Research Article

Evaluation of Different Carbon Sources for Biofloc Production in Tilapia (*Oreochromis niloticus* L.) Culture

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Abstract

This study aimed to investigate the appropriate carbon sources combined with molasses in a biofloc system for tilapia culture. Three carbon sources including rice bran, ground bread crumb and corn meal were selected to induce a biofloc system. This experiment was conducted in the concrete tank size 1 × 1 × 1 m^3 with high aeration to achieve the dissolved oxygen at least 4 mg/L. The stocking density was 30 tilapia fingerlings/m^3. Fish were fed with pellet feed followed tilapia feeding program and this experiment was run for six months. The water quality parameters were monitored twice a day. The results showed that there were no significant differences ($P > 0.05$) in weight gain, survival rate, average daily weight gain and specific growth rate among treatments while using molasses with ground bread crumb gave the highest feed conversion ratio and total suspended solid ($P < 0.05$). In summary, molasses combined with ground bread crumb was the best carbon source for biofloc production in tilapia culture.

Key Words**:** Biofloc; Tilapia; Carbon sources

Introduction

Nowadays, the intensive aquaculture has been expanded and intensified because of the demand for aquatic food are highly required. Increase in productivity per unit space is needed to perform by increasing the rearing density of fishes. This condition negatively affected on environment resources particularly the basic natural resources of water and land (Avnimelech, 2009). In addition, there is a need to establish the culture system which wisely uses limited natural resources, nonpolluting and economically sustainable. Thus, the both activities should be considered for balancing between the water usage and environment friendly. All activities must be developed for the environment damage avoidance. An intrinsic feature of recent intensive aquaculture system is shown the rapid accumulation of feed residues, organic matter and toxic inorganic nitrogen that generates two major problems including water quality deterioration and the low feed utilization in cases when high water exchange.

The biofloc technology (BFT) has begun since 1990. It was first developed to solve water quality problems based on controlling density of heterotrophic bacteria within culture component by converting the organic nitrogenous waste into bacterial biomass (Schneider et al., 2005) for water quality improvement. The BFT component was identified by the forming of bacteria aggregation with living and dead particulate organic matter, other micro-organisms, protozoa, phytoplankton and zooplankton (Hargreaves, 2006). The process is adding carbohydrates, elevated carbon content of the feed or extra carbon source in the culture pond to promote nitrogen uptake by bacterial growth that decreases the ammonium concentration along with control the C/N ratio in aquaculture water. This technology is ecological farming technique used to reduce nitrogen concentration serving as

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in situ food to aquatic animals. Moreover, one of the most important factors to implement the BFT is to mix water by continuously aeration or agitation the water throughout the culture.

There are numerous reports about the tilapia (*Oreochromis niloticus*, L.) cultured in BFT system. The main carbohydrate source in BFT system is molasses (Schrader et al., 2011; Ekasari et al., 2014; Ekasari et al., 2015; Bakar et al., 2015; Pérez-Fuentes et al., 2016; Xu et al., 2016). Hence, this study aimed to investigate the other carbon sources used as the exogenous carbon source combined with molasses which designed for main combination included rice bran, ground bread crumb and corn meal for their effect on enhancing growth, biofloc concentration and water quality for tilapia culture.

Materials and Methods

Experimental facilities and design

This experiment was conducted at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand. The twelve concrete square tanks dimension $1 \times 1 \times 1$ m^3 were prepared and located outdoors under 70% shade nets. Each tanks contained 0.8 – 1.0 m^3 working volume of water. The aeration was continuously aerated using compressed air root blowers delivered through a stone diffuser to maintain the dissolved oxygen (DO) of ≥ 4 mg/L and no water exchange was applied during the experiment. The completely randomize experimental design (CRD) with four treatments and three replicates each was applied consisting of molasses (Control), molasses and rice bran (T1), molasses and ground bread crumb (T2), molasses and corn meal (T3). The carbon sources particularly molasses, rice bran and corn meal were obtained from local agriculture store and the other, ground bread crumb was obtained from local bakery shop. The experiment was conducted over a period of 6 months (180 days).

Fish stocking and management

All sex-reversal male tilapia (*Oreochromis niloticus*) fingerlings were obtained from a commercial fish farm and reared at a circular tank for 15 days to acclimatize to the experimental environment. Thirty tilapia fingerlings were stocked in each concrete tank with 0.5 g initial body weight at the beginning of the experiment. The fish were fed a commercial-pellet diet with 40% crude protein (CP) in the beginning month and then reduced to 35% CP in the second month and 30% CP from the third month until the sixth month. The feeding rates were 8-10 % body weight (BW) per day at the beginning month and gradually reduced 5 – 8 % BW per day in the second month as well as 3-5 % BW per day from the third month to the end of experiment. Each month, 10 tilapias from each treatment were randomly captured and excess water was removed; these individuals were weighed (Kumar et al., 2014) fortnightly to adjust the feeding rate of total body weight. Feed inputs were recorded daily for each tank.

Biofloc technology production

The biofloc culture was established 30 days before the experiment with adult tilapias and then these fish were removed before the beginning of the experiment. Tilapia were fed with seven to eight times a day in the beginning month and gradually reduced with twice a day at the end of experiment. Molasses were selected as the main carbon source. The combinations between molasses with rice bran, grind bread and corn meal in 50: 50 proportion were added to the culture water when total ammonia nitrogen (TAN) concentrations were above 1 mg/L. During the beginning phase carbon supplementation was based on the actual level of TAN in the culture water requiring the addition of 6 g of carbon for each 1 g of TAN found in the water (Ebeling et al., 2006). All carbon sources were added daily to obtain an estimated C/N ratio of 10 to promote biofloc formation. Moreover, the pH was maintained at a range of 7.0 – 7.5 using Sodium bicarbonate ($NaHCO_3$) (Furtado et al., 2012).

The volume of the biofloc was determined using an Imhoff cone, taken the volume in by the flocs in 1 L of pond water after 15 – 20 minutes sedimentation and defined as Total Suspended Solids (TSS, mg/L).

Assessment of water quality parameters

The water quality parameters consisting of dissolved oxygen (DO), pH, alkalinity, temperature and total ammonia nitrogen (TAN) (NH_3-N), Nitrite-N (NO_2-N) and Nitrate-N (NO_3-N) were monitoring on daily (twice a day, particularly in the morning and afternoon) basis using titration technique according to standard methods (APHA, 1998).

Growth parameters of tilapia

At the end of the experiment, the weight gain, survival rate (SR), average daily weight gain (ADG), specific growth rate (SGR) and feed conversion ratio (FCR) were used to calculate growth performances.

Statistical analysis

All data were analyzed by one-way ANOVA. The differences were considered as statistically significant at a probability value of $P < 0.05$. The Duncan's multiple comparisons test (DMRT) was used to decide differences between treatment groups. The relationship between growth performance and biofloc production (TSS) analyzed by Pearson's product moment correlation coefficient (r). All data were analyzed using SPSS software version 17.

Results

Tilapia growth parameters

At harvest, the results of tilapia cultured in different carbon sources are showed in Table 1. The mean weight gain of fish in treatment 3 (molasses with corn meal) was highest at 127.38±1.08 g. and treatment 2 (molasses with ground bread crumb) attained rather lowest at 126.42±0.13 g. while mean survival rate (SR) has an excellent at higher than 96 percent in all treatments, the mean average daily weight gain (ADG) and specific growth rate (SGR) were rather low also although they had higher than 0.07 g/fish/day and 3.05 %/fish/day in all treatments. However, all above did not differ significantly between treatments ($P > 0.05$). On the other hand, the mean feed conversion ratio (FCR) was significantly different ($P < 0.05$) with the lowest through the highest values at 1.56 ± 1.12, 1.58±0.41, 1.59 ± 0.06 and 1.60 ± 0.05 in fish treatments 2, 1, 3, and control, respectively.

Biofloc production

The trend of biofloc density over six months was slightly increasing. The mean volume of biofloc within six months (180 days) is shown in Table 2. All the biofloc treatment groups were found to be significantly ($P < 0.05$) higher than control. Compared with all treatments, treatment 2 (molasses + ground bread crumb) was obtained the highest mean of biofloc density with 147.00 ± 75.11 mg/L followed by treatment 1 (molasses + rice bran) with 135.34 ± 30.76 mg/L and treatment 3 (molasses + corn meal) with 130.50 ± 21.09 mg/L and control obtained the lowest density with 119.00 ± 29.68 mg/L.

The relationship between growth performances of tilapia and biofloc density during six months is shown in Table 3. There was very low positive correlation (r = 0.18) and no significant difference ($P > 0.05$).

Table 1 Growth parameters of tilapia cultured in BFT during six months (180 days)

Parameters	Treatment				P-value
	Control Molasses	T1 Molasses + rice bran	T2 Molasses + ground bread crumb	T3 Molasses + corn meal	
Initial weight (g)	0.50±3.81	0.51±0.19	0.50±0.45	0.50±0.72	-
Final weight (g)	127.45±1.25	127.61±3.41	126.92±0.39	127.88±0.24	-
Weight gain (g)	126.95±0.49^{a}	127.10±0.65^{a}	126.42±0.13^{a}	127.38±1.08^{a}	0.68
ADG (g/fish/day)	0.70±0.51^{a}	0.71±0.46^{a}	0.70±0.35^{a}	0.71±0.76^{a}	0.51
Survival rate (%)	97.67±1.25^{a}	97.11±0.54^{a}	96.90±0.83^{a}	96.80±0.33^{a}	0.32
FCR	1.60±0.05^{a}	1.58±0.41^{ab}	1.56±1.12^{c}	1.59±0.06^{ab}	0.05
SGR (%/fish/day)	3.05±0.74^{a}	3.05±0.09^{a}	3.05±0.69^{a}	3.05±0.44^{a}	0.44

Remark: Each value represents mean ± S.E. Values in the same row with the different superscript letters are significant differences ($P < 0.05$).

Table 2 Volume of biofloc in tilapia pond over six months (180 days)

Parameters	Treatment				P-value
	Control Molasses	T1 Molasses + rice bran	T2 Molasses + ground bread crumb	T3 Molasses + corn meal	
TSS (mg/L)	119.00±29.68^{d}	135.34±30.76 ^{b}	147.00±75.11^{a}	130.50±21.09^{c}	0.39

Remark: Each value represents mean ± S.E. Values in the same row with the different superscript letters are significant differences (P < 0.05).

Table 3 The relationship between growth performance of tilapia and biofloc density over six months (180 days)

Relationship	*r*	P-value	Interpretation
TSS and Growth performance	0.18	0.128^{ns}	Very low positive correlation

^{ns} = non significant

Water quality analysis

The water quality analysis is shown in Table 4. The mean water temperatures were 26.12 ± 0.45, 26.80 ± 1.24, 27.21± 1.83 and 27.74 ± 0.56 °C from treatments 2, 1, 3 and control, respectively. Moreover, mean dissolved oxygen (DO) was maintained at least 4 mg/L with 4.76 ± 1.35, 4.8 ± 0.60, 4.96 ± 0.34 and 5.01 ± 1.12 mg/L from treatments 2, 1, control and 3, respectively. The pH in all treatments were 7.34 ± 0.42, 7.41 ± 0.11, 7.50 ± 1.07 and 7.58 ± 0.35 from treatments 2, control, 3 and 1, respectively by adding sodium bicarbonate ($NaHCO_3$). In case of alkalinity, there were 119 ± 0.30, 120 ± 1.89, 121 ± 0.18 and 121 ± 0.22 mg/L from treatments control, 2, 3 and 1, respectively. The mean TAN were 0.019± 0.37, 0.021±0.63, 0.027±1.77 and 0.03 ± 0.55 from treatments 3, control, 2 and 1, respectively. However, there were no significant differences in water quality parameters (P > 0.05) because they were maintained in normal conditions during experiments.

Table 4 Water quality parameters in tilapia ponds in BFT over six months (180 days)

Parameters	Treatment				P-value
	Control Molasses	T1 Molasses + rice bran	T2 Molasses + ground bread crumb	T3 Molasses + corn meal	
temperature (◦C)	27.74 ± 0.56 ^{a}	26.80 ± 1.24^{a}	26.12 ± 0.45^{a}	27.21± 1.83^{a}	0.13
DO (mg/L)	4.8 ± 0.60^{a}	5.01 ± 1.12^{a}	4.76 ± 1.35^{a}	4.96 ± 0.34^{a}	0.36
pH	7.41 ± 0.11^{a}	7.58 ± 0.35^{a}	7.34 ± 0.42^{a}	7.50 ± 1.07^{a}	0.20
Alkalinity (mg/L)	119 ± 0.30^{a}	121 ± 0.22^{a}	120 ± 1.89^{a}	121 ± 0.18^{a}	0.19
TAN (mg/L)	0.021±0.63^{a}	0.03 ± 0.55 ^{a}	0.027± 1.77^{a}	0.019± 0.37^{a}	0.27

Remark: Each value represents mean ± S.E. Values in the same row with the different superscript letters are significant differences (P < 0.05)

Discussions

Most studies of the cultivated fish particular tilapia species under biofloc technology report a significant increase in the growth performances (Avnimelech, 2007; Crab et al., 2009; Luo et al., 2014; Ekasari et al., 2015; Long et al., 2015; Pérez-Fuentes et al., 2016). Molasses, the common and effective factor was designed to be main source of organic matter in many reports. In the present study, after treat the tilapia with different external sources of carbon combined with molasses the production of biofloc was slightly increasing in all treatments. Ground bread crumb with molasses had a highest bi ofloc production followed by molasses with rice bran and molasses with corn meal and molasses was attained the lowest. This was possibly ground bread crumb, rice bran and corn meal were the long lasting carbon source for heterotrophic bacteria used for converting this carbon to increase their biomass. Molasses was more rapid converted by bacteria than others. In case of long lasting carbon source, the bacteria take more times to decompose them into simple sugars therefore the ammonia removal within the culture was slower than single molasses (Verma et al., 2016). The type of carbon source also influenced the availability, palatability and digestibility for the cultured organisms (Crab, 2010; Crab et al., 2010). For further study, the C/N ratio should be controlled higher than present study.

According to the results above, the growth performance of tilapia in this study was unsatisfied because the harvest size after 180 days culture was too small compared with the marketable size (300 – 500 g.). It is possible due to two obstacles; first, the quantity and quality component of microbial organisms combined to be biofloc in this experiment within temperature 26 – 27 °C may not contain high nutrition value particularly crude protein, etc. Then, there was no significant relationship between growth performance and biofloc density (TSS) although very low positive relationship was observed. This was agree with Zhao et al. (2014) described the temperature is major important environmental factor affecting microbial metabolism. Several studies showed that optimum temperature for bioflocs formation ranged from 18 to 25 °C. This was further demonstrated that there was significant positive correlation between biofloc volumes and water temperature suggesting that heterotrophic microorganism and plankton increased during high temperatures. Second, the NH_3 (TAN) accumulation in all experiments showed very low concentration. It could not reach the maximum risk point because it related to the size of tilapia that could not produce high NH_3 concentration. Therefore low NH_3 concentration could be converted to be high biofloc biomass. Therefore, the controlled C/N ratio was higher than present study together with increased water temperature should be more considered for high density of biofloc production. Moreover, this was possibly the fish was consumed biofloc inadequate. We observed that when feeding pellet feed by percent body weight per day the tilapia could finish within 15 minutes which contrast with Avnimelech (2007) described the feed contribution of microbial flocs contribute close to 50% of protein requirement then it was possible to reduce feed ratio. Fish uptake biofloc depends probably on fish species, feeding traits, fish size and floc density (Avnimelech, 2012). The tilapia size after harvest in present study was no significant in all treatment groups because it could access to feed equally proved by no significance among weight gain, ADG, survival rate and SGR but the survival rate and FCR in all treatments were clarified as main benefits. This was similar with Martin et al. (2005) described the variation in fish sizes within a population can be influenced by the accessibility to feed. One benefits of biofloc system, fish could be always accessible food source outside and the regular feeding moments (Luo et al., 2014). According to Verma et al. (2016) found the growth, feed conversion ratio (FCR) and specific growth rate (SGR) of *Labeo rohita* fingerlings raised in biofloc system using indirect or long lasting carbon sources consisting of tapioca, wheat, corn and sugar bagasse were significantly better. Luo et al. (2014) found the individual fish weight at harvest and FCR of tilapia cultured in the BFT were much greater than in the recirculating aquaculture system (RAS). The other benefit of BFT reveal higher survival rate, similar to this study. Ekasari et al. (2015), Long et al. (2015) and Pérez-Fuentes et al. (2016) were found the tilapia raised in biofloc technology had a higher survival and a few fish died during the entire experiment.

The control C/N ratio in present study was at the estimation of 10: 1 similarly with Ekasari et al. (2015) that found the survival rate of the tilapia larvae from BFT was higher than no BFT. Pérez-Fuentes et al. (2016) also found the tilapia production obtained the highest in BFT and provided good survival and growth of tilapia with no water exchange at 10: 1 C/N ratio. However, Wang et al. (2015), Long et al. (2015) and Bakar et al. (2015) found C/N ratio at 15: 1 using glucose given 100 percent survival at harvest. Moreover, 15: 1 C/N was suitable with *Clarias gariepinus* culture and effectively reduced ammonia nitrogen, nitrite and nitrate concentration in *Carassius auratus* culture. While weight gain rate (WGR), specific growth rate (SGR) were considerately increased when C/N = 20: 1 or 25: 1. In contrast with Pérez-Fuentes et al. (2016) found greater 12.5:1 and 17.5: 1 C/N ratios attained the lowest production. Compared with this study, an increase C/N ratio at higher than 10 might increase weight gain and SGR of tilapia.

In the present study, the water quality parameters in each experiment were within the optimal range for normal growth for raising tilapia (Popma and Masser, 1999; El-Sayed, 2006; Azim and Little, 2008). This was similar with Verma et al. (2016) that found the different sources of organic carbon consisted of tapioca, wheat, corn and sugar bagasse could be adequate to maintain the major water quality parameters within the normal range for fish growth. DO was maintained at a level greater than 4 mg/L. However, the DO level in all treatments was well within the acceptable range for the survival and growth of fish. The TSS levels in this study increased gradually throughout the experimental period and reached around 100 – 150 mg/L in all experiments. Although it was significant in TSS but when compared with suggestion of Avnimelech (2007) and Avnimelech (2012) the TSS in tilapia culture should be approximate 400 mg/L or ranging from 460 to 643 mg/L, while Azim and Little (2008) presented average levels of 597 and 560 mg/L. Thus, the further study should improve TSS to reach 400 – 600 mg/L. The TAN concentrations in all biofloc groups slightly increased and remained stable from the beginning to the end of the experiment by controlled C/N ratio within 10:1 because the nitrogen waste accumulation in the ponds was not much then the TAN could be maintained within normal range easily. Control TAN can be used and stored by the formation of biofloc microbes (Asaduzzaman et al., 2008; Avnimelech, 1999; Ebeling et al., 2006; Emerenciano et al., 2012).

Adding different carbon sources from this study could be induced biofloc systems to serve as an alternative to standard intensive farming of tilapia by reducing environmental impact, eating natural primary producers rather than commercial feed, and using far less water. The results obtained in this work may be site specific or may vary with ponds and other variables.

Conclusions

The current study investigated the different kinds of carbon sources combined with molasses to produce microbial flocs (biofloc) developing in tilapia culture within 180 days. Molasses with grind bread was suggested the best carbon source to produce highest biofloc density compared with others in this study. However, the tilapia grew rather slow even though it could reach the marketable size (especially more than 500 g in Thailand) and the survival rate was very high under the BFT. More or less, it was demonstrated to be an effective potential food source for tilapia. Rice bran, ground bread crumb and corn meal could provide a low cost external carbon source for application in biofloc technology. It was possible to apply the other kinds of local carbon sources that available at the moment instead of 50 percent of the molasses to improve the best of cost and benefit in biofloc technology

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References

APHA. (1998). *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington D.C.

Asaduzzaman, M., Wahab, M. A., Verdegem, M. C. J., Huque, S., Salam, M. A., and Azim, M. E. (2008). C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*, *280,* 117–123.

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture, 176*, 227–235.

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture*, *264*, 140–147.

Avnimelech, Y. (2009). *Biofloc Technology — A Practical Guide Book*. pp.182, The World Aquaculture Society, Baton Rouge, LA, USA.

Avnimelech, Y. (2012). Bioflocs technology — *A Practical Guide Book*, 2nd ed., pp. 73-91. The World Aquaculture Society, Baton Rouge, LA, USA.

Azim, M. E. and Little, D. C. (2008). The bioflocs technology (BFT) in indoor tanks: water quality, bioflocs composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *283*, 29–35.

Bakar, N. S. A., Nair N. M., Lananan, F., Hamid, S. H. A., Lam, S. S., and Jusoh, A. (2015). Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (*Clarias gariepinus*) utilizing Bioflocs. *International Biodeterioration and Biodegradation*, *102*, 100-106.

Crab, R. (2010). *Bioflocs Technology: an Integrated System for the Removal of Nutrients and Simultaneous Production of Feed in Aquaculture*. PhD thesis, Ghent University, pp. 178

Crab, R., Kochva, M., Verstraete, W., and Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering*, *40*, 105–112.

Crab, R., Chielens, B.,Wille, M., Bossier, P., and Verstraete,W. (2010). The effect of different carbon sources on the nutritional value of biofloc, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture*, *41*, 559–567.

Ebeling, J. M., Timmons, M. B., and Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, 257, 346–358.

Ekasari, J., Crab, R., and Verstraete, W. (2010). Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *HAYATI Journal of Bioscience*, *17*(3), 125–130.

Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P., and Schryver, P. D. (2014). The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture*, 426–427, 105–111.

Ekasari, J., Rivandi, D. R., Firdausi, A. P., Surawidjaja, E. H., Zairin, M. Jr., Bossier, P., and Schryver, P. D. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture*, 441, 72–77.

El-Sayed, A. E. M. (2006). Tilapia culture. CAB International, Oxfordshire, UK, pp. 33–46.

Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., and Wasielesky, W. (2012). Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture*, Res. *43*, 447–457.

Furtado, P. S., Poersch, L. H., and Wasielesky, W. (2011). Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zoo technical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) system. *Aquaculture*, *321*, 130–135.

Hargreaves, J. A. (2006). Photosynthetic suspended-growth systems in aquaculture. *Aquaculture Engineering*, *34*, 344–363.

Kumar, S., Anand, P. S. S., De, D., Sundaray, J. K., Raja, R. A., Biswas, G., Ponniah, A. G., Ghoshal, T. K., Deo, A. D., Panigrahi, A., and Muralidhar, M. (2014). Effects of carbohydrate supplementation on water quality, microbial dynamics and growth performance of giant tiger prawn (*Penaeus monodon*). *AquaC*, *22*, 901–912.

Long, L., Yang, J., Li, Y., Guan, C., and Wu, F. (2015). Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*, *448*, 135–141.

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L., and Tan, H. (2014). Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture*, 422–423, 1–7.

Martin, C. I. M., Scharma, J. W., and Verreth, J. A. J. (2005). Inherent variation in growth efficiency of African catfish *Clarias gariepinus* (Burchell, 1822) juveniles. *Aquaculture Research*. *36*, 868–875.

Pérez-Fuentes, J. A., Hernández-Vergara, M. P., Pérez-Rostro, C. I., and Fogel, I. (2016). C/N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture*, *452*, 247–251.

Popma, T. and Masser, M. (1999). *Tilapia life history and biology. Southern Regional Aquaculture Center*, SRAC Publication No. 283, Stoneville, Ms, USA.

Schneider, O., Sereti, V., Eding, E. H., and Verreth, J. A. J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquaculture Engineering*, *32*(3), 379–401.

Schrader, K. K., Green, B. W., and Perschbacherc, P. W. (2011). Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). *Aquacultural Engineering*, *45*, 118– 126.

Verma, I. A. H. A.K., Rani, A.M. B., Rathore, G., Saharan, N., and Gora A. H. (2016). Growth, non-specific immunity and disease resistance of *Labeo rohita* against *Aeromonas hydrophila* in biofloc systems using different carbon sources. *Aquaculture*, 457, 61–67.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L., and Zheng, Z. (2015). Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture*, *443*, 98–104.

Xu, W. J., Morris, T. C., and Samocha, T. M. (2016). Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. *Aquaculture*, *453*, 169–175.

Zhao, Z., Xu, Q., Luo, L., Wang, C., Li, J., and Wang, L. (2014). Effect of feed C/N ratio promoted bioflocs on water quality and production performance of bottom and filter feeder carp in minimum-water exchanged pond polyculture system. *Aquaculture*, *434*, 442–448.