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AQUACULTURE

Dietary supplementation with soybean oil has not favored the tilapia growth in BFT tanks submitted to feeding restriction

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ABSTRACT. The present work aimed at assessing the possibility of compensating the notorious deficiencies of bioflocs in lipids by supplementing the tilapia commercial diet with soybean oil. In the positive control, there was no feeding restriction nor dietary supplementation with soybean oil. In the experimental treatments, the commercial diet was restricted by 25% over the positive control level. In the negative control tanks, there was feeding restriction and the artificial diet had no oil supplementation. In the experimental tanks, soybean oil was mixed daily with the commercial diet at the levels of 0.6%, 1.2 and 2.4%. Additionally, there were fed-restricted tanks that received a daily supplementation of 1.2% soybean oil mixed with dry molasses, and not with the commercial diet. In general, only the restriction of the commercial diet affected the water quality. The supplementation of the artificial diet with soybean oil up to 2.4% has not improved the proximate composition of bioflocs, nor the fish growth performance. It was concluded that the strategy of supplementing the Nile tilapia juveniles' commercial diet with increasing levels of soybean meal, in BFT tanks submitted to 25%-feeding restriction, was not capable of avoiding the fish growth performance deterioration.

Keywords: aquaculture; fish culture; fish nutrition; Bioflocs; lipids.

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Introduction

Responsible aquaculture employs production systems that combine economic and environmental sustainability. Among such systems, the biofloc technology (BFT) applied to aquaculture performs minimum or even zero water exchange. Bioflocs are composed of different organisms, such as bacteria, plankton, nematodes, invertebrates; and of organic and mineral debris (Azim & Little, 2008; De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008). The application of supplementary organic carbon sources adjusts the C: N ratio of water and it promotes the development of heterotrophic bacteria which remove ammonia and produce microbial protein. Therefore, bioflocs improve water quality and serve as a source of nutrients for the animals (Avnimelech, 2009).

In aquaculture BFT tanks, the expenses with commercial diets could be reduced if part of the animal nutrition came from the ingestion of bioflocs (Xu & Pan, 2012). For that purpose, the chemical composition of bioflocs should satisfy, at least partially, the nutritional requirements of the species. However, bioflocs are not complete foods for aquaculture species, being deficient in some essential amino acids and fatty acids (Crab, Chielens, Wille, Bossier, & Verstraete, 2010; Bauer, Prentice-Hernandez, Tesser, Wasielesky, & Poersch, 2012; Ekasari et al., 2014; Dantas et al., 2016). Nile tilapia needs all essential amino acids, lysine being the most limiting (Richter et al., 2021). This species has a requirement for linoleic acid in the range of 0.5 to 1% (National Research Council [NRC], 2011; Jiao et al., 2020). The nutritional value of bioflocs will depend on some factors, such as the composition of its organisms, particle size and digestibility. The protein and lipid contents of bioflocs vary widely between studies (Ekasari et al., 2014; Wei, Liao, & Wang, 2016), and their lipid concentration might be very low, varying between 1.3 - 7.5% (Luo et al., 2014; Ekasari et al., 2014; Khanjani, Sajjadi, Alizadeh, & Sourinejad, 2017; Promthale, Pongtippatee, Withyachumnarnkul, & Wongprasert, 2019; Martinez-Porchas et al., 2020).

The tilapia commercial diet supplementation with soybean oil is a feeding strategy which has not been evaluated yet. It has been hypothesized that it could compensate, at least partially, for some of the lipidic deficiencies of bioflocs. Vegetable oils are good sources of linoleic acid for tilapia. Soybean oil has 52.6% linoleic acid in its composition (Rostagno et al., 2011), and the oil digestibility is 89.85% for Nile tilapia (Boscolo, Hayashi, & Meurer, 2002). If successful, that strategy could reduce the production costs for Nile tilapia rearing in BFT tanks submitted to feeding restriction. This study aimed at evaluating the supplementation of Nile tilapia's commercial diet with soybean oil as a strategy to partially compensate for the deficiencies of bioflocs in lipids. For this, the water quality of the rearing tank was monitored systematically.

Material and methods

Juvenile Nile tilapia (*Oreochromis niloticus*) with a body weight of 1.74 ± 0.06 g were obtained from a regional producer and transported to the laboratory facilities. In the laboratory, the fish were submitted to prophylactic treatment with potassium permanganate at a concentration of 4 mg L^{-1}. After 6 days of acclimatization, the animals were transferred to 30 indoor tanks of 100 L served by constant mechanical aeration provided by one 2.5-hp air blower. Each tank was supplied with 70 L of freshwater and 30 L of bioflocs-rich water taken from one 1000-L outdoor maturation tank. The biofloc maturation tank, from which the necessary volumes for inoculation in the experimental tanks were removed, was filled with 1000 L of fresh water. Twenty Nile tilapia juveniles, with an average body weight of 55.1 ± 8.1 g, were stored in the maturation tank to accelerate biofloc development. The animals were fed with commercial powdered food for omnivorous tropical fish (41.2% CP; Aquamix PL-0, Integral Mix, Fortaleza, Ceará), which was offered four times a day, at 8:00 am, 11:00 am, 2:00 pm and 5:00 pm, at a feed rate of 7.5% of the biomass stored per day. Molasses powder was added to the culture waters to adjust the C:N ratio of the water to 15:1, following the recommendations of Avnimelech (1999). After twenty-three days, 30 L volumes of water from the biofloc maturation tank were transferred to each experimental unit. At the study onset, twenty-three Nile tilapia juveniles (2.2 ± 0.04 g) were stocked in each of the tanks and kept for 6 weeks.

The experimental design was completely randomized with six treatments and five repetitions. Fish have been submitted or not to feeding restriction based on the regular chart rates. There was no food restriction in the positive-control tanks, nor dietary supplementation with soybean oil. The negative-control tanks had a 25% feeding restriction and no dietary supplementation. In the experimental tanks, the allowance of commercial feed to fish was restricted by 25% and the diet was supplemented daily with 0.6, 1.2 and 2.4% of soybean oil. There were also tanks submitted to feeding restriction but supplemented with soybean oil at 1.2% mixed daily with dry molasses. This percentage of 2.4% corresponds to half the level of inclusion of soybean oil used in the basal diet of the work by Sá, Pezzato, Lima, and de Magalhães Padilha (2004). From there, the other two treatments were obtained as half and 1/4 of the initial reference.

Fish were manually fed four times a day, at 8, 11, 14 and 17h with a commercial powdered diet for tropical fish (41% crude protein, Aquamix PL-0, Integral Mix, Fortaleza, Ceará). The soybean oil was mixed daily with the commercial diet or dry molasses. The dietary allowances were adjusted every two weeks after fish weightings. The initial feeding rate was 11.3% which was reduced to 6.0% at the end. The C: N ratio of water was adjusted daily to 15: 1 by the application of dry molasses (Indumel, Biosev, Sertãozinho, São Paulo) to the tanks. The amounts of molasses applied in each tank were calculated according to Avnimelech (1999), which considers the dietary crude protein level and the daily allowances to the animals.

Twenty percent of the tank volume were removed for sedimentation for 30 minutes when the settleable solids exceeded 30 mL L^{-1}. The superficial water was then returned to its respective tank. Sodium bicarbonate was applied to maintain the total alkalinity and pH of water at values equal to or greater than 60 mg L^{-1} $CaCO_3$ and 7.0, respectively. Hydrogen peroxide was used as an emergency source of O_2 during electricity failures (Furtado, Serra, Persch, & Wasielesky, 2014). There was no water exchange in the tanks over the experimental period only the replenishment to maintain the initial level.

The water quality variable monitored throughout the experiment were the followings: pH (pH meter mPA210 - MS Tecnopon®), temperature (digital thermometer), dissolved oxygen (YSI 55 oximeter) and specific conductance (SC; CD- 850 conductivity meter), daily at 9 h; total ammonia nitrogen (TAN, indophenol method), nitrite ($N\text{-}NO_2^-$; sulfanilamide method), nitrate ($N\text{-}NO_3^-$; Cd reducing column) and total alkalinity (TA; titration with a standard H_2SO_4 solution), weekly; free CO_2 (titration with a Na_2CO_3 standard solution), total hardness (TH; titration with a EDTA standard solution), reactive phosphorus (molybdenum blue method), dissolved iron (thiocyanate method) and organic matter (oxygen consumed method), fortnightly. Those determinations were carried out according to Clesceri, Greenberg, and Eaton (1998). The concentrations of total suspended solids (TSS) and settleable solids (SS) in the water were determined weekly (Boyd & Tucker, 1992). The bioflocs' proximate composition for moisture, crude protein, ether extract and ash were performed according to the Association of Official Analytical Chemistry (AOAC, 2000)'s methods. Fish growth performance was monitored through the following variables: survival, final body weight, specific growth rate (SGR = [(ln final body weight - ln initial body weight)/number of days] * 100), fish yield, feed conversion ratio (FCR = feed allowance/fish weight gain) and protein efficiency ratio (PER = fish weight gain/dietary crude protein allowance).

The water quality, bioflocs proximate composition and growth performance results were submitted to the One-way ANOVA for completely randomized experiments. The means were compared two by two using the Tukey's test when the differences between the treatments were significant ($p < 0.05$). The normality and homogeneity of variance assumptions were checked by the Shapiro-Wilk and Levene tests, respectively. Data in percentage and ratio were transformed to arccosine before analyses. The statistical analyzes were performed with the aid of the SigmaPlot for Windows V.12 (Systat Software, Inc.) and Excel 2016 software.

Results and discussion

Water quality

There were no significant differences for the water temperature between treatments (26.8 ± 0.3°C). The pH and concentrations of total alkalinity and O_2 of water did not differ between the tanks with or without feeding restriction and supplemented or not with soybean oil ($p > 0.05$; Table 1). The specific conductance of the water was higher in tanks with no feeding restriction ($p < 0.05$; Table 1).

The physical-chemical stability of BFT tanks for aquaculture will largely depend on maintaining favorable O_2 and alkalinity concentrations in water. The demand for O_2 increases with feeding intake and molasses applications, and bioflocs intensely consume dissolved O_2 (Avnimelech, 2009; Hargreaves, 2013; Lara, Krummenauer, Abreu, Poersch, & Wasielesky, 2017). The pH and alkalinity of water tend to decrease over time and, thereby, deserve careful monitoring. Tilapia resists a wide variation in alkalinity and pH values, being indicated to maintain values above 60 mg L^{-1} and 6 - 9, respectively (Boyd; Tucker; Somridhivej, 2016; Rebouças, Lima, Cavalcante, & Sá, 2016). The heterotrophic bacteria activity reduces the bicarbonates and carbonates concentrations in water. Nitrifying bacteria, which also develop in BFT tanks, consume O_2 and water alkalinity (Ebeling, Timmons, & Bisogni, 2006; Hargreaves, 2013). Therefore, frequent liming is required in BFT tanks to adjust their pH and alkalinity.

Table 1. Dissolved oxygen, pH, electrical conductivity and total water alkalinity, after 6 weeks of cultivation of juvenile Nile tilapia, *Oreochromis niloticus*, in 100 L BFT tanks, submitted or not to food restriction and supplemented with increasing levels of soybean oil (mean ± sd; n = 5).

Restriction in the supply of commercial feed	Supplementation with soybean oil	Dissolved oxygen (mg L^{-1})	pH	Electric conductivity (μS cm^{-1})	Total alkalinity (mg L^{-1} $CaCO_3$)
0%	-	6.22 ± 0,18	7.25 ± 0.15	1209 ± 57 a[1]	122.2 ± 15.7
25%	-	6.60 ± 0.33	7.38 ± 0.26	1110 ± 35 b	129.3 ± 13.2
25%	0.6% feed	6.74 ± 0.36	7.38 ± 0.22	1099 ± 40 b	136.3 ± 19.2
25%	1.2% feed	6.51 ± 0.24	7.43 ± 0.28	1088 ± 38 b	128.3 ± 10.5
25%	2.4% feed	6.47 ± 0.25	7.36 ± 0.28	1101 ± 49 b	124.2 ± 29.6
25%	1.2% molasses	6.60 ± 0.27	7.39 ± 0.16	1072 ± 53 b	128.3 ± 12.2
P-valor		ns[2]	ns	< 0.01	ns
CV (%)[3]		4.14	3.04	4.07	13.10

[1]In the same column, means with different letters are significantly different from each other by the Tukey test ($p < 0.05$); [2]Not significant ($p > 0.05$); [3]CV (%) - Coefficient of variation.

In the present work, the specific conductance (SC) was affected only by the feeding restriction carried out. The lowering of water SC was due to the reduced feeding allowances which produced fewer organic wastes. Fish feces and uneaten feed release ions to the water after their decomposition and increase SC. The results of the present work were similar to those obtained by Lima, Apoliano, Cavalcante, and Sá (2021).

The concentrations of total hardness, reactive phosphorus, dissolved iron and organic matter in the water were affected only by the dietary restriction performed. The total hardness of the water was higher in the tanks with no feeding restriction ($p < 0.05$) and there were no significant differences for free CO_2 between the experimental tanks (Table 2). The tanks with no feeding restriction had higher concentrations of reactive phosphorus in the water than the other tanks, except for the units receiving 1.2% soybean oil blended with the commercial diet ($p < 0.05$; Table 2). The concentration of dissolved iron was higher ($p < 0.05$) in the tanks with no feeding restriction. As expected, the tanks with no feeding restriction had higher concentrations of organic matter in water (Table 2).

The hardness of water was higher in the non-restricted tanks because the commercial diet contains calcium and magnesium. Dos Santos et al. (2021) observed significantly higher total hardness values in BFT tanks of tambaqui (*Colossoma macropomum*) cultivation in relation to cultures in water, regardless of the stocking density used. These authors used dolomitic limestone to increase the alkalinity of the water, also affecting the total hardness. The high concentrations of reactive phosphorus observed in the tanks with no feeding restriction was due to their regular feed intake. Similar results were observed by Cavalcante, Lima, Rebouças and Sá (2017) who attributed that to the greater mineralization of organic matter. According to Da Silva, Wasielesky, and Abreu (2013), BFT tanks tend to accumulate phosphorus which could favor the growth of cyanobacteria.

Table 2. Total hardness, free CO_2 concentration, reactive phosphorus, dissolved iron and organic matter in the water, after 6 weeks of cultivation of juvenile Nile tilapia, *Oreochromis niloticus*, in 100 L BFT tanks, whether or not subjected to food restriction and supplemented with increasing levels of soybean oil (mean ± sd; n = 5).

Restriction in the supply of commercial feed	Supplementation with soybean oil	Total hardness (mg L^{-1} $CaCO_3$)	Free CO_2 (mg L^{-1})	Reactive phosphorus (mg L^{-1})	Dissolved iron (mg L^{-1})	Organic matter (mg L^{-1})
0%	-	221.6 ± 11.5 a[1]	14.4 ± 1.8	3.37 ± 0.30 a	2.15 ± 0.27 a	602 ± 22 a
25%	-	183.0 ± 8.9 b	11.2 ± 1.9	2.64 ± 0.34 b	1.73 ± 0.13 b	541 ± 29 b
25%	0.6% feed	182.3 ± 13.2 b	11.4 ± 1.9	2.65 ± 0.21 b	1.74 ± 0.13 b	547 ± 18 b
25%	1.2% feed	178.9 ± 11.3 b	11.8 ± 2.1	2.74 ± 0.28 ab	1.72 ± 0.17 b	550 ± 21 b
25%	2.4% feed	181.0 ± 9.7 b	11.8 ± 2.6	2.72 ± 0.26 b	1.71 ± 0.12 b	559 ± 30 ab
25%	1.2% molasses	180.6 ± 12.0 b	12.1 ± 2.0	2.73 ± 0.35 b	1.66 ± 0.31 b	552 ± 18 b
P-valor		< 0.001	ns[2]	< 0.01	< 0.01	< 0.05
CV (%)		5.94	17.03	10.32	10.54	4.15

[1]In the same column, means with different letters are significantly different from each other by the Tukey test ($p < 0.05$); [2]Not significant ($p > 0.05$). [3]CV (%) - Coefficient of variation.

The concentrations of dissolved iron were lower in the non-restricted tanks because their daily feed allowances were diminished. The commercial fish feeds are regularly supplemented with macro and microminerals. In closed rearing systems, such as in BFT tanks, the accumulation of minerals in water favors their retention on the animal tissues and bioflocs (Bakhshi, Najdegerami, Manaffar, Tukmechi, & Farah, 2018; Li, Ren, Liu, Dong, & Zhu, 2018). On the contrary, Prangnella, Castro, Ali, Browdy, and Samocha (2020) observed that feeding rations with different protein levels did not lead to problematic accumulation of metals in the cultured water or shrimp tissues, kept in BFT tanks.

In the present study, the concentration of organic matter in water decreased with the commercial diet input. As the fish submitted to feed restriction exhibited reduced body growth, the feed daily allowances and concentrations of suspended solids were lower in those units. Similar results were obtained by Cavalcante et al. (2017) and Lima et al. (2021) in BFT tanks. Pérez-Fuentes, Pérez-Rostro, Hernández-Vergara, and Monroy-Dosta (2018) indicated a significant reduction in the concentration of solids in the water of Nile tilapia culture in BFT tanks, when levels of feed restriction greater than 30% were used, resulting in a lower concentration of organic matter.

No significant differences were found for the final concentrations of TAN, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ between the experimental tanks ($p > 0.05$; Table 3). Since bioflocs uptake ammonia and, consequently, improve water quality in BFT tanks (De Schryver et al., 2008; Avnimelech, 2009), the development of bioflocs was similar between the treatments.

Table 3. Concentrations of total ammoniacal nitrogen (TAN), nitrite (NO_2^-), nitrate (NO_3^-), sedimentable solids (SS) and total suspended solids (TSS) in the water, after 6 weeks of cultivation of juvenile Nile tilapia, *Oreochromis niloticus*, in 100 L BFT tanks, submitted or not to food restriction and supplemented with increasing levels of soybean oil (mean ± SD; n = 5).

Restriction in the supply of commercial feed	Supplementation with soybean oil	TAN (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})	SS (mL L^{-1})	TSS (mg L^{-1})
0%	-	0.08 ± 0.06	0.91 ± 0.26	14.98 ± 0.78	180.0 ± 27.4 a[1]	744 ± 26.1
25%	-	0.06 ± 0.05	0.75 ± 0.29	15.47 ± 0.79	130.0 ± 44.7 ab	684 ± 48.3
25%	0.6% feed	0.05 ± 0.05	0.82 ± 0.36	14.88 ± 0.67	113.6 ± 34.3 ab	678 ± 43.2
25%	1.2% feed	0.10 ± 0.03	0.78 ± 0.35	14.78 ± 0.58	111.6 ± 36.9 ab	698 ± 32.7
25%	2.4% feed	0.06 ± 0.04	0.80 ± 0.24	14.48 ± 0.51	101.6 ± 30.4 b	684 ± 16.7
25%	1.2% molasses	0.10 ± 0.06	0.76 ± 0.37	15.05 ± 0.59	114.0 ± 49.8 ab	680 ± 46.4
P-valor		ns[2]	ns	ns	< 0.05	ns
CV (%)		67.09	39.37	4.37	29.77	5.12

[1]In the same column, means with different letters are significantly different from each other by the Tukey test ($p < 0.05$); [2]Not significant ($p > 0.05$); [3]CV (%) - Coefficient of variation.

Therefore, the ammonia concentrations were not affected by feeding restriction or the dietary soybean oil supplementation carried out. The producer must keep the TAN concentration in the water always below 1 mg L^{-1}. Elevated levels of ammonia in the water can affect the central nervous system and gills, impairing fish growth and survival. The bioflocs that were inoculated at the start of the experiment might have contributed to the rapid maturation of the tank microbiota. Ferreira et al. (2021) suggested that supplemental organic C applications should be made only if the concentrations of ammonia exceeded stressful levels. Such strategy would allow a better establishment of nitrifying bacteria and result in less accumulation of organic matter. The concentrations of nitrite in water exceeded the acceptable threshold of 0.3 mg L^{-1} (Sá, 2012). Nitrite affects the O_2 transport by hemoglobin and the application of common salt in water could prevent nitrite toxicity in fish farming. The accumulation of nitrate in water indicates the occurrence of nitrification in the tanks. Nitrification in BFT tanks is a desirable activity for removing nitrite from water. The occasions of interruption of the energy supply and the use of hydrogen peroxide may have affected the autotrophic bacteria, preventing further reduction of the nitrite concentration in the water. With the maintenance of optimal system conditions, that is, adequate levels of oxygen, alkalinity and pH, the nitrifying bacteria will act effectively in the removal of nitrogenous compounds. Nitrifying bacteria develop in BFT tanks with moderate applications of organic carbon sources (Azim & Little, 2008; Xu, Morris, & Samocha, 2016). Other studies have also noted no significant effects of increasing levels of dietary lipids on nitrate (Toledo, Silva, Vieira, Mouriño, & Seiffert., 2016; Hamidoghli et al., 2020).

The tanks with no feeding restriction had higher concentrations of settleable solids (SS), with the exception of the food restriction treatment and addition of 2.4% soybean oil, but their concentrations of total suspended solids (TSS) were not affected by the dietary restriction or oil supplementation. The management of solids in BFT tanks is fundamental for their correct operation. Fed-restricted tanks had lower SS concentrations in water and their TSS concentrations exhibited a decreasing trend. Hamidoghli et al. (2020) and Toledo et al. (2016) also have found no significant effects of increasing dietary lipid levels on TSS. The allowance of artificial diet is the main factor affecting the concentrations of SST in aquaculture tanks. Restricted feeding rates favored lower SS and SST concentrations in BFT tanks (Da Silva et al., 2020; Pérez-Fuentes et al., 2018). In general, the water quality of the tilapia BFT tanks was affected only by the feeding restriction carried out. On the other hand, the dietary supplementation with increasing levels of soybean oil has not affected any of the water quality variables. Therefore, the producer might use that food strategy safely because there was not any water quality deterioration.

Proximate composition of bioflocs

No significant effects due to feeding restriction and dietary oil supplementation were observed on the bioflocs' proximate composition (Table 4). Just over 8% of the bioflocs biomass was dry matter. The average moisture present in the bioflocs was 91.9 ± 1.4%. Their concentrations of crude protein and total lipids were similar between the treatments (18.36 ± 0.32%; 0.80 ± 0.03%, respectively; dry matter basis). Similarly, the feeding restriction and soybean oil supplementation have not affected the ash content of the bioflocs (8.29 ± 0.47%).

Table 4. Proximate composition of bioflocs, after 6 weeks of cultivation of juvenile Nile tilapia, *Oreochromis niloticus*, in 100 L BFT tanks, submitted or not to food restriction and supplemented with increasing levels of soybean oil (mean ± sd; n = 5).

Restriction in the supply of commercial feed	Supplementation with soybean oil	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash (%)
0%	-	91.24 ± 1.79[1]	18.56 ± 0.48	0.90 ± 0.10	8.45 ± 0.48
25%	-	91.99 ± 1.42	18.33 ± 0.41	0.80 ± 0.10	8.18 ± 0.54
25%	0.6% feed	92.45 ± 1.11	18.31 ± 0.39	0.81 ± 0.11	8.28 ± 0.56
25%	1.2% feed	92.11 ± 1.52	18.37 ± 0.24	0.80 ± 0.13	8.30 ± 0.62
25%	2.4% feed	91.37 ± 1.60	18.31 ± 0.33	0.83 ± 0.08	8.38 ± 0.38
25%	1.2% molasses	92.20 ± 1.76	18.26 ± 0.30	0.75 ± 0.09	8.13 ± 0.63
P-valor		ns[1]	ns	ns	ns
CV (%)		1.67	1.94	12.57	6.46

[1]In the same column, means with different letters are significantly different from each other by the Tukey test ($p < 0.05$); [2]Not significant ($p > 0.05$); [3]CV (%) - Coefficient of variation.

The nutritional composition of bioflocs can be influenced by their microbial community biodiversity (Ebeling et al., 2006; Crab et al., 2010) and the source of supplemental organic carbon (Wei et al., 2016; Bakhshi et al., 2018). In general, bioflocs have low and lacking lipid concentrations (Luo et al., 2014; Khanjani et al., 2017; Rajkumar et al., 2016; Kuhn, Lawrence, Crockett, & Taylor, 2016). In a previous work carried out in the laboratory, higher concentrations of lipids were observed in bioflocs (2.0-2.2%; Lima et al., 2021). The concentrations of lipids in bioflocs observed were like those found by Rajkumar et al. (2016) with 0.57–0.92%, but higher than the ones reported by Bauer et al. (2012; 0.3%). Da Silva et al. (2020) reported that the 50% reduction in the allowances of commercial diet have decreased the bioflocs protein but not affected their lipid content. Similar results were obtained by Toledo et al. (2016) and Hamidoghli et al. (2020). The first authors have found no significant differences in the bioflocs' proximate composition in a *L. vannamei* study that evaluated the use of increasing dietary lipid levels. Toledo et al. (2016), however, obtained higher concentrations of protein (20.9 - 21.4%) and lipid (4.2 - 4.4%) in bioflocs. Hamidoghli et al. (2020) observed a reduction in the bioflocs lipid concentration from 3.5 to 0.41% over the experimental period.

Fish growth performance

In general, the tilapia growth performance in the fed-restricted BFT tanks was not favored by the dietary supplementation with soybean oil. That meant an increase in the production costs with no additional benefits. Since the expenses with artificial diets comprise more than 50% of the operational costs, lower dietary allowances would increase the aquaculture economic sustainability (Kuhn et al., 2016). Some authors have observed that moderate feeding restriction levels could be used in BFT tanks with no significant effect on the tilapia growth performance (Hisano, Parisi, Cardoso, Ferri, & Ferreira, 2020; Da Silva et al., 2020; Pérez-Fuentes et al., 2018). In that case, however, bioflocs should have an adequate nutritional profile with high levels of protein and lipids.

Fish survival varied between 81 and 88% with no significant effects of the feeding restriction and dietary soybean oil supplementation ($p > 0.05$; Table 5). Since the fish survival was not affected by feeding restriction and oil supplementation in the present work, it is suggested that at least part of the animal nutrition came from the ingestion of bioflocs. That inference was also made by Da Silva et al. (2020) and Pérez-Fuentes et al. (2018).

Table 5. Survival, final body weight (FBW), specific growth rate (SGR) and weekly weight gain (WWG) of juvenile Nile tilapia, *Oreochromis niloticus*, after 6 weeks of cultivation in 100 L BFT tanks, submitted or not to food restriction and supplemented with increasing levels of soybean oil (mean ± SD; n = 5).

Restriction in the supply of commercial feed	Supplementation with soybean oil	Survival (%)	FBW (g)	SGR (% day^{-1})	WWG (g)
0%	-	87.83 ± 7.14	12.92 ± 0.69 a[1]	4.19 ± 0.18 a	1.78 ± 0.12 a
25%	-	80.87 ± 5.83	11.52 ± 0.50 b	3.95 ± 0.10 ab	1.56 ± 0.08 b
25%	0.6% feed	81.74 ± 3.64	11.40 ± 0.64 b	3.89 ± 0.18 b	1.53 ± 0.11 b
25%	1.2% feed	82.61 ± 4.35	11.56 ± 0.55 b	3.97 ± 0.14 ab	1.56 ± 0.09 b
25%	2.4% feed	80.87 ± 3.89	11.57 ± 0.32 b	3.95 ± 0.11 ab	1.56 ± 0.06 b
25%	1.2% molasses	82.61 ± 5.32	10.93 ± 0.81 b	3.82 ± 0.17 b	1.46 ± 0.13 b
P-valor		n[2]	< 0.001	< 0.05	< 0.01
CV (%)		6.05	5.04	3.66	6.42

[1]In the same column, means with different letters are significantly different from each other by the Tukey test ($p < 0.05$); [2]No significant ($p > 0.05$); [3]CV (%) - Coefficient of variation.

The feeding restriction reduced the final body weight of fish, negatively affecting the weekly weight gain and the specific growth rate ($p < 0.05$; Table 5). Supplementation of diets with soybean oil, in tanks where there was feed restriction, was not effective to recover fish growth. Fish yield was significantly higher in tanks with no feeding restriction (Table 6). The commercial diet and dry molasses supplemented with increasing levels of soybean oil had no effect on fish yield ($p > 0.05$).

The tilapia growth performance decreased significantly in the tanks with feeding restriction. Therefore, the dietary supplementation with increasing levels of soybean oil has not recovered the animal growth deterioration in tanks that received less commercial diet. Because the bioflocs concentrations of protein and lipids were low, their ingestion by fish did not provide the necessary amino acids and fatty acids to provide a better animal performance. Contrarily, Lima et al. (2021) observed that tilapia juveniles kept in BFT tanks submitted to the same level of feeding restriction had regular body growth rates when the animals were fed with a diet supplemented with 1% or more of DL-methionine. Since bioflocs are usually deficient in lipids (Luo et al., 2014; Dantas et al., 2016; Khanjani et al., 2017), it has been speculated about the strategy of dietary supplementation with soybean oil in the present work. Such alternative feeding management, however, has not been successful because it was not capable of supplying the nutrients needed by fish maintained in fed-restricted tanks.

Table 6. Fish yield, feed conversion ratio (FCR) and protein efficiency ratio (PER) for the cultivation of juvenile Nile tilapia, *Oreochromis niloticus*, after 6 weeks of cultivation in 100 L BFT tanks, whether or not subjected to food restriction and supplemented with increasing levels of soybean oil (mean ± sd; n = 5).

Restriction in the supply of commercial feed	Supplementation with soybean oil	fish yield (g m^{-3} day^{-1})	FCR	PER
0%	-	61.93 ± 2.60 a[1]	1.56 ± 0.10	1.61 ± 0.10
25%	-	50.95 ± 2.89 b	1.41 ± 0.12	1.79 ± 0.14
25%	0.6% feed	51.11 ± 4.66 b	1.39 ± 0.17	1.82 ± 0.23
25%	1.2% feed	52.21 ± 2.14 b	1.36 ± 0.10	1.85 ± 0.14
25%	2.4% feed	51.27 ± 3.29 b	1.38 ± 0.15	1.82 ± 0.19
25%	1.2% molasses	49.27 ± 1.96 b	1.47 ± 0.09	1.71 ± 0.11
P-valor		< 0.001	ns[2]	ns
CV (%)		5.58	8.68	8.47

[1]In the same column, means with different letters are significantly different from each other by the Tukey test (p < 0.05); [2]Not significant (p > 0.05); [3]CV (%) - Coefficient of variation.

The feed conversion ratio (FCR) and protein efficiency ratio (PER) were similar between treatments, being equal to 1.43 ± 0.07 and 1.77 ± 0.09 ($p > 0.05$). Although the bioflocs nutritional composition is generally lower than those observed in commercial diets, part of the fish and shrimp nutrition may come from the ingestion of bioflocs (Avnimelech, 2007). Pérez-Fuentes et al. (2018) evaluated four increasing levels of feeding restriction (0, 10, 20, 30 and 40%) in a Nile tilapia BFT study, having observed that the restriction levels of 30 and 40% significantly reduced fish body weight gain. Therefore, tilapia was able to keep its body growth at feeding restriction levels lower or equal at 20%. Pérez-Fuentes et al. (2018) obtained better FCR and PER results (1.07 and 2.69, respectively), for the same level of feeding restriction carried out in the present work (25%). These results suggest that tilapia make better use of bioflocs as food when the artificial diets allowances are lowered. In addition, restrictive feeding rates could increase the digestive enzyme activities and, thus, improve the use of artificial diets (Baloi et al., 2017).

The supplementation of the tilapia commercial diet with up to 2.4% soybean oil has not improved the proximate composition of bioflocs, especially on their protein and lipid concentrations. The fish growth performance in the fed-restricted tanks was not improved by dietary supplementation with soybean oil. The bioflocs biomass, therefore, was not capable of compensating for the reduction on the commercial diet allowances. It is speculated that the protein and lipid contents of bioflocs were negatively affected by the applications of hydrogen peroxide carried out in the present work. Those H_2O_2 applications were necessary to avoid fish death after the mechanical aeration interruptions which happened over the electricity failures.

Conclusion

The supplementation of the Nile tilapia commercial diet with soybean oil was not able to avoid the deterioration of fish growth performance in BFT tanks submitted to 25% feeding restriction. No lipidic enrichment was observed in the bioflocs biomass by supplementing the artificial diet with soybean oil up to 2.4%.

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