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Growth Performance of Catfish (*Clarias gariepinus*) in Biofloc-Based Super Intensive Culture Added with *Bacillus* sp.

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ABSTRACT

This study was conducted to study growth performance of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added *Bacillus* sp. cells addition. Biofloc cultured in separated aquarium (100 L) was used as source of inocula for cultivation for *C. gariepinus* (average body weight of 2.3 ± 0.12 g fish⁻¹). Different concentration of *Bacillus* sp., cells was included in diets and in the media, i.e., (A) 10^2 CFU mL⁻¹, (B) 10^4 CFU mL⁻¹, (C) 10^6 CFU mL⁻¹, biofloc without *Bacillus* sp., cells addition (K⁺), without biofloc as well as *Bacillus* sp., cells addition (K⁻, control). Results showed that the Survival Rate (SR) of catfish showed significantly different between *Bacillus* sp., cells addition and control ($p < 0.05$). Growth and feed conversion ratios in *Bacillus* sp., cells addition were better than that of the controls. Nutrient content of biofloc containing 10^6 CFU mL⁻¹ showed the highest protein value of 34.06%. Total bacterial cells abundance were ranging from 10^6 - 10^8 CFU mL⁻¹ cells, with or without the addition of *Bacillus* sp., as a control. The highest performance of catfish growth was obtained using biofloc treatment by 10^6 CFU mL⁻¹ *Bacillus* sp., addition compare to other treatment.

Key words: *Bacillus* sp., bioflocs, catfish, growth

INTRODUCTION

Catfish is one of widely cultivated fish in Indonesia, with 613,120 t production rate in 2014, 12.75% higher from previous year. The increasing production rate of catfish from super intensive culture unfortunately brings the negative impact on environment quality which then might affect the fish health. Organic waste from remaining feed, feces, as well as fish metabolic waste is among several causes for environmental degradation. Previous study suggested that biofloc aquaculture technology is a promising alternative to overcome the problems. Moreover, application of this technology is more profitable by decreasing inorganic nitrogen waste as well as providing additional feed for cultured fish, thus increasing fish growth and feed efficiency. Biofloc technology can be applied using addition of organic carbohydrate into the culture media to increase C/N ratio and to stimulate the growth of heterotrophic bacteria (Crab *et al.*, 2007).

Heterotrophic bacteria are able to rapidly assimilate Total Ammonia Nitrogen (TAN) in water environment, converted into protein in optimum C/N ratio for bacteria growth (Avnimelech, 1999), which then form the biofloc. De Schryver *et al.* (2008) studied that the biofloc consists of phytoplankton, bacteria, living aggregates, organic matter, cation and dead cells. This mixture of microbial communities growing well in the media containing nitrogen waste, recycles excreted nitrogen from the cell biomass which can be consumed by fish (Avnimelech, 2007).

According to Irianto (2003), *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus polymyxa* were among those which can be used to improve water quality of culture ponds. Probiotic is also functioned as immunostimulant, able to increase feed conversion ratio, able to inhibit the growth of pathogenic bacteria, produce antibiotic and improve water quality (Kesarcodi-Watson *et al.*, 2008). This study was aimed to evaluate growth performance of catfish (*Clarias gariepinus*) cultivated in biofloc-based super intensive culture added with *Bacillus* sp.

MATERIALS AND METHODS

Tested material: Catfish (*Clarias gariepinus*) with size approximately 2.3 ± 0.12 g fish⁻¹ was used as tested material with stocking density of 50 fish/container. For seven days prior to treatment, catfish was cultured in aquarium for adaptation. Size of each container was 90×40×50 cm, filled with 100 L well water, equipped with aerator system with minimum water exchange.

Feed preparation, strain of bacteria and biofloc culture system: Commercial pellet with protein content of 31.77% was used as feed. Before being fed to the fish, feed was fermented using *Bacillus* sp., cells for two days, with the cells concentration of 10² CFU mL⁻¹ (treatment A), 10⁴ CFU mL⁻¹ (treatment B) and 10⁶ CFU mL⁻¹ (treatment C). Approximately 10 mL m⁻³ *Bacillus* sp., cells was added into culture media for once a week according to each treatment dosage. Molasses containing 35% C was added into biofloc media with final concentration C:N ratio of 15:1.

Pisciculture: Four days prior to treatment (D-4), 10 mL m⁻³ *Bacillus* sp., was inoculated according to designated bacterial concentration for each treatment into culture media, together with 10 g liquid molasse. Daily growth rate of bacteria in media was monitored until D-0, just before catfish stocking. The fish was farmed for 42 days, fed twice a day with feeding rate of 5% from fish biomass. Sampling to measure fish growth was conducted once every 2 weeks, with once a week fish fasting in all treated groups, beside non-biofloc control group.

Experiment design: Completely randomized design was applied on 5 treatments, which were 10² CFU mL⁻¹ (A), 10⁴ CFU mL⁻¹ (B), 10⁶ CFU mL⁻¹ (C), biofloc without *Bacillus* sp., cells addition (K⁺), without biofloc as well as *Bacillus* sp., cells addition (K⁻, control). Each data was taken triplicate.

Survival rate: Survival Rate (SR) was calculated in D-42 using equation by Effendi (2004):

$$SR (\%) = \frac{N_t}{N_o} \times 100$$

where, SR expressed as percentage (%), survived fishes in final stage (N_t) and initial stage (N_o) expressed as number per fish.

Specific growth rate: This parameter was calculated as the weight of the fish after 42 days, calculation was conducted using equation by Huisman (1987):

$$\text{SGR (\%)} = \left[t \sqrt{\frac{W_t}{W_o}} - 1 \right] \times 100$$

where, SGR expressed as %/day, W_o and W_t were the weight of the fish during the day of measurement (t) and initial stage (o) expressed as gram (g), while experiment period was expressed as day (t).

Feed Conversion Ratio (FCR): The FCR is the amount of feed consumed by fish (g) to increase 1 g of fish weight, calculated to measure fish growth rate and consumed feed. Equation used was based on Zonneveld *et al.* (1991):

$$\text{FCR} = \frac{F}{W_t + W_m + W_o}$$

where, F is the amount of feed given during treatment (g), W_o is initial fish biomass (g), W_m is biomass of dead fish during treatment (g) and W_t is final biomass of the fish (g).

Proximate analysis: The analysis was conducted on fermented feed and formed biofloc in the final stage of experiment, consisted of; protein content, lipid content, free-nitrogen extract material, moisture content and ash content (AOAC., 1995).

Amount of bacterial population: Total bacterial population was measured once every 7 days using total plate count method at serial dilution from 10^{-1} - 10^{-8} CFU mL⁻¹. Culture was incubated at 28-30°C for 24-28 h. The colony number was calculated using following formula by Madigan *et al.* (2014):

$$\text{Bacteria colony number (CFU mL}^{-1}\text{)} = N \times \frac{1}{f_p} \times \frac{1}{S}$$

where, N is bacteria number in petridish (koloni), f_p is dilution factor and S is the amount of sample taken from bacteria suspension (mL).

Data analysis: Data from specific growth rate, survival rate, Feed Conversion Ratio (FCR) and biofloc proximate analysis was statistically measured using one-way analysis of variance (Steel and Torrie, 1980) using SPSS software (versi 18) at confidence interval of 95% ($p < 0.05$). Turkey test was following when significant difference present. Data of bacteria amount was analyzed in description.

RESULTS

Survival rate: Survival rate of the fish during experiment was presented in Fig. 1. Survival rate of treatment C of $89.33 \pm 2.4\%$ was higher ($p < 0.05$) than negative control (K^-) and positive control (K^+) groups, but not significant different compare to treatment A ($87.33 \pm 6.59\%$) and B ($86.67 \pm 9.42\%$). Results from both treatment A and B were significantly different ($p < 0.05$) to negative control ($50.67 \pm 3.77\%$) but not significant to positive control ($75.33 \pm 3.77\%$).

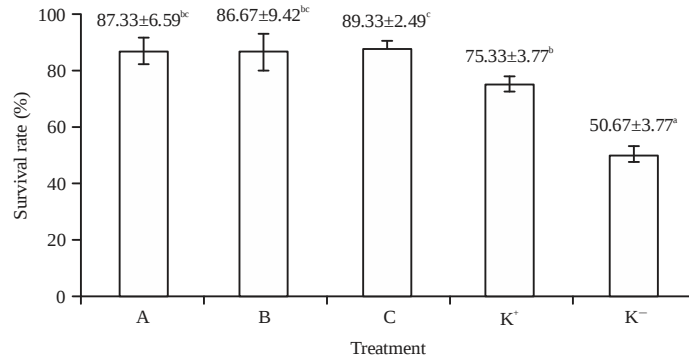


Fig. 1: Survival rate of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp., Different letters indicate different results ($p < 0.05$), A: Treatment dose of 10^2 CFU mL^{-1} , B: Treatment dose of 10^4 CFU mL^{-1} , C: Treatment dose of 10^6 CFU mL^{-1} , K⁺: Positive control, K⁻: Negative control

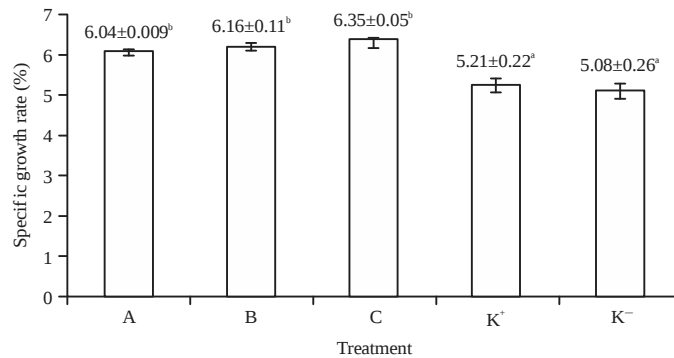


Fig. 2: Specific growth rate of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp., Different letters indicate different results ($p < 0.05$), A: Treatment dose of 10^2 CFU mL^{-1} , B: Treatment dose of 10^4 CFU mL^{-1} , C: Treatment dose of 10^6 CFU mL^{-1} , K⁺: Positive control, K⁻: Negative control

Specific growth rate: As presented in Fig. 2, daily growth rate during 42 days experiment showed that treated groups were significantly different to control groups ($p < 0.05$). Highest SGR was obtained by treatment C ($6.35 \pm 0.05\%$), which not significantly different ($p > 0.05$) to treatment A ($6.04 \pm 0.09\%$) and B ($6.16 \pm 0.11\%$). However, results from those 3 treatments were all significantly different to K⁺ (positive control) and K⁻ (negative control) of 5.21 ± 0.22 and $5.08 \pm 0.26\%$, respectively.

Feed Conversion Ratio (FCR): Feed conversion ratio of catfish grown in biofloc culture was presented in Fig. 3. It was indicated that results from treated groups were significantly different ($p < 0.05$) to K⁻ (negative control). Highest FCR was obtained by treatment C (0.91 ± 0.01), followed by B (0.98 ± 0.11), A (0.99 ± 0.06), then K⁺ (1.01 ± 0.07), whereas, FCR for negative control (K⁻) was 1.42 ± 0.26 .

Proximate composition of biofloc and fermented feed: Proximate composition of biofloc during experiment was presented in Table 1. Results showed that protein content, nitrogen free

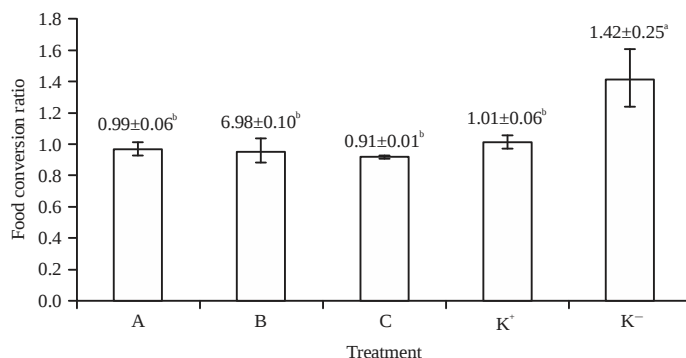


Fig. 3: Feed conversion ratio of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp., Different letters indicate different results ($p < 0.05$), A: Treatment dose of 10^2 CFU mL⁻¹, B: Treatment dose of 10^4 CFU mL⁻¹, C: Treatment dose of 10^6 CFU mL⁻¹, K⁺: Positive control, K⁻: Negative control

Table 1: Proximate analysis of biofloc

Treatments	Proximate biofloc (% on dry weight)				
	Crude protein	Crude lipid	NFE	Ash	Crude fiber
A	31.99±2.70 ^a	7.87±1.69 ^b	30.10±9.65 ^a	17.85±3.89 ^a	12.17±7.00 ^a
B	31.81±1.51 ^a	6.47±0.86 ^b	39.46±4.17 ^a	15.29±1.29 ^a	6.95±3.67 ^a
C	34.06±1.70 ^a	9.17±4.53 ^b	33.37±4.18 ^a	18.73±6.51 ^a	4.65±6.63 ^a
K ⁺	31.64±4.31 ^a	14.92±2.87 ^a	30.54±12.78 ^a	13.92±2.58 ^a	8.95±10.23 ^a

A: Treatment dose of 10^2 CFU mL⁻¹, B: Treatment dose of 10^4 CFU mL⁻¹, C: Treatment dose of 10^6 CFU mL⁻¹, K⁺: Positive control, NFE: Nitrogen free extract

Table 2: Proximate analysis of fermented feed

Treatments	Proximate fermented feed (% on dry weight)				
	Crude protein	Crude lipid	NFE	Ash	Crude Fiber
A	31.83	7.01	50.28	1.45	9.42
B	31.96	6.95	49.31	2.65	9.12
C	33.48	8.32	47.59	1.59	9.00
K ⁺	31.77	5.88	51.47	9.34	1.53
K ⁻	31.77	5.88	51.47	9.34	1.53

A: Treatment dose of 10^2 CFU mL⁻¹, B: Treatment dose of 10^4 CFU mL⁻¹, C: Treatment dose of 10^6 CFU mL⁻¹, K⁺: Positive control, K⁻: Negative control, NFE: Nitrogen free extract

extract, ash and crude fiber of biofloc from all probiotic treatments were not significantly different ($p < 0.05$) to K⁺. Protein content of biofloc from treatment C was 34.06 ± 1.70 , A and B were 31.99 ± 2.70 and 31.81 ± 1.51 , respectively, while K⁺ was 31.64 ± 4.31 . In the other hand, lipid content of biofloc from all probiotic treatments were significantly different ($p < 0.05$) to K⁺. Highest lipid content obtained from K⁺ (14.92 ± 2.87), followed by C (9.17 ± 4.53), A (7.87 ± 1.69) and B (6.47 ± 0.86).

Proximate composition of fermented feed was presented in Table 2. Highest protein content was obtained from treatment C (33.48), followed by B (31.96), A (31.83), then K⁺ and K⁻ (31.77). Highest lipid content was also obtained by treatment C (8.32), followed by A (7.01), B (6.94), then K⁺ and K⁻ (5.88).

Total bacterial population: Bacterial population was measured once a week during 42 days experiment, results were presented in Fig. 4. Bacterial population during treatment was dynamics, for all treatments were ranging from 10^5 up to 10^8 CFU mL⁻¹, from initial to the final stage of

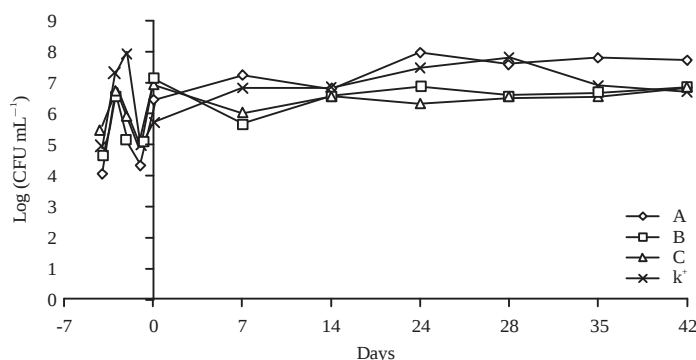


Fig. 4: Dynamics of total bacterial populations on culture catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp., A: Treatment dose of 10^2 CFU mL⁻¹, B: Treatment dose of 10^4 CFU mL⁻¹, C: Treatment dose of 10^6 CFU mL⁻¹, K⁺: Positive control

Table 3: Parameters of water quality

Water quality							
Treatments	DO (mg L ⁻¹)	pH	Temperature (°C)	TAN (mg L ⁻¹)	Nitrite (mg L ⁻¹)	Nitrate (mg L ⁻¹)	Ammonia (mg L ⁻¹)
A	5.1-8.1	5.74-7.95	30.5-31.7	0.16-0.68	0.16-0.99	0.16-0.77	0.000-0.010
B	5.9-7.6	5.58-7.71	30.3-32.1	0.17-0.74	0.09-1.49	0.17-0.74	0.000-0.028
C	5.6-7.6	5.30-7.67	30.2-32.2	0.22-0.88	0.09-1.08	0.22-0.73	0.000-0.011
K ⁺	3.8-7.9	5.68-7.78	30.5-32.6	0.21-1.11	0.03-1.49	0.21-1.11	0.000-0.026
K ⁻	4.0-8.0	5.52-7.79	30.3-32.3	0.14-1.23	0.02-1.40	0.14-1.23	0.000-0.027

A: Treatment dose of 10^2 CFU mL⁻¹, B: Treatment dose of 10^4 CFU mL⁻¹, C: Treatment dose of 10^6 CFU mL⁻¹, K⁺: Positive control, K⁻: Negative control, DO: Dissolved oxygen, TAN: Total ammonia nitrogen

experiment. During initial stage, total bacteria was ranging between 10^5 - 10^7 CFU mL⁻¹, while during final stage, total bacterial population was 10^6 - 10^8 CFU mL⁻¹. It was indicated that total bacterial population from all treatments was not much different.

Observation on water quality: Water quality parameters observed in the study were including of temperature, Dissolved Oxygen (DO), pH, Total Ammonia Nitrogen (TAN), nitrite and nitrate. Those were measured once every 7 days on catfish culture media. The results were presented in Table 3.

DISCUSSION

The survival rate of treatment C group was higher ($p < 0.05$) than negative control (K⁻) and positive control (K⁺). Both treatment A and B were significantly different ($p < 0.05$) compare to negative control ($50.67 \pm 3.77\%$) but not significant compare to positive control ($75.33 \pm 3.77\%$). This result indicated that *Bacillus* sp., cells addition into biofloc-based culture media for catfish was able to increase the survival rate. Similar result also indicated by another research using *Staphylococcus lentus* on biofloc-based catfish culture, resulted higher cumulative mortality than control (Salamah *et al.*, 2015). Research by Decamp *et al.* (2008), Tseng *et al.* (2009) and Verschuere *et al.* (2000) also noted that *Bacillus* sp., addition was able to increase growth performance and survival rate of fish culture.

Azim and Little (2008) mentioned that, the presence of optimum concentration microbial cell in biofloc was able to increase fish health status. Bacterial cells in the biofloc accumulate the poly- β -hydroxybutyrate (PHB) with alleged role in microbial pathogens inhibition of fish culture. The PHB content in biofloc consumed by fish was able to increase its immune system so that the fish can be more resistant to environmental interference during treatment (Michaud *et al.*, 2006).

The non-biofloc control (K^-) had low survival rate due to diverse rate of catfish growth, which later increasing fish cannibalism. The main contribution of fish cannibalism in non-biofloc culture was 49.33%. Baras and D'Almeida (2001) noted that irregular diverse size of the fish affected the rate of survival as well as cannibalism rate during larvae catfish culture. During 7 weeks of *C. gariepinus* culture, cannibalism reported as the main cause for mortality up to 60-93% (Adamek *et al.*, 2011).

The Specific Growth Rate (SGR) of A, B and C treated groups significantly higher than control groups. In this study, protein content of biofloc was also increased in line with *Bacillus* sp., concentration, the highest was 34.06% in treatment C. It is indicated that the catfish effectively consume the biomass of biofloc with *Bacillus* sp., cells addition. Several previous studies suggested that biofloc was able to enhance the growth of tilapia (Avnimelech, 1999, 2007), *Macrobrachium rosenbergii* (Asaduzzaman *et al.*, 2008), vanname shrimp (Xu *et al.*, 2012; Ju *et al.*, 2008b; Kuhn *et al.*, 2009, 2010; Burford *et al.*, 2004), tiger shrimp (Anand *et al.*, 2014) and crucian carp (Wang *et al.*, 2015).

The addition of *Bacillus* sp., cells into the feed had positive impact on fish digestive system due to exogenous enzymes released by the cells, thus expenditure energy was more effective during digestion. Energy difference resulting from this process can be used for the growth. Liu *et al.* (2009) mentioned that the increasing growth rate of aquatic organisms provided with probiotic in their feed was related to higher enzymatic digestion activity together with vitamin synthesis, therefore, digestibility and organism weight were also increased.

Feed conversion ratio from treatment A, B and C were all significantly different ($p < 0.05$) with negative control (K^-), but not significantly different to the positive control (K^+). This result indicated that biofloc system was able to increase feed efficiency indicated by lower feed conversion ratio. Several previous studies suggested that biofloc was able to substitute conventional fish feed and reduce FCR value in tiger shrimp (Anand *et al.*, 2014). It was also indicated that *Bacillus* sp., addition into cultivation media had positive contribution on feed efficiency, significantly different compared to control. This was cause by rich content of bioactive compound of biofloc such as carotenoids, chlorophyll, phytosterol, bromophenols, amino sugar (Ju *et al.*, 2008a) and anti-bacteria compounds (Crab *et al.*, 2010). *Bacillus* sp., cells addition into biofloc resulted in better growth rate and FCR. Utilized feed could be reduced by 10-20% by biofloc technology application (De Schryver *et al.*, 2008). Verschuer *et al.* (2000) noted that probiotic cells contributed to higher immune response by increasing intestinal microflora balance through increasing feed quality, enzymatic role during digestion, inhibition of pathogenic microorganisms and growth factor.

Protein content of feed is an important factor for aquatic organism nutrition. Nutritional contents in feed adequate for catfish were 24-26% protein (Halver and Hardy, 2002) and 3-6% lipid (Webster and Lim, 2002). In this study, average protein content of biofloc in treated groups was $32.81 \pm 1.25\%$ protein and $7.83 \pm 1.35\%$ lipid (Table 1). High content of protein and lipid in the biomass of biofloc was presumably related to concentration of Extracellular Polymer Substances (EPS), approximately of 80-95% of organic matter in biofloc (Wilen *et al.*, 2008).

Previous study presented by Tacon *et al.* (2002) mentioned that 35-38% protein, 5-9% lipid, 7-10% ash and 18-19 kJ g⁻¹ energy were contained in biofloc produced in closed-system shrimp culture using commercial feed. This result indicated that protein, lipid, carbohydrate and ash content in biofloc can be utilized as fish culture feed (Crab *et al.*, 2010). Biofloc can be considered as complete nutrition source and contained several bioactive compounds and probably unidentified factors as well (Ju *et al.*, 2008b). With similar protein content with commercial feed, biofloc is beneficial for fish growth.

The amount of total bacteria of the treatments from the initial stage to the final ranged between 10⁶ and 10⁸ CFU mL⁻¹, with dynamic pattern throughout culture period (Fig. 4). Increasing rate of bacterial cells population occurred 3 days after *Bacillus* sp., cells inoculation. It was also observed before treatment. In the first day after inoculation (D-4 prior to treatment), cells population was ranging from 10⁴ up to 10⁵ CFU mL⁻¹ and in the next day (D-3 prior to treatment), it was increased in the range of 10⁶-10⁷ CFU mL⁻¹. And the next day (D-2 prior to treatment), it was 10⁵-10⁷ CFU mL⁻¹, whereas, day-4 (D-1 prior to treatment), total bacterial cells started to decrease into 10⁴-10⁵ CFU mL⁻¹.

The carbohydrate is an important carbon source for the growth of bacterial cells. The carbohydrate addition into culture media was able to rapidly stimulate the growth of heterotrophic bacterial cells (Avnimelech, 1999). Therefore, molasses addition as carbon source was able to promote bacterial cell propagation in the culture medium. As mentioned by Burford *et al.* (2004), carbon source addition was able to support transformation of inorganic nitrogen into microbial protein through increasing population of heterotrophic bacterial cells.

Water quality parameters were considered in the range values of normal for all treated groups. Measurement results showed that water temperature, DO, pH, TAN, nitrite, nitrate and ammonia in all treatment groups were in optimum condition for catfish culture. Dissolved oxygen ranged from 4.0 up to 8.1 ppm, while pH ranged from 5.30 up to 7.95 for all treatments. During experiment, the temperature ranged from 30.2-32.6°C. Ammonia content for all treatments ranged from 0.001 up to 0.028 mg L⁻¹ which was considered as standard quality. According to Boyd (1982), 0.1 mg L⁻¹ ammonia content was able to reduce fish growth and causing damage to the gills of channel catfish. Nitrite content ranged from 0.002 up to 1.49 mg L⁻¹ and nitrate ranged from 0.16 up to 1.23 mg L⁻¹ for all treatments. Nitrite level should be less than 1 mg L⁻¹ for proper aquaculture condition (Timmons and Ebeling, 2007).

CONCLUSION

The highest performance of catfish growth was obtained using biofloc treatment by adding *Bacillus* sp., cells of 10⁶ CFU mL⁻¹, with survival rate of 89.33±2.49%, the specific growth rate of 6.35±0.05% and the feed conversion ratio of 0.91±0.01.

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