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Dietary supplementation of tilapia juveniles reared in bft (bioflocs) tanks with dl-methionine

Suplementação da dieta de juvenis de tilápia criados em tanques bft (bioflocos) com dl-metionina

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Abstract

The present study aimed at determining the effects of dietary DL-methionine supplementation on the water quality, bioflocs composition and Nile tilapia juvenile's (initial body weight = 2.76 ± 0.06 g) growth performance in BFT rearing tanks (18 fish/100-L tank). Fish were or not subjected to artificial feed restriction. The experimental treatments consisted of two control groups: 1 – no feed restriction, no methionine supplementation; 2 – feed restriction at 25%, no methionine supplementation. There were also four treated groups: 1 - feed restriction at 25%, dietary DL-methionine supplementation at 0.5%; 2 - feed restriction at 25%, dietary DL-methionine supplementation at 1.0%; 3 – feed restriction at 25%, dietary DL-methionine supplementation at 2.0%; 4 – feed restriction at 25%, DL-methionine supplementation of molasses at 1.0. Supplementation of the commercial diet with DL-methionine has not affected either the water quality of the BFT Nile tilapia rearing tanks or the proximate composition of the bioflocs. After 8 weeks, weight gain of fish reared in tanks with feed restriction and dietary DL-methionine supplementation at 1% or 2% has not differed (P>0.05) from the tanks without feed restriction. In conclusion, it is possible to restrict the daily feed allowances of Nile tilapia juveniles reared in BFT tanks at 25%, with no growth performance impairment, if a minimal dietary DL-methionine supplementation of 1.0% is given.

Keywords: Bioflocs. Amino acid. Aquaculture. Water quality.

Resumo

O presente estudo teve por objetivo determinar os efeitos da suplementação da ração balanceada de juvenis de tilápia do Nilo (peso médio inicial = 2,76 ± 0,06 g), submetidos à restrição na oferta de alimento artificial em tanques BFT de cultivo (18 juvenis/tanque 100 L), com DL-metionina, sobre a qualidade da água, composição dos bioflocos e desempenho zootécnico. Os tratamentos experimentais foram constituídos por dois grupos controle: 1 – sem restrição alimentar, sem suplementação com metionina; 2 – com restrição alimentar de 25%, sem suplementação com metionina. Os quatro

tratamentos experimentais foram os seguintes: 1 – restrição alimentar de 25%, suplementação da ração com 0,5% de DL-metionina misturada à ração; 2 – restrição alimentar de 25%, suplementação da ração com 1,0% de DL-metionina misturada à ração; 3 – restrição alimentar de 25%, suplementação da ração com 2,0% de DL-metionina misturada à ração; 4 – restrição alimentar de 25%, suplementação do melaço em 1,0% de DL-metionina. A suplementação da ração balanceada com DL-metionina não afetou a qualidade da água de cultivo de juvenis de tilápia do Nilo, em tanques BFT, nem a composição centesimal do bioflocos. Após 8 semanas, o ganho em peso corporal dos peixes mantidos nos tanques com restrição alimentar e suplementação da ração com 1% ou 2% de DL-metionina não diferiu (P>0,05) do observado nos tanques sem restrição alimentar. Concluiu-se que é possível restringir as taxas de arraçoamento de juvenis de tilápia do Nilo, mantidos em tanques BFT de cultivo, em 25%, sem prejuízo zootécnico, desde que se faça a suplementação da dieta com, pelo menos, 1% de DL-metionina.
Palavras-chave: Bioflocos. Aminoácido. Aquicultura. Qualidade de água.

Introduction

Bioflocs are aggregates made up of different microorganisms, animal feces and other organic particles. In addition to improving water quality, bioflocs also serve as a food source for some species of fish and shrimp, such as Nile tilapia, *Oreochromis niloticus*, and marine shrimp, *Litopenaeus vannamei*(1,2). The biofloc technology (BFT) applied to aquaculture aims to control water quality by favoring the development of heterotrophic and nitrifying bacteria capable of removing ammonia and nitrite in tanks with minimal or zero-water exchange(1,3,4). The BFT technology is applied to superintensive closed aquaculture systems, being advantageous both economically and epidemiologically. Bioflocs may have a probiotic role, acting to prevent the development of pathogenic bacteria and improve the activity of digestive enzymes in fish and shrimp(4,5).

Ingestion of bioflocs can partially meet the nutritional requirements of fish and shrimp and reduce commercial feed costs(6). In studies carried out with tilapia and *L. vannamei*, bioflocs allowed a decrease in dietary protein without growth performance impairment(1,7,8,9). Therefore, the amino acids, fatty acids, vitamins, and minerals of bioflocs might reduce the need of nutritionally complete diets(10).

However, the bioflocs value as a food source for farmed fish and shrimp is unpredictable because there is great variability in its chemical composition. The source of organic carbon used to adjust the C: N ratio of water and the water quality are some factors that affect the bioflocs proximate composition(2,11). While Ballester *et al*.(12) observed circa of 30% and 5% proteins and lipids, respectively, in the bioflocs biomass, Durigon *et al*.(13) reported only 17% and 1%, respectively, in tilapia tanks.

Although bioflocs might be an important source of essential amino acids, some studies have shown they are generally deficient in methionine to meet tilapia and *L. vannamei*'s requirements[11,14,15,16]. The development of a feeding strategy to correct the bioflocs' methionine deficiency would be of importance for economic sustainability of the activity. The present study aimed at determining the effects of the supplementation of a commercial feed with DL-methionine on water quality, composition of bioflocs and Nile tilapia growth performance, in BFT tanks under feed restriction.

Material and methods

Nile tilapia (*Oreochromis niloticus*) juveniles were obtained from a regional producer (Itaitinga, Ceará), transported to the laboratory facilities, and transferred to an acclimation tank. The study was carried out in the lab indoor culture system, which consists of 30 circular polyethylene 100-L tanks. Each tank was initially supplied with 70 L freshwater and 30 L bioflocs-rich water obtained from one 1000-L outdoor tank. The bioflocs tank had 30 juveniles of Nile tilapia with an average weight of 31.6 ± 3.4 g. Fish were fed with a commercial powdered diet for omnivorous tropical fish (40% crude protein), four times daily, at 8, 11, 14 and 17 h, at a rate of 7.5%. The bioflocs were harvested after 21 days. The adjustment of the water C: N ratio to 15: 1 was performed by applying dry molasses to water, following the recommendations of Avnimelech[17].

Tanks were mechanically aerated with a radial compressor (air blower) with a nominal power of 2.5 hp. Tanks were continuously aerated throughout the study. Each tank was provided with an air adjuster and a microporous hose (25 cm long; 2.5 cm in diameter), linearly arranged at the tank center for air dispersion in water. There was no water exchange over the entire experiment, only the replacement to maintain the initial water level.

At the beginning of the study, each rearing tank was stored with 18 juveniles of Nile tilapia with a body weight of 2.76 ± 0.06 at 49.6 ± 1.0 g fish per tank of 100 L (≈ 500 g fish m^{-3}). The completely randomized design consisted of two control groups and four experimental groups with five replications each (30 tanks in total). Fish in the positive control were fed regularly, completely following the feeding rates of the laboratory (≈ 8% biomass day^{-1}). Besides, the commercial diet had no supplementation with DL-methionine 99% (MetAMINO®, Evonik Animal Nutrition Ltda). Fish in the negative control group received only 75% dietary allowance of the positive group and no methionine supplementation. Fish in three experimental groups received 75% daily dietary allowance[18], but their commercial diet was supplemented with increasing levels of DL-methionine (0.5; 1.0 and 2.0%). The DL-methionine was blended to the amounts of artificial diet offered in each tank as follows: the designed amounts of DL-methionine (0.5%; 1.0% 2.0%) were manually mixed with the artificial diet with the aid of a glass rod; the daily amounts of diet supplemented with DL-methionine were weighed and stored in plastic bags until use. In one of the experimental groups, fish received 75% artificial diet, but the 1.0% DL-methionine was supplemented to the dry molasses instead of the diet.

Fish were fed for eight weeks, four times a day at 8, 11, 14 and 17h, with commercial powdered diet for omnivorous fish (Aquamix PL-0, Integral Mix, Fortaleza, Ceará). After weighing fish fortnightly, the amounts of feed delivered to the animals were adjusted. Feeding rates ranged from 10.5% (initial) to 5.3% (final). Daily, dry molasses (Indumel, Biosev, Sertãozinho, SP) was applied to adjust the C: N ratio of water to 15: 1. The amount of molasses applied in each experimental unit was obtained using the formula presented by Avnimelech(17), which consider the tank feeding management as follows: total ammonia nitrogen (g/tank/day) = commercial diet allowed (g/tank/day) * % N diet * 0.5.

Suspended solids were removed twice a week by sedimentation of 20% tank volume. That procedure was always performed when the concentrations of settleable solids exceeded 30 mL L^{-1}. The supernatant 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} $CaCO_3$ eq. and 7.0, respectively.

Water quality was evaluated as follows: pH (pH meter mPA210 - MS Tecnopon@), temperature, specific conductance (conductivity meter CD-850) and dissolved oxygen (oximeter YSI55), daily at 9h; total alkalinity (titration with a standard H_2SO_4 solution), total ammonia nitrogen (TAN; indophenol method), nitrite (sulfanilamide method) and nitrate (Cd-column reduction method), weekly; free carbon dioxide (free CO_2; titration with a standard Na_2CO_3 solution), total hardness (titration with a standard EDTA solution), reactive phosphorus (molybdenum blue method), dissolved iron (thiocyanate method) and organic matter (oxygen consumed method), fortnightly. The determinations of water quality were carried out according to Clesceri et al.(19). The concentrations of total suspended solids were weekly determined, following the recommendations of Boyd and Tucker(20).

Growth performance variables were monitored over the study: survival, final body weight, weekly weight gain, specific growth rate (SGR = [ln (final weight) - ln (initial weight)]/days of cultivation) x 100), fish yield, feed conversion ratio (FCR = dietary allowance/body weight gain) and protein efficiency ratio (PER = fish weight gain/dietary protein allowance).

The bioflocs' proximate composition was determined by the procedures indicated by AOAC(21) to crude protein (Kjeldahl method), ether extract (Soxhlet method), ash (muffle oven incineration) and moisture (drying at 105°C for 24 h).

Results were tested by One-way ANOVA for completely randomized experiments. When there was a significant difference ($p<0.05$) between treatments, the means were compared pairwise using the Tukey's test. Assumptions of normal distribution and homogeneity of variance were checked. Polynomial regression was applied on the final body weight and feed conversion ratio (FCR) results, in which the DL-methionine supplementation levels was the independent variable. The optimal supplementation of DL-methionine was estimated for each growth performance variable using the expression $x = -b/2a$, where a and b were the regression coefficients. Statistical analyses were run with the aid of the softwares SigmaPlot for Windows v.12 (Systat Software, Inc.) and Microsoft Excel 2016.

Results and discussion

Water quality

No significant differences were detected between the treatments for dissolved oxygen, pH, temperature, and total alkalinity of water (p>0.05; Table 1). The specific conductance (SC) of the water was significantly higher in tanks with no feed restriction, except for the tanks subjected to 2% DL-methionine supplementation. There was an increase in mineralization of the organic matter with greater feed intake in tanks supplemented with 2% methionine due to their greater fish growth. The input of nutrients raises the SC of the water, which could be used as an indicator of eutrophication[22]. BFT tanks tend to have high SC because they accumulate organic matter in water.

Table 1. Dissolved oxygen, pH, specific conductance, temperature, and total alkalinity of water after 8 weeks rearing Nile tilapia juveniles subjected or not to feed restriction and supplemented with increasing levels of DL-methionine (mean ± sd.; n = 5)

Feed restriction (%)	DL-methionine (%)	Dissolved oxygen (mg L^{-1})	pH	Specific conductance (µS cm^{-1})	Temperature (°C)	Total alkalinity (mg L^{-1} eq. $CaCO_3$)
0	0.0	5.65 ± 0.21	7.28 ± 0.10	1643 ± 36 a	26.2 ± 0.11	141.9 ± 9.0
25	0.0	5.74 ± 0.14	7.40 ± 0.11	1528 ± 40 b	26.2 ± 0.13	150.4 ± 11.0
25	0.5	5.69 ± 0.22	7.42 ± 0.11	1530 ± 39 b	26.1 ± 0.16	151.3 ± 10.7
25	1.0	5.72 ± 0.15	7.39 ± 0.12	1568 ± 35 b	26.1 ± 0.13	152.2 ± 7.8
25	2.0	5.64 ± 0.18	7.35 ± 0.12	1581 ± 40 ab	26.1 ± 0.15	144.7 ± 8.4
25	1.0*	5.71 ± 0.12	7.37 ± 0.08	1558 ± 27 b	26.2 ± 0.15	149.4 ± 12.1
P-value		NS	NS	<0.001	NS	NS

DL-methionine was mixed with the artificial diet in all treatments except for that marked with an asterisk, in which methionine was mixed with dry molasses. In the same column, means with different letters are significantly different by Tukey's test (P<0.05). NS = Non-significant (P>0.05).

The free CO_2 and dissolved iron concentrations did not significantly differ between the treatments (p>0.05; Table 2). Total hardness (TH) of water in the tanks under no feed restriction was significantly higher, except for the tanks supplemented with 2% DL-methionine. Allowances of feed and molasses were greater in tanks with greater fish growth, which increased their TH. Da Costa et al.[23] state that molasses is a product used for ruminant animal nutrition and that it receives mineral additives such as calcium. As the water TH expresses the concentrations of calcium and magnesium, the higher input of molasses increased TH.

Table 2. Total hardness, concentration of free CO_2, reactive phosphorus, dissolved iron and organic matter in water after 8 weeks rearing Nile tilapia juveniles subjected or not to feed restriction and supplemented with increasing levels of DL-methionine (mean ± sd.; n = 5).

Fee restriction (%)	DL-methionine (%)	Total hardness (mg L^{-1} eq. $CaCO_3$)	Free CO_2 (mg L^{-1})	Reactive phosphorus (mg L^{-1})	Dissolved iron (mg L^{-1})	Organic matter (mg L^{-1})
0	0.0	331.3 ± 18.1 a	14.8 ± 2.8	4.59 ± 0.34 a^2	3.19 ± 0.34	806 ± 24 a
25	0.0	287.3 ± 12.8 b	11.8 ± 2.9	3.78 ± 0.43 b	2.79 ± 0.18	743 ± 19 b
25	0.5	293.9 ± 10.6 b	11.8 ± 2.8	3.81 ± 0.21 b	2.75 ± 0.15	748 ± 14 b
25	1.0	298.3 ± 16.9 b	12.2 ± 3.0	3.94 ± 0.38 ab	2.84 ± 0.25	747 ± 28 b
25	2.0	302.8 ± 11.4 ab	13.4 ± 3.2	4.10 ± 0.40 ab	2.88 ± 0.29	762 ± 27 ab
25	1.0	288.4 ± 17.3 b	13.1 ± 3.6	3.91 ± 0.39 ab	2.79 ± 0.36	751 ± 20 b
P-value		<0.001	NS	0.019	NS	0.002

DL-methionine was mixed with the artificial diet in all treatments except for that marked with an asterisk, in which methionine was mixed with dry molasses. In the same column, means with different letters are significantly different by Tukey's test (P<0.05). NS = Non-significant (P>0.05).

The concentrations of reactive phosphorus were higher in tanks with no feed restriction (P<0.05), except by the tanks supplemented with 1.0 and 2.0% of DL-methionine (Table 2). Commercial fish diets contain considerable concentrations of phosphorus (0.5 - 1.0%), mostly of phytic nature. As the feeding allowances were higher in tanks with 2% DL-methionine supplementation, the P excretion to water was proportionally higher in those tanks. Similar results were reported by Caldini et al.(18), who attributed the higher concentrations of reactive phosphorus to the greater allowances of commercial diets.

Concentrations of organic matter were higher in tanks with no feed restriction (p<0.05), except for the tanks supplemented with 2% DL-methionine (Table 2). Since the fish growth was higher in the latter tanks, feed intake was greater in those units because the amounts of artificial diet were proportional to fish biomass.

As the regression slope between the level of DL-methionine supplementation (%) and the concentration of TAN (mg L^{-1}) was close to zero, there was no significant effects of methionine supplementation on ammonia (Figure 1).

This result suggests that the dietary supplementation with DL-methionine did not stimulate the formation of bioflocs, serving only as an extra nutrient for fish. Otherwise, it would be expected a TAN reduction caused by DL-methionine supplementation. The organic carbon source and the C: N ratio of water influence the bioflocs formation and structure, having an important impact on the absorption of TAN(11,24).

Concentrations of nitrite concentrations were not significantly affected by DL-supplementation (Figure 2), suggesting that extra methionine did not stimulate the growth of *Nitrobacter*. This result strengthens the suggestion that DL-methionine served only as a nutrient for fish growth, not contributing to improve the water quality.

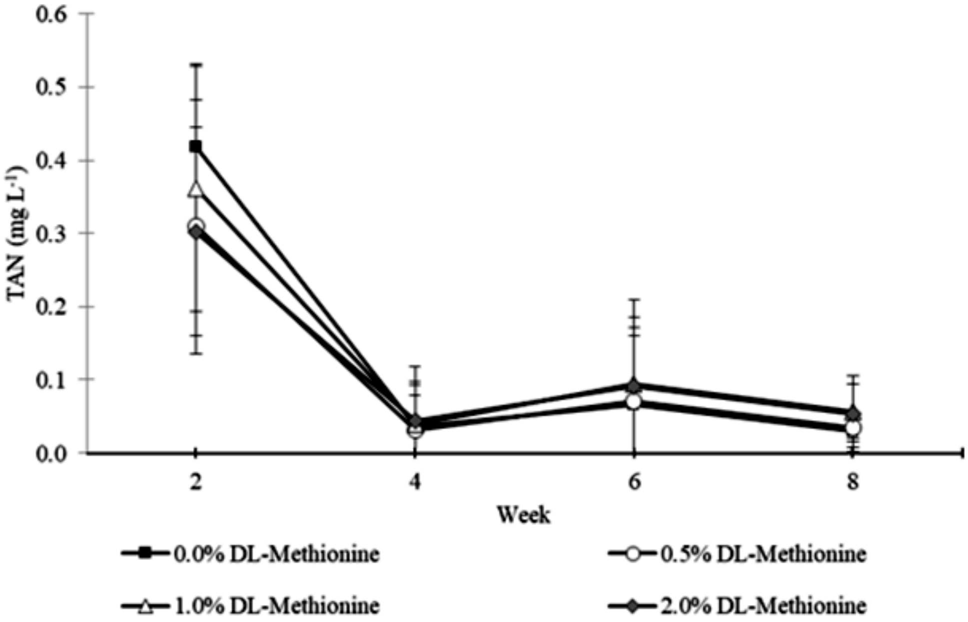

Figure 1. Concentration of total ammonia nitrogen in Nile tilapia BFT tanks subjected or not to feed restriction and supplemented with increasing levels of DL-methionine. At each time, the differences between the treatments are not significant (P>0.05).

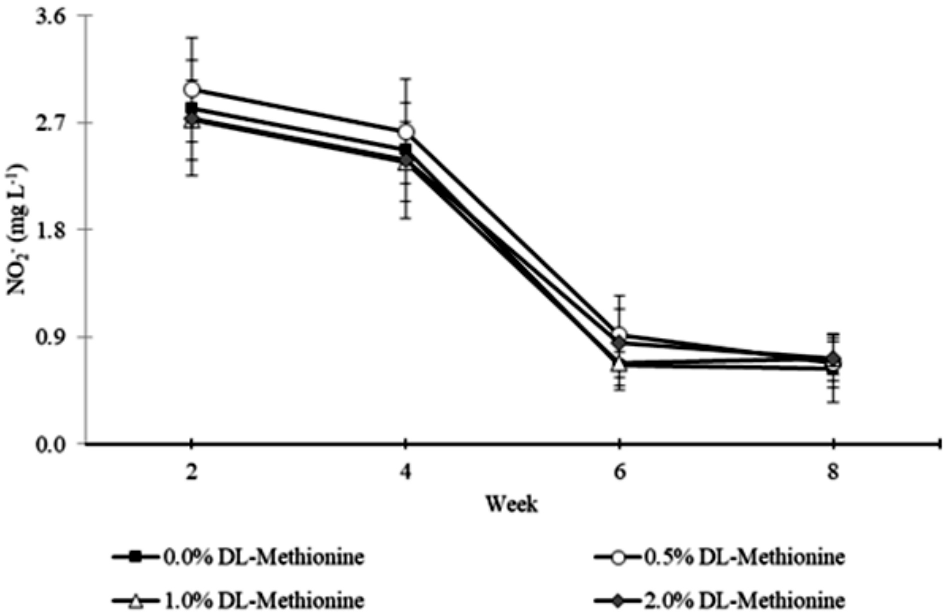

Figure 2. Nitrite in Nile tilapia BFT tanks subjected or not to feed restriction and supplemented with increasing levels of DL-methionine. At each time, the differences between the treatments are not significant (P>0.05).

Heterotrophic and nitrifying bacteria coexist in well-developed bioflocs tanks[1,25]. Those bacteria, however, might compete for ammonia, adhesion surface and micronutrients[24]. Heterotrophic bacteria develop quickly after the addition of a highly biodegradable organic carbon source to the tank, assimilating most of the ammonia. Nitrification, however, may have an increased importance and be responsible for removing up to 25 - 50% TAN[26]. The main factors affecting nitrification in BFT systems are the concentrations of dissolved O_2, ammonia and nitrite; the ratio C: N, pH, temperature, and total alkalinity of water[27,28].

Nitrate concentrations increased in all tanks over the experimental period and there was no influence of DL-methionine supplementation on nitrate (Figure 3).

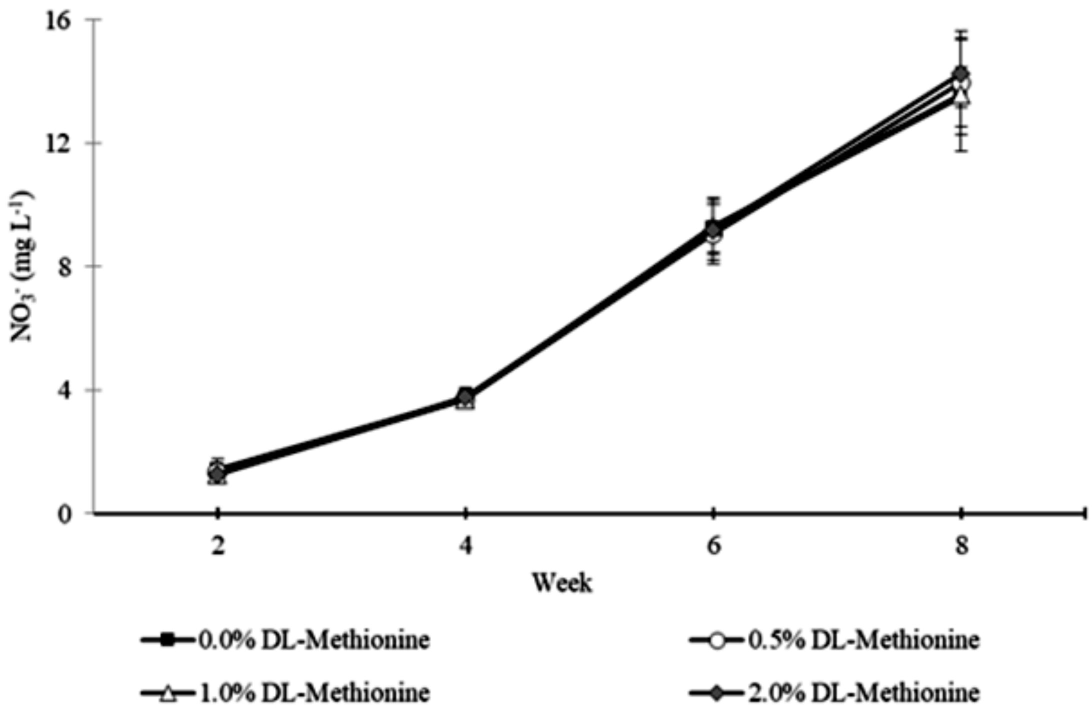

Figure 3. Nitrate in Nile tilapia BFT tanks subjected or not to feed restriction and supplemented with increasing levels of DL-methionine. At each time, the differences between the treatments are not significant (P>0.05).

Nitrification occurs under aerobic conditions and consumes inorganic carbon in the form of bicarbonates[27]. Nitrate tends to accumulate in BFT and RAS tanks[29]. No significant effects on TAN, NO_2^- and NO_3^- were found in BFT *L. vannamei*[7] and Nile tilapia[30] tanks when the animals fed diets with different protein levels.

The concentrations of total suspended solids (TSS) were significantly affected by the supplementation with DL-methionine (Figure 4).

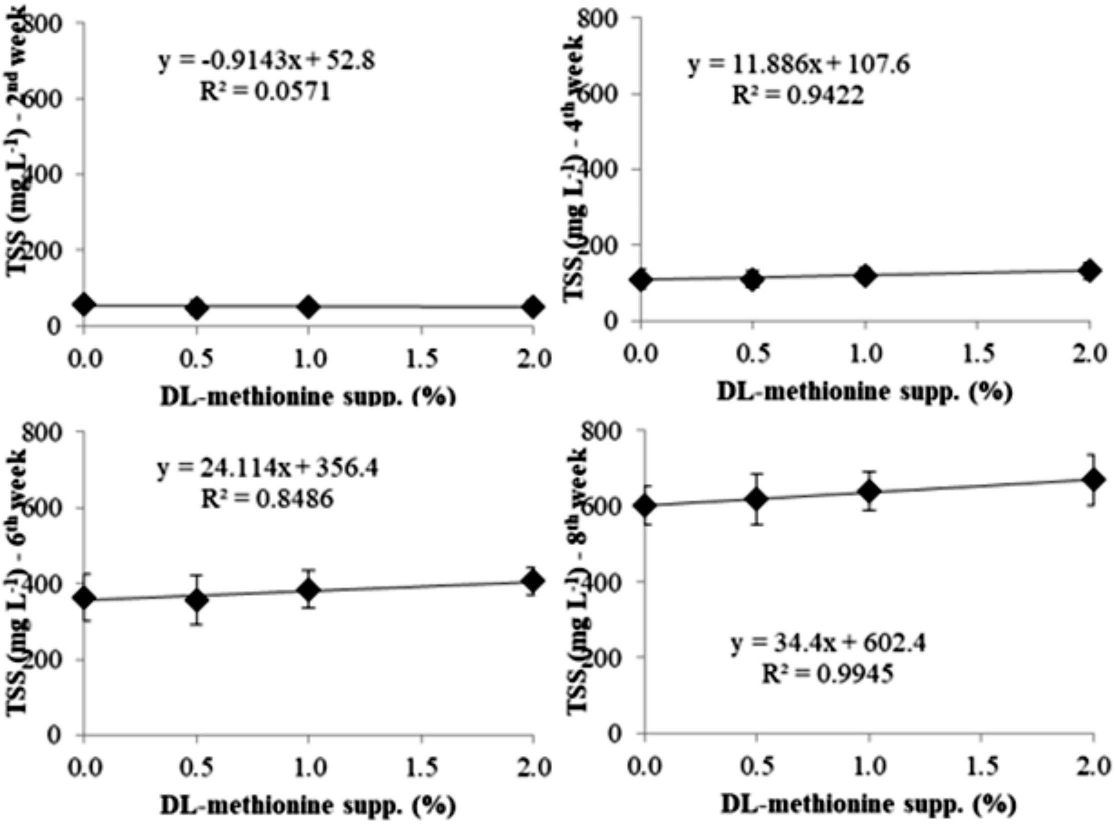

Figure 4. Concentrations of total suspended solids (TSS) in Nile tilapia, *Oreochromis niloticus*, BFT tanks. The commercial diet was supplemented with increasing levels of DL-methionine (n = 5).

DL-methionine supplementation favored fish weight gain in the present study (Table 4). Daily commercial diet and dry molasses inputs were higher in tanks with greater methionine supplementation because those actions were carried out in a way directly proportional to fish growth. More artificial diet produced more feces and more molasses and produced more bioflocs. As a result, there was a significant increase in TSS concentrations in tanks receiving more DL-methionine.

TSS concentrations should not exceed 500 mg L^{-1} in tilapia BFT tanks[26]. The solids concentration in water should be managed to prevent fish gill clogging[31]. As a preventive measure, it is recommended to use clarifiers to remove excess solids in BFT tanks.

Chemical composition of bioflocs

Bioflocs proximate composition was not affected by the dietary feed restriction or DL-methionine supplementation (Table 3; P>0.05). The bioflocs moisture did not differ between the treatments (P>0.05). Similarly, the different DL-methionine supplementation levels have not affected the bioflocs' crude protein, ether extract and ash contents.

Table 3. Proximate composition of bioflocs in Nile tilapia BFT tanks subjected or not to feed restriction and supplemented with increasing levels of DL-methionine (dry matter basis)

Feed restriction (%)	DL-methionine (%)	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash (%)
0	0.0	88.87 ± 0.84	31.49 ± 1.60	2.07 ± 0.17	10.78 ± 0.71
25	0.0	89.42 ± 0.85	31.31 ± 1.87	2.04 ± 0.24	10.33 ± 0.52
25	0.5	89.90 ± 0.88	32.43 ± 1.81	2.18 ± 0.15	10.66 ± 0.72
25	1.0	89.56 ± 0.96	31.45 ± 1.79	2.02 ± 0.10	10.72 ± 0.88
25	2.0	89.99 ± 0.70	32.33 ± 1.50	2.07 ± 0.18	10.58 ± 0.51
25	1.0*	89.79 ± 1.09	31.94 ± 1.99	2.11 ± 0.11	10.60 ± 0.63
P-value		NS	NS	NS	NS

DL-methionine was mixed with the artificial diet in all treatments except for that marked with an asterisk, in which methionine was mixed with dry molasses. Mean ± standard deviation (n = 3). NS = Non-significant (P> 0.05).

The bioflocs' crude protein, moisture and ash values were similar to those observed by Gallardo-Collí, Pérez-Rostro and Hernández-Vergara[32]. These authors found 36%, 86% and 11%, respectively, for the same variables. Durigon et al.[13] and Rajkumar et al.[33] observed levels of ether extract of 1.2% and 0.9%, respectively, values lower than in the present study. Therefore, the supplementation of DL-methionine mixed with the artificial diet or dry molasses did not nutritionally enrich the bioflocs. However, it would be important to determine the methionine concentration in bioflocs to check if there had not been an increase in that content.

As there was a positive effect of DL-methionine supplementation on fish growth in tanks subjected to feed restriction, the DL-methionine was probably used by fish as a nutrient. In general, bioflocs are deficient in methionine to meet the nutritional requirements of Nile tilapia and *L. vannamei*. The bioflocs reported by Wei, Liao and Wang[11] and Ekasari et al.[15] were deficient in methionine with concentrations below 20 μmol g^{-1}. One cause for the animal growth impairment when the individuals are given bioflocs biomass is their methionine deficiency[11,15,16]. Valle et al.[34] recommended that the *L. vannamei's* diet should be supplemented with methionine when bioflocs meal is used as animal feed. A decrease in fish weight gain was expected in the tanks subjected to feed restriction. Since it has not occurred in tanks that receiving DL-methionine at 1% or 2%, the intake of supplemental DL-methionine by fish was important in maintaining unchanged rates of animal growth.

Fish growth performance

Fish survival was equal to 91.3 ± 1.8% with no significant difference between the treatments ($p>0.05$; Table 4). The final body weight and SGR in tanks with no feed restriction were significantly higher than in the other treatments, except for the units with 1.0% or 2.0% DL-methionine. Those same variables were significantly lower in tanks with 1% DL-methionine mixed with molasses. Therefore, the optimal DL-methionine supplementation of Nile tilapia BFT subjected to feed restriction would be 1%. Above that level there would be no clear growth performance advantages. Feed restriction aimed at increasing the bioflocs intake by fish. If well succeeded, a significant part of the fish nutrition would come from the ingestion of bioflocs. He et al.[35] reported that the optimal inclusion level of L-methionine in Nile tilapia diets for maximum growth was 0.91% with 0.83 g kg^{-1} cysteine in the diet. According to the authors, methionine requirement for immunological integrity is greater than for maximum growth. In BFT tanks, fish are subjected to various stressors, such as high concentrations of nitrite and suspended solids in the water. Therefore, the requirements of tilapia for methionine might be even higher in BFT tanks.

DL-methionine supplementation should be via the commercial diet and not the molasses. Hence, DL-methionine was probably important only for fish nutrition and not for bioflocs' development. While the DL-methionine-artificial diet blend aimed at tilapia nutrition, the DL-methionine-molasses blend aimed at the bioflocs nutrition. The DL-methionine-molasses blend may have affected the amino acid stability, causing their loss to water by leaching, since the dry molasses absorbs moisture. Because crystalline DL-methionine is soluble in water its use is limited in aquatic diets. Leaching of 20% DL-methionine may occur 30 minutes after its contact with water[36]. According to Guo et al.[37], the dipeptide DL-methionyl-DL-methionine (Met-Met) has an extremely low solubility in water and a better absorption than the other available sources of synthetic methionine, such as DL-methionine and L-methionine.

There were significant effects of feed restriction and supplementation with DL-methionine on fish weight gain and fish yield (Table 5). The highest fish yields were observed in tanks with no feed restriction and with feed restriction but 2% DL-methionine supplementation. The lowest fish yields values were observed in tanks with feed restriction and not supplemented with DL-methionine or supplemented with 0.5% and 1.0% methionine, the latter case when mixed with dry molasses.

Fish subjected to feed restriction was forced to ingest a greater amount of bioflocs to meet their nutritional requirements. It is assumed that bioflocs were able to partially meet the nutritional requirements of tilapia juveniles because no significant differences were detected on fish growth performance between the tanks with no feed restriction and the tanks under 25% feed restriction but supplemented with 1% DL-methionine. Therefore, dietary supplementation with DL-methionine played a key role because fish in tanks subjected to feed restriction but with no methionine supplementation grew significantly less. It is speculated that bioflocs' deficiency in methionine was indirectly offset by the dietary DL-methionine supplementation. A more economical feed management for tilapia BFT systems might have been found if the present results were confirmed by further studies carried out on a larger scale. This finding would contribute to give greater economic sustainability for tilapia aquaculture in BFT tanks.

Table 4. Survival, final body weight and specific growth rate (SGR) of Nile tilapia juveniles (initial body weight of 2.76 ± 0.06 g) maintained for 8 weeks in BFT tanks subjected or not to feed restriction and supplemented with increasing levels of DL-methionine (mean ± sd; n = 5)

Feed restriction (%)	DL-methionine (%)	Survival (%)	Final body weight (g)	SGR (% day^{-1})
0	0.0	90.0 ± 6.0	20.1 ± 1.2 a	3.53 ± 0.11 a
25	0.0	90.0 ± 7.2	17.5 ± 0.4 b	3.30 ± 0.07 b
25	0.5	94.4 ± 5.5	17.3 ± 0.7 b	3.29 ± 0.10 b
25	1.0	92.2 ± 6.3	18.7 ± 1.5 ab	3.41 ± 0.15 ab
25	2.0	91.1 ± 8.4	20.0 ± 0.7 a	3.56 ± 0.08 a
25	1.0*	90.0 ± 4.6	17.9 ± 1.2 b	3.35 ± 0.13 b
P-value		NS	<0.001	<0.001

DL-methionine was mixed with the artificial diet in all treatments except for that marked with an asterisk, in which methionine was mixed with dry molasses. SGR = [ln (final weight) - ln (initial weight)] / days of rearing x 100). Means with different letters, in the same column, are significantly different by Tukey's test (P<0.05). NS = Non-significant (P>0.05).

Table 5. Weekly weight gain, fish yield and protein efficiency ratio (PER) of Nile tilapia juveniles maintained in BFT tanks subjected to feed restriction and supplemented with increasing levels of DL-methionine (mean ± SD; n = 5).

Feed restriction (%)	DL-methionine (%)	Weekly weight gain (g)	Fish yield (g m^{-3} day^{-1})	PER
0	0.0	2.17 ± 0.15 a	58.7 ± 1.4 a	2.23 ± 0.03 c
25	0.0	1.84 ± 0.06 b	50.6 ± 3.7 c	2.61 ± 0.18 b
25	0.5	1.82 ± 0.09 b	52.5 ± 4.1 c	2.67 ± 0.22 b
25	1.0	1.99 ± 0.19 ab	55.1 ± 2.4 b	2.83 ± 0.08 ab
25	2.0	2.17 ± 0.09 a	58.7 ± 5.2 a	3.10 ± 0.25 a
25	1.0*	1.90 ± 0.16 b	51.8 ± 2.9 c	2.72 ± 0.09 b
P-value		<0.001	0.004	<0.001

DL-methionine was mixed with the artificial diet in all treatments except for that marked with an asterisk, in which methionine was mixed with dry molasses. PER = fish weight gain (g)/dietary protein allowance(g). Means with different letters, in the same column, are significantly different by Tukey's test (P<0.05).

Better FCR and PER results were observed in tanks with feed restriction and supplementation of 2% DL-methionine, except for tanks receiving 1% DL-methionine mixed with the commercial diet (Table 5 and Figure 5). The worst results of FCR and PER were found in tanks with no feed restriction. These results differ from Cavalcante et al.[38], who have not observed significant differences for FCR and PER in BFT tanks with and without feed restriction. The better FCR and PER results in tanks with feed restriction could be explained by their greater bioflocs intake. Therefore, the best treatment in the present study was the dietary supplementation with 1% DL-methionine. A still unanswered question is whether the DL-methionine and commercial diet blend would favor fish performance if larger size pellets were used. The powdered artificial diet allowed a homogeneous blend with DL-methionine.

A significant and direct effect of the DL-methionine supplementation level on fish final body weight was observed (Figure 5A). Besides, DL-methionine supplementation showed an inverse relationship with FCR ($P<0.05$; Figure 5B). These trends were also observed by Figueiredo-Silva et al.[39] in a study with hybrid tilapia, *O. niloticus* × *O. mossambicus*. The authors have reported that dietary methionine increase resulted in higher final body weight, specific growth rate and protein efficiency ratio. Therefore, the dietary supplementation of DL-methionine could improve the Nile tilapia growth performance in BFT tanks and allow savings with commercial diet purchases.

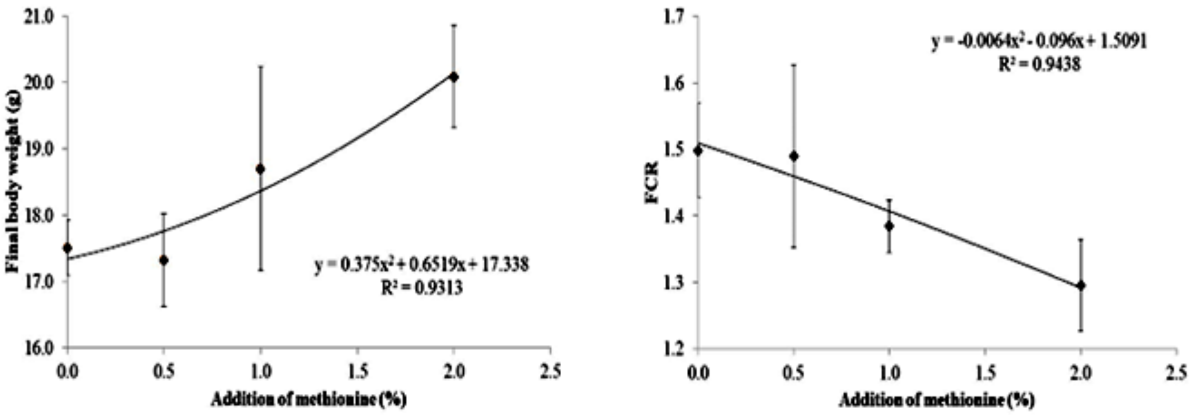

Figure 5 - Final body weight and feed conversion ratio (FCR) of Nile tilapia juveniles maintained in BFT tanks subjected to feed restriction and supplemented with increasing levels of DL-methionine (mean ± sd; n= 5).

Dietary DL-methionine supplementation in tilapia BFT systems subjected to moderate feed restriction has a favorable cost: benefit ratio, as could be seen by the following example: 1 - commercial diet's cost: R$ 5.60 kg^{-1}; 2 - DL-methionine's cost: R$ 20.00 kg^{-1}; 3 - fish biomass: 1.0 kg m^{-3}; fish body weight: 2.0 g. In this case, the daily feeding rate would be 11.3%. Accordingly, the daily feed allowance and cost with commercial diet would be equal to 113 g m^{-3} and R$ 0.63 m^{-3} day^{-1}, respectively. There would be savings of R$ 0.16 m^{-3} day^{-3} with the 25% feed restriction. On the other hand, DL-methionine supplementation at the 1% and 2% would result in increases of R$ 0.02 and R$ 0.04 m^{-3} day^{-1}, respectively. Therefore, there would be final savings of R$ 0.14 and R$ 0.12 m^{-3} day^{-1}, respectively, when the strategies of feed restriction and the DL-methionine supplementation are employed simultaneously.

Conclusions

Supplementation of commercial diets for Nile tilapia with DL-methionine did not affect the water quality of BFT tanks, nor the bioflocs' proximate composition. It may be possible to reduce the tilapia's feeding rates by 25% with no growth performance impairment if the commercial diets are supplemented with 1% or more DL-methionine.

Conflict of interest

The authors declare no conflict of interest.

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