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Supplementation of the commercial diet of Nile tilapia (*Oreochromis niloticus*) with DL-methionine and soybean oil under feed restriction

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ABSTRACT: This study aimed to assess the strategy of supplementing the commercial diet of Nile tilapia juveniles with DL-methionine and soybean oil, in BFT culture tanks, by monitoring the water quality, bioflocs composition, and growth performance. The commercial diet daily allowances were restricted by 25% and different levels of DL-methionine (0 and 1) and soybean oil (0, 0.6, and 1.2%) supplementation were carried out, having the delivery of artificial diet as reference. The tanks submitted to feeding restriction exhibited lower concentrations of organic matter, settleable solids and total suspended solid in water. The supplementation of the commercial diet with DL-methionine and soybean oil did not affect the centesimal composition of bioflocs. The combined use of DL-methionine and soybean oil improved the tilapia juveniles' survival submitted to artificial diet restriction. It was concluded that the supplementation of the Nile tilapia's commercial diet with 1.0% DL-methionine and 0.6% soybean oil may prevent the reduction of fish growth performance when the artificial diet is allowed restrictively.

Key words: aquaculture; bioflocs; feeding restriction; water quality

Suplementação da dieta de juvenis de tilápia do Nilo (*Oreochromis niloticus*) com DL-metionina e óleo de soja, sob restrição alimentar

RESUMO: Este estudo teve por objetivo avaliar a estratégia de suplementação da dieta comercial de juvenis de tilápia do Nilo, mantidos em tanques BFT sob restrição alimentar, com DL-metionina e óleo de soja, pela observação da qualidade da água, composição dos bioflocos e o desempenho animal. Foram aplicadas taxas restritivasde 25% na oferta de ração comercial e diferentes níveis de suplementação de DL-metionina (0 e 1,0%) e óleo de soja (0; 0,6 e 1,2%), em relação à quantidade de ração fornecida. Os tanques sob restrição alimentar apresentaram menores concentrações de matéria orgânica, sólidos sedimentáveis e sólidos suspensos totais na água. A suplementação da ração comercial com DL-metionina e óleo de soja não afetou a composição centesimal dos bioflocos. O uso combinado de DL-metionina e óleo de soja melhorou a sobrevivência dos juvenis de tilápia sob restrição na oferta da dieta artificial. Conclui-se que a suplementação da ração comercial, fornecida de modo restritivo aos juvenis de tilápia criados em tanques BFT, com 1,0% de DL-metionina e 0,6% de óleo de soja, pode prevenir a queda no desempenho animal.

Palavras-chave: aquicultura; bioflocos; restrição alimentar; qualidade de água

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Introduction

The BFT system (biofloc technology) for aquaculture gives the culture greater biosecurity and environmental sustainability. In BFT tanks, it is possible to obtain high yields, with minimal water changes (Krummenauer et al., 2014), and with the development of probiotic microorganisms that provide greater sanity to the culture medium. The bioflocs present in the water can be ingested by farmed fish and shrimp, thus reducing the consumption of artificial diets (Kumar et al., 2018). The ingestion of bioflocs by farmed animals can reduce the supply of commercial feed by up to 25%, without zootechnical damage (Najdegerami et al., 2016), or decrease in the protein content of the artificial diet, and without altering the feed supply (Green et al., 2019).

Bioflocs can be a good source of proteins, lipids, amino acids, fatty acids, vitamins, and minerals for farmed animals (Promthale et al., 2019). However, the nutritional value of bioflocs varies depending on the composition of microorganisms, particle size, source of organic C added to the tanks, and physico-chemistry of the water (Ekasari et al., 2014; Wei et al., 2016).

The protein concentration in bioflocs can vary widely, ranging from 9.6% to 53.7% (Sabry-Neto et al., 2015; Rajkumar et al., 2016). For the lipid content, very different values were also found between the studies (0.6 – 0.9% in Rajkumar et al. (2016); 7.2 – 9.1% in Sabry-Neto et al. (2015)). The nutritional value of bioflocs will therefore depend, among other factors, on their amino acid and essential fatty acid profile.

Methionine concentrations of bioflocs are generally below the levels required nutritionally by fish and shrimp (Ekasari et al., 2014). Wei et al. (2016) and Li et al. (2018), corroborating the conclusions of Ekasari et al. (2014), who reported that bioflocs are strongly deficient in methionine and therefore cannot be used as complete feeds for aquaculture. In addition to some amino acids, bioflocs can also be deficient in essential fatty acids (Dantas et al., 2016).

Therefore, there is the possibility of nutritionally enriching bioflocs by supplementing the commercial diet, or the source of organic C applied to the water, with sources of amino acids and essential fatty acids. This productive strategy had not yet been evaluated by science until now. The present study aimed to evaluate the effects of supplementation of the commercial diet of juvenile Nile tilapia, Oreochromis niloticus, kept in BFT tanks, with DL-methionine and soybean oil, on the proximate composition of bioflocs, water quality, and zootechnical performance.

Material and Methods

The work was carried out at the Laboratory of Aquaculture Science and Technology - LCTA, Department of Fisheries Engineering, Center for Agricultural Sciences, Federal University of Ceará, Fortaleza, Ceará. Male juvenile Nile tilapia (*Oreochromis niloticus*), with a body weight of 2.06 ± 0.08 g, were obtained from a producer in that region and transported to the laboratory facilities. After the acclimatization period, twenty tilapia juveniles, with an initial body weight of 2.62 ± 0.14 g, were stored in each 100 L tank, with a total biomass of 52.3 ± 2.9 g tank^{-1}. The animals were kept in the laboratory's indoor cultivation system for eight weeks. This system is formed by 30 circular polyethylene tanks, supplied with 70 L of fresh water and 30 L of water rich in bioflocs, coming from a 1000 L outdoor maturation tank. This tank was stocked with 30 juvenile Nile tilapia, with body weight of 50.6 ± 10.5 g, which were fed commercial powdered food for omnivorous tropical fish (40% CP; 8% EE) four times a day, at 8, 11, 14, and 17 h, at the rate of 7.5% of the total biomass per day.

Mature bioflocs were collected after 25 days. The adjustment of the C:N ratio of water to 15:1 was performed by applying powdered molasses to water, following the recommendations of Avnimelech (1999). The water in the indoor tanks was continuously aerated by a radial compressor, with a power of 2.5 hp. In each experimental tank, a microporous hose (25 cm in length and 2.5 cm in diameter) was installed and arranged in a rectilinear and central manner. There was no water change during the entire experimental period, only replacement to maintain the initial level.

The experimental design was completely randomized and consisted of the following treatments: 1 - positive control: no restriction in the supply of commercial feed and no supplementation with DL-methionine and soybean oil; 2 - negative-control: with 25% restriction in the supply of commercial feed and without DL-methionine and soybean oil supplementation; 3 - restriction of 25% in the supply of commercial feed, supplementation of 0.6% of soybean oil in the feed and without addition of DL-methionine; 4 - restriction of 25% in the supply of commercial feed, supplementation of 1.2% of soybean oil in the feed and without addition of DL-methionine; 5 - 25% restriction in the supply of commercial feed, supplementation of 1.0% of DL-methionine 99% (MetAMINO®, Evonik Animal Nutrition Ltda.) and no addition of soybean oil to the feed; 6 - 25% restriction in the supply of commercial feed, supplementation of 1.0% of DL-methionine and 0.6% of soybean oil in the feed; 7 - 25% restriction in the supply of commercial feed, supplementation of 1.0% of DL-methionine and 1.2% of soybean oil in the feed.

The corresponding amounts of DL-methionine to be mixed with the powdered feed were incorporated weekly, by manual mixing, with the aid of a glass rod. Subsequently, the daily amount of feed with DL-methionine, individualized for each tank, was weighed and packed in plastic bags. Corresponding amounts of soybean oil were added to the feed shortly before feeding, and mixed into the feed using a glass rod. The ration with DL-methionine and/or soybean oil was offered in the place with the least movement of water, being supplied gradually to avoid wastage for water. Except for the positive control, which had six replicates, all other treatments had four replicates each, totaling 30 experimental units.

The fish were fed with a commercial diet containing 40% protein and 1.1% methionine. The ration was offered daily to the animals, four times a day (8, 11, 14 and 17h). The feeding rates used varied between 10.5% (initial) and 6.0% (final). Biweekly, partial biometrics of the fish were performed to adjust the amounts of feed offered. Adjustment of the water C:N ratio to 15:1 was performed daily by applying liquid molasses to all experimental tanks (Avnimelech, 1999). Part of the solids suspended in the water were removed when its concentration exceeded 30 mL L^{-1}. Periodically, sodium bicarbonate was applied to the water to maintain the total alkalinity and pH at values equal to or greater than 60 mg L^{-1} $CaCO_3$ eq. and 7.0, respectively.

Water quality determinations were performed as follows: pH (mPA210 pH meter - MS Tecnopon@), temperature, specific conductance (CD-850 conductivity meter) and dissolved oxygen (55 oximeter - YSI), daily at 9 am; total alkalinity (titration with standard H_2SO_4 solution), total ammoniacal nitrogen (TAN; indophenol method), nitrite (sulfanilamide method), and nitrate (Cd reducing column method), weekly; free carbon dioxide (free CO_2; titration with standard solution of Na_2CO_3), total hardness (titer with standard solution of EDTA), reactive phosphorus (molybdenum blue method), dissolved iron (thiocyanate method), and organic matter (consumed oxygen method), fortnightly. Water quality determinations were performed according to methodologies described by Clesceri et al. (1998). Total suspended solids concentrations were determined weekly, following the recommendations of Boyd & Tucker (1992).

The zootechnical performance variables that were monitored throughout the study were the following: survival, final body weight, weekly weight gain, specific growth rate (SGR = [ln (final weight) - ln (initial weight)]/days of cultivation) x 100), fish productivity, feed efficiency rate (FCR = feed offered/gain in body weight), and of protein efficiency ratio (PER = gain in weight/protein offered). The proximate composition of bioflocs was determined for crude protein (Kjeldahl method), lipids (Soxhlet method), ash (incineration in a muffle furnace), and moisture (drying at 105°C/24 h; AOAC (2000)).

The results of water quality and chemical composition were submitted to two-way ANOVA and to the zootechnical performance to one-way ANOVA, respectively. The means were compared with each other using the Tukey test when there was a significant difference between them ($p < 0.05$). The assumptions of normal distribution and homogeneity of variance were checked before applying ANOVA. Statistical analyzes were performed using SigmaPlot for Windows V.12 (Systat Software, Inc.) and Excel 2016 software.

Results and Discussion

Water quality

The average water temperature was 27.1 ± 0.5 ºC, with minimum and maximum of 26.2 and 27.8 °C, respectively, with no significant differences between treatments ($p > 0.05$). No significant differences were observed for water pH, concentrations of O_2 and free CO_2, neither between the experimental treatments, nor between those and the tanks not subjected to feed restriction ($p > 0.05$; Table 1). The water temperature, pH and O_2 concentrations were within the respective optimal ranges for tropical fish farming (Azim & Little, 2008).

DL-methionine and soybean oil supplementation did not affect the specific conductance (EC) of water ($p > 0.05$; Table 1). The EC of water in ponds without feed restriction was higher than in ponds with commercial feed restriction, in which were without DL-methionine and soybean oil supplementation ($p < 0.05$). In the first tanks, the greater supply of feed, and the consequent mineralization of organic debris, resulted in an increase in the concentration of dissolved ions in the water. In addition, larger amounts of sodium bicarbonate were applied to the tanks without feed restriction to maintain alkalinity and pH. Moreover, feed supplementation with DL-methionine and/or soybean oil did not influence the results of water O_2, pH, free CO_2 and EC. These findings corroborate those obtained by Lima et al. (2021).

At the end of the experimental period, the total alkalinity of the water did not differ between the experimental treatments ($p > 0.05$; Table 1). The total alkalinity of BFT tanks should be maintained above 100 mg L^{-1} $CaCO_3$ for better development of heterotrophic and nitrifying bacteria (Furtado et al., 2015). When the alkalinity of the water drops below 100 mg L^{-1} and the pH becomes less than 7, there is a loss in the bacterial nitrification process in BFT aquaculture tanks (Furtado et al., 2011).

Dietary supplementation with DL-methionine and/or soybean oil did not significantly affect total water hardness (Table 1). The water hardness of the tanks without feed restriction was higher than that observed in the tanks with restriction and not supplemented with DL-methionine, regardless of soybean oil supplementation ($p < 0.05$). Over the course of the cycle, BFT tanks, without water change, tend to have very high water hardness due to the daily intake of feed and molasses.

The concentrations of reactive phosphorus and dissolved iron in the water did not differ between the experimental treatments ($p > 0.05$; Table 1). The concentration of reactive P in the tanks without feed restriction was higher than that observed in the tanks with restriction and no supplementation ($p < 0.05$). Although orthophosphate tends to accumulate in water in BFT systems for aquaculture, concentrations up to 20 mg L^{-1} do not affect the performance of farmed animals (Emerenciano et al., 2017).

Organic matter concentrations in water were not influenced by dietary supplementation with DL-methionine and/or soybean oil (Table 1). As the BFT tanks have minimal water exchange, the concentration of organic matter rises rapidly and can exceed safe levels. To prevent this from happening, the producer must control suspended solids through the use of decanters or clarifiers.

The concentrations of total ammoniacal nitrogen (TAN) and nitrite (NO_2^-) did not differ between treatments ($p > 0.05$; Table 1). On average, the final concentrations of TAN and NO_2^- were equal to 0.05 ± 0.02 mg L^{-1} and 1.28 ± 0.08 mg L^{-1}, respectively.

Table 1. Water quality of the cultured of juvenile Nile tilapia, *Oreochromis niloticus*, after 8 weeks in BFT tanks (mean ± sd; n = 4)[1].

Variable	With feed restriction				No feed restriction [2]	p-value[3]	CV (%)[6]
	DL-methionine supplementation (%)	Soy oil supplementation (%)					
		0.00	0.60	1.20			
O_2 (mg L^{-1})	0.0	6.04 ± 0.33	5.95 ± 0.41	6.12 ± 0.32	5.64 ± 0.31	ns[4]	5.51
	1.0	5.81 ± 0.25	5.83 ± 0.31	5.97 ± 0.35			
pH	0.0	7.66 ± 0.30	7.64 ± 0.20	7.59 ± 0.18	7.44 ± 0.23	ns	3.07
	1.0	7.58 ± 0.19	7.62 ± 0.26	7.57 ± 0.27			
Specific conductance (µS cm^{-1})	0.0	1266 ± 51*[4]	1284 ± 57	1299 ± 44	1389 ± 44	<0.05	4.19
	1.0	1298 ± 71	1288 ± 62	1310 ± 53			
Free CO_2 (mg L^{-1})	0.0	12.6 ± 2.3	12.8 ± 1.7	12.9 ± 2.5	14.0 ± 2.9	ns	19.34
	1.0	13.1 ± 2.4	12.9 ± 3.2	13.5 ± 2.6			
Total alkalinity (mg L^{-1} eq. $CaCO_3$)	0.0	171 ± 13	170 ± 17	161 ± 17	148 ± 16	ns	7.91
	1.0	161 ± 21	164 ± 21	157 ± 20			
Total hardness (mg L^{-1} eq. $CaCO_3$)	0.0	262 ± 29*	260 ± 29*	265 ± 35*	330 ± 31	<0.05	10.41
	1.0	280 ± 24	272 ± 25	292 ± 28			
Reactive phosphorus (mg L^{-1})	0.0	3.79 ± 0.66*	3.94 ± 0.53	4.08 ± 0.74	5.03 ± 0.46	<0.05	13.82
	1.0	4.12 ± 0.60	4.27 ± 0.54	4.42 ± 0.58			
Dissolved iron (mg L^{-1})	0.0	2.88 ± 0.34	3.19 ± 0.31	3.33 ± 0.36	3.81 ± 0.53	ns	12.78
	1.0	3.37 ± 0.49	3.15 ± 0.41	3.60 ± 0.54			
Organic matter (mg L^{-1})	0.0	756 ± 57*	773 ± 54*	788 ± 42	905 ± 63	<0.05	6.40
	1.0	799 ± 61	794 ± 35	823 ± 49			
Total ammoniacal N (mg L^{-1})	0.0	0.05 ± 0.04	0.03 ± 0.03	0.07 ± 0.03	0.07 ± 0.04	ns	63.61
	1.0	0.04 ± 0.04	0.06 ± 0.05	0.06 ± 0.02			
NO_2^- (mg L^{-1})	0.0	1.37 ± 0.41	1.32 ± 0.22	1.16 ± 0.38	1.24 ± 0.40	ns	27.26
	1.0	1.33 ± 0.36	1.19 ± 0.30	1.36 ± 0.37			
NO_3^- (mg L^{-1})	0.0	6.9 ± 0.5*	7.2 ± 0.5*	7.1 ± 0.3*	8.49 ± 0.53	ns	6.68
	1.0	7.3 ± 0.6*	7.2 ± 0.6*	7.4 ± 0.5*			
Sedimentable solids (mL L^{-1})	0.0	76 ± 20*	80 ± 17*	82 ± 20*	133 ± 13	<0.01	22.26
	1.0	86 ± 26*	81 ± 17*	94 ± 27			
Total suspended solids (mg L^{-1})	0.0	640 ± 51*	670 ± 58 *	697 ± 78*	922 ± 63	<0.01	8.63
	1.0	667 ± 54*	687 ± 61*	710 ± 66*			

ANOVA bifactorial														
Factor	O_2	pH	SC	Free CO_2	AT	DT	Reactive P	Fe^{+2}	OM	TAN	NO_2^-	NO_3^-	SS	TSS
DL-Met	ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns
OS	ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns
DL-Met X OS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]No significant differences were observed between the variables for the levels of DL-methionine and soybean oil supplementation, nor interaction between these factors ($p > 0.05$); [2]No restriction in the supply of artificial diet and no supplementation with DL-methionine and soybean oil; [3]For comparison between treatments and control; [4]Not significant ($p > 0.05$); [5]The asterisk indicates that there is a significant difference in relation to the control; [6]Coefficient of variation.

In aquaculture BFT tanks, TAN and NO_2^- concentrations should always be kept below 1 mg L^{-1}. Nitrate concentrations (NO_3^-) also did not vary significantly between treatments (Table 1). NO_3^- concentrations of up to 20 mg L^{-1} generally do not cause toxicity to cultured animals (Emerenciano et al., 2017). In the end, NO_3^- concentrations in the tanks without feed restriction were higher than in the others ($p < 0.01$). In aquaculture systems with minimal water exchange, bacterial nitrification leads to the accumulation of nitrate in the water (Azim & Little, 2008; Rajkumar et al., 2016). If water quality control measures are not carried out, nitrate can impair the performance of animals kept for long periods in BFT systems. Increasing water salinity (Kuhn et al., 2010) and removing suspended solids (Souza et al., 2019) can decrease toxicity and reduce nitrate levels in water, respectively.

Dietary supplementation with DL-methionine and soybean oil did not significantly affect the concentrations of sedimentable solids (SS) and total suspended solids (TSS) in the water (Table 1). SS concentrations were higher in tanks without feed restriction, except for units supplemented with 1% DL-methionine and 1.2% soybean oil ($p < 0.01$). The tanks without feed restriction had higher concentrations of TSS, in relation to the other treatments. To rear Nile tilapia in BFT tanks, SS concentrations in the water must be maintained between 25 and 50 mL L^{-1}; and TSS concentrations below 500 mg L^{-1} (Hargreaves, 2013). Therefore, it is possible that the excess of solids in the water, in the tanks of the present work, has harmed the growth of the fish by the clogging of the gills. The work of removing suspended solids from the water was not efficient because it did not reduce the concentrations of SS and TSS to adequate levels.

The concentrations of inorganic nitrogen compounds (TAN, NO_2^- and NO_3^-) and particulate matter (SS and TSS) in the water were not influenced by the supplementation of DL-methionine and/or soybean oil in the diet. Other studies also found no effects of amino acid (Yun et al., 2017; Lima et al., 2021) and lipid (Hamidoghli et al., 2020) supplementation on these same variables. Therefore, supplementation of commercial aquaculture diets with DL-methionine and/or soybean oil can be performed with limnological safety, as it does not affect water quality.

Centesimal composition of bioflocs

Supplementation of commercial feed with DL-methionine and soybean oil did not affect the proximate composition of bioflocs. Likewise, the restriction in the supply of artificial diet to the fish did not change the chemical composition of the bioflocs (Table 2; p > 0.05). The dry biomass of bioflocs contained 24.76 ± 0.42% and 1.39 ± 0.14% of crude protein and ether extract, respectively. Unlike the present study, Silva et al. (2020) observed that the feeding rate of the animals influenced the chemical composition of the bioflocs. In this work, halving the feed rate reduced the crude protein content of bioflocs from 25% to 21%. On the other hand, the ether extract content of the bioflocs was not affected by the different feeding rates.

The great variability in their chemical composition and the deficiencies in some amino acids and essential fatty acids make the isolated use of bioflocs as food for aquaculture unfeasible. Thus, it would be important to evaluate the use of different combinations between artificial diets and bioflocs, in fish and shrimp tanks, to obtain the best possible productive results, in each case (Silva et al., 2020).

In the present work, the commercial diet had minimum concentrations of 40% of protein, 8% of ether extract, 1.1% of methionine and 1.4% of phosphorus. These protein and lipid values are well above those observed in biofloc biomass. For the creation of Nile tilapia in BFT tanks, it would be possible to use a commercial diet with less protein, without zootechnical damage, as long as there was supplementation with some essential amino acids, such as lysine, methionine and threonine (Bomfim et al., 2008). Lipid concentrations of less than 1% have already been found in bioflocs extracted from BFT tanks for aquaculture (Rajkumar et al., 2016).

Lima et al. (2021) evaluated the supplementation of DL-methionine and soybean oil, alone, in diets for juvenile Nile tilapia kept in BFT tanks, submitted to feed restriction. In both studies, the supplementation did not influence the proximate composition of the bioflocs. In Lima et al. (2021), the concentrations of crude protein and ether extract of bioflocs were equal to 32% and 2.1%, respectively, in tanks supplemented with DL-methionine. These values are higher than those of the present study (Table 2). It is likely that the interruption of mechanical aeration of the water, due to lack of electrical energy in the laboratory, and the application of a commercial product containing sodium percarbonate in the water have caused changes in the chemical composition of the bioflocs in the present study.

More detailed studies could be carried out to determine whether or not bioflocs are able to incorporate methionine and fatty acids into their biomass and to what extent this strategy is economically viable. Higher levels of soybean oil supplementation could be evaluated to identify possible changes in lipid and fatty acid concentrations of bioflocs and the benefits of this for the growth of Nile tilapia juveniles.

Zootechnical performance

Fish survival was affected by soybean oil supplementation; not being influenced, however, by the inclusion of DL-methionine (p < 0.05; Table 3). Soybean oil supplementation provided greater fish survival, starting at the first inclusion level (0.6%). These results differ from previous work carried out in laboratory which did not observe an effect of soybean oil supplementation on the survival of juvenile Tilapia kept in BFT tanks. As the bioflocs of the present work contained higher lipid concentration when compared to the previous work, the survival of the animals may have been positively affected by such conditions.

Except for the treatment with a higher level of soybean oil supplementation, the survival of Nile Tilapia tended to increase when DL-methionine was included in the diets, in tanks subjected to restriction in the supply of artificial diet. This was also observed by Lima et al. (2021), in which the survival of the animals was increased with the supplementation of the balanced diet with DL-methionine. These results suggest that

Table 2. Bromatological composition of biofloc biomass extracted from Nile tilapia juveniles, *Oreochromis niloticus*, after 8 weeks in BFT tanks (mean ± sd; dry basis; n = 4)[1].

Variable	With feed restriction				No feed restriction[2]	p-value	CV (%)[4]
	DL-methionine supplementation (%)	Soybean oil supplementation (%)					
		0.00	0.60	1.20			
Crude protein (%)	0.0	24.72 ± 0.32	24.56 ± 0.38	24.66 ± 0.42	25.13 ± 0.57	ns[3]	1.76
	1.0	24.81 ± 0.51	24.88 ± 0.56	24.60 ± 0.31			
Ether extract (%)	0.0	1.37 ± 0.13	1.35 ± 0.13	1.33 ± 0.14	1.48 ± 0.15	ns	10.67
	1.0	1.38 ± 0.10	1.32 ± 0.18	1.48 ± 0.20			
Ash (%)	0.0	21.74 ± 1.29	21.93 ± 0.90	22.19 ± 0.94	22.53 ± 1.31	ns	5.14
	1.0	22.05 ± 1.27	22.07 ± 1.16	21.85 ± 1.06			

ANOVA bifactorial			
Factor	CP	EE	AS
DL-Met	ns	ns	ns
OS	ns	ns	ns
DL-Met X OS	ns	ns	ns

[1]No significant differences were observed between the variables for the levels of DL-methionine and soybean oil supplementation, nor were interaction between these factors (p > 0.05); In addition, no significant differences were observed between treatments with and without dietary restriction; [2]No restriction in the supply of artificial diet and no supplementation with DL-methionine and soybean oil. [3] Not significant (p > 0.05); [4]Coefficient of variation.

Table 3. Zootechnical performance of juvenile Nile tilapia (*Oreochromis niloticus*) after 8 weeks in BFT tanks (mean ± sd; n = 4).

Variable	With feed restriction				No feed restriction[1]	p-value[2]	CV (%)[8]
	DL-methionine supplementation (%)	Soybean oil supplementation (%)					
		0.00	0.60	1.20			
Survival (%)	0.0	86.2 ± 7.5 a[3]	96.2 ± 2.5 b	98.7 ± 1.2 b	95.0 ± 4.5	ns[4]	4.14
	1.0	88.7 ± 2.5 a	97.5 ± 2.5 b	93.7 ± 4.8 ab			
Final body weight (g)	0.0	14.4 ± 0.7*[5]	15.2 ± 1.1*	15.1 ± 1.1*	17.2 ± 1.5	<0.01	5.77
	1.0	15.6 ± 0.6*	15.1 ± 0.5*	15.7 ± 0.7*			
SGR[6] (% dia^{-1})	0.0	3.05 ± 0.14	3.13 ± 0.13	3.13 ± 0.19	3.36 ± 0.16	ns	3.99
	1.0	3.18 ± 0.06	3.18 ± 0.14	3.18 ± 0.07			
Weekly weight gain (g semana^{-1})	0.0	1.48 ± 0.09*	1.57 ± 0.13*	1.56 ± 0.14*	1.83 ± 0.19	<0.01	6.71
	1.0	1.62 ± 0.06*	1.57 ± 0.07*	1.63 ± 0.07*			
Fish yield (g m^{-3} dia^{-1})	0.0	44.4 ± 3.1*a	52.2 ± 4.2 b	53.3 ± 4.9 b	58.5 ± 7.0	<0.01	7.74
	1.0	49.5 ± 2.4 a	52.6 ± 2.5 a	52.5 ± 4.0 a			
FCR[7]	0.0	1.58 ± 0.17 aA	1.38 ± 0.08*bA	1.36 ± 0.11*bA	1.72 ± 0.22	<0.01	8.22
	1.0	1.42 ± 0.06 aB	1.34 ± 0.11*aA	1.37 ± 0.09*aA			
PER[8]	0.0	1.59 ± 0.18 a	1.82 ± 0.11*ab	1.85 ± 0.14*b	1.48 ± 0.19	<0.01	7.94
	1.0	1.76 ± 0.08 a	1.87 ± 0.15*a	1.83 ± 0.12*a			

ANOVA bifactorial							
Factor	Survival	Final body weight	SGR	Weekly weight gain	Fish yield	FCR	PER
DL-Met	ns	ns	ns	ns	ns	<0.05	ns
OS	<0.01	ns	ns	ns	<0.01	<0.05	<0.05
DL-Met X OS	ns	ns	ns	ns	<0.05	<0.05	ns

[1]No restriction in the supply of artificial diet and no supplementation with DL-methionine and soybean oil; [2]For comparison between treatments and control (no feed restriction); [3]For the same variable, means in the same row or column with different lowercase and uppercase letters, respectively. They are different from each other by the Tukey test (p < 0,05). Absence of letters indicates that the differences between the means are not significant; [4]Not significant (p > 0,05); [5]The asterisk indicates that there is a significant difference in relation to the control (no feed restriction); [6]SGR: specific growth rate = [ln (final weight) - ln (initial weight)] / days of cultivation) x 100); [7]FCR = ration offered (g)/body weight gain (g); [8]PER = body weight gain (g)/protein offered (g); [8] Coefficient of variation.

supplementation of commercial diets with DL-methionine and soybean oil can increase the survival of Nile tilapia juveniles kept in BFT tanks subjected to feed restriction.

Supplementation of commercial feed with DL-methionine and soybean oil did not affect final body weight, specific growth rate and weekly weight gain of cultured fish ($p > 0.05$; Table 3). On the other hand, Lima et al. (2021) reported improvement in body weight and weight gain of Tilapia when the commercial feed was supplemented with 1% of DL-methionine.

The lower nutritional quality of the bioflocs in the present study possibly affected the growth of the fish, as it did not provide the necessary nutrients for satisfactory growth. Fish kept in ponds with balanced feed restriction, regardless of DL-methionine or soybean oil supplementation, exhibited lower final body weight and weekly weight gain when compared to animals stored in ponds without feed restriction. Similarly, Pérez-Fuentes et al. (2018) observed lower final weight and weight gain of Nile tilapia when the animals' feeding rate was reduced by 30%. These results suggest that the higher intake of bioflocs, in tanks with feed restriction, was not able to compensate for the drop in nutritional support caused by the lower supply of balanced ration. In addition, the success of the commercial feed supplementation strategy with DL-methionine and soybean oil will depend on the nutritional value of the bioflocs present in the breeding tanks.

The productivity of the ponds, in grams of fish per m³ per day, was significantly affected by soybean oil supplementation (Table 3). In ponds that did not receive DL-methionine supplementation, the addition of soybean oil, regardless of the level used, resulted in fish productivity. In tanks that received DL-methionine, soybean oil supplementation did not affect this variable. These results suggest that supplementation of a balanced diet, offered in a restricted way to juvenile Tilapia kept in BFT tanks, with soybean oil, allows for keeping the productivity of the tanks unchanged. In addition, if the ration is supplemented with 1.0% DL-methionine, the productivity of the ponds will not be significantly affected, even without the application of soybean oil.

The feed efficiency rate (FCR) of the animals was affected by both DL-methionine and soybean oil supplementation, with a significant interaction between these factors (Table 3). In the tanks without soybean oil supplementation, the application of 1.0% DL-methionine, together with the supply of a balanced ration, resulted in better FCR ($p < 0.05$). When DL-methionine was not supplemented, the incorporation of 0.6 and 1.2% of soybean oil in the diet significantly improved FCR.

On the other hand, FCR did not differ between the different levels of soybean oil inclusion when supplementing with 1.0% DL-methionine. The FCR results of all treatments were better than those observed in the tanks without restriction in the supply of balanced feed ($p < 0.01$).

When the diet was not supplemented with DL-methionine, the protein efficiency ratio (PER) of the fish was higher in the tanks with the highest level of soybean oil inclusion. When supplementing the diet with 1% DL-methionine, the addition of soybean oil did not affect PER ($p > 0.05$). The PER of the fish kept in the tanks without feed restriction was lower when compared to the PER of the tanks that received some soybean oil supplementation, with or without DL-methionine.

Therefore, the results of FCR and PER improved with the supplementation of the balanced diet with soybean oil, only when there was no supplementation with DL-methionine. The effects of artificial diet supplementation, restricted to animals, with DL-methionine and soybean oil, on the feed efficiency of tilapia reared in BFT tanks will depend on the nutritional composition of the bioflocs. Bigger success of these production strategies is expected when the nutritional richness of bioflocs, in terms of protein and lipid concentrations, is greater.

The nutrients not available to the fish, due to the restriction in the supply of balanced feed, could be recovered, at least partially, by the consumption of bioflocs enriched with DL-methionine and soybean oil. If this occurs, the cost of producing tilapia would be reduced, contributing to giving greater economic sustainability to the activity. According to Dong et al. (2017), fish digest and assimilate dietary proteins and lipids better when under feed restriction. The use of lipids from bioflocs as a source of energy by fish can allow a reduction in the amount of feed offered to animals, without zootechnical damage.

Conclusions

Feed restriction of 25% improved the quality of Nile tilapia cultured water in biofloc tanks. Supplementation of artificial diets with DL-methionine and soybean oil did not impair water quality. Therefore, the producer can make use of these measures without prejudice to water quality.

Supplementation of the artificial diet did not cause changes in the chemical composition of the bioflocs.

The combined use of DL-methionine and soybean oil in artificial diets improves the survival of Nile tilapia under feed restriction conditions. Supplementation of 1.0% DL-methionine and/or 0.6% soybean oil in artificial diets, with a 25% restriction, prevents worsening of zootechnical performance indices.

Compliance with Ethical Standards

Author contributions: Conceptualization: FRSL, MVCS; Data curation: FRSL, MLSA, DHC; Formal analysis: FRSL, MLSA, DHC; Funding acquisition: MVCS; Methodology; MVCS: FRSL; Investigation: FRSL, MLSA, DHC; Project administration: MVCS; Resources: MVCS; Supervision: MVCS; Visualization: FRSL; Writing – original draft: FRSL; Writing – review & editing: MVCS.

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