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Original Article

Consequential study on different levels of C/N ratios used in biofloc-based aquaculture system

Estudo consequente sobre diferentes níveis de proporções C/N utilizadas no sistema de aquicultura à base de biofloc

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Abstract

Biofloc technology is much highlighted these days because of its tremendous effects on aquaculture. Microbes were enriched on cheapest organic carbon source i. e., powdered banana peels and were incorporated in different aquaria rearing grass carp fingerlings under different C/N treatments (10:1, 15:1 and 20:1) and 10% water daily water exchange. The initial growth of fingerlings was recorded. The experiment was settled in triplicates for 60 days and run parallel to control group provided with commercial feed and daily water exchange. Its effect was evaluated by measuring the growth of fingerlings and water parameters of each aquarium. The average % gain in weight and length of fingerlings was obtained significantly highest (28.12 ± 0.30g and 17.29 ± 0.46cm respectively) in aquaria containing pure powdered banana peels with 10% water exchange and C/N ratio was adjusted at 20: 1 (T3) than other treatments and control. Ammonia and other water parameters were also under control in T3 than other experimental and control groups. By all counts, it was concluded that the highest C/N ratio in biofloc system had the potential to increment *C. idella* growth rate by reducing toxicity and could be used as fish meal substitute.

Keywords: biofloc technology, C/N ratio, microbial flocs, metagenomics, aquatic toxicity.

Resumo

A tecnologia Biofloc é muito destacada hoje em dia por causa de seus tremendos efeitos na aquicultura. Os micróbios foram enriquecidos com a fonte de carbono orgânico mais barata, i. e., cascas de banana em pó, e foram incorporadas em diferentes aquários de criação de alevinos de carpa-capim sob diferentes tratamentos C/N (10: 1, 15: 1 e 20: 1) e 10% de troca diária de água. O crescimento inicial dos alevinos foi registrado. O experimento foi resolvido em triplicatas por 60 dias e executado paralelamente ao grupo controle fornecido com ração comercial e troca diária de água. Seu efeito foi avaliado medindo o crescimento dos alevinos e os parâmetros da água de cada aquário. O% de ganho médio em peso e comprimento dos alevinos foi obtido significativamente mais alto (28,12 ± 0,30g e 17,29 ± 0,46 cm respectivamente) em aquários contendo cascas de banana em pó puro com 10% de troca de água e a relação C/N foi ajustada em 20: 1 (T3) do que outros tratamentos e controle. A amônia e outros parâmetros da água também estavam sob controle no T3 mais do que nos outros grupos experimentais e de controle. Por todas as contagens, concluiu-se que a maior razão C/N no sistema de bioflocos tem o potencial de incrementar a taxa de crescimento de *C. idella* reduzindo a toxicidade e pode ser usada como substituto da farinha de peixe.

Palavras-chave: tecnologia biofloc, razão C/N, flocs microbianos, metagenômica, toxicidade aquatic.

1. Introduction

Biofloc is a protein rich system in which different microorganisms are combined together in an organic matrix, helpful in improving water quality, waste treatment and disease prevention in aquaculture. The microorganisms in the system utilize the provided carbon source and nitrogenous wastes excreted by fish. The nitrogenous waste is being converted by microorganisms into usable nitrates which are assimilated as their protein part. As long as there is sufficient mixing and aeration to maintain an active floc in suspension, water quality can be controlled (Hargreaves, 2015). Biofloc not only recycles the nutrients in water but also aggravates growth rate, feed conversion ratio and weight gain in shrimps and tilapia (Burford et al., 2004; Wasielesky Junior et al., 2006), hence, biofloc technology

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offers a low-cost sustainable technique in terms of minimal water exchange, reduced feed input and maximum output (Avnimelech and Kochba, 2009). Many of the agri-wastes such as fruit peels can act as sole nutritional source for the microbial growth (Maller et al., 2011). An optimal C/N ratio can improve production and recycling of nutrients. According to Burford et al. (2003) and Pérez-Fuentes et al. (2016), C/N ratio must be above 10:1, however, according to Schneider et al. (2005), the best C/N ratio is approximately 15:1. Wasielesky Junior et al. (2006) found optimal C/N ratios for microbial floc formation from 14:1 to 30:1, with predominance of heterotrophic bacteria. Molasses were used for tilapia rearing by Widanarni et al. (2012) (C/N ratio of 15:1) and Lima et al. (2015) (C/N ratio of 6:1).

Aquaculture is emerging as the most popular agro industry but imparts to produce huge amount of wastes such as suspended solids, total solids and total nitrogen. Recycling of these waste products is important to reduce toxicity especially in intensive aquaculture systems. (Brown et al., 1999; Neori et al., 2004). Another most notable problem is the daily exchange of water. Toxicity and wastes by fish are accumulated in water. This water when dispend into rivers, streams or lakes pollutes the aquatic environment. Therefore there is a dire need of recirculation aquaculture system which minimizes the damage to the environment (Avnimelech, 2007; Stokstad, 2010).

Biofloc technology offers a low-cost sustainable technique in terms of minimal water exchange, reduced feed input and maximum output (Avnimelech and Kochba, 2009). Many of the agri-wastes such as fruit peels can act as sole nutritional source for the microbial growth (Maller et al., 2011). Biofloc grown on different carbon sources (glucose, starch and glycerol) have different nutritional requirements and microbial community structures (Wei et al., 2016). Hu et al. (2017) observed that C-source determine the type, quantity and community of the bioflocs which improves the health status of the fish because addition of these substances not only changes the microbial community structure but also affect the microbial pathogenic activity (Wei et al., 2020). However, fruit peels' as C source have not yet been employed in BFT based aquaculture. This study pursued the incorporation of powdered banana peels as carbon substrate in place of fish meal along with minimum water exchange. The C/N ratio was also adjusted to the extent that minimized the toxicity. Hence it is quite important in establishing an inexpensive sustainable aquaculture system.

2. Material and Methods

2.1. Enrichment of bacteria from soil on cheapest organic source

The soil sample was taken from fertile pond for this study from University of Agriculture, Faisalabad. Soil was collected in an autoclaved air tight jar following standard sampling methods (US EPA, 1989) and was brought to the laboratory for inoculation. The sample were stored in refrigerator and inoculated within 48 hours. Powdered banana peels were collected from different fruit shops and house garbage and were washed, sun dried and ground. The powdered organic substrate was stored in air tight glass jars at room temperature for use. The study was carried out in Microbiology research laboratory, GC Women University, Faisalabad. The powdered banana peels were used as substrate to enrich microbes from the soil sample. For enrichment of bacteria three glass jars (1000 mL) each containing 1000 mL of distilled water and 100gm powdered banana peels were autoclaved for 15 minutes was used to develop biofloc (Hargreaves, 2006). Soil (20 gm/1000 mL) was inoculated in each autoclaved bottle for enrichment of microbes utilizing powdered banana peels. Oxygen pumps were attached with aeration pipes to provide oxygen. The whole experiment was settled for two weeks for the development of biofloc. The enriched medium was then shifted to aquaria to rear *Ctenopharyngodon idella*. Aquaria of 90L capacity were used in triplicates. Four experimental setups for the powdered banana peels were established having varied treatments of C/N ratios (T1, T2 and T3) and control (T4) group. Each aquarium was filled with 60L water with a ratio of 40 liters' filtered water and 20 liters' tap water. Aeration was provided for continuous mixing of oxygen. It was then allowed for the growth of bacteria at 10% inoculums. Enrichment of bacteria was checked by taking water samples weekly and the colony forming unit (C.F.U) was calculated. When the C.F.U reached close to $1x10^5$/mL, it was considered ready for stocking fish. Experimental setups (T1, T2 and T3) were run with control (T4) in which commercial feed was used and water was exchanged on daily basis.

2.2. Collection of fish fingerlings

Fingerlings of grass carp (*Ctenopharyngodon idella*), 3.5 cm to 4.5 cm in size were brought from hatchery situated at Satiana road, Faisalabad. They were stocked in a tub for 24 hours to acclimatize. After acclimatization of 24 hours, fish were distributed in bio-flocculated aquaria and in control aquaria after recording their weight and length. Each aquarium was stocked with seven fish carefully. The average weight and average length of fish in each aquarium was noted as first reading.

2.3. Rearing and feeding of fish

Fish were reared at room temperature (30 °C). They were fed twice a day with respective amount of agri-wastes (6% of average weight of fish) and commercial feed for control. The whole experiment was continued for 60 days. The average length and weight of fish as well as colony forming unit (C.F.U) of each aquarium was calculated weekly to observe the effects of biofloc on fish growth. The general formula followed for C.F.U was (Formula 1):

$$C.F.U / mL = \left(no.\ of\ colonies \times dilution\ factor \right) / volume\ of\ inoculums \quad (1)$$

C.F.U or colony-forming-unit is the measure of viable cells of bacteria.

2.4. Rearing of grass carp under different C/N ratios

The powdered banana peels as carbon source had been added in experimental aquaria at different concentration levels. Each treatment was monitored in triplicates having organic source and 10% water exchange. Carbon to nitrogen ratios varied in each condition. First treatment group (T1) had at (10: 1) C/N ratio. The second treatment (T2) had (15: 1) C/N ratio. Third treatment (T3) had (20: 1) C/N ratio. All these treatments were run parallel to the control (T4) which contained pure commercial diet and a daily water exchange with no C/N ratio adjusted.

2.5. Fish growth analysis

Fish growth analysis was done by measuring the average length and average gain in fish weight. The percent gain in weight (%GW), percent gain in length (%GL), FCR, %SGR and %survival was calculated by applying Formulas 2 to 6 below.

$$\textit{Percent gain in weight } (\%GW) = \left[(W2 - W1) \ / \ (W1) \right] x100 \tag{2}$$

$$\textit{Percent gain in length } (\%GL) = \left[(L2 - L1) \ / \ (L1) \right] x100 \tag{3}$$

$$\textit{Feed conversion ratio } (FCR) = \textit{feed intake} / \% \textit{ weight gain} \tag{4}$$

$$\textit{Percent specific growth rate } (\%SGR) = \textit{final weight} - \textit{initial weight} / \ t \ x \ 100 \tag{5}$$

$$\textit{Percentage survival } (\% \textit{ survival}) = \textit{final number of fish} \ / \ \textit{initial number of fish } x100 \tag{6}$$

2.6. Physico-chemical analyses

During the whole time period of experiment, all the physiochemical parameters were monitored and adjusted where needed. These parameters included ammonia concentration mg/L (kit method), free CO_2 estimation chlorides, nitrates, phosphates and turbidity. TDS (total dissolved solids), dissolved oxygen, pH, electrical conductivity and salinity was checked with "JENWAY meter 4520" for all aquaria and compared with the control experiment.

2.7. Statistical analyses

All the obtained data was subjected to One-way ANOVA to check the significant differences of the means followed by Tukey's test to compares the means of all treatments to the mean of every other treatment among the growth parameters and water parameters at degree of freedom 6 and 95% level of confidence.

3. Results

Following observations were noted down during the experiment. Growth parameters and water parameters were recorded in Table 1 and Table 2 respectively. It was obvious from the results that T3 showed significant enhancement (28.12±0.30) in percent increase in fish weight, than T1 (18.72±0.36) and T2 (22.12±0.37) but it was non- significant from control (T4) i.e., (29.13±0.51). The percent increase in length was also enhanced in T3 (17.29±0.46) than T2 and T1 but lowered than control T4 (18.77±0.10). FCR recorded for T3 was (0.04±0.001) significantly better from T1 (0.07±0.001) and T2 (0.06±0.001) but much closer to control T4 (0.04±0.0005). Similarly the results for %

Table 1. Growth of *Ctenopharygodon idella* fingerlings under various C/N ratios after a rearing period of 60 days.

Treatment groups	Varied C/N ratios in aquaria fed with agri waste and 10% water exchange	Average Initial weight (g)	Average Final weight (g)	%gain in weight (g)	Average Initial length (cm)	Average Final length (cm)	%gain in length (cm)	FCR	%Specific growth rate	% survival
T1	Pure agri waste and 10% water exchange at C/N = 10:1	22.46	26.66	18.72±0.36[c]	25.55	27.40	7.22±0.25[c]	0.07±0.001[c]	7.01±0.13[c]	100
T2	Pure agri waste and 10% water exchange at C/N = 15:1	21.81	26.64	22.12±0.37[b]	24.76	27.96	12.91±0.49[b]	0.06±0.001[b]	8.04±0.09[b]	100
T3	Pure agri waste and 10% water exchange at C/N = 20:1	22.07	28.28	28.12±0.30[a]	24.86	29.16	17.29±0.46[a]	0.04±0.001[a]	10.34±0.04[a]	100
T4	Controlled (commercial feed and daily water exchange)	22.08	28.52	29.13±0.51[a]	25.65	30.46	18.77±0.10[a]	0.04±0.0005[a]	10.72±0.22[a]	100
p-value		<0.05								

Means within columns having different superscripts are significantly different at P < 0.05. Means sharing same superscripts are non significant to each other. Values are means ±SD of triplicates.

Table 2. Physico-chemical parameters of water under various C/N ratios in the BFT tank.

Treatment groups	Treatments under C/N ratios vs control	Physicochemical parameters of experimental and control tanks at 30ºC temperature										
		NH_3 conc. (mg/L)	Dissolved Oxygen (mg/L)	Salinity (g/L)	pH	Free carbon dioxide	Cl (ppm)	Nitrate	Phosphate	Electrical conductivity (ms)	TDS (mg/L)	Turbidity
T1	Pure agri waste and 10% water exchange at C/N = 10:1	$0.03^{a} \pm 0.002$	$33.08^{c} \pm 0.21$	$1.8^{a} \pm 0.04$	$8.3^{a} \pm 0.04$	$2.63^{b} \pm 0.01$	$1346^{a} \pm 2.05$	$0.563^{c} \pm 0.001$	$0.233^{d} \pm 0.003$	$5.19^{b} \pm 0.03$	$3258^{a} \pm 2.49$	$23.40^{c} \pm 0.02$
T2	Pure agri waste and 10% water exchange at C/N = 15:1	$0.02^{b} \pm 0.004$	$34.21^{bc} \pm 0.38$	$1.7^{a} \pm 0.04$	$8.2^{a} \pm 0.17$	$2.54^{b} \pm 0.05$	$1323^{b} \pm 1.70$	$0.757^{b} \pm 0.006$	$0.347^{c} \pm 0.002$	$5.35^{a} \pm 0.02$	$3252^{a} \pm 4.55$	$23.67^{b} \pm 0.03$
T3	Pure agri waste and 10% water exchange at C/N = 20:1	$0.01^{b} \pm 0.00$	$34.89^{ab} \pm 0.08$	$1.8^{a} \pm 0.04$	$8.3^{a} \pm 0.04$	$2.59^{b} \pm 0.01$	$1315^{b} \pm 1.24$	$0.768^{b} \pm 0.001$	$0.598^{b} \pm 0.00$	$5.46^{a} \pm 0.01$	$3265^{a} \pm 3.56$	$23.92^{a} \pm 0.01$
	T4 Controlled	$0.02^{b} \pm 0.004$	$35.54^{a} \pm 0.05$	$1.2^{b} \pm 0.04$	$8.1^{a} \pm 0.04$	$2.87^{a} \pm 0.004$	$1234^{c} \pm 1.24$	$0.889^{a} \pm 0.001$	$0.765^{a} \pm 0.00$	$1.92^{c} \pm 0.009$	$926^{b} \pm 1.70$	$10.75^{d} \pm 0.008$

Means within columns having different superscripts are significantly different at $P < 0.05$. Means sharing same superscripts are non-significant to each other. Values are means ±SD of triplicates.

specific growth rate were increased according to % gain in weight and recorded highest in T3 i.e., 10.34±0.04 among other treatments but lower than control i.e., 10.72±0.22. However, there was 100% survival in all treatments (T1, T2 and T3) and control (T4).

Similarly water parameters were recorded and given in Table T2. Ammonia (NH_3) levels were noted highest in T1 (0.03±0.002), followed by T2 (0.02±0.004), T4 (0.02±0.004) and T3 (0.01±0.00). The levels of NH_3 recorded in T1 were significantly highest than T2, T3 and T4 while the levels in T2, T3 and T4 were non-significant to each other.

Dissolved Oxygen (DO) levels were recorded highest in T4 (35.54±0.05), followed by T3 (34.89±0.08), T2 (34.21±0.38) and T1 (33.08±0.21). It was observed that the levels of dissolved oxygen were noted significantly highest in T4 while significantly lowest in T1. The levels obtained in T3 were noted non-significant with T4 and T2 while T2 was highly significant with T4. Similarly, the levels in T2 were non-significant with T1 and T3 while T1 was highly significant with T3.

Salinity was recorded highest in T1 and T3 which was (1.8±0.04) and (1.8±0.04) respectively, followed by T2 (1.7±0.04) and T4 (1.2±0.04). The levels of salinity in T1, T2 and T3 were significantly higher than T4 but non-significant to each other. T4 showed the significantly lower level amongst all.

pH was observed highest in T1 and T3 which was (8.3±0.04) and (8.3±0.04) respectively, followed by T2 (8.2±0.17) and T4 (8.1±0.04). The levels of pH in T1, T2, T3 and T4 were noted non-significant to on anther.

Free carbon dioxide (CO_2) levels were noted highest in T4 (2.87±0.004) followed by T1 (2.63±0.01), T3 (2.59±0.01) and T2 (2.54±0.05). The levels of free CO_2 obtained in T4 were significantly higher amongst all while the levels in T1, T2 and T3 were noted non-significant to each other.

The levels of chloride (Cl) were highest in T1 (1346±2.05) followed by T2 (1323±1.70), T3 (1315±1.24) and T4 (1234±1.24). The levels of chloride in treatments T1 were significantly highest than T2, T3 and T4 while the levels in T4 were significantly lowest amongst all. The levels in T2 and T3 were significantly lower than T1 and higher than T4 but they were non-significant to each other.

Nitrate (NO_3) levels were noted highest in T4 (0.889±0.001) followed by T3 (0.768±0.001), T2 (0.5757±0.006) and T1 (0.563±0.001). The values of nitrate obtained in treatments T4 were significantly highest amongst all. The values in T2 and T3 were lower than T4 but highest than T1. T2 and T3 were noted non-significant to each other while T1 was significantly lowest amongst all.

Phosphate (PO_4) levels were recorded highest in T4 (0.765±0.00) followed by T3 (0.598±0.00), T2 (0.347±0.002) and T1 (0.233±0.003). The levels of phosphate in treatments T1, T2, T3 and T4 were significantly different from one another.

Electrical conductivity (EC) levels were recorded highest in T3 (5.46±0.01) followed by T2 (5.35±0.02), T1 (5.19±0.03) and T4 (1.92±0.009). The levels of electrical conductivity in T2 and T3 were significantly higher than T1 and T4. The levels in T1 were significantly lower than T2 and T3 but higher than T4. The levels in T4 showed significantly lowest values.

Total dissolved solids (TDS) were noted highest in T3 (3265±3.56), followed by T1 (3258±2.49), T2 (3252±4.55) and T4 (926±1.70). All the values obtained for total dissolved solids in treatments T1, T2 and T3 were noted non-significant to each other but significantly higher than T4. The levels of TDS were significantly lowest amongst all.

Turbidity levels were highest in T3 (23.92±0.01) followed by T2 (23.67±0.03), T1 (23.40±0.02) and T4 (10.75±0.008). All the values obtained for turbidity in treatments T1, T2, T3 and T4 were significantly different from one another.

The significant differences among them were expressed in different superscripts.

4. Discussion

The research study was executed to assess the outcomes of carbon to nitrogen ratio (C/N ratio) adjusted at different levels in biofloc based aquaculture system. It was concluded from the experiment that higher amount of carbon against nitrogen used helped in reducing toxicity and had pronounced effects on fish growth. Linan-Cabello et al. (2002) and Ju et al. (2008) revealed that bioactive compounds found in the floc cultures were scrutinized to be powerful enhancers of growth and immunity in aquatic organisms. The inter connection between addition of carbohydrates, lowering down of ammonium and generation of microbial proteins rely upon the microbial conversion coefficient, the C/N ratio in the microbial biomass and the added carbon contents (Avnimelech, 1999).

Better results were come out in aquaria T3 having highest amount of carbon against nitrogen i.e., C/N (20: 1). T3 showed significant enhancement in fish growth, than T1and T2 but it was non- significant from control (T4). FCR recorded for T3 was significantly better from T1 and T2 but much closer to control (T4). Lower value of FCR stipulated the better up picking of ingredients given to fish. Sahzadi et al. (2006) observed the highest fish growth reared on sunflower meal with lowest FCR other than cottonseed and bone meal as well. The value of %SGR for T1 was significantly higher than T1 and T2 but non-significant with control (T4). From the account it was to be in view that if the carbon source is used in greater amount, the results came out closer to control condition which was far expensive than treatment groups. It was clearly explained that fish and other aquatic organisms could be reared under low cost conditions as in T3 to achieve the goals particularly great production of aquaculture products at cheaper prices and least utilization of water to save environment.

The physicochemical parameters such as ammonia (NH^3), dissolved oxygen (DO), salinity, pH, free carbon dioxide, chlorides (Cl), nitrates, phosphates, electrical conductivity, total dissolved solid (TDS) and turbidity were recorded at 30 °C temperature and compared among agri wastes and with controlled. The compatible results were obtained in aquaria and recorded at 30 °C. All the parameters under range for growth but T3 showed optimal values for each of the parameters shown in Table 2.

Toxic ammonia nitrogen excreted in aquaculture systems is checked by carbohydrates supplementation to grow bacteria resulting in microbial protein synthesis by subsequent removal of nitrogen from water that ultimately reduced toxicity. Naylor et al. (2000) also reinforced to develop sustainable aquaculture systems that least suffers the habitat. Da Silva et al. (2013) and Zhao et al. (2014) also fortified that it is necessary to augment the carbon source in order to increase the C/N ratio for bioflocs production in aquaculture systems, especially for treating pond wastewater with high nitrogen concentration.

Metabolic activities of organisms are highly influenced by temperature which is another important environmental factor. In this study the average optimal temperature recorded was 30 °C suitable for growth of C. *idella* reared on biofloc closed to the study done by Kurup and Prajith (2009) who recommended 31 °C to 36 °C optimal temperature for the growth of Nile tilapia. Santhosh and Singh (2007) also revealed that optimum water temperature for carp culture is between 24 and 30 °C.

5. Conclusion

Biofloc system works wonder where cheapest carbon sources are used to replenish the toxic nitrogen and produce high protein food. It not only provides low cost diet but also improves nutritional efficiency of culture system. Hence, with proven results, the main objective of the study was achieved. The conditions owned in treatment T3 (powdered banana peels, 10% water exchange and C/N ratio) are highly recommended to fish farmers to maintain a sustainable aquaculture system in low budgets.

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