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Evaluation of Different Carbon Sources for Polyculture of Hybrid Catfish (*Clarias gariepinus x Clarias macrocephalus)* and Nile Tilapia (*Oreochromis niloticus*) under Biofloc Technology

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This study aimed to investigate the appropriate carbon sources for produce biofloc and growth performance of hybrid catfish (*Clarias gariepinus x Clarias macrocephalus*) and nile tilapia (*Oreochromis niloticus*) under biofloc technology. Molasses was designed to be main combination between wheat flour and rice bran with 50:50 ratio and its individual as different carbon sources. The plastic tanks contain 3.5 ton of water was prepared. The stocking density of hybrid catfish and nile tilapia fingerlings were prepared for 40 and 30 fishes per cubicmeters moreover fishes were fed with feed pellet under catfish and tilapia program within 4 and 6 months, respectively. The C/N ratio was controlled within 10 ratio. The water quality particularly dissolved oxygen (DO) and NH_3-N were maintained at 5 ppm minimum and 1 ppm maximum, respectively. The results showed that there were no significant of biofloc production between different carbon sources included the single molasses, molasses with wheat flour and molasses with rice bran with 53±1.85, 57±1.24 and 60±1.77 mg/liters, respectively (P>0.05). While, weight gains, average daily weight gains (ADG), feed conversion ratios (FCR) and survival rates of hybrid catfish and nile tilapia were no significant too (P>0.05). This was indicated that wheat flour and rice bran can be used to be the other carbon sources for develop biofloc production and it could effected growth performance of hybrid catfish and nile tilapia (*r*=0.37 and 0.51, respectively) (P<0.05) with high survival rates (97.60±1.73 and 95.11±0.59, respectively) and low FCR (1.65±0.82 and 1.68±0.96, respectively).

Keywords: carbon sources, hybrid catfish (*Clarias gariepinus x Clarias macrocephalus*), nile tilapia (*Oreochromis niloticus*), biofloc

Introduction

The intensive aquaculture has been expanded and intensified worldwide because of the demand for aquatic food are highly required. Inland aquaculture increase in productivity per unit space is needed to perform by increasing the rearing density of fishes. This condition were negatively affected on

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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) 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 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 successful numerous reports about the tilapia (*Oreochromis niloticus*) cultured in BFT system but in opposite few reports about walking catfish particularly hybrid catfish (*Clarias gariepinus x Clarias macrocephalus*). This both species, a main commercial freshwater fishes designed to be polyculture. The hybrid catfish was considered to be major sources of organic matter for biofloc combination with tilapia species. The sugar particularly molasses was selected to be popupar carbohydrate source in BFT system (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). According to Avnimelech (2015) there still many carbon sources such as sugar, starch, cassava meal etc. Hence, the altenative choice to producing biofloc concentration for polyculture still have to examine.

Objectives: 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, and wheat flour for their effect on enhancing growth, biofloc concentration and water quality for polyculture between hybrid catfish and nile tilapia.

Materials and methods

Experimental facilities and design

The experimental was conducted at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand. The twelve plastic tanks contained 3,500 Litres working volume of water were prepared and located outdoors under 70% shade nets. The aeration was continuously aerated using compressed air root blowers delivered through a stone diffuser to maintain the dissolved oxygen (DO) of ≥ 5 mg/L and no water exchange was applied during the experiment. The completely randomize experimental design (CRD) with four treatment and three replicate was designed consist of molasses (Control), molasses and wheat flour (T1), molasses and rice bran (T2). The experiment was conducted over a period of 6 months (180 days).

Fish stocking and management

All male tilapia (*Oreochromis niloticus*) and hybrid catfish (*Clarias gariepinus x Clarias macrocephalus*) 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 and forty hybrid catfish fingerlings were stocked in each concrete tank with 3.50±3.81 and 3.45±0.21 g initiate body weight each at the beginning of the experiment. The tilapia was fed a commercial-pellet diet with 40% crude protein (CP) in the beginning month and gradually reduce 35% CP on second month and 30% CP since third month until sixth month and hybrid catfish was fed the same program with tilapia within four months. The feeding rates were 8-10 % body weight (BW) per day at the beginning month and gradually reduce 5 – 8 % BW per day in the second month whereat 3-5 % BW per day since third month until sixth month. Each two weeks 10 tilapias and hybrid catfishs 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 hybrid catfish and were removed before the beginning of the experiment. Tilapia and hybrid catfish were fed with seven to eight times a day in the beginning month and gradually reduce with twice a day at the end of experiment. Molasses were selected as the main carbon source. The other one were the combination between molasses with rice bran, grind bread and corn meal in 50 : 50 proportion were added to the culture water when measured 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 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.*, 2011).

The volume of the biofloc was determined using an Imhoff cone, taken the volume in by the flocs in 1 Litre of pond water after 15–20 minutes sedimentation.

Assessment of water quality parameters

The water quality parameters consists 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 basis using titration technique according to standard methods (APHA, 1998).

Growth parameters of tilapia

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

Calculations and 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

After added single molasses, molasses with wheat flour and molasses with ricebran. The trend of suspended biofloc during six month was increasing rapidly within first 4 months (120 days) and slightly decreasing at last 2 months (180 days) in all different carbon sources (Figure 1).

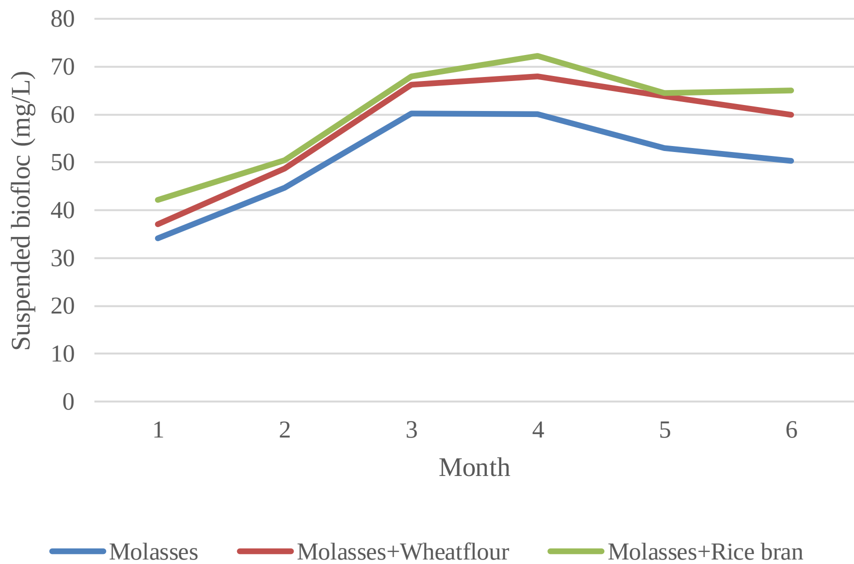

Figure 1 suspened biofloc (mg/L) from each carbon sources in nile tilapia and hybrid catfish culture within 6 months

The mean suspended biofloc within six months (180 days) was shown in Table 1. All the biofloc based treatment groups were found to be non significantly (P>0.05). Compared with all treatment, treatment 2 (molasses + rice bran) was obtained the highest mean of suspended biofloc with 60.00± 1.77 mg/L followed by treatment 1 (molasses + wheat flour) with 57±1.24 mg/L and control (single molasses) obtained the lowest suspended with 53.00±1.85 mg/L.

Table 1. Means suspended biofloc under the different carbon sources on polyculture (Nile tilapia and hybrid catfish) within 6 months (180 days)

Carbon sources	**suspended biofloc (mg/L)**	**P-value**
Molasses	53±1.85[a]	0.571
Molasses and Wheat flour (50 : 50)	57±1.24[a]	0.848
Molasses and Rice bran (50 : 50)	60±1.77[a]	0.803
Mean	**56.67±0.93**	

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

There are non significant between weight gain, average daily weight gain (ADG), survival rate, feed conversion ratio (FCR) and specific growth rate (SGR) of nile tilapia culture in biofloc added by different carbon sources during six months (Table 2). The survival slightly high when added molassed with wheat flour (T1) with 83.76±0.54 followed by single molasses (control) and molasses with rice bran (T2) with 85.60±1.25 and 87.65±1.23 percent, respectively moreover FCR was found low when added single molasses (control) with 1.68±0.44 followed by molasses with rice bran (T2) and molassed with wheat flour (T1) by 1.70±0.82 and 1.72±1.01, respectively.

Table 2. Growth parameters of nile tilapia cultured in BFT on different carbon sources during six months (180 days)

Parameters	**Treatment**			**P-value**
	Control Molasses	**T1 Molasses + wheat flour**	**T2 Molasses + rice bran**	
Initial weight (g.)	3.50±2.77[a]	3.51±0.69[a]	3.50±1.02[a]	0.562
Final weight (g.)	327.30±1.33[a]	325.87±2.11[a]	329.18±0.85[a]	0.329
Weight gain (g.)	323.80±0.56[a]	322.36±0.73[a]	325.68±1.64[a]	0.885
ADG (g./fish/day)	1.80±1.59[a]	1.79±0.42[a]	1.81±0.35[a]	0.734
Survival rate (%)	85.60±1.25[a]	83.76±0.54[a]	87.65±1.23[a]	0.671
FCR	1.68±0.44[a]	1.72±1.01[a]	1.70±0.82[a]	0.336
SGR (%/fish/day)	2.52±0.60[a]	2.52±0.79[a]	2.52±0.72[a]	0.902

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

There are non significant between weight gain, average daily weight gain (ADG), survival rate, feed conversion ratio (FCR) and specific growth rate (SGR) of hybrid catfish culture in biofloc added by different carbon sources during four months (Table 3). The survival slightly high when added single molasses (control) with 82.68±0.66 followed by molassed with wheat flour (T1) and molasses with rice bran (T2) with 85.10±0.66 and 86.92±1.27 percent, respectively moreover FCR was found low when added molasses with rice bran

(T2) with 1.69±1.22 followed by single molasses (control) and molassed with wheat flour (T1) by 1.73±0.85 and 1.74±1.40, respectively.

Table 3. Growth parameters of hybrid catfish cultured in BFT on different carbon sources during four months (120 days)

parameters	Treatment			P-value
	Control Molasses	**T1 Molasses + wheat flour**	**T2 Molasses + rice bran**	
Initial weight (g.)	3.52±0.12 [a]	3.45±0.26 [a]	3.38±0.45 [a]	0.773
Final weight (g.)	247.12±0.99 [a]	251.16±0.64 [a]	256.04±0.23 [a]	0.518
Weight gain (g.)	243.60±0.51[a]	247.71±0.44[a]	252.66±0.68[a]	0.498
ADG (g./fish/day)	2.03±0.97[a]	2.06±1.13 [a]	2.10±0.76 [a]	0.692
Survival rate (%)	82.68±0.66 [a]	85.10±0.66 [a]	86.92±1.27 [a]	0.444
FCR	1.73±0.85 [a]	1.74±1.40 [a]	1.69±1.22 [a]	0.381
SGR (%/fish/day)	2.36±1.66 [a]	2.38±1.59 [a]	2.40±1.42 [a]	0.421

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

The relationship between growth performance of nile tilapia and suspended biofloc during six months is shown in Table 4. There was non significant different (P>0.05) between suspened biofloc added by single molasses (control), molasses with wheat flour (T1) and molasses with rice bran (T2) by 0.34, 0.38 and 0.41, respectively.

Whileas, the relationship between growth performance of hybrid catfish and suspended biofloc during four months is shown in Table 4. There was non significant different (P>0.05) between suspened biofloc added by single molasses (control), molasses with wheat flour (T1) and molasses with rice bran (T2) by 0.21, 0.25 and 0.30, respectively.

Table 4. The relationship between growth performance of tilapia and biofloc density during six months (180 days)

Relationship Suspended biofloc and Growth performance	***r***	**P-value**	**Interpretation**
Nile Tilapia			
Molasses	0.34 [ns]	0.418	Positive and weak correlation
Molasses + wheat flour	0.38 [ns]	0.462	Positive and weak correlation
Molasses + rice bran	0.41 [ns]	0.243	Positive and moderate correlation
Hybrid catfish			
Molasses	0.21 [ns]	0.712	Positive and weak correlation
Molasses + wheat flour	0.25 [ns]	0.651	Positive and weak correlation
Molasses + rice bran	0.30 [ns]	0.558	Positive and weak correlation

The water quality in this experiments under strickly controlled by monitoring daily. Particularly, dissolved oxygen (DO), aerator was blow to maintained the minimum, 5 ppm through culture period. The temperature was under normal range and suitable for nile tilpia and hybrid catfish growth. Observation, alkalinity in all experiment slightly high. Then diurnal fluctuation of pH should be monitor to maintain pH fluctuation within 2 units per day (Table 5).

Table 5. Water quality parameters in nile tilapia and hybrid catfish culture in BFT on different carbon sources during six months (180 days)

Parameters	**Treatment**		
	Control Molasses	**T1 Molasses + wheat flour**	**T2 Molasses + rice bran**
Temperature (°C)	27.74 ±0.56 [a]	26.80 ±1.24 [a]	23.38±0.45 [a]
DO (mg/l)	5.10 ±1.35 [a]	5.51 ±1.12 [a]	4.96 ±0.34 [a]
pH	7.51 ±0.11 [a]	8.01 ±0.35 [a]	7.80 ±1.07 [a]
Alkalinity (mg/l)	119 ±0.30 [a]	121 ±0.22 [a]	121 ±0.18 [a]
TAN (mg/l)	0.021±0.63 [a]	0.02 ±0.55 [a]	0.019±0.37 [a]
NO_2-N (mg/l)	0.42±0.45 [a]	0.60±0.64 [a]	0.55±0.12 [a]

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

Discussion

The different external carbon sources particularly molasses, rice bran and wheat flour can induced mass suspended biofloc in nile tilapia and hybrid catfish culture. The single molasses and combination between molasses and rice bran and wheat flour were possibly carbon source suitable for heterotrophic bacteria used for converted to increase their biomass. Molasses, the simple sugars form was more rapid converted by bacteria than wheat flour and rice bran, the long lasting carbon source because the bacteria take more times for decomposed them into simple sugars therefore adding simultaneous simple and long lasting supported the ammonia removal within the culture the low ammonia nitrogen level was maintained similarly with Verma *et al.* (2016) found using indirect or long lasting carbon sources consist of tapioca, wheat, corn and sugar bagasse were significantly better growth for *Labeo rohita* fingerlings raised in biofloc system.

The organic wastes from hybrid catfish could be lead floc biomass more than nile tilapia because it was contained high amount of nitrogenous wastes, high consumption and excretion according their behaviour. The reason above pointed ammonia could be remove rapidly cause the increasing of heterotrophic bacteria combination with adding carbon sources. They were convert to increase their biomass observed from the first four monts the floc biomass increasing but after harvest hybrid catfish the floc decreasing immediately.

However, after harvested the hybrid catfish reached marketable size but nile tilapia did not. According to Avnimelech (2015) explained fish uptake biofloc depends probably on fish species, feeding traits, fish size and floc density. In case of hybrid catfish probably fish take all kind of feed without consideration mostly in this experiment fish consumed the pellet feed and cause high FCR. While nile tilapia is plankton or filter feeder, fish consumed biofloc more than hybrid catfish since the fingerling to adult different with study Avnimelech (2007); Crab *et al.* (2009); Luo *et al.* (2014); Long *et al.* (2015) found successful in tilapia growth. The floc density in all experiment found lower than many reported probably the C/N ratio at 10:1 is good enough for tilapia fingerling trough starter or juvenile but not good enough for tilapia grow up or adult similarly with Ekasari *et al.* (2015) that found the survival rate of the tilapia larvae from BFT was higher than no BFT moreover Pérez-Fuentes *et al.* (2016) also found the tilapia production obtained the highest in BFT and provides good survival and growth of tilapia 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 suitable with *Clarias gariepinus* culture and effectively reduce 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 if tried to increase C/N ratio at higher than 10:1 it might be increase weight gain and SGR of nile tilapia together and fish will be reach maketable size (>600 g.).

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