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Dietary restriction associated with inorganic fertilization in fishponds on growth of Nile tilapia fingerlings

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ABSTRACT

The study evaluated muscle growth and performance in Nile tilapia fingerlings fed a restricted diet combined with phytoplankton in fertilized ponds. Four treatments were tested: 100% feed daily, 100% feed on alternate days, 50% feed daily + fertilization, and 50% feed on alternate days + fertilization. Fish were stocked at 19 fish/m^2 and fed a 45% protein diet. Ponds were fertilized weekly with superphosphate and ammonium sulfate, and water parameters were monitored. Growth, morphometrics, and histological analyses of dorsal and caudal muscles were performed at 0, 30, and 60 days. The 50% feed daily + fertilization treatment yielded similar weight gain, growth curves, and final lengths to the 100% feed daily group. Muscle growth through hyperplasia and hypertrophy was significantly higher ($p < 0.05$) in the 50% feed daily + fertilization group, supporting diverse phytoplankton communities, especially Chlorophyceae, Bacillariophyceae, and Cyanobacteria. These findings indicate that restricting feed to 50% of body weight with weekly fertilization maintains fish development, reduces feed costs, and promotes muscle growth while enhancing phytoplankton diversity in ponds. This approach offers a sustainable and cost-effective strategy for tilapia farming.

Keywords: Tilapia; Feed restriction; Natural feed; Hyperplasia and hypertrophy; Growth performance; Morphometrics measurement.

Restrição dietética associada à fertilização inorgânica em viveiros de peixes no crescimento de alevinos de tilápia-do-nilo

RESUMO

O estudo avaliou o crescimento muscular e o desempenho de alevinos de tilápia-do-Nilo alimentados com uma dieta restrita combinada com fitoplâncton em viveiros fertilizados. Quatro tratamentos foram testados: 100% da ração diária, 100% da ração em dias alternados, 50% da ração diária + fertilização e 50% da ração em dias alternados + fertilização. Os peixes foram estocados a uma densidade de 19 peixes/m^2 e alimentados com uma dieta contendo 45% de proteína bruta. Os viveiros foram fertilizados semanalmente com superfosfato e sulfato de amônio, e os parâmetros da água foram monitorados. O crescimento, a morfometria e as análises histológicas dos músculos dorsal e caudal foram realizados nos dias 0, 30 e 60. O tratamento com 50% da ração diária + fertilização apresentou ganho de peso, curva de crescimento e comprimento final similares ao grupo alimentado com 100% da ração diária. O crescimento muscular por hiperplasia e hipertrofia foi significativamente maior ($p < 0,05$) no grupo com 50% de ração diária + fertilização, promovendo comunidades diversificadas de fitoplâncton, especialmente Chlorophyceae, Bacillariophyceae e Cyanobacteria. Esses resultados indicam que a restrição alimentar a 50% do peso corporal com fertilização semanal mantém o desenvolvimento dos peixes, reduz custos com ração e favorece o crescimento muscular, além de promover a diversidade de fitoplâncton nos viveiros.

Palavras-chave: Tilápia; Restrição alimentar; Alimentação natural; Hiperplasia e hipertrofia; Desempenho de crescimento; Medição morfométrica.

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INTRODUCTION

Worldwide, tilapia production represents 9% of total fish output, and, in Brazil, it surpasses 60%, leading to the most produced species nationally (FAO, 2022; Peixe BR, 2022). Nonetheless, expanding production is curtailed by the high costs, particularly feed at 70% of the total. Therefore, strategies, such as feed restriction and fertilization in the tanks, may be alternatives to reduce the cost of feed, but without affecting batch performance (De Oliveira, 2015; Schalch, 2013).

Feed restriction involves the reduction of daily servings, suspension of daily feed, or feeding on alternate days (Afram et al., 2021; Da Palma et al., 2010; Lui, 2016; Salger et al., 2020). Even in the natural habitat, fish encounter food shortages during seasonal periods, implicating the ability of these organisms to develop strategies to overcome these challenges, in turn suggesting that producers could similarly turn to alternate feeding regimes with no loss of growth or performance (Camargo et al., 2008).

Natural feed is an essential part of the development of tilapias, especially during the first phases of fingerling and brood. This enables the optimization of animal feed since tilapias are endowed with excellent plankton and zooplankton filtration ability (El Sayed, 2006). This means that organic and/or inorganic substrates in known concentrations (Kubitza, 2006; Salger et al., 2020) can be used to cultivate desirable plankton communities for tilapia nutrition. (De Araújo, 2010).

Since reduction in feed costs could, in turn, increase pond production of tilapia in Brazil, this work aimed to compare muscle growth and performance of Nile tilapia fingerlings based on a restricted manufactured diet plus a natural diet of phytoplankton in fishponds fertilized with inorganic compounds.

MATERIAL AND METHODS

Locality, installation, managing and sampling

The experiment was carried out at the Agricultural Technology State Agency in Pindamonhangaba, state of São Paulo, Brazil (latitude 22°55'S, longitude 45°30'W, and altitude of 550 m) between September and November 2021.

Male tilapia fingerlings weighing 1.16 ± 0.37 g were distributed in 16-m^2 brickwork tanks with a density of 19 fish/m^2 (Ayroza & Ayroza, 2011) in a completely randomized design with four treatments in three repetitions for the purpose of evaluating the following feed strategies:

- T1: fish feed with 100% feed every day;
- T2: fish feed with 100% feed on alternate days;
- T3: Fish feed with 50% feed every day + fertilization;
- T4: Fish feed with 50% feed on alternate days + fertilization.

Fish were fed four times a day with extruded feed that contained 45% protein in 10% live fish. Biometrics were performed at the start of experimentation and after 15, 30, and 60 days. Feed amount was adjusted according to growth up to the end of experimentation at 60 days (Senar, 2019).

Ponds were completely drained over a period of three days. During this time, each tank was treated with quicklime for disinfection and pH correction, proportional to 10,000 kg/ha in the wet pond, one week before fertilization in order to avoid phosphorus precipitation and any rise in pH levels (De Queiroz, 2012).

The ponds were partially filled (25 cm in depth) with water, later adding simple superphosphate fertilizer and ammonium sulphate, both in the proportion of 130 kg/ha (Emater, 2000). After five days, the ponds were filled with water for ideal functionality (50 cm in depth), adding fingerlings three days later to begin the experiment. Fertilization of 75 kg/ha was then performed weekly to maintain the plankton community (Emater, 2000).

Biometrics were performed at the start of experimentation and after 15, 30 and 60 days by capturing 30 fish from each tank through a fishing net and evaluating weight and amount of feed. Weight gain (WG) and feed conversion (FC) were determined as Eqs. 1 and 2:

$$\text{Weight gain (WG)} = \text{final weight} - \text{initial weight} \qquad (1)$$

$$\text{Feed conversion ratio (FCR)} = \text{total feed consumption} / \text{batch weight gain} \qquad (2)$$

At the end of the experiment, WC and FCR were determined, considering the experimental period and survival. Survival (S) was calculated by Eq. 3:

$$S = \text{initial quantity of fish} / \text{final quantity of fish} \qquad (3)$$

Every day at 7 a.m., temperature (°C) and dissolved oxygen (mg/L) were measured. pH, water transparency (Secchi disk), as well as ammonia, nitrate, and nitrite levels were measured weekly (Silva et al., 2012).

For morphometric measurement during biometry, 10 fish from each tank were individually collected, weighed and measured at the start of experimentation and after 30 and 60 days. They were stunned with eugenol in accordance with Inoue and Moraes (2007), who recommended the dilution of 30 mg/L of eugenol in 1 mL alcohol A.P. in 1 L of water. After that, five fish were returned, and another five were used for the extraction of muscle tissues.

Individual samples consisted of both weight and body morphometric measurements, such as standard length, height, and width, as well as perimeter in the middle portion of the body and in the caudal peduncle.

Tissue extraction and histologic techniques

After individual samples were collected and stunned as previously described, they were sacrificed by vertical column disruption. Muscle tissue samples were removed below dorsal fin for histological analysis of the white fiber and caudal peduncle for red fiber analysis. Samples were fixed with 10% buffered formaldehyde, and the histological protocol followed Dos Santos et al. (2021) and Nunes and Cinsa (2016).

Phytoplankton community composition

To collect phytoplankton, a plankton net with a mesh opening of 20 μm was thrown into the culture tanks and dragged from the center to the system's water outlet with the net opening not totally submerged. After removing the net and total draining, remaining plankton were transferred for collection. The water portion with phytoplankton material was collected in 50-mL glass flasks and preserved by adding 4% of formaldehyde (Marín et al., 2004).

Qualitative taxonomic analysis was done based on morphological and morphometric examinations of the organs using the Olympus model BX51 photonic microscope with reticle in micrometer scale. Samples were examined in 400 and 1,000x. Several slides were analyzed for a population of 20 to 30 for each taxon per sample. Identification was done at genus and infragenus level. Specialized bibliography was used, including flora, reviews, and monographies. To identify genera and groups, the keys of Bicudo and Menezes (2017) and Wehr and Sheath (2003) were used. To identify names and taxonomic categories, the Algae Base Listing the World's Algae website was used.

Frequency of occurrence (F) was expressed in percentage, taking into account the quantity of samples in which each taxon occurred and total amount of samples analyzed, applying Eq. 4:

$$F\% = 100A/a \tag{4}$$

Where: A= the number of samples identified for each taxon; a= the total number of samples.

Taxa were classified as:

- Very frequent (more than 75% occurrence);
- Frequent (occurrence between 50 and 75%);
- Not so frequent (occurrence between 25 and 50%);
- Occasional (less than 25% occurrence).

Statistical analysis

Analysis of variance was done using the general linear model (SAS OnDemand for Academics, SAS Institute). Data normality and deviation homogeneity tests were done. Medians of treatments were compared by the Tukey's test. Every analysis was performed at 95% significance level. Growth study was done according to Santos et al. (2008) by adjusting all data from fingerling weights to the following exponential model (Eq. 5):

$$yi = Ae^{kxi} + ei \tag{5}$$

Where: y= the weight observed in each fish; i= 1, 2, ..., N; A= the initial estimated weight and the base over natural logarithm; k= specific growth tax; xi= age of fish and ei= the error associated with each observation, which by hypothesis is NID (0, σ^2).

The curve parameters for each feed strategy were compared to confidence intervals at the level of 95%. Adjusted equations and coefficient determination were provided through R^2adj, which indicates how good the data is fitting the regression model such that a high value is more significant. Estimates were obtained using weighted least squares (Draper & Smith, 1998; Santos et al., 2008).

Different muscle fiber diameters were separated by size, so a histogram of frequency was plotted (%) in order to characterize the occurrence of both hyperplasia and hypertrophy. Cell frequency < 20 μm in diameter indicated the occurrence of hyperplasia, whereas the frequency of cells > 80 μm in diameter showed hypertrophy.

Average fiber diameter is shown in μm and fiber density in fibers/mm^2, and both of which were obtained directly from Leica Application Software 3.0.

To analyze the similarity of composition in the phytoplankton community among treatments, cluster analysis was applied from a matrix of presence and absence. The analysis was carried out considering paired groups, using the Jaccard index with cophenetic coefficient correlation based on presentation of a 999-fold bootstrap. PC-ORD 6.0 for Windows (McCune & Mefford, 2011) was used for the analysis.

RESULTS AND DISCUSSION

Limnological variables

The limnological variables of temperature, pH, and ammonia did not differ statistically ($p > 0.05$) at 22.03 ± 0.22°C, 7.00 ± 0.24 and 0.19 ± 0.04 mg/L, respectively. Nitrate and nitrite

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concentrations were below the detection limit. Among the treatments, a significant difference was observed in dissolved oxygen and transparency ($p < 0.05$). The average dissolved oxygen of the tanks submitted to fertilization was 5.25 ± 0.37 mg/L, and transparency median was 42.9 cm, while in nonfertilized tanks not only the average was 4.72 ± 0.23 mg/L, but the median was 50 cm ($p < 0.05$).

The mean values of limnological parameters, including dissolved oxygen in all off the rearing tanks, are in acceptable levels for tilapia according to El Sayed (2006). It should be noted that the dissolved oxygen values were higher than expected in treatments in which fertilization was performed, owing to the greater photosynthetic activity of phytoplankton and resultant lower transparency rates because of the presence of these organisms (Kunlasak et al., 2013).

Zootechnical performance

The mean and standard deviation values of the zootechnical performance measurements are shown in Table 1. Treatment with 100% of feed offered daily without fertilization showed no significant difference ($p > 0.05$) relative to the treatment in which 50% of feed was offered daily, along with fertilization of the nurseries, based on weight gain (38.83 ± 3.22 and 35.13 ± 5.94 g, respectively). In these treatments, weight gain was higher ($p < 0.05$) than treatments with 100% feed of live weight on alternate days without fertilization and 50% feed of live weight on alternate days with fertilization (25.86 ± 6.40 and 14.20 ± 0.74 g, respectively). Feed conversion was higher in the treatment with 100% daily feed at 1.72 ± 0.33 compared to the other treatments ($p < 0.05$). Higher biomass was found in the treatment in which 50% of the daily ration of live weight with fertilization was offered, but no significant differences were found in survival.

For an experimental period lasting 12 weeks, Salger et al. (2020) reported that complete feed restriction every other day in fertilized tanks provided a 50% reduction in feeding costs with little impact on survival, growth, and carcass yield of tilapia fingerlings compared to treatments with daily feeding, either with or without weekly fertilization. Afram et al. (2021) evaluated the supply of feed on alternate days for Nile tilapia with weekly fertilization of tanks for five months and found low production costs, but also low economic returns when compared to treatments with 66.7 and 75% of daily ration calculated according to biomass of the tanks. Finally, Diana et al. (2004) reported that fingerlings reared at the density of 3 fish/m^2 in tanks fertilized with chemical fertilizers had better results in terms of growth, survival, and water quality when compared with higher densities (6 and 9 fish/m^2), also in fertilized tanks. Thus, they all recommended intensive use of feed and fertilization to achieve this performance.

However, these previous surveys differed from the present study by both running time and factors such as density. Unlike studies from both Diana et al. (2004) and Salger et al. (2020), who used 5 fish/m^2, and Afram et al. (2021), who used 2 fish/m^2, the present study used an initial density of 19 fish/m^2 as representative of the Brazilian commercial production of fingerlings (Ayroza & Ayroza, 2011).

Alal (2018), Daupota et al. (2016), and Villaroel et al. (2011) all performed studies whereby feed was restricted once a week, reducing the frequency from six to three or four times a day. They found that the regimen had no effect on growth, survival or feed conversion in juvenile tilapia. However, in the present work, the adoption of more day in the week to impose a simple 50% reduction in feed supplied daily resulted in feed savings, which indicated and improved effect on growth, feed conversion, and survival.

Fertilization is an excellent strategy to enhance productivity in culture tanks. Duodu et al. (2020) obtained outcomes analogous to those in the present study with respect to weight gain, specific growth, and feed conversion in fertilized tanks compared to those tanks not fertilized. Our research also demonstrated that it is feasible to achieve similar zootechnical performance results when adopting feed restriction on alternate days associated with fertilization, allowing even more feed economy.

Table 1. Mean and standard deviation of weight gain of tilapia submitted to varied feeding strategies*.

Treatment	Weight gain (g)	Final Biomass (g)	FCR	Survival (%)
T1	38.83 ± 3.22a	4,683.67 ± 870.25ab	1.72 ± 0.33a	40.78 ± 11.00a
T2	25.86 ± 6.40ab	3,791.67 ± 146.97b	0.82 ± 0.08b	51.56 ± 16.31a
T3	35.13 ± 5.94a	5,781.67 ± 232.08a	0.72 ± 0.07b	55.67 ± 7.22a
T4	14.20 ± 0.74b	1,779.67 ± 782.65c	0.61 ± 0.08b	52.50 ± 4.00a

T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization; FCR= feed conversion ratio; *means followed by different lowercase letters in the column are not equal by Tukey's test at 5%.

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Growth model

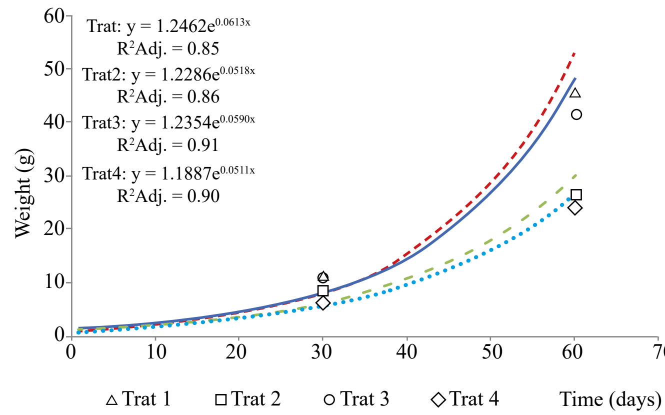
Table 2 presents the estimated parameters of the exponential growth model. Weight adjustment data for all dietary strategies were adequate (high R^2adj) with applicable estimates and reliability. On the one hand, the estimation of A parameters was similar among treatments; on the other hand, the growth rate was higher in treatments 1 and 3 ($p < 0.05$). The final weight estimate by the exponential growth model at 60 days was 38.45% higher in treatments 1 and 3 (49.31 and 42.58 g, respectively) when contrasted with treatments 2 and 4 (27.49 and 25.50 g, respectively). The model fit is shown in Fig. 1.

Table 3 shows the averages of body morphometric measurements, and the weight/length ratio of tilapia fingerlings submitted to varied feeding strategies at 30 and 60 days of cultivation. Both standard length and head length were greater in treatments 1 and 3 compared to treatments 2 and 4 after 30 days of cultivation, with differences only intensifying by 60 days ($p < 0.05$). Additionally, at 60 days, differences were also found in the height, width, and weight/length ratio of these fingerlings, which were all greater in treatments 1 and 3 ($p < 0.05$).

Fish feed with 100% feed on alternate days and fish feed with 50% feed on alternate days + fertilization showed lower weight, as previously mentioned, but also smaller size in contrast to treatments with daily feeding by a significant statistical difference ($p < 0.05$).

Nile tilapia fingerlings subjected to severe feed restriction did not achieve the same growth in size in comparison to the control group fed daily from one to three weeks in separated groups, with full refeeding of both in 10 weeks (Nebo, 2015).

Lui et al. (2020) did not recommend feed restriction because such practices negatively influenced weight gain and standard length of tilapia juveniles at the end of 60 days, either on two consecutive or alternate days of feed restriction, when compared to treatments without restriction. However, Gao et al. (2015) reported that feed restriction at an interval of two consecutive days, followed by refeeding for five days without interruption for the total of 185 days, had little effect on the size of tilapia fingerlings if analyzing those that received feed every day in their treatments. Araujo et al. (2020) opted only to lessen the daily amount of feed from 20 to 30% of live weight per day. Hence, at the end of 154 days, they obtained sizes that were very close to those of treatments with no feed restriction for tilapia fingerlings. This indicates that reduction itself would not be sufficient to significantly affect growth by size.



T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization.

Figure 1. Exponential growth model of tilapia fingerlings reared under different feeding strategies. Each point represents the average of thirty fish.

Table 2. Estimates and confidence intervals of parameters of the exponential growth model of tilapia fingerlings cultivated in different feeding strategies*.

Treatment	Estimated parameters		Confidence limits			
			A (g)		K (g/day)	
	A (g)	K (g/day)	Inferior	Superior	Inferior	Superior
T1	1.2462 A	0.0613 A	1.0853	1.4071	0.0584	0.0642
T2	1.2286 A	0.0518 B	1.0698	1.3873	0.0491	0.0545
T3	1.2354 A	0.0590 A	1.0730	1.3979	0.0565	0.0616
T4	1.1887 A	0.0511 B	1.0505	1.3270	0.0487	0.0536

T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization; *means followed by different uppercase letters in the column are not equal by Tukey's test at 5%.

Table 3. Mean and standard deviation of morphometric measurements of tilapia submitted to varied feeding plans at 30 and 60 days of cultivation*.

Measurement	Treatment	30 days		60 days	
Standard length (cm)	T1	7.89	(0.08)a	12.83	(0.44)a
	T2	7.34	(0.45)b	11.14	(0.61)b
	T3	8.02	(0.45)a	12.80	(0.43)a
	T4	7.20	(0.26)b	10.46	(0.87)b
Head length (cm)	T1	1.94	(0.07)a	3.43	(0.20)a
	T2	1.75	(0.12)a	3.03	(0.12)b
	T3	1.91	(0.10)a	3.30	(0.20)a
	T4	1.65	(0.05)a	2.80	(0.26)b
Body width (cm)	T1	0.84	(0.09)a	1.44	(0.04)a
	T2	0.85	(0.01)a	1.13	(0.06)b
	T3	0.87	(0.07)a	1.40	(0.04)a
	T4	0.71	(0.03)a	1.16	(0.16)b
Body height (cm)	T1	2.71	(0.03)a	4.14	(0.05)a
	T2	2.37	(0.24)a	3.46	(0.28)b
	T3	2.67	(0.20)a	4.04	(0.22)a
	T4	2.18	(0.10)a	3.29	(0.38)b
W/SL	T1	1.40	(0.06)a	3.54	(0.68)a
	T2	1.14	(0.14)a	2.34	(0.24)b
	T3	1.36	(0.19)a	3.23	(0.14)a
	T4	0.91	(0.03)a	2.37	(0.24)b

W= weight; SL= standard length ratio; T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization; *means followed by the same letter in the column are not different by Tukey's test at 5%.

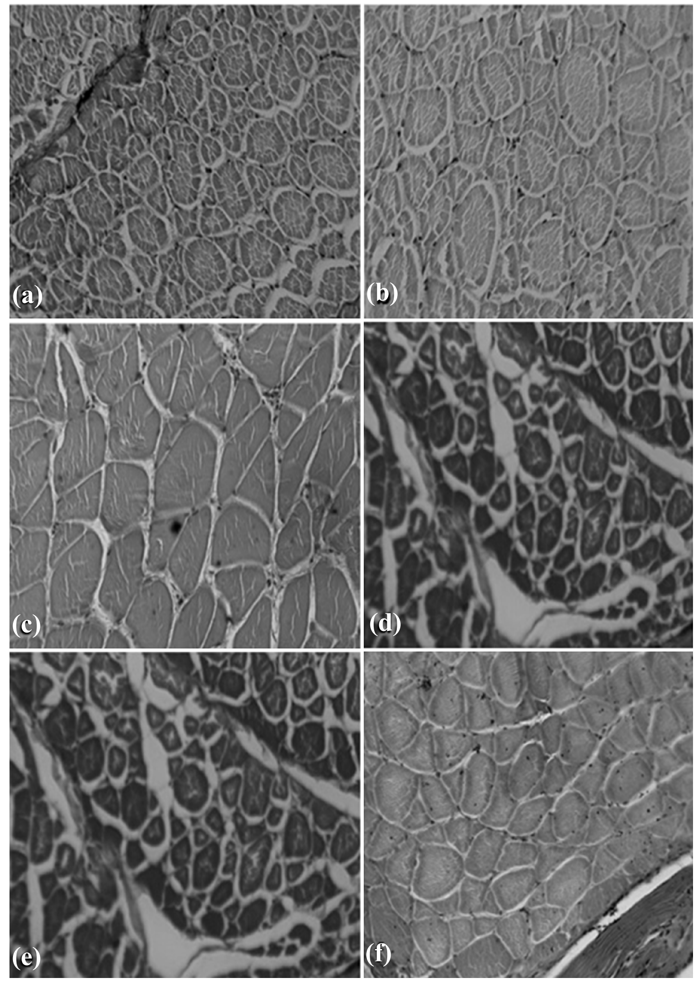


Figure 2. Cross-sectional images of tilapia fingerling muscles. Muscle samples from the dorsal region at (a) the beginning, (b) at 30, and (c) 60 days. Muscle samples from the caudal region at (d) the beginning, (e) at 30, and (f) 60 days. 400x magnification.

Muscle growth

Figure 2 shows cross-sectional images of the musculature of the dorsal and caudal regions of tilapia fingerlings at 30 and 60 days of cultivation under varied feeding strategies. Tables 4 and 5, respectively, show the mean measurements by diameter and density of muscle fibers in the dorsal and caudal regions of tilapia fingerlings submitted to different feeding strategies at 30 and 60 days of cultivation. Diameter and density were higher in treatment 3 when compared to the other treatments after 60 days of cultivation ($p < 0.05$).

The frequency of distribution of muscle fibers in the dorsal region of tilapia fingerlings after 30 and 60 days is shown in Fig. 3. At 30 days, the frequency of distribution of fibers in different diameter classes was similar. However, after 60 days of experimentation, a lower frequency of fibers was observed in a smaller diameter classes (< 20 µm and 20 to 40 µm) and a higher occurrence/regularity of fibers in the larger diameter classes (40 to 60 µm, 60 to 80 µm and > 80 µm) in the treatment with 50% of feed supplied daily plus tanks of fertilization. In addition, the frequency distribution of muscle fibers in the caudal region of tilapia fingerlings after 30 and 60 days is shown in Fig. 4. At 30 days of experimentation, a lower occurrence/regularity of fibers was seen in the smallest diameter class (< 20 µm), and a higher occurrence/regularity of fibers was noted in the larger diameter class (20 to 40 µm) in treatments that received fertilization. At 60 days, the fiber frequency distribution in different diameter classes was similar among treatments.

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Table 4. Mean and standard deviation of the diameter and density of muscle fibers in the dorsal region of tilapias at 30 and 60 days of cultivation under different feeding plans.

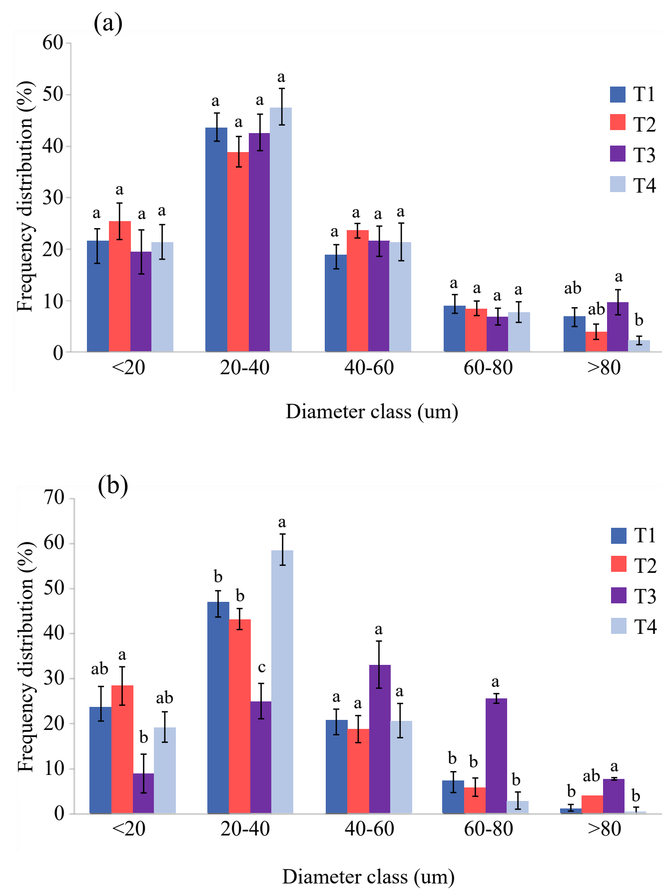
Time (days)	Treatment	Diameter (um)	Density (fibers/mm²)
30	T1	38.77 ± 5.86 a*	1,202.98 ±188.09 a
	T2	36.42 ± 4.25 a	981.52 ±123.30 a
	T3	38.92 ± 8.08 a	1,014.23 ±197.32 a
	T4	35.03 ± 4.36 a	968.11 ± 217.75 a
60	T1	33.53 ± 5.65 b	1,001.63 ± 260.70 b
	T2	32.21 ± 6.46 b	1,071.13 ± 257.95 b
	T3	49.81 ± 2.90 a	1,445.01 ±142.86 a
	T4	31.23 ± 5.41 b	964.69 ± 165.45 b

T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization; *Means followed by the same letter in the column are not different by Tukey's test at 5%.

Table 5. Mean and standard deviation of the diameter and density of muscle fibers in the caudal region of tilapias at 30 and 60 days of cultivation in different feeding plans.

Time (days)	Treatment	Diameter (um)	Density (fibers/mm²)
30	T1	21.97 ± 2.89 b*	1,750.706 ± 360.45 a
	T2	23.06 ± 3.27 ab	1,704.762 ± 390.12 a
	T3	29.39 ± 2.54 a	1,877.141 ± 211.17 a
	T4	27.96 ± 3.84 ab	2,075.530 ± 208.59 a
60	T1	24.977 ± 7.36 a	1,999.08 ± 645.34 a
	T2	24.108 ± 5.78 a	2,651.48 ± 928.98 a
	T3	26.143 ± 4.50 a	2,354.36 ± 938.14 a
	T4	22.416 ± 6.77 a	2,700.61 ± 853.50 a

T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization; *Means followed by the same letter in the column are not different by Tukey's test at 5%.

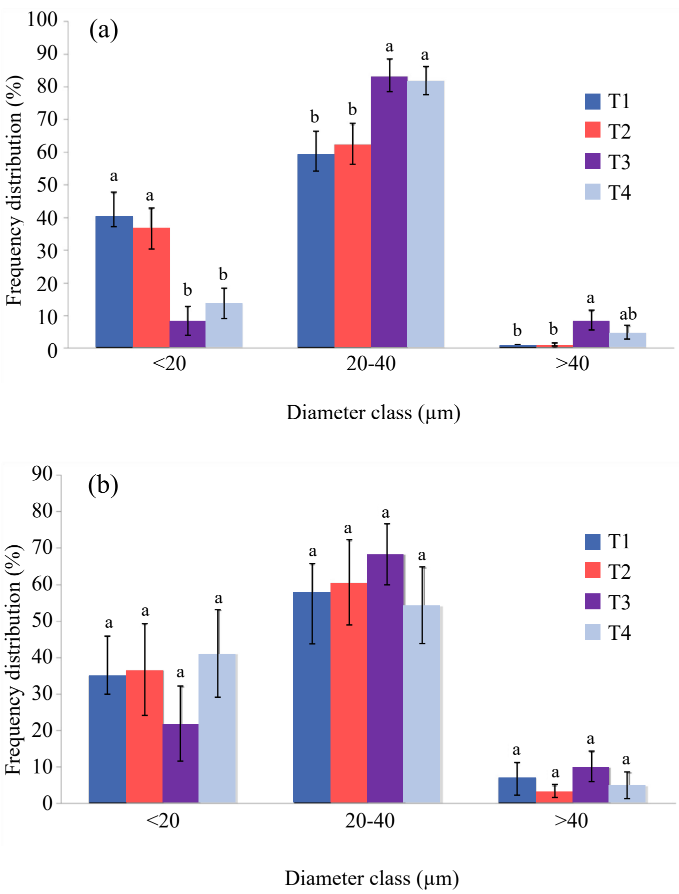


T1= fish feed 100% of live weight with daily feed without fertilization; T2= fish feed at 100% live weight with feed on alternate days without fertilization; T3= fish feed 50% of live weight with feed plus daily fertilization; T4= fish feed with 50% of live weight with feed plus fertilization; *different letters present statistical difference among treatments ($p < 0.05$).

Figure 3. Frequency distribution of muscle fiber sizes in tilapia dorsal region: (a) 30 days of experimentation; (b) 60 days of experimentation*.

At 30 days, fish feed with 100% feed on alternate days and fish feed with 50% feed on alternate days + fertilization with feed restriction showed smaller muscle fiber sizes and densities when compared to treatments without feed restriction. However, at the end of the experiment, the densities were higher, indicating greater growth owing to hyperplasia during this period (Fig. 4). This result is similar to that reported by Lui et al. (2020), who obtained less growth at the end of 60 days owing to hypertrophy in juveniles fed on alternate days by total live weight.

Fava et al. (2022) compared tilapia muscle fiber growth among different feeding frequencies for 30 days. They reported that the lowest frequency (four times a day) resulted in less fiber growth between 10–20 μm. Although they adopted a decrease



T1= fish feed 100% of live weight with daily feed without fertilization; T2= fish feed at 100% live weight with feed on alternate days without fertilization; T3= fish feed 50% of live weight with feed plus daily fertilization; T4= fish feed with 50% of live weight with feed plus fertilization; *different letters present statistical difference among treatments ($p < 0.05$).

Figure 4. Frequency distribution of muscle fiber sizes in tilapia caudal region (a) 30 days of experimentation, and (b) 60 days of experimentation*.

in frequency instead of reduction in the daily amount of feed by 50%, the results indicated a negative impact on growth owing to hyperplasia. However, in the present study, 50% feed reduction provided greater growth as a result of hyperplasia and hypertrophy of white muscle fibers at the end of 30 days and then up to 60 days, indicating the contribution of fertilization and phytoplankton production.

Braz et al. (2022) also investigated the impact of feed restriction on the growth of muscle fibers in tilapia fingerlings, evaluating the influence of low temperatures. Their results showed that dietary restriction of 15 days of feeding followed by 15 days of fasting showed a high frequency of fibers smaller than 20 µm, indicating delay in growth because of hypertrophy when compared to treatments without restriction.

Robisalmi et al. (2021) found that red tilapia submitted to a seven-day fast and re-fed in 83 days had a higher frequency of muscle fibers larger than 50 µm, indicating compensatory growth after this period. This was not observed at the end of our experiment in treatment 4, which consisted of alternate days of feeding. With this treatment, the lowest rate of hypertrophy was obtained in fibers of the dorsal region at the end of the experiment.

After 60 days, fish feed with 100% feed every day and fish feed with 50% feed every day + fertilization remained close in mean size and density of muscle fibers, indicating this are similar. The natural food may have played an interesting role in supplying the 50% savings in feed offered by fish feed with 50% feed every day + fertilization. Khalil et al. (2022) demonstrated that deficiency of the amino acid lysine in the tilapia diet was attenuated through the selectivity of plankton abundantly present in the tanks. According to our analyses, no significant differences in the size and density of red fibers (caudal muscle) occurred between the treatments during 30 and 60 days. Salomão et al. (2018) found the most frequent size to be between 20 and 40 µm.

Analysis of phytoplankton community composition

Images of the taxa of some species found in the experiment are shown in Fig. 5.

In the treatments carried out, a total of 97 phytoplankton taxa was identified and then distributed into 14 taxonomic classes in the following order: Chlorophyceae (35), Cyanobacteria (18), Bacillariophyceae (13), Trebouxiophyceae (11), Xanthophyceae (4),

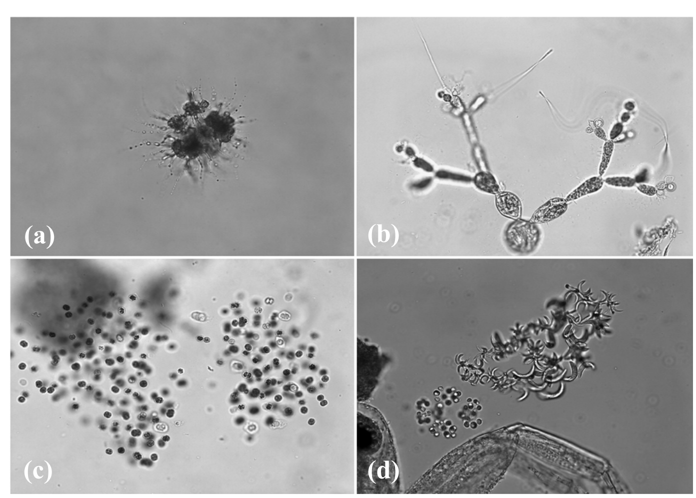


Figure 5. Images of phytoplankton taxa found in tilapia fingerling nurseries submitted to different feeding strategies. (a) *Botryococcus terribilis*. (b) *Batrachospermum* sp. (c) *Microcystis* sp. (d) *Selenastrum* sp. Magnification 400x.

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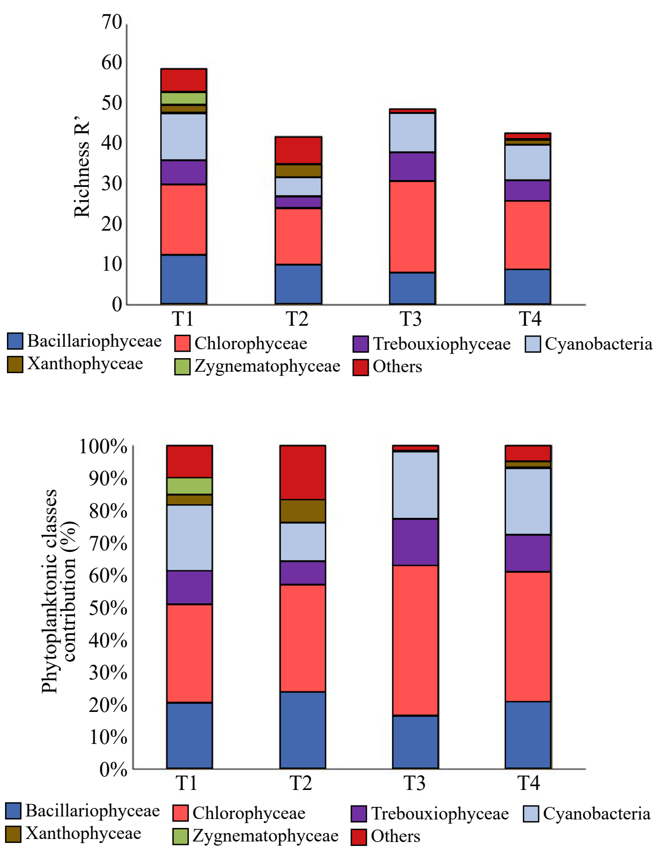
Zygnematophyceae (3), Coscinodiscophyceae (2), Mediophyceae (2), Euglenophyceae (2), Florideophyceae (2), Chrysophyceae (2), and Cryptophyceae (1), Coleochaetophyceae (1), and Dinophyceae (1) (Fig. 6a). Chlorophyceae was the most representative taxon among the treatments, followed by the Cyanobacteria and Bacillariophyceae (Fig. 6b). Specimens of Zygnematophyceae were registered only in treatment 1, and the percentage contribution of Chlorophyceae was higher in treatment 3.

Fourteen taxa were considered very frequent and were registered in the four treatments. Among them, six taxa belong to Chlorophyceae, whereas only one belongs to the Cyanobacteria group (*Aphanocapsa delicatissima*). Another 14 taxa were classified as frequent, 25 as uncommon, and 44 as sporadic.



Bacillariophyceae= sum Bacillariophyceae, Coscinodiscophyceae and Mediophyceae; Others= sum Chrysophyceae, Cryptophyceae, Dinophyceae, Coleochaetophyceae, Euglenophyceae, and Florideophyceae; T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization.

Figure 6. (a) Mean values of richness of the phytoplankton community in four Nile tilapia feed plans. (b) Percent contribution values of the phytoplankton community in four Nile tilapia feed plans.

Cluster analysis applied to phytoplankton community abundance showed the formation of three groups among treatments with a cophenetic coefficient of 0.9268, suggesting that the cluster was consistent. In the first one, formed by treatments daily feeding at 100% body weight and alternate-day feeding at 100% body weight, the similarity of community composition was high, above 64%. The second group was formed by treatments alternate-day feeding at 100% body weight and four with a similarity of 10%. As for treatment daily feeding at 50% body weight with pond fertilization, part of the composition of the phytoplankton community is unlike the composition of other treatments (Fig. 7).

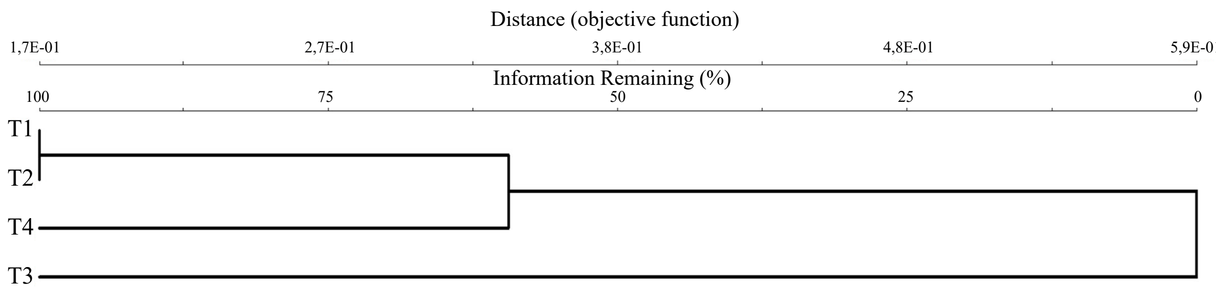
Similar results were found by Chukwu and Afolabi (2017), Mohammed et al. (2017), Osti et al. (2018), Peng et al. (2021), Tikue and Workagegn (2023), and Vieira et al. (2009). These groups evaluated the changes in phytoplankton communities in tilapia ponds reared in a semi-intensive system and also in reservoirs subjected to hydrological physicochemical influences. In these cases, the families Chlorophyceae, Bacillariophyceae, and Cyanobacteria were also the most representative.

Studies evaluating the impact of inorganic fertilization on the abundance and distribution of phytoplankton communities are scarce in the literature. Yet, Tawwab (2003) investigated the selectivity of tilapia in the ingestion of these microorganisms in tanks fertilized by NPK (20:20:5). The most representative families found in tilapia stomachs were also Chlorophytas, Bacillariophyta, and Cyanobacteria. He pointed out that genera like *Scenedesmus* and *Chlorella*, for example, were the most common among the Chlorophyta, as also identified in our work.

As it has been demonstrated in this paper, treatment 3 with marked differences in the composition of phytoplankton showed dissimilarities when compared to the other treatments. This may explain the noteworthy outcomes of this treatment, including zootechnical performance (fewer feed consumption), morphometry, and growth by hyperplasia and hypertrophy of the formerly discussed muscle fibers.

CONCLUSION

Tilapia fingerlings can be fed with 50% less ration of live weight per day in conjunction with chemical fertilization practices in the nurseries without causing deleterious effects on zootechnical and morphometric indicators. This feeding strategy provided greater growth of white muscle fibers by hyperplasia and hypertrophy at the end of 60 days of tilapia culture and could bring more sustainability advantages. The same treatment also demonstrated the dissimilarity of phytoplankton communities in



T1= fish feed with 100% feed every day; T2= fish feed with 100% feed on alternate days; T3= fish feed with 50% feed every day + fertilization; T4= fish feed with 50% feed on alternate days + fertilization.

Figure 7. Dendrogram of similarity of phytoplankton composition in four Nile tilapia feed plans.

composition, diversity and frequency among families and species in the different treatments. Feed restriction adopted on alternate days, either with 50 or 100% of feed offered by live weight, was not compelling for these indicators, even when associated with natural feeding, thus discouraging the use of these practices.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTIONS

Writing – original draft: Rossi, G.H., Santos, V.B.; **Data curation:** Rossi, G.H., Santos, V.B.; **Conceptualization:** Rossi, G.H., Santos, V.B.; **Resources:** Schalch, S.C.; **Investigation:** Schalch, S.C., Osti, J.A.S., Martins, A.M.C.R.P.F.; **Writing – review & editing:** Suzuki, G.T.; **Methodology:** Osti, J.A.S., Martins, A.M.C.R.P.F.; **Formal Analysis:** Osti, J.A.S., Santos, V.B. ; **Final approval:** Santos, V.B.

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