



Imbalanced C/N - controlled, periphyton-based system has hampered tilapia growth in stagnant experimental tanks

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ABSTRACT. The simultaneous use of periphyton and controlled C/N ratio of water may improve water quality and fish growth. The current assay investigated the interaction between periphyton and C/N ratio of water in rearing tanks with Nile tilapia juveniles. The study was carried out in 20 outdoor stagnant 250-L tanks. A wooden structure for periphyton development was submerged in five of the tanks. A completely randomized 2 x 2 factorial mode design was employed to evaluate the following factors: (1) substrate for periphyton and (2) the C/N ratio of water. Dry molasses were applied weekly in the tanks to raise C/N ratio of water to 20:1. The addition of molasses to the culture water significantly lowered DO₂ and pH levels of water, and raised nitrite concentration. Fish stocked in the control tanks (no periphyton, no C/N ratio balance) attained a final body weight significantly higher than those observed for other treatments after 6 weeks of culture. In spite of the correction of C/N ratio of water to 20: 1, low DO₂ concentrations avoided the suitable development of bioflocs.

Keywords: fish culture, bioflocs, natural food, bioturbation effect.

Mau dimensionamento de sistema baseado em perifíton com relação C/N controlada prejudicou o crescimento da tilápia em tanques experimentais estáticos

RESUMO. O uso simultâneo de perifíton e de relação C/N balanceada pode elevar a qualidade da água e o crescimento dos peixes. O presente trabalho investigou a interação existente entre o perifíton e a relação C/N da água em tanques de cultivo de juvenis de tilápia do Nilo. O estudo foi realizado em 20 tanques estáticos de 250 L. Em cinco dos tanques, foi submersa uma estrutura de madeira para o desenvolvimento de perifíton. O delineamento completamente casualizado em arranjo fatorial 2 x 2 foi empregado na avaliação dos seguintes fatores: (1) substrato para perifíton e (2) relação C/N da água. Aplicações semanais de melaço em pó foram feitas em determinados tanques para elevar a relação C/N da água para 20:1. A adição de melaço na água de cultivo diminuiu significativamente o O₂D e o pH da água e elevou a concentração de nitrito. Os peixes estocados nos tanques controle (sem perifíton e sem correção da relação C/N) obtiveram peso corporal final significativamente maior em relação ao observado nos demais tratamentos, após seis semanas de cultivo. Apesar da correção da relação C/N da água para 20:1, as baixas concentrações de O₂D da água impossibilitaram o desenvolvimento adequado de bioflocos.

Palavras-chave: piscicultura, bioflocos, alimento natural, bioturbação.

Introduction

There is currently a great concern on the possible environmental impacts of aquaculture. It is well known that fish farm's effluents are nutrient rich, especially for nitrogen and phosphorus. If released directly into the receiving water bodies, fish farm effluents may cause eutrophication and, consequently, deterioration and loss of biodiversity (LEFRANÇOIS et al., 2010).

Among the proposed solutions to the eutrophic fish farm effluents problem, the employment of culture systems based on minimal or even no water

exchange should be highlighted. These systems rely on the self-cleaning capacity of tanks which should be suitably stimulated by appropriate methods. Accordingly, the biological processes that remove nutrients from the culture water, such as photosynthesis and heterotrophic bacteria (bioflocs) growth, are suitably promoted in those systems (AZIM; LITTLE, 2008).

Although photosynthesis may significantly remove ammonia and phosphorus from water, its effects may be insufficient to obtain the desired water quality. The use of submerged substrates for periphyton, a cluster of algae, bacteria, protozoa etc,

increases the removal of nutrients from the water and function as an efficient biofilter (MILSTEIN et al., 2008). Another management able to achieve the same results is the water's C/N ratio balance. It has been reported that when the water's C/N ratio is close to 20:1, the formation of heterotrophic bacteria biomass (bioflocs) is boosted. Bioflocs remove ammonia from the culture water and, additionally, provide high-quality food for the farmed animals (AVNIMELECH, 1999).

Several researches have been carried out on substrates- and bioflocs-based aquaculture systems (ASADUZZAMAN et al., 2009a and b; AVNIMELECH; KOCHBA, 2009; AZIM et al., 2002; CRAB et al., 2007, 2009). However, very few assays have studied the simultaneous use of the two managements and their effects on the water quality and growth performance of Nile tilapia farmed in stagnant water tanks. Knowledge on the organic matter fish-driven re-suspension effect and the optimum fish stocking density/feeding rate ratio is especially lacking. The C/N ratio controlled, periphyton-based technology is of interest to poor aqua-farmers in developing countries which cannot invest in intensive aeration systems (ASADUZZAMAN et al., 2010b). The current research investigated the interaction between periphyton and the C/N ratio of water in Nile tilapia juvenile's rearing tanks.

Material and methods

Fish and culture system

One thousand sex-reversed Nile tilapia juveniles, *Oreochromis niloticus*, Chitralada strain, were obtained from a commercial fish producer and transported by road to the laboratory facilities. Fish were initially stocked at the laboratory for four days in one 1,000 L-polyethylene tank with constant aeration for acclimatization. During this phase, fish were fed on a commercial 45% crude protein diet for tropical fish at a feeding rate of 10% stocked biomass per day (Nutreco Fri-Ribe Animal Nutrition, Maracanaú, Ceará State, Brazil). The daily ration was split into even meals at 0800, 1100, 1400 and 1700h.

The assay was carried out in the outdoor culture system of the laboratory. This system has 20 round polyethylene 250 L-tanks. First, de-chlorinated tap water was used to fill the tanks. A wooden structure for periphyton development was submerged in ten of the culture tanks (Figure 1). The available surface area for periphyton in the structures was equivalent to 100% of the tank surface area ($\approx 0.7 \text{ m}^2$).

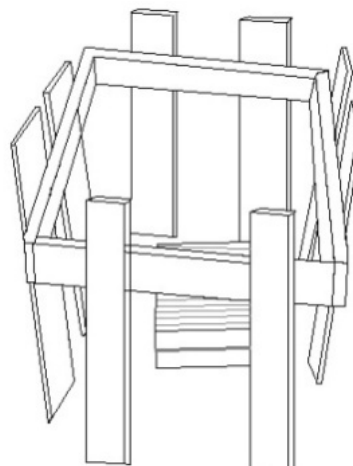


Figure 1. Submerged wooden structure for periphyton colonization.

All tanks received 50 mL of phytoplankton-rich water per day, starting from two weeks before fish stocking, to promote microalgae growth. When the color of the culture water was faint green, the phytoplankton inoculations stopped altogether. Moreover, a small quantity of a commercial diet for fish was used to fertilize the water ($1 \text{ g tank}^{-1} \text{ day}^{-1}$). Afterwards, ten Nile tilapia juveniles ($0.43 \pm 0.03 \text{ g}$) were stocked in each tank and maintained for six experimental weeks. No water exchange was performed during the entire experimental period. New freshwater was added just to keep the water level unchanged.

Experimental design and feed management

A completely randomized design arranged in a 2×2 factorial mode was employed to evaluate simultaneously the following factors: (1) substrate for periphyton (absence or presence) and (2) the C/N ratio of water (free or adjusted to 20:1). The control tanks had no substrate for periphyton and no balance of the C/N ratio of water. The 'Perip' (periphyton) tanks had one submerged structure per tank for periphyton but no balance of the C/N ratio of water. The 'C/N' tanks received weekly applications of molasses to correct their C/N ratio of water to 20:1 but no substrate for periphyton. Finally, the 'Perip + C/N' tanks had one submerged structure per tank and received weekly applications of dry molasses to balance their C/N ratio of water to 20: 1. Each control or treatment group had five replicates (20 tanks).

Although the standard method to raise the C/N ratio of culture water is based on the chemical composition of feeds given to fish and on the supplemental C source used (NOOTONG; PAVASANT, 2011), an alternative methodology was employed in the current assay for simplicity sake.

This new method adjusted weekly the C/N ratio of water based solely on its total ammonia nitrogen (TAN) concentration. Since the final disposal of most ($\pm 75\%$) of the added organic nitrogen is the culture medium, as inorganic nitrogen, mainly as TAN (CRAB et al., 2012), it seemed reasonable to consider TAN as a useful index to estimate the concentration of total organic nitrogen in water. Accordingly, the concentrations of TAN of culture waters were determined weekly with a spectrophotometer at 640 nm by the phenate method (APHA, 1999). Next, dry molasses were applied to the C/N and Perip + C/N tanks to add 20 times more organic carbon in water in relation to their TAN concentrations. For example, if the TAN concentration in water was 1 mg L^{-1} , there would be $250 \text{ mg TAN tank}^{-1}$ ($1 \text{ mg} \times 250 \text{ L}$). In this case, the required application of organic carbon to raise the C/N ratio of water to 20:1 would be $250 \text{ mg} \times 20 = 5,000 \text{ mg}$ or $5 \text{ g organic C tank}^{-1}$. Since 40% of dry molasses is approximately carbon (LIMA-SILVA et al., 2009), a simple cross-multiplication was carried out to obtain the total quantity of molasses to be added in the tank (12.5 g tank^{-1} , in the above example). The dry molasses were evenly scattered over the tank surface and mixed with a stick.

Throughout the experiment, fish were fed on balanced commercial diets formulated for tropical fish nutrition (FriAcqua Inicial and Alevinos, Nutreco Fri-Ribe Animal Nutrition, Maracanaú, Brazil), at daily feeding rates ranging between 11.9 and 4.0% of fish body weight, and split in four meals (0800, 1100, 1400 and 1700h).

Experimental variables

Water transparency was observed at 0900 h daily using a Secchi disk. Twice a week, at 0900 and 1600h, water temperature, electrical conductivity (EC) and pH were monitored by portable equipments (Instrutherm digital thermometer, Lutron CD-4301 water conductivity meter and Marconi MA522 pH-meter, respectively). Concentrations of total ammonia nitrogen (TAN) of culture waters were determined by the phenate method at 0900h, every week. Water samples were taken fortnightly from the experimental tanks to determine their concentrations of dissolved oxygen (DO_2 ; Winkler method with azide modification), nitrite (diazotizing and coupling method) and reactive phosphorus (ammonium molybdate method). The water quality variables were determined according to standard methods (APHA, 1999).

Growth performance variables reported in the present assay were the following:

- Fish survival;
- Final body weight;
- Specific growth rate (SGR):

$$SGR = \left(\frac{\text{Ln final body weight} - \text{Ln initial body weight}}{\text{rearing days}} \right) \times 100 \quad (1)$$

- Yield (Y):

$$Y = \frac{\text{Final biomass of fish (g)}}{\text{Tank volume (m}^3\text{)}} \quad (2)$$

- Food conversion ratio (FCR):

$$FCR = \frac{\text{weight of feed offered (g)}}{\text{fish weight gain (g)}} \quad (3)$$

Statistical analysis

Water quality results were analyzed by two-way ANOVA. Since no significant interactions were detected between the experimental factors (periphyton and C/N ratio of water), the growth performance variables were analyzed by one-way ANOVA. The significantly different means were compared two-by-two by Tukey's test. Normal distributions and homogeneity of variances were checked before analysis. Percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA analyses were carried out at 5% level of significance using SigmaStat for Windows 2.0 (Jandel Statistics, USA).

Results and discussion

Water quality

The temperature of culture water at 0800 and 1600h during the experimental period ranged between 25.4 and 26.9°C and between 27.4 and 29.9°C , respectively. These temperatures were within the range for normal tilapia growth (AZAZA et al., 2008). Water electrical conductivity (EC) progressively increased during the experimental period, rising from $674 \mu\text{S cm}^{-1}$ to $1,051 \mu\text{S cm}^{-1}$ in the end. No significant difference in EC was reported between the tanks.

The application of molasses to the culture water significantly reduced the Secchi disk depth ($p < 0.05$), valid for tanks with and without substrate for periphyton. No significant effect was observed on Secchi disk depth due to periphyton growth (Table 1). Instead of promoting biofloc growth, the molasses applications carried out in the C/N and Perip + C/N tanks probably boosted only phytoplankton and periphyton, as indicated by the

color of the culture water in these tanks, deep green from the midterm up to the end. Low levels of dissolved oxygen (DO₂) in water probably avoided the flourishing of bioflocs in the present assay. Therefore, the application of molasses without a correspondent increase in the rate of water aeration and circulation was a useless and maybe harmful water quality management in aquaculture.

The water samplings to determine DO₂ were taken between 0800 and 0900h. Early morning concentrations of DO₂ were low, generally below 4 mg L⁻¹. The addition of molasses to culture water significantly lowered DO₂ levels in the non-periphyton tanks. The opposite effect was seen in the periphyton tanks, albeit with no statistical significance (Table 1). The applications of molasses in the C/N and Perip + C/N tanks increased their organic carbon load and raised their biological demand for oxygen. Therefore, the application of molasses or other sources of organic carbon to the culture water demand caution because it may lead to environmental hypoxia.

The application of molasses significantly lowered the pH of water in all tanks (Table 1). The decomposition of molasses released CO₂ into the water and lowered its pH. The same effect was observed by Tendencia et al. (2012) in a study on *Penaeus monodon*. Therefore, it is advised to lime fish ponds before or after the application of molasses to avoid water acidification.

No significant differences for TAN were reported between tanks. On average, the concentration of TAN in water was 0.55 ± 0.02 mg L⁻¹. These results disagree with those from Asaduzzaman et al. (2008, 2010b) who found a significant decrease in the inorganic N concentrations of water when C/N ratio was

manipulated. In the present assay, the high stocking density of fish employed and the use of a disproportionally big submerged substrate for periphyton (Figure 1) have probably not allowed the necessary re-suspension of fish-driven particulate organic matter in the C/N and Perip + C/N tanks.

Significant effects of periphyton and C/N ratio balance were observed on nitrite concentration in water. The application of molasses significantly increased the concentrations of nitrite, especially in the periphyton tanks. Further, nitrite concentrations in the periphyton tanks were significantly higher than those in the non-periphyton ones (Table 1). Since very few, if any, bioflocs grew in the culture tanks, the molasses applications carried out in the present work only increased the biological demand for dissolved oxygen. Consequently, the nitrification process might have stopped at the nitrite point due to the water's low DO₂ levels (HARGREAVES, 1998).

In the non-periphyton tanks, the correction of the C/N ratio of water to 20:1 increased the concentration of reactive phosphorus. The same effect, however, was not observed in the periphyton tanks. Moreover, the periphyton increased the concentration of reactive phosphorus in tanks with free C/N ratio. Contrastingly, the periphyton did not significantly affect the concentration of phosphorus when compared to that of the non-periphyton tanks in the 20:1 C/N ratio tanks. The addition of organic carbon (molasses) to the water acted as a good fertilizer and boosted phytoplankton. That effect probably increased the organic and inorganic phosphorus load of the culture water. A similar effect was elicited by the submerged structure for periphyton growth.

Table 1. Water quality in 250 L outdoor culture tanks, supplied or not with substrate for periphyton, and with free or balanced C/N ratio (to 20:1). Ten Nile tilapia juveniles (0.4 g) were stocked in each tank for 6 weeks (mean ± s.d.; n = 5).

Variable	C/N ratio of water	Substrate for periphyton				
		No	Yes			
Secchi's depth	Free	30 ± 3.3 A ¹	31 ± 4.5 A			
(cm)	20:1	21 ± 3.8 B	17 ± 3.4 B			
DO ₂	Free	3.8 ± 0.9 Aa	3.2 ± 0.2 Aa			
(mg L ⁻¹)	20:1	2.6 ± 0.5 Ba	4.0 ± 0.7 Ab			
pH ²	Free	8.8 ± 0.1 Aa	8.3 ± 0.2 Ab			
	20:1	8.5 ± 0.4 Ba	8.1 ± 0.2 Bb			
TAN	Free	0.52 ± 0.08	0.55 ± 0.09			
(mg L ⁻¹)	20:1	0.57 ± 0.12	0.58 ± 0.10			
NO ₂ ⁻	Free	0.04 ± 0.01 Aa	0.09 ± 0.02 Ab			
(mg L ⁻¹)	20:1	0.07 ± 0.02 Ba	0.55 ± 0.03 Bb			
Reactive phosphorus	Free	0.147 ± 0.04 Aa	0.239 ± 0.05 Ab			
(mg L ⁻¹)	20:1	0.255 ± 0.05 Ba	0.227 ± 0.06 Aa			
Two-way ANOVA P						
Factor	Secchi	DO ₂	pH	TAN	NO ₂ ⁻	Rc-P ⁴
C/N ratio of water	< 0.001	< 0.05	< 0.05	ns	< 0.001	< 0.05
Substrate for periphyton	ns ³	< 0.05	< 0.01	ns	< 0.001	< 0.05
Interaction	ns	< 0.05	ns	ns	ns	< 0.05

¹In each variable, means in the same row or column without the same small or capital letter, respectively, are statistically different by the Tukey's test (p < 0.05); absence of letters means no significant difference; ²pH of water at 1600h; ³Not significant (p > 0.05); ⁴Reactive phosphorus.

Growth performance

Fish survival was very high in all tanks ($\geq 98\%$) with no significant difference between treatments. However, the interventions made in the culture tanks, namely, the installation of substrates for periphyton and the correction of the C/N ratio of water to 20:1, significantly impaired fish growth.

Starting from a body weight of 0.43 ± 0.03 g, fish stocked in the control tanks (no periphyton, no C/N ratio correction) attained, after 6 weeks of culture, a final body weight (15.3 ± 1.14 g) significantly higher than that observed for the other treatments (Figure 2). The differences observed between the treatments Perip (13.2 ± 2.1 g), C/N (13.7 ± 1.9 g) and Perip + C/N (13.1 ± 1.5 g) for fish's final body weight were not significant between themselves (ANOVA $p > 0.05$).

The substrate-based aquaculture system proved to be valuable to produce fish at a low cost (ASADUZZAMAN et al., 2008). However, its advantages become notorious only if the proper combination of fish stocking density and feeding rate is achieved (AZIM; LITTLE, 2006; REBOUÇAS et al., 2012). The growth performance of fish will be probably poor if there

is an imbalance in the fish stocking density/feeding rate ratio (SD/FR ratio). A high SD/FR ratio would result in growth delay due to fish malnourishment. On the other hand, a low SD/FR ratio would impair fish growth due to poor water quality (low DO_2 + high NH_3). In the current assay, the feeding rates employed ($11.9 - 4.0\%$ of the stocked biomass day^{-1}) revealed themselves to be excessive to a stocking density of 40 fish m^{-3} or 268 mg fish L^{-1} . It was a typical case of low SD/FR ratio (not due to low SD but high FR). In such a situation, the use of periphyton by fish as a feed source had minor importance. Besides, the water quality in the periphyton tanks with low SD/FR ratio was poor. Better growth performance results in the periphyton tanks would probably have been attained if a higher SD/FR ratio had been used in the present assay (by lowering feeding rates). The fish growth impairment observed in the periphyton tanks might have been due to their low DO_2 and high ammonia levels. Asaduzzaman et al. (2010a) also observed under-utilization of the natural food (plankton, periphyton and bacteria) in ponds with *Macrobrachium rosenbergii*. Similarly to the presente work, the above authors probably employed a low SD/FR ratio too.

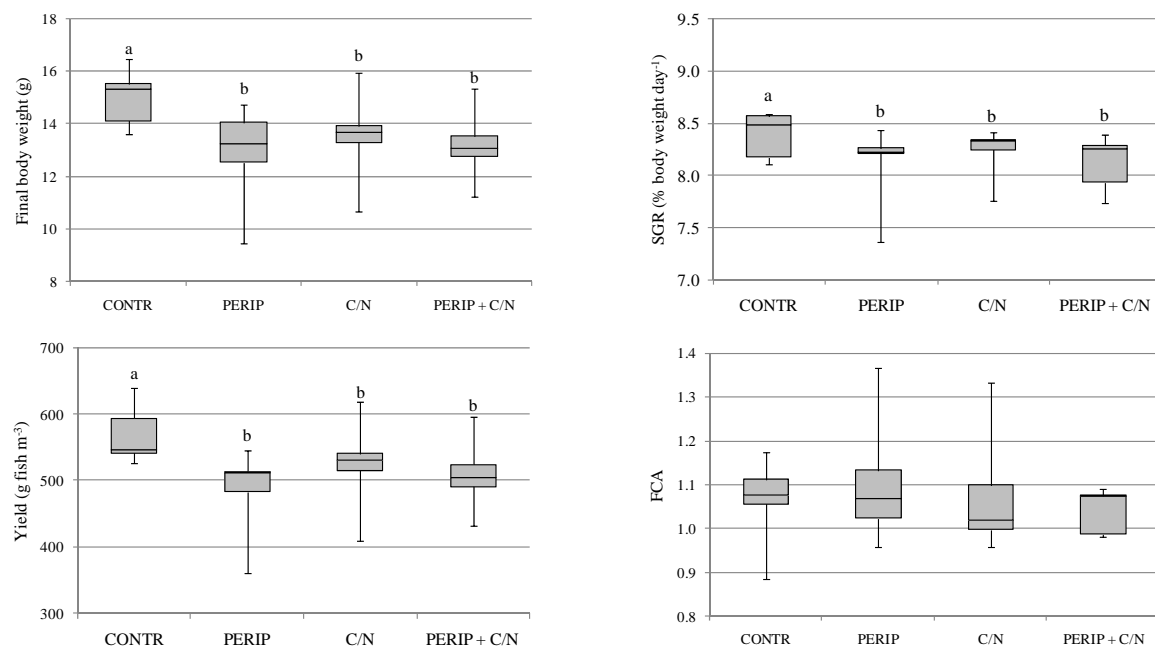


Figure 2. Box plots of Nile tilapia juveniles' final body weight, specific growth rate (SGR), tank yield and food conversion ratio (FCR). Ten fish were stocked for six weeks in twenty 250 L outdoor tanks ($n = 5$). CONTR: no periphyton and no C/N ratio correction; PERIP: tanks with substrate for periphyton only; C/N: tanks with correction of C/N ratio of water to 20:1 only; PERIP + C/N: tanks with periphyton plus C/N ratio correction. Boxes with different letters represent medians significantly different between themselves by Tukey's test ($p < 0.05$). No letters mean absence of statistical significance.

The substrate-based systems for aquaculture are classified as semi-extensive. The substrates added for periphyton development allow that higher stocking densities of fish be employed when compared to densities in the extensive systems (ASADUZZAMAN et al., 2009a). It is possible in the substrate-based systems to attain acceptable fish growth with very low usage of artificial food by employing the right stocking density of fish or a correctly balanced SD/FR ratio, as discussed above.

The addition of molasses to the culture water failed to stimulate the formation of bioflocs as initially expected. In spite of the correction of the C/N ratio of water to 20:1, the low DO₂ concentrations in the culture tanks probably avoided the suitable development of bioflocs. Further, it is also suggested that the fish swimming activity in the culture tanks (bioturbation effect) was not sufficient to re-suspend successfully the particulate organic matter to the water column, as required by the biofloc technology. Although the movements of tilapia in the tank bottom might have raised up some organic matter to a more oxygenated water layer (JIMÉNEZ-MONTEALEGRE et al., 2002), the proportionally oversize wooden structure in the periphyton tanks made the broad swimming movements of fish very difficult during the current assay.

Conclusion

It is of paramount importance in C/N ratio controlled, periphyton culture systems, carried out in stagnant tanks, that the farmed fish have enough free space to swim and efficiently re-suspend the settled particulate organic matter to the water column. Accordingly, the submerged substrates for periphyton development should occupy just a small area of the pond or tank's total area. Besides, it is necessary to pay great attention to the fish stocking density/feeding rate ratio employed in the C/N ratio controlled, periphyton system for aquaculture be successful. Imbalances in the fish stocking density/feeding ratio may cause poor growth performance.

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