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Nile tilapia culture under feeding restriction in bioflocs and bioflocs plus periphyton tanks

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ABSTRACT. Intensive aquaculture systems may cause pollution in water bodies because their effluents have high levels of nutrients. The use of substrates for periphyton and the manipulation of the C: N ratio of water are technologies that can be employed to increase aquaculture yield with environmental sustainability. The present study has aimed at determining whether feeding restriction could stimulate a greater use of natural food in three different Nile tilapia rearing systems (green water, bioflocs and biophyton), without growth performance impairment. There were nine treatments with four replicates each one (36 experimental units). The animals were raised in conventional (green water) tanks, C: N-ratio adjusted tanks (bioflocs) and bioflocs + periphyton integrated tanks (biophyton). In each culture system, the artificial diet was delivered regularly or under two levels of restriction (15 and 30%). In conventional tanks, fish growth performance was reduced by feeding restriction. Ammonia and nitrite were reduced in bioflocs tanks. Underwater structures for periphyton were not able to increase ammonia and nitrite removal. In bioflocs tanks, feeding restriction of 15% did not lessen fish weigh gain. Underwater structures for periphyton have not improved fish growth performance in any aspect.

Keywords: aquaculture, water quality, eutrophication.

Cultivo de juvenis de tilápia do Nilo, *Oreochromis niloticus* em sistemas convencional, bioflocos e biofíton sob restrição alimentar

RESUMO. Sistemas intensivos de aquicultura podem causar poluição de corpos hídricos, pois os efluentes gerados nesses sistemas são caracterizados pelas altas concentrações de nutrientes. O uso de substratos para perifíton e a manipulação da relação carbono: nitrogênio da água são tecnologias que podem ser empregadas no aumento da produtividade aquícola, com sustentabilidade ambiental. O presente trabalho teve por objetivo determinar se a restrição alimentar pode propiciar um maior aproveitamento do alimento natural, em três diferentes sistemas de cultivo (águas verdes, bioflocos e biofíton), sem prejuízo zootécnico. Foram constituídos nove tratamentos com quatro repetições cada um, totalizando 36 unidades experimentais. Os animais foram cultivados em tanques convencionais (águas verdes), com ajuste da relação C: N da água (bioflocos) e com integração entre bioflocos e perifíton (biofíton). Em cada sistema de cultivo, houve oferta regular ou sob restrição (15 e 30%) de ração artificial. Nos tanques convencionais, o desempenho produtivo dos peixes foi reduzido pela restrição alimentar. A presença de bioflocos reduziu a concentração de amônia e nitrito na água. A instalação das estruturas submersas em tanques com bioflocos não foi capaz de elevar a remoção de amônia e nitrito da água. Nos sistemas com bioflocos, o nível de restrição alimentar de 15% não causou prejuízo ao ganho em peso animal. A instalação de estruturas submersas para perifíton não afetou de modo significativo o peso corporal final dos peixes cultivados.

Palavras-chave: aquicultura, qualidade de água, eutrofização.

Introduction

Intensive aquaculture systems can cause eutrophication of nearby water bodies because their effluents have a high load of nutrients, especially nitrogen and phosphorus (Crab, Avnimelech, Defoirdt, Bossier, & Verstraete, 2007). The major part of the nitrogen that comes into these systems remains inside the water. The decomposition of wasted feed and animal feces release ammonia (Hu, Lee, Chandran, Kim, & Khanal, 2012). Therefore, the quality of aquaculture effluents deteriorate as the culture intensifies. Two possible ways to increase the aquaculture yield with environmental sustainability are (1) use of underwater substrates for periphyton and (2) correction of the C: N ratio of water (Avnimelech, 2006).

The adjustment of the C: N ratio of water is carried out by adding a carbon source to water, usually dry molasses, to boost the development of heterotrophic bacteria in water (bioflocs). These bacterial bioflocs may be used as a protein source by the rearing animals, immobilizing nitrogenous compounds and decreasing the likelihood of disease outbreaks (Emerenciano, Cuzon, Arievalo, & Gaxiola, 2014; Khatoon et al., 2016). On the other hand, bioflocs technology for aquaculture requires high aeration rates and high capital investment for its installation (Vilani et al., 2016).

Aquaculture systems based on substrate seek the development of algae and bacteria (periphyton) on the underwater structures. Periphyton is capable to filter the water and reduce the concentrations of toxic compounds such as ammonia. It can also be ingested by the rearing animals as food (Ferragut & Campos, 2010; Richard et al., 2010). Aquaculture systems based on substrate, however, are most suited for semi-extensive culture, that one with minimal artificial feed allowances and low levels of fish production (Azim, Beveridge, Van Dam, & Verdegem, 2005; Liu et al., 2016).

A new possibility is the use of tanks supplied with underwater structures and adjustment of the C: N ratio of water. In this mixed "biophyton" system, it is expected a higher provision of natural food and more water filtration. This new technology, however, has not been fully evaluated by the science until now. The present study has aimed at determining whether feeding restriction could stimulate a greater use of natural food in three different Nile tilapia rearing systems (green water, bioflocs and biophyton), without growth performance impairment.

Material and methods

One thousand sex-reversed Nile tilapia juveniles with 1.22 ± 0.08 g were obtained from a regional producer and transported to the laboratory facilities. After a 1-week acclimation period, the fish were transferred to thirty-six 250-L polyethylene circular outdoor tanks. After a 24 hours period of aeration to remove residual chloride, tap water was used to fill in the tanks. No water exchange was carried out over the entire study, just replenishment to keep up the initial level. All tanks had non-stop aeration provided by one air blower of 2 HP. The rearing tanks were intensely aerated to keep the particulate material suspended in water.

Culture tanks were populated with nine fish per tank (36 fish m^{-3}) for 10 weeks. Initially, the fish were fed with one commercial powdered diet for tropical fish (48% CP), allowed daily at 10.5% of the stocked biomass. Feeding rate was adjusted fortnightly according to fish growth. From 5 g body weight, a commercial diet measuring 0.8 – 1.2 mm (40.6% CP) was delivered daily at 4.7% of the biomass stored per day. The fish were fed every day at 8 a.m., 11 a.m., 2 p.m. and 5 p.m.

The experimental design was completely randomized with nine treatments and four replicates per treatment. There were four control groups and five experimental treatments (Table 1). The control groups were the followings: GW0: conventional culture of tilapia in green waters; GW15: the same as carried out in GW0 except by feeding restriction of 15%; GW30: the same as carried out in GW0 except by feeding restriction of 30%; and BF0: culture of tilapia in tanks with adjustment of C: N ratio of water to allow development of bioflocs. The experimental treatments were the followings: BF15: the same as carried out in BF0 except by feeding restriction of 15%; BF30: the same as carried out in BF0 except by feeding restriction of 30%; BP0: the same as carried out in BF0 except by installation of underwater structures for periphyton; BP15**:** the same as carried out in BP0 except by feeding restriction of 15%; BP30: the same as carried out in BP0 except by feeding restriction of 30%.

The C: N ratios of water in BF (0, 15 and 30) and BP (0, 15 and 30) tanks were adjusted daily to 15: 1 by application of dry molasses to water, following the guidelines presented by Schryver and Verstraete (2009). Two plain polyethylene boards were installed into BP (0, 15 and 30) tanks for periphyton growth. These boards were positioned vertically in the water column, having a superficial area of 0.90 m^2.

Table 1. Experimental design.

Treatment	Acronym	Culture system	Feeding restriction (%)
1	GW0	green waters	-
2	GW15	green waters	15
3	GW30	green waters	30
4	BF0	bioflocs	-
5	BF15	bioflocs	15
6	BF30	bioflocs	30
7	BP0	bioflocs + periphyton	-
8	BP15	bioflocs + periphyton	15
9	BP30	bioflocs + periphyton	30

The pH of water was recorded daily at 0800 and 1500 (MS Tecnopon, mPA210). Concentrations of dissolved oxygen (DO_2; Winkler method (with azide modification) and total ammonia nitrogen (TAN; indophenol method) were determined weekly (0800 – 0900). Non-ionized ammonia (NH_3) was estimated by the Emerson's formula according to El-Shafai, El-Gohary, Nasr, Steen, and Gijzen (2004). Total

alkalinity (titration with H_2SO_4 standard solution), total hardness (titration with EDTA standard solution), reactive phosphorus (molybdenum blue method), nitrite (sulfanilamide method) and nitrate (cadmium reduction method) were monitored fortnightly. All chemical analyses were carried out according to the guidelines provided by (American Public Health Association [APHA], 1999).

Growth performance variables were monitored as follows: survival, final body weight, specific growth rate (SGR = [(Ln final body weight – Ln initial body weight)/days of culture] x 100, fish yield [fish biomass gain (g)/tank volume (m^3)/day], feed conversion ratio (FCR = artificial diet allowance/body weight gain) and protein efficiency ratio (PER = body weight gain/feed protein allowance).

Initially, water quality and growth performance were submitted to normality (Shapiro-Wilk) and homogeneity of variance (Levene) tests. The conformist results were submitted to analysis of variance (one-way ANOVA) for completely randomized experiments. The significantly ($p < 0.05$) different means were compared two by two using Tukey's test. SPSS v.15 and Windows Excel 2010 software were used for statistical analyses.

Results and discussion

Water quality

The pH of water was significantly higher in tanks without bioflocs (GW0, GW15 and GW30). Feeding restriction did not affect pH in any treatment (GW, BF and BP). The pH of water remained always above 7 (Table 2). It is probable that more CO_2 has been released due to a greater bacteria activity in BF and BP tanks (Azim, Little, & Bron, 2008). BF and BP tanks require a stricter monitoring to avoid acidosis caused by CO_2 (Abbink et al., 2011). Dissolved oxygen (DO_2) did not differ among treatments ($p > 0.05$). DO_2 levels exceeded 6.4 mg L^{-1}in all tanks (Table 2).

The alkalinity was lower ($p < 0.05$) in BF and BP (Table 2). Since great amounts of free CO_2 are released by heterotrophic bacteria in bioflocs tanks, they frequently require regular liming to keep alkalinity high (Martins, Tarouco, Rosa, & Robaldo, 2017). If liming is not performed as required, alkalinity might decrease quickly (Xu, Morris, & Samocha, 2016). Feeding restriction raised alkalinity in bioflocs tanks (BF and BP). Lower variations of pH would be expected in tanks with greater feeding restriction levels. The better conditions of the water could have improved fish growth, but it did not happen. Feeding restriction at 30% had adverse nutritional effects not compensated by high water quality. Underwater structures for periphyton did not affect alkalinity. The hardness was greater ($p < 0.05$) in C: N-ratio adjusted tanks because dry molasses added calcium to water (Rostagno, 2011) (Table 2). Hardness was lower in green water tanks submitted to feeding restriction.

There was less organic matter in tanks without bioflocs ($p < 0.05$; Table 2). The concentration of total suspended solids is usually much higher in bioflocs tanks than in green water tanks (Rocha et al., 2012). BF and BP tanks submitted to feeding restriction presented less organic matter than the non-restricted ones. Feeding restriction reduced organic matter in BF slightly more than in BP. The differences in this regard, however, were not significant (BF15 x BP15; BP30 x BP30; $p > 0.05$). Periphyton might have been detached from the underwater structures, adding a little more organic matter to water.

Higher TAN and nitrite were observed in green water tanks when compared to BF and BP (Table 3). Bioflocs remove ammonia and nitrite from water in C: N ratio adjusted tanks (Lorenzo et al., 2016). The underwater structures did not affect TAN and nitrite in BP tanks. Similar results were obtained by Schveitzer et al. (2013). Feeding restriction did not significantly affect TAN, NH_3 and nitrite in any treatment (GW, BF, BP; Table 3). Therefore, no benefits in regard to TAN, NH_3 and NO_2^- were observed due to feeding restriction. These results disagree with Rebouças, Caldini, Cavalcante, Silva, and Sá (2012) who observed less TAN and NO_2^- in 30%-fed restricted tanks.

Table 2. pH, dissolved oxygen (DO_2; mg L^{-1}), total alkalinity (TA; mg L^{-1} $CaCO_3$), total hardness (TH; mg L^{-1} $CaCO_3$) and organic matter (mg L^{-1}) of Nile tilapia culture tanks (mean ± S.D.; n = 4).

Treatment[1]	Variable				
	pH	DO_2	TA	TH	Org matter
GW0	8.05 ± 0.23 a[2]	6.71 ± 1.34	110.6 ± 6.6 a	181.3 ± 5.9 b	94.4 ± 7.6 c
GW15	8.15 ± 0.31 a	6.70 ± 1.56	105.9 ± 4.1 a	169.1 ± 2.8 c	91.0 ± 7.3 c
GW30	8.09 ± 0.25 a	6.43 ± 1.43	104.3 ± 3.4 a	169.7 ± 9.3 c	85.9 ± 4.3 c
BF0	7.45 ± 0.41 b	7.82 ± 0.48	73.3 ± 12.8 c	276.8 ± 9.3 a	143.4 ± 11.5 a
BF15	7.9 ± 0.39 b	7.91 ± 0.77	87.8 ± 12.0 bc	256.3 ± 10.7a	129.0 ± 9.0 b
BF30	7.80 ± 0.61 b	7.85 ± 0.61	95.3 ± 9.7 b	248.1 ± 14.6a	127.4 ± 10.2 b
BP0	7.56 ± 0.53 b	8.02 ± 0.54	77.9 ± 14.6 bc	280.8 ± 49.6a	147.5 ± 11.8 a
BP15	7.76 ± 0.37 b	7.82 ± 0.72	84.2 ± 10.1 bc	276.1 ± 36.5a	137.9 ± 8.3 ab
BP30	7.47 ± 0.58 b	7.99 ± 0.43	93.6 ± 7.4 b	267.7 ± 35.4a	135.1 ± 10.8 ab
ANOVA P	<0.05	ns[3]	<0.001	<0.001	<0.001

[1]Please see Table 1; [2]In each column, means with distinct letters are significantly different among themselves by Tukey's test ($p < 0.05$). Absence of letters indicates no significant differences ($p > 0.05$).[3]Not significant ($p > 0.05$).

Table 3. Concentrations of total ammonia nitrogen (TAN), NH_3, nitrite, nitrate and reactive phosphorus, in mg L^{-1}, in Nile tilapia culture tanks (mean ± S.D.; n = 4).

Treatment[1]	Variable				
	TAN	NH_3	Nitrite	Nitrate	React phosphorus
GW0	0.68 ± 0.14 a[2]	0.23 ± 0.07 a	0.38 ± 0.09 a	23.4 ± 1.8 b	0.11 ± 0.01 c
GW15	0.54 ± 0.15 a	0.31 ± 0.06 a	0.27 ± 0.05 a	26.1 ± 2.8 b	0.11 ± 0.01 c
GW30	0.52 ± 0.09 a	0.26 ± 0.06 a	0.28 ± 0.10 a	20.1 ± 3.2 b	0.06 ± 0.01 d
BF0	0.17 ± 0.06 b	0.06 ± 0.04 b	0.20 ± 0.05 b	39.4 ± 2.3 a	0.39 ± 0.03 a
BF15	0.12 ± 0.08 b	0.05 ± 0.03 b	0.19 ± 0.07 b	42.2 ± 3.2 a	0.35 ± 0.03 a
BF30	0.16 ± 0.07 b	0.06 ± 0.03 b	0.18 ± 0.06 b	52.7 ± 4.2 a	0.30 ± 0.03 b
BP0	0.15 ± 0.04 b	0.06 ± 0.02 b	0.18 ± 0.07 b	50.2 ± 3.1 a	0.37 ± 0.03 a
BP15	0.20 ± 0.05 b	0.08 ± 0.04 b	0.23 ± 0.08 b	39.6 ± 4.2 a	0.37 ± 0.03 a
BP30	0.16 ± 0.08 b	0.07 ± 0.02 b	0.16 ± 0.05 b	48.5 ± 3.7 a	0.30 ± 0.03 b
ANOVA P	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Please see Table 1; [2]In each column, means with distinct letters are significantly different among themselves by the Tukey's test ($p < 0.05$).

The nitrate was higher in bioflocs tanks than in green water tanks ($p < 0.05$; Table 3). Probably, nitrifying bacteria grew along with heterotrophic bacteria in bioflocs tanks. More nitrate is generally found in nitrifying bacteria rich tanks (Zhao et al., 2012). The underwater structures in BP tanks did not affect nitrate ($p > 0.05$).

There was less reactive phosphorus in green water tanks than in BF and BP (Table 3). Reactive phosphorus was greater in bioflocs tanks (BF and BP) probably due to their higher rate of organic matter mineralization. Similar results were also found by Nancharaiah, Reddy, Mohan, and Venugopalan (2015) and Lorenzo et al. (2016). In the present study, the underwater structures installed in BP tanks did not remove more phosphorus from water in relation to the BF tanks. The same was observed by Schveitzer et al. (2013). Therefore, installation of artificial substrates in BFT tanks aiming a cleaner effluent seems unfeasible. Reactive phosphorus was significantly lowered by feeding restriction in all tanks (GW, BF, BP), only at the highest level of restriction (30%) that was a direct effect of reduced input of phosphorus through feeding.

Growth performance

Survival of fish was high in all treatments with no significant differences among them (Table 4). Fish raised in green water tanks exhibited a lower ($p < 0.05$) final body weight when compared to BF and BP. Improved fish growth in BP and BF was probably due to their higher feed availability and better water quality (less TAN and NH_3).

Regardless the treatment, tanks submitted to 30% feeding restriction level had lower final body weight ($p < 0.05$). While fish body weight decreased in green water tanks submitted to 15% or more feeding restriction, the same was only observed in BF and BP for 30% restriction (Table 4). Feeding restriction of 15% did not cause any damage in BF and BP growth performance. On the other hand, the underwater structures brought no benefit when installed in BF tanks.

Fish submitted to 30% feeding restriction had a poor SGR in all tanks (Table 4; $p < 0.05$). Correia et al. (2014) observed similarly a lower SGR in BFT tanks submitted to protein restriction. The lowest fish yield was seen in green water tanks. In bioflocs tanks (BF and BP), the best fish yields were observed for 0 and 15%-fed restricted tanks (Table 4).

FCR was not significantly different among treatments (Table 4). This result disagrees with Jatobá et al. (2014) who found a better FCR in bioflocs tanks. In the present work, a poor FCR was expected in the 30% fed-restricted tanks due to their lower fish body growth. However, as the feed allowances were adjusted fortnightly according to fish growth and biomass, lower amounts of feed were delivered to the 30%-fed restricted tanks. That has probably avoided an even poorer FCR results in these tanks. On the other hand, FCR deterioration is expected in commercial bioflocs tanks because their adjustments in feeding allowance are less precise.

Protein efficiency ratio (PER) was significantly improved due to feeding restriction (Table 4). However, PER considers only artificial protein, not counting the natural protein derived from bioflocs and periphyton. This explains why the best PER in each treatment was seen in the highest feeding restriction level. In these tanks, despite the lower artificial protein allowance, the fish had natural food available. From an environmental standpoint, a high PER is important because less ammonia is released to water. However, as fish growth was hampered by 30% feeding restriction, the best solution to compromise growth performance and environmental protection is the adoption of the 15% feeding restriction management. Periphyton protein was not able to improve fish growth when present in bioflocs tanks.

Table 4. Growth performance of Nile tilapia juveniles (initial body weight = 1.22 ± 0.08 g; mean ± S.D.; n = 4).

Tr[1]	Variable[2]					
	Survival (%)	FBW (g)	SGR (% day^{-1})	FY (g m^{-3} day^{-1})	FCR	PER
GW0	91.7 ± 11	27.3 ± 2.5 b[3]	5.4 ± 0.2 b	14.6 ± 1.7 c	1.17 ± 0.1	3.5± 0.1 c
GW15	91.7 ± 6	23.3 ± 2.3 c	5.2 ± 0.2 b	12.5 ± 1.7 d	1.03 ± 0.1	3.6± 0.2 c
GW30	97.2 ± 6	20.6 ± 0.8 c	4.7 ± 0.1 d	11.0 ± 0.4 d	1.06 ± 0.1	3.8±0.2 b
BF0	77.8 ± 9	35.6 ± 1.8 a	5.6 ± 0.1 a	19.7 ± 2.4 a	1.01 ± 0.1	3.9± 0.2 b
BF15	86.1 ± 11	33.1 ± 2.3 a	5.5 ± 0.2ab	17.7 ± 2.3 ab	1.03 ± 0.1	4.3± 0.2 ab
BF30	91.7 ± 11	29.1 ± 1.2 b	5.0 ± 0.1 c	15.6 ± 1.54bc	1.10 ± 0.1	4.6± 0.3 ab
BP0	92.6 ± 5.7	34.4 ± 2.0 a	5.67 ± 0.13 a	18.43 ± 1.05 a	1.04 ± 0.1	3.9± 0.1 b
BP15	84.4 ± 16.9	33.3 ± 3.4 a	5.50 ± 0.24 a	17.84 ± 1.33 ab	1.13 ± 0.1	4.8 ± 0.3 a
BP30	91.1 ± 9.3	30.4 ± 2.7 b	5.00 ± 0.26 c	16,30 ± 1,21 b	1.03 ± 0.1	4.9± 0.3 a
P	ns[4]	<0.001	<0.05	<0.001	ns	<0.001

[1]Please see Table 1; [2]FBW: final body weight, SGR: specific growth rate = [(ln final body weight – ln initial body weight)/days of culture] x 100; FY: fish yield; [3]In each column, means with distinct letters are significantly different among themselves by the Tukey's test ($p < 0.05$). Absence of letters indicates no significant differences ($p > 0.05$);[4]Not significant ($p > 0.05$).

Conclusion

In conventional green water tanks, tilapia growth performance may be reduced by feeding restriction. In bioflocs tanks, it is possible to reduce feeding rates up to 15% without damage in fish growth performance.

Underwater structures for periphyton installed in bioflocs tanks may not improve water quality and growth performance.

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