N ratio 12.5:1	0	0	0.25	0	0	0.25	0	0	0.25	0
	A2 : C/N ratio 15:1	0	0	0	0	0	0.25	0	0	0.25	0
	A3 : C/N ratio 17.5:1	0	0	0	0	0.25	0	0	0.25	0	0
	A4 : C/N ratio 20:1	0	0	0	0	0	0	0	0.25	0	0
	Different Feeding Rate										
	B1 : Feed at 1% body weight	0	0.25	0	0	0	0	0	0	0	0
	B2 : Feed at 2.5% body weight	0	0.25	0	0	0	0	0	0	0	0
	B3 : Feed at 4% body weight	0	0.25	0	0.25	0	0	0	0	0.25	0
	B4 : Feed at 7% body weight	0	0.25	0.25	0.25	0.25	0.25	0.5	0	0.25	0.25
	Different Stocking Densities										
	C1 : 20 fish/m ³	0	0.25	0.25	0	0	0	0	0.5	0	0
	C2 : 40 fish/m ³	0	0.25	0.25	0	0	0.25	0	0	0	0
	C3 : 60 fish/m ³	0	0.25	0.25	0.25	0	0.5	0	4	0.5	0
	C4 : 80 fish/m ³	0	0.25	0.25	0	0.5	0	1	0.25	0	0

Table 3 shows all of the total ammonium nitrogen (TAN) concentrations at different levels of the C/N ratio, feeding rates and stocking densities. The TAN concentrations

were kept at a non-toxic level along the experiment period which is less than 1 ppm of ammonia in the water [14] except set C3 and C4 with the second-highest and the

highest stocking densities at the last quarter of the experiment period. This may be due to the accumulation of fish faces (high stocking density) and the inefficiency of heterotrophic microbes in the biofloc system to convert the ammonia from the faeces [9].

By comparing the different carbon: nitrogen ratios, the highest C/N ratio (20:1) shows the best result in controlling the ammonia level. However, we found that the lowest C/N ratio (10:1) still able to keep the ammonia level at the non-toxic level for a moderate stocking density (50 fish m⁻³). Therefore, it is not necessary to use a higher C/N ratio in the bioflocs system. A higher C/N ratio that exceeds 15:1 may enhance the microbes' activity and promote too many bioflocs in the aquaculture system. This condition may lower the dissolved oxygen

concentration and negatively affect the growth of tilapia [7].

The table also shows that the higher the feeding rate, the worsened the ammonia level in the bioflocs system. The higher feeding rate leads to higher ammonia levels due to the excess feed and more excrement of fish because of stomach fullness and feeds directly passing through the intestine then be excreted as faces [15].

The stocking densities of 60 fish m⁻³ and 80 fish m⁻³ have exceeded the toxic level at once and twice respectively. The ammonia level of 60 fish m⁻³ treatment has reached 4 ppm at week 10 which may be caused by the dead fish in the tank which is late to be removed out. The ammonia level of 80 fish m⁻³ treatment always shows 0.25 ppm and above due to more feed and more excrement of fish [16].

3.2 Nitrite and nitrate level

Table 4 Nitrite Level (ppm)

Week		1	2	3	4	5	6	7	8	9	10
Nitrite Level (ppm)	C (control set)	0.00	0.00	0.00	0.50	2.00	0.50	0.00	0.00	0.00	1.00
	Different Carbon: Nitrogen ratio										
	A1 : C/N ratio 12.5:1	0.00	0.00	0.00	0.50	1.00	0.50	0.25	0.00	0.50	1.00
	A2 : C/N ratio 15:1	0.00	0.00	0.00	0.50	1.00	0.50	0.50	0.50	0.00	0.50
	A3 : C/N ratio 17.5:1	0.00	0.00	0.00	0.50	0.50	0.50	0.25	0.25	0.50	0.50
	A4 : C/N ratio 20:1	0.00	0.00	0.00	0.50	1.00	0.50	0.00	0.00	0.00	0.50
	Different Feeding Rate										
	B1 : Feed at 1% body weight	0.00	0.00	0.00	0.50	1.00	0.25	0.00	0.00	0.00	0.50
	B2 : Feed at 2.5% body weight	0.00	0.00	0.00	0.50	1.00	0.50	0.00	0.00	0.00	1.00
	B3 : Feed at 4% body weight	0.00	0.00	0.00	0.50	2.00	0.50	0.00	0.00	0.00	1.00
	B4 : Feed at 7% body weight	0.00	0.00	0.00	0.50	2.00	0.50	0.00	0.00	0.50	2.00
	Different Stocking Densities										
	C1 : 20 fish/m ³	0.00	0.00	0.00	0.50	2.00	0.25	0.00	0.00	0.25	0.50
	C2 : 40 fish/m ³	0.00	0.00	0.00	0.50	1.00	0.50	0.00	0.00	0.25	1.00
C3 : 60 fish/m ³	0.00	0.00	0.00	0.50	1.00	0.50	0.00	0.00	0.25	1.00	
C4 : 80 fish/m ³	0.00	0.00	0.00	0.50	2.00	1.00	0.00	0.00	0.50	1.00	

Table 5 Nitrate Level (ppm)

Week		1	2	3	4	5	6	7	8	9	10
Nitrate Level (ppm)	C (control set)	0	0	0	5	10	40	80	80	80	40
	Different Carbon: Nitrogen ratio										
	A1 : C/N ratio 12.5:1	0	0	0	5	10	20	80	80	80	40
	A2 : C/N ratio 15:1	0	0	0	5	10	40	80	80	80	40
	A3 : C/N ratio 17.5:1	0	0	0	5	20	40	80	80	40	20
	A4 : C/N ratio 20:1	0	0	0	20	40	80	80	80	40	10
	Different Feeding Rate										
	B1 : Feed at 1% body weight	0	0	0	0	5	20	40	80	80	40
	B2 : Feed at 2.5% body weight	0	0	0	0	5	40	80	80	80	40
	B3 : Feed at 4% body weight	0	0	0	5	10	40	80	80	80	40
	B4 : Feed at 7% body weight	0	0	0	5	10	40	80	80	80	40
	Different Stocking Densities										
	C1 : 20 fish/m ³	0	0	0	0	5	10	60	60	60	40
	C2 : 40 fish/m ³	0	0	0	5	10	20	60	60	80	40
C3 : 60 fish/m ³	0	0	0	5	20	40	80	80	80	40	
C4 : 80 fish/m ³	0	0	0	5	10	40	80	80	80	80	

From Tables 4 and 5, the results revealed that the values of nitrite and nitrate value only increased starting from week 3. The decrease of ammonia level between the 3rd and 4th weeks in Table 3 and the increase of nitrite and nitrate values at week 3 indicates the autotrophic bacteria becoming active [17]. The decreasing concentration of nitrate level in the 8th week or 9th week indicates the start of heterotrophic bacteria activity which converts the nitrate ions into nitrogen gas [14].

By comparing the C/N ratio in Table 4, sets A3 and A4 show the nitrite values decrease faster than other treatments, and set A4 shows the lowest nitrate level for the 10th week. These indicate that heterotrophic bacteria are very active in higher C/N ratios. The previous study stated that the increase of the C/N ratio in the biofloc systems aids the growth of heterotrophic bacteria for absorbing the nitrogenous waste for the synthesis of microbial proteins [7].

Table 3 also shows that the nitrite level was low at the lower feeding rate compared to the higher feeding rate. This indicates the experimental tanks with a low feeding rate produced a low level of the nitrogenous compound. However, from Table 5, all of the feeding rates show a

similar trend on the nitrate value indicating the feeding rate didn't show a significant effect on the activity of the heterotrophic bacteria.

For different stocking densities in Table 5, nitrate value set C1 is the lowest followed by set C2. Control set and set C3 show a similar nitrate value trend while set C4 shows the highest nitrate value. This indicates the higher the stocking densities, the higher the nitrogenous compound produced [10].

3.3 Dissolved Oxygen and pH

The pH value is relatively constant for all 10 weeks. The pH values of all treatments were within the range of 7.4-8.0 which is slightly alkaline and suitable for floc formation.

For dissolved oxygen, the value showed a downtrend throughout the experiment period because of the increase in the biofloc volume in the tank. The presence of the bioflocs utilizes the dissolved oxygen to convert the ammonia to biomass [7]. However, the values of the dissolved oxygen of all tanks were still kept above 4ppm.

3.4 Biofloc Volume

Table 6 Biofloc Volume (ml/l)

Week		1	2	3	4	5	6	7	8	9	10	
Biofloc Volume (ml/l)	C (control set)	0	5	7	15	10	6	6	8	10	8	
	Different Carbon: Nitrogen ratio											
	A1 : C/N ratio 12.5:1	0	6	7	15	15	16	16	25	20	24	
	A2 : C/N ratio 15:1	0	6	6	13	8	4	6	8	10	14	
	A3 : C/N ratio 17.5:1	0	4	4	4	4	3	4	4	3	4	
	A4 : C/N ratio 20:1	0	2	3	8	15	9	9	10	15	22	
	Different Feeding Rate											
	B1 : Feed at 1% body weight	0	2	3	5	6	10	5	8	7	10	
	B2 : Feed at 2.5% body weight	0	1	4	8	6	9	9	8	12	14	
	B3 : Feed at 4% body weight	0	1	4	8	14	14	8	12	8	10	
	B4 : Feed at 7% body weight	0	2	5	7	13	8	6	15	20	30	
	Different Stocking Densities											
	C1 : 20 fish/m ³	0	0	0	1	1	2	6	8	13	16	
	C2 : 40 fish/m ³	0	0	0	1	11	26	26	24	28	36	
	C3 : 60 fish/m ³	0	0	0	1	2	13	15	17	24	30	
	C4 : 80 fish/m ³	0	0	0	2	2	8	10	16	14	22	

From Table 6, it is observed that the formation of floc does not show a significant relationship with the different carbon-to-nitrogen ratios, different feeding rates, and different stocking densities. However, the floc volume showed an increasing trend throughout the experiment period as the conversion of the ammonia to the heterotrophic bacteria with the presence of carbon sources and dissolved oxygen [18].

3.5 Growth performance

Table 7 Growth Performance

	WG(g)	WG(%)	SGR (%/day)	FI, (g/fish)	FCR	PER	Survival (%)
C (control set)	16.10	730.34	2.52	26.59	1.65	1.73	100.00
Different Carbon: Nitrogen ratio							
A1 : C/N ratio 12.5:1	11.99	543.81	2.22	21.33	1.78	1.61	100.00
A2 : C/N ratio 15:1	17.06	773.70	2.58	26.71	1.57	1.83	100.00
A3 : C/N ratio 17.5:1	7.82	354.74	1.80	16.82	2.15	1.33	100.00
A4 : C/N ratio 20:1	17.80	807.03	2.63	28.97	1.63	1.76	99.00
Different Feeding Rate							
B1 : Feed at 1% body weight	5.50	249.30	1.49	2.95	0.54	5.31	100.00
B2 : Feed at 2.5% body weight	8.56	388.07	1.89	8.45	0.99	2.89	99.00
B3 : Feed at 4% body weight	14.63	663.36	2.42	18.99	1.30	2.20	100.00
B4 : Feed at 7% body weight	16.15	732.43	2.52	36.35	2.25	1.27	100.00
Different Stocking Densities							
C1 : 20 fish/m ³	11.96	542.18	2.21	18.86	1.58	1.81	80.00
C2 : 40 fish/m ³	12.64	573.15	2.27	21.61	1.71	1.67	90.00
C3 : 60 fish/m ³	13.02	590.25	2.30	21.19	1.63	1.75	90.00
C4 : 80 fish/m ³	16.25	736.96	2.53	22.08	1.36	2.10	87.50

For the results of the carbon-to-nitrogen ratio, the weight gain, FI, and SGR of the control set, set A2 and set A4 are almost the same while the FCR and PER of set A2 are the lowest and the highest respectively. The survival for set A2 is 100%. Therefore, the C/N ratio of 15:1 was found adequate for tilapia development and water quality control without affecting survival and growth which contrasts with the results of Pérez-Fuentes, Hernández-Vergara, Pérez-Rostro, & Fogel [7] which state the lowest C/N (10:1) is the best C/N ratio.

Sets B1 and B2 show the FCR value less than 1 which means less than 1 kg of feed able to produce 1 kg of fish and it does seem not logical. This may be because the samples used for weighing are bigger than average, we should take more samples for weighing to get more accurate results. Besides, it may be also because of too much natural food such as algae in the experimental tank since the experiments were conducted outdoors [18].

The experiment of different feeding rates shows that set B3 has better overall results which are a high weight gain and protein efficiency ratio, and a lower food conversion ratio compared with others. Thus, set B3 with the feeding rate of 4% of body weight is the appropriate feeding rate for the biofloc system which the feeding rate is lower than the optimal feeding rate (5%) stated in Liu, Wen, & Luo [11].

From the different stocking densities, we found that all of the treatment shows low survival rates (80-90%) compared to the other treatments (99-100%). This is mostly because of the use of smaller experimental tanks for these 4 treatments. By ignoring the survival rate, set C4 (80 fish m⁻³) is the best treatment among them which has the highest weight gain, specific growth rate, feed intake, and protein efficiency ratio and lowest food conversion rate. The appropriate stocking density (80 fish m⁻³) differs from the stocking density stated in Zaki, et al. [10] which is 40 fish m⁻³.

4 Conclusion

In conclusion, the treatment with the highest carbon-to-nitrogen ratio (20:1) promotes the best water quality which shows a low nitrogenous compound level. Besides, the lower the feeding rate, the higher the protein efficiency ratio, but the lower the weight gain, and higher feeding rates lead to worse water quality. Biofloc aquaculture system with a high stocking density (more than 50 fish m⁻³) produced lower water quality and survival rate. However, the survival rate of high stocking density in biofloc systems still can be considered high. The results of the present study show that biofloc technology has a positive effect on controlling water quality even in high feeding rate and high stocking density.

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