



## Communication

[Comunicação]

### Dietary cost of Nile tilapia subject to food restriction in a biofloc system

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[Custo com alimentação de tilápia-do-nilo submetidas à restrição alimentar em sistema de bioflocos]

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The biofloc system (BFT) is a sustainable alternative for fish production because it reduces water use more than 150 times over that of conventional systems. Furthermore, since water is not renewed, the load of nutrients discarded in adjacent environments is reduced (Jatobá *et al.*, 2019); instead, they are reused by microorganisms to form bioflocs (Avnimelech, 2015; Ebeling *et al.*, 2006). The presence of microbiota in BFT, in turn, contributes to good growth performance, while reducing feed conversion (Jatobá *et al.*, 2019). According to the literature, BFT can reduce the feed supply for Nile tilapia by up to 20% (Pérez-Fuentes *et al.*, 2018), while maintaining the same productivity as that of conventional and clear water culture systems, respectively. Thus, BFT becomes an attractive alternative as food is considered the most expensive cost in Nile tilapia production (more than 60%), while management, including labor, represents the second highest cost of fish farming, around 14% (Trombeta *et al.*, 2017).

To further reduce these costs, fish farm operators are looking to food restriction, which has already been tested in some studies with Nile tilapia at different feeding frequencies. Nile tilapia reared in BFT showed compensatory growth after feeding cycles for 36 days and 12 subsequent days of restriction (Gallardo-Collí *et al.*, 2020). Therefore, it is possible to apply food restriction in BFT, but it is still necessary to determine the appropriate frequency (Correa *et al.*, 2020).

Even for Nile tilapia reared in BFT, production costs are still driven mainly by the cost of feed and labor. Dietary restriction at the proper frequency could allow for further reduction of these costs, while maintaining performance.

However, the feasibility of implementing such a food management plan requires further study. Thus, this work aimed to evaluate the effects of food restriction for one day out of seven on juvenile Nile tilapia (*Oreochromis niloticus*) reared in a biofloc system (BFT). Specifically, dietary costs, growth performance, water quality and body indexes were all determined.

The experiment and analyses were carried out at the Aquaculture Laboratory of the Instituto Federal Catarinense – *Campus* Araquari and approved by the animal ethics committee (protocol no. 308/2019 CEUA/IFC-Araquari).

Fourteen days before settlement, water in the experimental units was fertilized with a carbon source (sugar) and a nitrogen source (powdered feed), resulting in an initial solids concentration of 200.00 (mg.L<sup>-1</sup>). Fertilization was carried out within a week of introducing fish into the system in order to maintain the carbon:nitrogen ratio at 10:1. Over the next 15 days, sugar fertilization was performed to neutralize 40% of nitrogen in the feed and to keep ammonia lower than 1.0mg.L<sup>-1</sup> (Avnimelech, 2015; Ebeling *et al.*, 2006).

The experimental design was completely randomized and consisted of two groups: 1) fish without food restrictions (7A/0R) and 2) fish fed six days a week (6A/1R) (no feeding on Sundays). Thus, 180 Nile tilapia (*O. niloticus*) were distributed in six rectangular polyethylene boxes with a useful volume of 250L. Each box was equipped with a constant aeration system and heater to maintain the temperature. Fish were fed with commercial food (Nutricol®, containing 32% crude protein, manufacturer's guaranteed

levels), and fish in both groups were fed twice a day (08:00 and 15:00).

Biometrics were carried out weekly to monitor the growth of fish and adjust the amount of feed, between 3 and 6% of the total biomass of each experimental unit. Dissolved oxygen, water temperature (YSI PRO20 Oximeter), settleable solids (Imhoff cone), and water turbidity (Secchi disk) were measured daily. The pH was measured weekly with a pH meter, pHep®), while total ammonia, toxic ammonia, nitrite and nitrate in the water were measured using a photocolorimeter (Alfakit®) and applying the

$$TC \text{ of feed } \left( \frac{R\$}{kg \text{ of fish}} \right) = \text{feed cost} + \text{cost of fertilization} + \text{labor cost}$$

Feed cost was calculated by adding the cost of all ingredients used in the composition of feed (R\$1.17) throughout the entire experimental

$$\text{Feed cost} \left( \frac{R\$}{kg \text{ of fish}} \right) = \text{feed conversion} \times \text{feed price}$$

The cost of fertilization was estimated by the consumption of sugar, bicarbonate and powdered feed in kilograms multiplied by R\$2.22, R\$0.95 and R\$1.17, respectively, throughout the

$$\text{Cost of fertilization} \left( \frac{R\$}{kg \text{ of fish}} \right) = \frac{(\text{sugar cost} + \text{bicarbonate cost} + \text{powdered feed cost})}{(\text{initial biomass} - \text{final biomass})}$$

Labor cost was calculated according to Correa et al. (2020). Thus, an employee takes one hour to feed one hectare of fish farming. In this study, animals were fed twice a day, and the cost of labor to feed each experimental unit was estimated accordingly. Pay scale was calculated according to the daily rate of rural workers in Santa Catarina, the state in which the work was carried out, and based on data collected by Epagri (Mercado..., 2023). In addition, wages were doubled for Sunday treatment without dietary restrictions because Brazilian legislation requires that workers be paid a 100% bonus or

$$HSI (\%) = 100 \times \frac{\text{liver weight (g)}}{\text{fish weight (g)}}$$

$$VSI (\%) = 100 \times \frac{\text{viscera weight (g)}}{\text{fish weight (g)}}$$

Data were previously subjected to Bartlett analysis to verify homogeneity. Subsequently,

Nessler, alpha-naphthylamine and brucine sulfate methods, respectively. Total suspended solids were measured weekly, according to the methodology described in Apha (Standard..., 1995).

After six weeks, mean weight (g), survival (%), feed conversion, yield (Kg.m<sup>-3</sup>), protein efficiency rate and specific growth rate (%.day<sup>-1</sup>) were all determined. The total cost of feed per kg of fish (TC feed) was then calculated by adding the cost of feed, the cost of fertilization used in the biofloc system and the cost of labor for feeding, as follows:

period to arrive at the final cost of feed, according to the following equation:

experimental period. Thus, the individual cost of each component was obtained, according to the following equation:

compensation for hours worked on rest days (Brasil, 2021). This value was not included in the restricted treatment since no labor was needed.

For the evaluation of body indices, three fish from each experimental unit were subjected to anesthesia with eugenol (50mg.L<sup>-1</sup>) and subsequently euthanized by spinal section. The allometric condition factor (k) followed the description by Santos (1978). From collection and weighing of the viscera and liver with bile, the hepatosomatic (HSI) and viscerosomatic (VSI) indices were calculated, as follows:

they were subjected to the t test. All analyses were considered significant at 5%.

Water quality variables did not differ between the food restriction group and control (Table 1), despite observing a greater presence of

nitrogenous compounds in the control group, possibly related to greater feed intake, even without significant difference.

Table 1. Water quality variables (mean ± standard deviation) of BFT used for culture of Nile tilapia (*Oreochromis niloticus*) subjected to food restriction

Variables	Control	Restriction (6F/1R) <sup>1</sup>	p-value
Temperature (°C)	28.49±0.14	28.63±0.32	0.306
Dissolved oxygen (mg.L <sup>-1</sup> )	5.35±0.08	5.36±0.24	0.464
pH	7.21±0.23	7.25±0.23	0.498
Total suspended solids (mg.L <sup>-1</sup> )	235.34±59.01	221.39±70.90	0.495
Sedimentable solids (ml.L <sup>-1</sup> )	59.34±6.00	60.05±3.32	0.446
Turbidity (cm)	7.47±0.11	7.26±0.29	0.193
Ammonia (mg.L <sup>-1</sup> )	1.86±2.39	1.17±2.49	0.309
NH <sub>3</sub> (mg.L <sup>-1</sup> )	0.12±0.18	0.09±0.17	0.289
Nitrite (mg.L <sup>-1</sup> )	0.07±0.02	0.08±0.04	0.375
Nitrate (mg.L <sup>-1</sup> )	13.24±0.71	9.59±0.82	0.123

<sup>1</sup>Fed for six days and one day of restriction. \*Statistical difference by t-test (P<0.05).

Fish fed under restriction showed lower average weight, productivity, protein efficiency rate and specific growth rate, but higher feed conversion,

compared to control, while survival did not differ between groups (Table 2).

Table 2. Growth performance (mean ± standard deviation) of Nile tilapia (*Oreochromis niloticus*) reared in BFT and subjected to food restriction

Variables	Control	Restriction (6F/1R) <sup>1</sup>	Significance (p)
Mean weight (g)	114.42±1.80*	99.03±4.58	0.006
Survival (%)	98.80±1.57	96.70±2.72	0.187
Feed conversion	1.84±0.14	2.15±0.04*	0.021
Yield (Kg.m <sup>-3</sup> )	13.57±0.51*	11.46±0.15	0.002
Protein efficiency rate	1.50±0.11*	1.28±0.02	0.025
Specific growth rate (%.day <sup>-1</sup> )	0.71±0.03*	0.56±0.05	0.008

<sup>1</sup>Fed for six days and one day of restriction. \*Statistical difference by t-test (P<0.05).

In intensive aquaculture production systems, the economic viability of production has a close relationship with feeding management (Oliveira, *et al.*, 2021). Food restriction for a certain period can be an interesting alternative to reduce labor cost, especially on weekends, as this cost is highest on these days. Correa *et al.* (2020) observed that removing food on Sundays for Nile tilapia fingerlings (4-40g) in BFT did not harm growth performance based on final weight, survival and yield. However, the opposite was observed in the present study wherein it was found that tilapia weighing 50 ~100g and reared in BFT with food restriction had lower final weight, resulting from less food provision. This difference may be related to culture stage. That is, younger animals have a greater capacity to take advantage of natural food from the aquatic

environment. Furthermore, with no significant difference in survival, the higher mean final weight of fish in the control group demonstrated greater yield than that of fish subjected to food restriction.

Similar to our results, a restriction of 25% (Santos Lima *et al.*, 2022) and 30% (Pérez-Fuentes *et al.*, 2018) resulted in lower final body weight of tilapia cultured in BFT. Fish like Nile tilapia can use bioflocs for their development, but in the present work, the biofloc available in suspension could not meet the nutritional demand caused by the lower supply of feed to these animals. However, another study with tilapia juveniles reared in BFT imposed food restriction over the course of twelve consecutive days with the aim of exploring

compensatory growth. After 36 days, satisfactory results were observed in that tilapia had similar weight gain with or without food restriction (Gallardo-Collí *et al.*, 2020). And Oliveira *et al.* (2021) did not observe a difference in feed absorption efficiency when comparing the same amount of feed supplied (10% of the body weight) to tilapia in a recirculation system and a biofloc system. Furthermore, when testing a reduction in feed levels supplied in a bft system, it was possible to observe that the contribution of bioflocs to animal nutrition reduced as the feed supply increased. In this work, the feed was completely removed for one day a week, perhaps this period was not enough to stimulate the use of bioflocs efficiently by the fish, to compensate for the feed restriction.

When reviewing the practice of food restriction, it should be noted that different studies are conducted in different growth phases under different food restriction strategies. This means that such experimental variability could easily account for either success or failure in improving the growth performance of Nile tilapia juveniles in BFT. However, irrespective of method adopted, no studies with tilapia in BFT fed under food restriction regime have reported a difference in survival at the end of the experiment (Pérez-Fuentes *et al.*, 2018; Correa *et al.*, 2020; Gallardo-Collí *et al.*, 2020; Santos Lima *et al.*, 2022), thus corroborating our results.

Feed conversion depends on the amount of feed ingested and the biomass produced, and this directly affects food cost. Unlike our study, Santos Lima *et al.* (2022) observed an improvement in the feed conversion of tilapia with a restriction of 25% of feed per day. This study was also performed in a BFT system, but these results could be explained by the availability of biofloc which mitigated the effects of food restriction. These authors recorded values of settleable solids (76 mL<sup>-1</sup>) and suspended solids (640mg.L<sup>-1</sup>) higher than those of the present work at 60 mL<sup>-1</sup>. 1 and 221mg.L<sup>-1</sup>, respectively, suggesting that the quantity and availability of bioflocs to fish can either limit or expand their performance under food restriction.

In another study (Oliveira *et al.*, 2023), a lower concentration of solids was observed when restricting feed supply to 3 and 4 days of the

week. Unlike this study in which there was no difference when restricting the food. But a milder restriction was also evaluated (1 day per week), compared to the study mentioned above (4 and 3 days per week). This may indicate a greater consumption of bioflocs by the animals, however the feed provided contributes to the maintenance of the system through the supply of nutrients. Therefore, it does not prove that there is a defined relationship between the increase in the use of flakes and the reduction in feed supply.

On the other hand, a trend of lower values for nitrogen compounds was observed, probably by the lower feed amount added in the culture system, even without showing significant differences. Since 18% less feed was supplied, less nitrogen was available for metabolism within the system. In studies that tested a 20% reduction in feeding over the course of 18 days, followed by 6 days of restriction for tilapia, the water in BFT showed lower concentrations of nitrite than fish fed all days (Pérez-Fuentes *et al.*, 2018; Gallardo-Collí *et al.*, 2020).

Specific growth rate and protein efficiency rate were not affected in tilapia fed with 25% food restriction and raised in BFT (Santos Lima *et al.*, 2022). In this experiment, food was restricted by 18%, and results showed lower values for specific growth rate and protein efficiency rate, in turn causing lower fish biomass. Gallardo-Collí *et al.* (2020) observed that short cycles of food restriction, even with normal feed consumption, result in lower growth. The stress to which these animals are subjected by the reduced availability of feed may explain this lower performance.

Diet cost, fertilization cost and total feed cost per kg of fish produced were higher in the 6F/1R treatment (Table 3). Initially, we hypothesized that the group subjected to restriction would result in lower total food costs per kg of fish produced based on the smaller amount of feed offered, but that fish would compensate for the absence of diet by the consumption of bioflocs, thus reducing the final cost and making cultivation more efficient and profitable. In fact, however, reduction in the growth of fish subjected to food restriction compromised the viability of this technique. Furthermore, even considering labor costs (an extra day of work in the control group), no positive effect was

achieved that would otherwise be reflected in lower production costs. Interestingly, Correa *et al.* (2020) did observe a 46.7% reduction in

production costs (feed, fish performance and labor) when applying food restriction for 3 days a week for Nile tilapia raised in BFT.

Table 3. Costs per kilogram (mean  $\pm$  standard deviation) of Nile tilapia (*Oreochromis niloticus*) reared in BFT and subjected to food restriction

Variables	Control	Restriction (6F/1R) <sup>1</sup>	Significance (p)
Dietary cost per kg of fish (R\$.kg <sup>-1</sup> )	2.15 $\pm$ 0.16	2.51 $\pm$ 0.04*	0.021
Fertilization cost per kg of fish (R\$.kg <sup>-1</sup> )	0.98 $\pm$ 0.16	1.37 $\pm$ 0.03*	0.001
Feed cost total per kg of fish (R\$.kg <sup>-1</sup> )	3.39 $\pm$ 0.29	4.08 $\pm$ 0.09*	0.008

<sup>1</sup> Fed for six days and one day of restriction. \*Statistical difference by t-test (P<0.05).

However, additional data from this work suggests that the practice of food restriction for tilapia in BFT is not viable because it compromised growth performance. Nonetheless, when necessary, such as labor shortage, food restriction can still be used without compromising the survival of the animals. Therefore, even with the lower performance shown in this study, food restriction can be a feasible management practice, while awaiting studies that show successful feeding frequencies relative to growth performance.

The condition factor in fish fed with food restriction was greater than that of control (Table 4). Unlike the present study, tilapia reared in a recirculation aquaculture system and subjected to food restriction did not differ from the control (Ali *et al.*, 2016; Pérez-Fuentes *et al.*, 2018). However, other studies showing different growth performance highlight the necessity of defining an appropriate range of dietary restrictions. Neither viscerosomatic nor hepatosomatic variables differed between groups (Table 4).

Table 4. Body variables (mean  $\pm$  standard deviation) of Nile tilapia (*Oreochromis niloticus*) reared in BFT and subjected to food restriction

Variables	Control	Restriction (6F/1R) <sup>1</sup>	Significance (p)
Viscerosomatic index	14.05 $\pm$ 0.42	13.64 $\pm$ 0.60	0.235
Hepatosomatic index	3.73 $\pm$ 0.22	3.68 $\pm$ 0.12	0.401
Condition factor (k)	0.97 $\pm$ 0.01	1.04 $\pm$ 0.02*	0.025

<sup>1</sup> Fed for six days and one day of restriction. \*Statistical difference by t-test (P<0.05).

Hepatosomatic and viscerosomatic indices were not altered by dietary restriction. Corroborating our study, Ali *et al.* (2016) also found no difference in these variables in fish subjected to 0, 1 or 2 days of food restriction per week in clear water. Gallardo-Collí *et al.* (2020) recorded a reduction in the viscerosomatic index in addition to observing an inversely proportional relationship such that more days of food restriction correlated with lower hepatosomatic index values. Similarly, when reducing the daily feeding rate for tilapia in biofloc system, the hepatosomatic index is also correspondingly detrimental. But with a rate of 10% supplied, hepatosomatic index values similar to those obtained in this experiment were obtained (Oliveira, *et al.*, 2021). It is likely that the fish in this study did not need to use energy reserves to supplement their nutritional needs, even with

restricted feeding, explaining the equality between experimental groups.

Food restriction for Nile tilapia raised in BFT impaired growth performance, but without changes in body indices. Contrary to our initial hypothesis, this result suggests that food restriction is still a practice that requires more in-depth assessment of both frequency and volume of restriction, as well as the effect of flake availability (quantity and quality) on food compensation. Therefore, testing different feeding and restriction intervals, considering growth stages, is still required for rearing Nile tilapia (*O. niloticus*) in BFT when considering its overall effects on reducing production costs.

Keywords: food deprivation, food cost, biofloc technology, performance, body indices

## RESUMO

O objetivo deste trabalho foi avaliar os efeitos da restrição alimentar de um dia ao longo da semana, quanto aos custos dietéticos, ao desempenho zootécnico e aos índices corporais de juvenis de tilápia-do-nylo (*Oreochromis niloticus*) cultivados em sistema de bioflocos (BFT). Os peixes do grupo controle foram alimentados sete dias na semana (7A/0R), enquanto os peixes do grupo restrição foram alimentados seis dias na semana (6A/1R), resultando em uma restrição alimentar de 18%. Em seis caixas retangulares com volume útil de 250 litros, foram alojados 180 alevinos de tilápia-do-nylo (*O. niloticus*), com peso médio de 57,81±0,02g. Os peixes submetidos à restrição alimentar obtiveram menor peso médio, produtividade, taxa de eficácia proteica e taxa de crescimento específico; e maior conversão alimentar. O custo com ração, fertilização e alimentação total, por kg de peixe produzido, foi maior com o uso da restrição. Para índices corporais, os índices hepatossomático e vicerossomático não apresentaram diferenças significativas entre os tratamentos, contudo o fator de condição foi maior no tratamento com restrição. Os resultados indicam prejuízos ao desempenho dos animais submetidos à restrição, sendo necessários mais estudos para definir a frequência da restrição alimentar adequada, o que permitirá reduzir custos sem prejuízos à espécie.

*Palavras-chave:* privação alimentar, custo com alimentação, tecnologia de bioflocos, desempenho, índices corporais

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