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APPLICATION OF BIOFLOC TECHNOLOGY IN INTENSIVE FARMING AFFECTED PRODUCTION AND BLOOD PERFORMANCES OF THE CATFISH [*Clarias gariepinus* (Burchell, 1822)]

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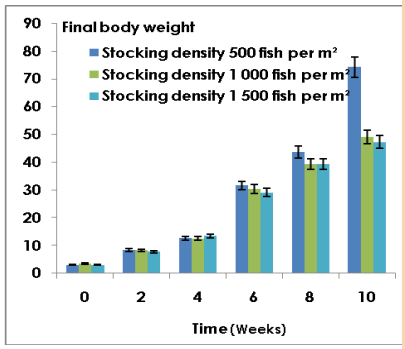
Graphical abstract



Abstract

The biofloc technology can control water quality under negligible water exchange. The aim of this study was to evaluate the effects of stocking density on production and blood performance of catfish (*Clarias gariepinus* [Burchell, 1822]). The catfish were reared in biofloc system (heterotrophic bacteria and addition of sugar for a period of 10 wk and used three levels of density i.e. (500, 1 000, and 1 500) fish per m^2. The production increased with the increasing of stocking density, relative growth rate decreased with the increasing of that. The stocking density of 1 500 fish per m^2 on the biofloc technology can support maximum catfish production, health and proper water quality.

Keywords: Biofloc technology, catfish [*Clarias gariepinus* (Burchell, 1822)], growth, intensive, production

1.0 INTRODUCTION

The African catfish (*Clarias gariepinus* [Burchell, 1822]) could be cultured at high density up to 500 kg m^{-3}, with spesific growth rate value were 3.8 %, and food convertion rate(FCR) 0.8 g g^{-1}. Stocking density of fish might affect various aspects of the health of farmed fish. The healthy of catfish, *Clariepinus burchell,* was influenced by growth cycle. The welfare indicators were growth performance, behavior and physiological condition [1]. Production, growth and survival of that fish are known to be influenced by stocking density [2]. High stocking density in commercial catfish aquaculture system can increase fish production [1, 2].

Intensive catfish aquaculture system can lead to ammonification from feed residue and fish excreation, in which organic waste and ammonia can be easily accumulated in water which can reach an unsafe level. High concentration of ammonia is toxic to most teleost. Ammonia in waters can be removed by biofloc system. The basic principle of biofloc technology is the retention of waste and its convertion to biofloc as a natural food within the culture system. This is done by constant aeration and agitation of water column and addition of carbon sources as organic substrate to allow aerobic decomposition and maintain high level of microbial floc in suspension in fed and or fertilized ponds [3-5]. The C: N ratio was balance of carbon to nitrogen as nutrient for develop and regeneration of the biofloc in the culrute tanks. Theoretically, increased C:N ratio through carbon addition enhances convertion of toxic inorganic nitrogen to microbial biomass available as food for culture catfish. The optimum C:N ratio in an aquaculture can be maintained by adding carbon source or reduction of protein contain in feed [6].

The basic prinsiples of activated suspension technique (AST), recently referred to as biofloc tecnology (BFT), is the retention of waste and its convertion to biofloc as a natural food within the

culture system. Biofloc technology has been widely applied in aquaculture. This system is not only an adequete approach in maintaining water quality in aquaculture system but also generate biomass that can contribute as a protein source for the cultured fish *in situ* [7]. Biofloc technology is useful for contributed to the growth and production of fish and it contains a good nutritional quality for herbivorous and omnivorous fish [5]. The principle of this system is assimilation of excreted dissolved nitrogen by heterotrophic bacteria by managing the C:N ratio in the water. Furthermore, the heterotrophic bacteria biomass forms aggregation, which is then called biofloc. Biofloc comprises not only the bacteria itself, but also other microorganism such as microalgae and zooplankton, which are trapped in organic particles or solid. The biofloc technology was good to control both water quality andl feed production *in situ*. Biofloc technology offers aquaculture a sustainable tool to simultaneously adress its environmental [8]. The biofloc technology was proposed as sustainable solution to catfish culture with high density, which can control water quality under negligible water exchange and sustain healthy culture of catfish. This study was conducted to evaluation the effects of stocking density in intensive aquaculture on blood and production performance of catfish (*Clarias gariepinus*) which were reared with biofloc technology.

2.0 EXPERIMENTAL

2.1 Experimental Design

The intensification experiement was done in one way design. Three level of catfish density i.e. 500 fish per m^2, 1 000 fish per m^2, and 1 500 fish per m^2 were stocked in fibre glass tank (2 × 1 ×1) m^3. The catfish were reared in zero–water exchange biofloc–base intensive culture tank for a period of 10 wk. Good water quality was maintained with promotion and development of biofloc through sugar addition during the experiment. Variables measured i.e. fish production, growth, survival rate, feed consumption, feeding efficiency, blood cell counting, bilirubin serum, blood glucose level, glutamat pyruvate aminotransferase (GPT), and glutamate oxaloasetate aminotransferase (GOT). Water quality and fish weight were determined every 2 wk. Blood variables were determined in the end of treatments. Values of variables assayed were presented in mean (± SD). One way ANOVA was used to determine significant difference by SPSS 16.0 program, If there were a significant F-test, subsequent comparisons of treatment means were performed using the Tukey's HSD test. The level significant was set at $P < 0,05$.

2.2 Biofloc

Biofloc were developed in the fibre glass tanks (2 000 L each) during the experiment. Biofoc were produced by inoculating heterotrophic bacteria in the experimental tanks with addition of sugar. The volume of water was initially equilibrated through exchange water beetwen the tanks. water was added to prevent evaporation. Tanks were aerated and stirred continously using aquarium pump.

2.3 Fish Stocking and Tanks Management

Eighteen thousand *C. gariepinus* fingerlings (3.18 g ± 0.79 g) were stocked to nine fibreglass tanks (2 × 1 ×1) m^3. within biofloc system. Fish were stocked at a density of (500, 1 000 and 1 500) fish per m^2 in each treatment (three treatments with three replications). The experiment was carried out over a 10 wk period to investigate the effects of stocking density on production and blood performance of catfish (*Clarias gariepinus*) which were reared with biofloc technology.

During experiment, fish were fed to satiation twice per day. Fish were fed with a floating pelleted feed, which contains 30 % of protein, 6 % of fiber, 5 % of fat and 13 % of ash, 13 % of water. The feed were weighed to calculate total food consumption. Water quality in the culture system was monitored every 2 wk for dissolved oxygen (DO), temperature, pH, and turbidity using water quality checker (HORIBA). Total ammonia nitrogen (TAN) was measured using Amonia Test Kit Microquant 114750, Merck, Jerman. Fish were weighed every 2 wk. Relative growth rate expressed in percentage was calculated by subtracting of the initial weight from the final weight and, dividing this by the initial weight and the total numbers of experimental days. Feed efficiency was caculated by dividing the total final biomass minus the total initial biomass by the total feed fed, and it was exprssed as a percentage.

In every 2 wk, five fishs were trawled randomly from each stocking density fibreglass tank. Chemical, enzyme and blood cell analysis were performed. Blood sample were collected from the caudal vein using heparinised syringes. Photometric blood cell were calculated using ABX Micros 60. Blood cells counting consist of leukocytes, erythrocytes, hemoglobin, hematocrit and platelet. Whole blood sample was centrifuged (5 min, 5 000 rpm)[1 rpm = 1/60 Hz] prior to hematocrite measurement. The reamining blood was centrifuged at 1 200 rpm for 15 min and the plasma was separated for analysis of glucose, bilirubin and aminotransferase enzyme. Blood glucose concentration was measured by the enzymatic reaction between glucose in blood samples with glucose oxidase and potassium ferric cyanide which produces potassium ferrocyanide. Potassium ferrocyanide was formed with a certain proportion of blood glucose levels. Oxidation of potassium ferrocyanide generates electricity which is

then converted into glucose concentration. Total bilirubin and direct bilirubin were measured by photometric method [9]. The principle of the method is that bilirubin reacted with DSA (diazotized sulfanilic acid) and forms a red color. Absorbance values at λ 546 nm represents direct proportion of the bilirubin concentration. Glucoronid bilirubin which is dissolved in water reacts directly with DSA, whereas indirect bilirubin conjugated with albumin will react with DSA only if there is an accelerator. Thus, total bilirubin = direct + indirect bilirubin. The reaction equation is as follows [9]:

Sulfanilic acid + sodium nitrite → DSA
Bilirubin + DSA → DIRECT Bilirubin
Bilirubin + DSA + accelerator →Total azobilirubin

Transaminase enzyme activity consisting of GPT and GOT in the blood serum and catfish sample from each treatment was measured using the kinetic method and UV-photometry method. The principle of measuring the reaction of GPT (ALAT) is as follows:

$$\text{2-oxaloglutarate + L alanine} \overset{\text{GPT}}{\rightleftharpoons} \text{L-glutamat + pyruvat}$$

$$\text{Pyruvat + NADH + H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{L-lactate + NAD}^+$$

Whereas GOT (ASAT) measured with principle reaction is as follows:

$$\text{2-oxaloglutarat+L-aspartate} \overset{\text{GOT}}{\rightleftharpoons} \text{L-glutamat+oxaloacetate}$$

$$\text{Oxaloacetate + NADH + H}^+ \overset{\text{MDH}}{\rightleftharpoons} \text{L-malate + NAD}^+$$

2.4 Statistical Analysis

In this study, fibreglass tanks were used as experimental unit. All calculations were made using the the SPSS system (SPSS 16.0). Data from the effects of stocking density were first analysed using one way analysis of variance (ANOVA) followed by the Tukey's HSD post-hoc test. Homogenity of variance and normality were tested to meet the assumptions of an ANOVA.

3.0 RESULTS AND DISCUSSION

3.1 Growth and Production Performance of Catfish

The increasing of body weight during the experiment was shown in Figure 1. The average of body weight of catfish decreased with the increasing of stocking density. Conversely, the production of catfish increased with the increasing of fish density. The response of fish production to stocking density of the catfish wich were reared with biofloc technology is presented in Table 1. The catfish density of (500, 1 000, and 1 500) fish per m^2 signicantly affected fish production ($p < 0.05$) in which the value of catfish production were (68.09 ± 1.00) kg; (87.41 ± 9.24) kg; and (127.29 ± 16.07) kg, respectively. Previously, study in Senegal sole showed that high stocking density might not alter growth [10]. High stocking density is considered a relevant issue concerning welfare of farmed fish [11] and it is also connected to production parameters when commercial farming conditions are developed, in line with this study stocking density affected to catfish production (Table 1).

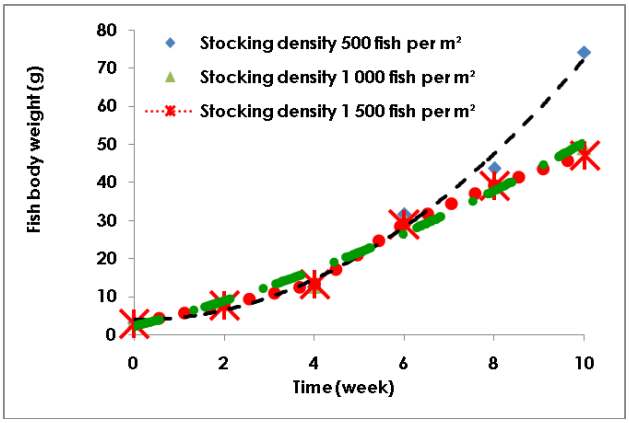


Figure 1 Catfish body weight during the experiment

The values of relative growth rate were (33.24 ± 2.450) % d^{-1}; (21.66 ± 1.82) % d^{-1}; and (21.40 ± 2.72) % d^{-1}, each for the stocking density of (500, 1 000, and 1 500) fish per m^2, respectively. The stocking density significantly affected to the relative growth rate ($P < 0.05$). The increasing of relative growth rate value was due to the total feed consumption. The feed compsumtion of stocking density treatments of (500,1 000, and 1 500) fish per m^2 were (63.63 ± 2.70) kg; (96.60 ± 2.49) kg; and (141.00 ± 14.17) kg, respectively. The value of relative growth rate dan total feed consumption in stocking density teatment (1 000 and 1 500) fish per m^2 was not significant different (Tukey'S HSD, $P > 0.05$), but value of relative growth rate and total feed consumption in stocking density treatment 500 fish per m^2 was significantly difference with that value in stocking density treatment (1 000 and 1 500) fish per m^2.

On the other hand, the values of feed efficiency decreased with the increasing of stocking density. The values of feed efficiency in stocking density (1 000 and 1 500) fish per m^2 were similar (Tuckey HSD, $P > 0.05$). However, the feed efficiency values of the two treatments were lower than the value of the stocking density 500 fish per m^2.

The values of survival rate for the stocking density of (500, 1 000, and 1 500) fish per m^2 were (96.07 ± 0.21) %; (95.38 ± 0.50) %; and (94.74 ± 1.30) %, respectively. Survival rate was not affected by stocking densities ($P < 0.05$). The high value of that survival rate coused by proper water quality parameters.

Table 1 Production performance of catfish were rearing with the biofloc technology

Stocking density (fish · m²)	500*)	1 000*)	1 500*)	*P-value*
Relative growth rate (% ·d⁻¹)	33.24 ± 2.45[a]	21.66 ± 1.82[b]	21.40 ± 2.76[b]	0.0010
Total feed consumtion (kg)	63.63 ± 2.70[c]	96.60 ± 2.49[b]	141.00 ± 14.17[a]	0.0001
Feed eficiencies (%)	107.20 ± 4.73[a]	90.36 ± 5.53[b]	90.27 ± 1.65[b]	0.0120
Biomas production netto (kg)	68.09 ± 1.00[b]	87.41 ± 9.24[b]	127.29 ± 16.07[a]	0.0100
Survival rate (%)	96.07 ± 0.21[a]	95.38 ± 0.50[a]	94.74 ± 1.30[a]	0.2190

Note : *)Value ± SD (Standard Deviation)

3.2 Water Quality

Water quality criteria were in the suitable range (Table 2). Average and range values of water temperature in stoking density of (500, 1 000, and 1 500) fish per m² were (29.55 °C, range 27.5 °C to 30 °C); (27.72 °C, range 27.3 °C to 28.5 °C); and (27.87 °C, range 27.2 °C to 28.5 °C), respectively. The values of dissolved oxygen concentration were 3.37 mg L^{-1}, range (3.2 to 3.69) mg L^{-1}; (3.36 mg L^{-1} range (3.22 to 3.47) mg L^{-1}); 3.27 mg L^{-1} range (2.85 to 3.53) mg L^{-1}), respectively for stocking density of (500, 1 000, and 1 500) fish per m², and pH were (7.54, range 7.35 to 7.8); (7.58, range 7.24 to 7.96); (7.51, range 6.88 to 7.92), respectively for stocking density of (500, 1 000, and 1 500) fish per m². The values of temperature, dissolved oxygen, and pH were within the suitable range for catfish calture. While the values of turbidity were (430.67 mg L^{-1}, range (47 to 627) mg L^{-1}); (667.79 mg L^{-1}, range (5.09 to 999) mg L^{-1}); and (667.82 mg L^{-1}, range (5.34 to 999) mg L^{-1}), respectively for stocking density of (500, 1 000, and 1 500) fish per m². The values of turbidity increased with the increasing of stocking density. Total ammonia nitrogen (TAN) values were (0.51 mg L^{-1}, range (0 to 1.03) mg L^{-1}); (0.51 mg L^{-1}, range (0.00 to 1.81) mg L^{-1}); (1.37 mg L^{-1}, range (0.25 to 3.87) mg L^{-1}), respectively for stocking density of (500,1 000, and 1 500) fish per m². This value were within safe range for catfish culture. The low level of total ammonia nitrogen was due to immobilization of ammonia to bacteria in biofloc technology. The nitrification proceses requires dissolved oxygen for oxidation of total ammonia nitrogen. Oxidating of 1 mg TAN required 4 mg oxygen and 8 mg HCO_3^-. While dissolved Oxygen values were within safe range for catfish culture. However, continuous vigorous aeration in biofloc technology ensured that oxygen was not limiting.

The biofloc systems lead to loss of buffering capacity and rates of nitrification [5]. The rate of nitrification in biofloc systems was coupled with ammonia immobilization into bacteria. It is reported that nitrification requires approximately 4 mg O_2 and 8 mg HCO_3^- for oxidizing 1 mg TAN. There are three principal pathways to remove hazardous Nitrogen species in aquaculture: (i) photoautotrophic removal by algae, (ii) immobilization by heterotrophic bacteria as proteinacious microbial biomass and (iii) chemo-autotrophic oxidation to nitrate by nitrifying bacteria [12]. Although the carbohydrate was added based on TAN:CHO of 1:20 [6], TAN concentrations were elevated, sometimes reaching critical levels in rearing tanks during the experiment. The CHO requirement to minimize TAN needs further evaluation, with addition of sugar.

3.3 Hematological and Bood Biochemical Parameters

The evaluation of hematological and biochemical parameters might be useful for the diagnosis of fish pathologies and physiological status [13]. Alteration of blood cell numbers, as well as biochemical and hormonal status might be indicative of unsuitable environmental conditions (temperature, pH, oxygen concentration) or the presence of stressing factors such as common farm operations. For instance, high stocking density is regarded as a chronic stressor eliciting an increase in plasma cortisol, glucose and osmolality levels [14,15]. The values of blood cell, blood biochemistry, blood glucose, and enzyme serum responses of catfish (*Clarias gariepinus*) to stocking density were presented in Table 3. In the present study, hematocrit, relative levels of circulating leucocytes and glucose levels remained were significantly different in densities treatments (Table 3), suggesting that catfish reared at both 500 and 1 500 fish per m² were not able to adapt to the increasing stocking densities under the particular culture conditions and during the experimental period described in this study. These results are in not agreement with that reported in other species, such as gilthead seabream (*Sparus aurata*), rainbow trout and European seabass (*Dicentrarchus labrax*) [16–18]. Furthermore, this hypothesis is supported by the lack of the immunosuppressive state typically observed in chronically stressed fish. When a given stressor is chronic, the immune response shows suppressive effects and therefore the chances of an infection may be enhanced [19].

Table 2 Value of water quality parameters during the experiment

Stocking density (Fish m²)		500	1 000	1 500
Temperature (°C)	Average ± SD	29.55 ± 1.14	27.72 ± 0.42	27.87 ± 0.51
	range	27.5 to 30.7	27.3 to 28.5	27.2 to 28.5
Dissolved Oxygen (mg ·L⁻¹)	Average ± SD	3.37 ± 1.14	3.36 ± 0.42	3.27 ± 0.51
	range	3.2 to 3.69	3.22 to 3.47	2.85 to 3.53
pH	Average ± SD	7.54 ± 0.15	7.58 ± 0.27	7.51 ± 0.39
	range	7.35 to 7.8	7.24 to 7.96	6.88 to 7.92
Turbidity (mg ·L⁻¹)	Average ± SD	430.67 ± 226.70	667.79 ± 513.10	667.82 ± 513.06
	range	47 to 627	5.09 to 999	5.34 to 999
Total Ammonia Nitrogen (mg ·L⁻¹)	Average ± SD	0.51 ± 0.45	0.73 ± 0.64	1.37 ± 1.33
	range	0.00 to 1.03	0.00 to 1.81	0.25 to 3.87

Table 3 Blood performances of catfish (*Clarias gariepinus*) response to different stocking density

Stocking density (fish per m²)	500*)	1 000*)	1 500*)	*P-value*
Blood cell parameters				
Total Leucocite (10³ sel per µl)	210.73 ± 0.6^{a}	108.36 ± 0.45^{b}	91.25 ± 0.35^{c}	0.0001
Eritrosit (10⁶ sel per µl)	1.83 ± 0.06	1.75 ± 0.20	1.57 ± 0.15	0.1770
Hemoglobine (gr ·dl⁻¹)	6.60 ± 0.26^{b}	7.43 ± 0.08^{a}	6.62 ± 0.12^{b}	0.0200
Hematocrite (%)	23.53 ± 0.50^{b}	26.56 ± 0.20^{a}	23.23 ± 0.23^{b}	0.0001
Trombocite (10³ sel per ul)	1.17 ± 0.29^{c}	16.25 ± 0.28^{a}	13.15 ± 0.14^{b}	0.0001
Blood biochemical parameters				
Glucose (mg ·dl⁻¹)	96.00 ± 2.00^{a}	76.20 ± 0.28^{b}	72.97 ± 0.03^{c}	0.0001
Total Bilirubin (mg ·dl⁻¹)	0.40 ± 0.10^{a}	0.08 ± 0.01^{b}	0.04 ± 0.01^{b}	0.0100
Direct bilirubin (mg ·dl⁻¹)	0.27 ± 0.06^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.0001
Indirect bilirubin (mg ·dl⁻¹)	0.13 ± 0.06^{a}	0.06 ± 0.01^{ab}	0.02 ± 0.02^{b}	0.0210
Serum enzyme parameter				
SGOT (U ·L⁻¹)	194.33 ± 4.04^{a}	145.04 ± 0.04^{b}	123.05 ± 0.05^{c}	0.0001
SGPT (U ·L⁻¹)	99.00 ± 3.61^{a}	57.02 ± 0.03^{b}	39.02 ± 0.03^{c}	0.0001

Note : *)Value ± SD

The values of blood cell counting which consists of total leucosit, hemoglobine, hematocrite, and trombocite were affected by stocking density ($P < 0.05$). On the other hand, the values of eritrocite was not affected by treatments. Surprisingly, several parameters in catfish such as total leucocite values, blood glucose, billirubin, SGOT, and SGPT declined in response to increasing stocking density. Fish stocking density has a significant effect on fish physiological responses, and the importance of evaluating density stress [20]. The respons of blood performances indicated that stocking of 1 500 fish per m² lead to healthy fish. The effects of stocking density on welfare African catfish was not negatively influenced by increasing density [1].

4.0 CONCLUSION

Stocking density in intensification catfish culture clearly contributed to the fish production, growth, feed efficiency and fish health. The biofloc technology can effectively remove total ammonia nitrogen (TAN) and thus prevent accumulation of TAN in the water. Optimum stocking density in catfish culture with biofloc technology was 1 500 fish per m². The stocking density in that system can support maximum catfish production, fish health and proper water quality.

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