Conjecturas

DOI: 10.53660/CONJ-855-P58

Addition of microalgae *Nannochloropsis* sp. and *Isochrysis* sp. in the feed of Nile tilapia larvae

Adição das microalgas Nannochloropsis sp. e Isochrysis sp. na alimentação de larvas de tilápia do Nilo

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ABSTRACT

Microalgae added to feed can increase the quality, production, and welfare of fish, although they contain antinutritive factors and allelopathic compounds that can be harmful. The aim of this work was to compare the effect of five feeds: Basic feed and enriched with two levels of *Nannochloropsis* sp. and *Isochrysis* sp. lyophilized (15 and 30 kg^{-1} of feed) in larval rearing of Nile tilapia. The larvae fed with *Isochrysis* sp. (15 and 30 g kg^{-1}) had some yield parameters slightly lower than those of the larvae fed with the basal feed. For larvae fed with *Nannochloropsis* sp., all yield parameters were similar to those of the control group, except for the weight and stress resistance of larvae fed with the 15 g kg^{-1} enriched feed, which were lower than those of the control group. Since the main objective of juvenile fish producers is survival rate, which was high and stable in larvae fed with *Nannochloropsis* sp. (30 g kg^{-1}), it is recommended that this feed should be used for rearing Nile tilapia larvae. The inclusion of *Isochrysis* sp. in the feed should be better evaluated.
Keywords: larviculture; lyophilized microalgae; *Oreochromis niloticus*; welfare.

RESUMO

As microalgas adicionadas à ração podem aumentar a qualidade, a produção e o bem-estar dos peixes, embora contenham fatores antinutritivos e compostos alelopáticos que podem ser prejudiciais. O objetivo deste trabalho foi comparar o efeito de cinco rações: ração básica e enriquecida com dois níveis de *Nannochloropsis* sp. e *Isochrysis* sp. liofilizados (15 e 30 kg^{-1} de ração) na criação de larvas de tilápia do Nilo. As larvas alimentadas com *Isochrysis* sp. (15 e 30 g kg^{-1}) apresentaram alguns parâmetros de rendimento ligeiramente inferiores aos das larvas alimentadas com a ração basal. Para as larvas alimentadas com *Nannochloropsis* sp., todos os parâmetros de produção foram semelhantes aos do grupo controle, exceto o peso e a resistência ao estresse das larvas alimentadas com ração enriquecida com 15 g kg^{-1}, que foram inferiores aos do grupo controle. Uma vez que o principal objetivo dos produtores de peixes juvenis é a taxa de sobrevivência, que foi alta e estável nas larvas alimentadas com *Nannochloropsis* sp. (30 g kg-1), recomenda-se que este alimento seja utilizado para a criação de larvas de tilápia do Nilo. A inclusão de *Isochrysis* sp. na ração deve ser melhor avaliada.

Palavras-chave: larvicultura; microalgas liofilizadas; *Oreochromis niloticus*; bem-estar.

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INTRODUCTION

The microalgae *Nannochloropsis* (Sarker et al., 2018) and *Isochrisys* (He et al., 2018) have been used to enrich feed for fish larvae. *Nannochloropsis* and *Issochrisys* are rich in n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Gbadamosi and Lupatsch, 2018, He et al., 2018). Although *Isochrysis* and *Nannochloropis* contain nutrients that are beneficial to fish, they also have antinutritional factors (Sims et al., 2019) and allelopathic compounds (Ammar et al., 2018) that may negatively affect culture. Therefore, the objective of this experiment was to investigate the effects of adding microalgae (*Isochrysis* sp. and *Nannochloropisis* sp.) at two levels in feed on the yield and welfare of Nile tilapia larvae.

MATERIAL AND METHODS

The experiment was conducted in Diamantina city, Minas Gerais, Brazil, for 48 days after being approved by CEUA UFVJM (n. 029/2016).

A total of 375 Nile tilapia larvae, weighing 0.015 g and a total length of 1.09 cm, with five days post-hatching were cultivated in 40 L aquaria with 15 individuals aquarium^{-1} in a recirculation system. The fish were fed with five feeds; the basic feed without the addition of microalgae and the others enriched with two species of microalgae, with two enrichment levels: with 15 g *Nannochloropsis* sp. kg^{-1} of feed; with 30 g *Nannochloropsis* sp. kg^{-1}; with 15 g *Isochrysis* sp. kg^{-1} and with 30 g *Isochrysis* sp. kg^{-1}. The experiment consisted of 25 aquaria with five treatments and five replicates in a completely randomized arrangement.

The composition of basal feed, commercial powder, and microalgae lyophilized is described in table 1. Feed was offered three times a day: 8, 12, and 16 h.

Table 1. Composition of the powder basal feed and lyophilized microalgae.

Powder feed base	Quantity		Unit
crude protein (max.)	550		g kg^{-1}
ether extract (min.)	80		g kg^{-1}
fibrous matter (max.)	30		g kg^{-1}
mineral matter (max.)	160		g kg^{-1}
calcium (max.)	30		g kg^{-1}
phosphorus (min.)	14		g kg^{-1}
moisture (max.)	100		g kg^{-1}
Microalgae	*Nannochloropsis* sp.	*Isochrysis* sp.	
cell size	2 to 4	4 to 8	µm
weight	2 to 3 x 10^{11}	4 to 8 x 10^{10}	cells g^{-1}
total neutral fat (min.),	150	150 to 200	g kg^{-1}
protein (min.)	350	350 to 450	g kg^{-1}
water (max.),	100	100	g kg^{-1}
ash (max.),	200	200	g kg^{-1}
Clostridium perfringens (max.).	10	10	cfu g^{-1}
mercury (max.),	0.1	0.1	mg kg^{-1}
lead (max.),	10	10	mg kg^{-1}
cadmium (max.)	1	1	mg kg^{-1}

Twice a week the aquaria were cleaned by siphoning, and 20% of the water volume did replace. Weekly water samples were collected from each aquarium to check the parameters: temperature (27.38±0.13 ^{o}C), pH (7.8±0.07), dissolved oxygen (3.36±0.44 mg L^{-1}), redox potential (231.4±9.32 mV), conductivity (27.94±4.33 mS cm-1), turbidity (25.94±4.32 NTU), and total dissolved solids (0.2±0.1g L^{-1}) using a HORIBA U10® and concentrations of ammonia (0.002±0.00 mg L^{-1}), nitrite (0.014±0.01 mg L^{-1}) and nitrate (0.016±0.01 mg L^{-1}) according to APHA (2012).

At the end of the experiment, two animals from each aquarium were subjected to osmotic stress in saline water with 40 ‰ of sodium chloride, measuring the moment when the fish began to fall sideways and swim unbalanced, which served to assess tolerance to saline stress. Subsequently, all specimens counted and were measured: weight, total and standard length, number of individuals, and total food consumption per sample unit. From these data were calculated, survival, weight gain, Fulton condition factor, feed conversion, biomass, biomass gain, and specific growth rate.

The values variables were tested for normality (Shapiro-Wilk) and homoscedasticity and then submitted to one-way ANOVA, and means were compared using Tukey's test ($P< 0.05$).

RESULTS

The performance parameters of Nile tilapia larvae fed different feeds are shown in Table 2.

Table 2 - Averages values and standard deviation of performance of Nile tilapia larvae fed basal feed enriched or not with microalgae *Isochrysis* sp. (15 and 30 g kg^{-1}) (Iso15 and Iso30) or *Nannochloropsis* sp. (15 and 30 g kg^{-1}) (Nanno15 and Nanno30).

	Basal feed	Iso15	Iso30	Nanno15	Nanno30
Weight (g)	0.60±0.07a	0.38±0.10b	0.40±0.11b	0.43±0.06b	0.52±0.07ab
WG (g)	0.6±0.07a	0.4±0.10b	0.4±0.11b	0.4±0.06ab	0.5±0.07ab
SL (cm)	2.14±0.09a	2.12±0.43a	1.95±0.20a	2.02±0.07a	2.15±0.07a
TL (cm)	2.84±0.09a	2.51±0.17b	2.59±0.20ab	2.67±0.07ab	2.76±0.12ab
K (%)	6.1±0.3a	4.5±1.7a	5.4±0.9a	5.2±0.6a	5.2±0.4a
Survival (%)	75.0±24.7a	66.7±24.9a	70.7±25.2a	80.0±4.7a	90.7±13.0a
Biomass (g)	6.6±1.9a	4.0±2.2a	4.4±2.1a	5.2±0.8a	7.0±1.3a
Biomass gain (g)	6.36±1.89a	3.73±2.17a	4.14±2.14a	5.00±0.84a	6.77±1.27a
Feed conversion	2.8±0.4b	4.7±1.1a	4.4±1.0a	3.9±0.6ab	3.3±0.5ab
SGR (%)	7.7±0.2a	6.7±0.5b	6.8±0.5b	7.0±0.3ab	7.4±0.3ab

Mean values on the same row followed by different letters differ statistically by Tukey's test ($p<0.05$). Weight gain = WG, Standard length = SL, Total length = TL, K= Fulton's condition factor, Specific growth rate = SGR.

Comparing larvae fed a basal feed with those enriched with *Nannochloropsis* sp. only the weight was higher for the basal feed than for the enriched with *Nannochloropsis*

sp. (15 g kg^{-1}). The other yield parameters did not differ from each other. Comparing the larvae fed with the basal feed with those fed on *Isochrysis* sp., the larvae fed with the basal feed showed greater total length than those fed with the feed with *Isochrysis* sp. 15 g kg^{-1} and higher weight, weight gain, and specific growth rate and lower feed conversion than those fed with *Isochrysis* sp. 15 and 30 g kg^{-1}. Standard length, Fulton condition factor, survival, biomass, and biomass gain did not differ between larvae fed the different foods.

Survival values of larvae fed *Nannochloropsis* sp. tended to be higher than those observed for larvae of the other food treatments (6.3% and 17.3% for 15 and 30 g of addition, respectively, when compared with basal treatment), although they were not significantly different. Moreover, they showed greater stability of results, as evidenced by the lower coefficient of variation (5.9 and 14.3% for 15 and 30 g of addition, respectively) compared to larvae fed basal feed (32.9%) and those fed 15 (37.3%) and 30 (35.6%) g of *Isochrysis* sp..

Tolerance to salt stress was similar in larvae fed the basal feed, feed containing *Nannochloropsis* sp. (30 g kg^{-1}) and *Isochrysis* sp. (15 g kg^{-1}) (Table 3). However, the tolerance of larvae fed on a basal feed was higher than those fed on a feed with *Isochrysis* sp. (30 g kg^{-1}) and *Nannochloropsis* sp. (15 g kg^{-1}).

Table 3 - Averages and standard deviation for the time of onset of tipping of fish in saline water at 40 ‰, tolerance to salt stress. Nile tilapia larvae fed with basal feed and enriched with microalgae *Isochrysis* sp. (15 and 30 g kg^{-1}) (Iso15 and Iso30) and *Nannochloropsis* sp. (15 and 30 g kg^{-1}) (Nanno15 and Nanno30).

	Basal feed	Iso15	Iso30	Nanno15	Nanno30
Time (min) when the fish starts to tip in water 40 ‰	13.23 ± 2.56a	10.29± 1.01ab	9.13± 1.36b	7.74± 2.05b	11.13± 2.90ab

Mean values on the same row followed by different letters differ statistically by Tukey's test (p<0.05).

DISCUSSION

These results are corroborated by the studies comparing basal feed with *Nannochloropsis* sp. supplementation offered to Nile tilapia juveniles, where the *Nannochloropsis* enriched feed resulted in slightly higher yields than the soybean meal-enriched feed, but lower than the fish meal-fed feed (Gbadamosi and Lupatsch, 2018).

Teuling et al. (2019), also, observed similar results depending on the processing undergone by the phytoplankton. Similary, Sarker et al. (2018) found that growth, feed conversion, and survival did not differ from those of the reference feed up to a certain level of *N. oculata* intake; thereafter, digestibility and growth were reduced due to higher levels of antinutrients.

Although tilapia larvae survive and grow when fed exclusively with feed from the first day of exogenous feeding (Al-Feky et al., 2016), and practically all enzymes are present in the gut on the first day pos- hatching, lipase appears only third-day post-hatching, and there is variation in the intensity and location in which enzymes appear (Tengjaroenkul et al., 2002). Therefore, the inferior performance of Nile tilapia larvae fed with *Isochrysis* was related to the developmental stage. However, this efficiency increases rapidly with increasing size (Lu et al., 2004). Another explanation for the lower results could be the antinutritional factors observed in *Isochrysis* (Sims et al., 2019), such as non-starch polysaccharides, pectin, cellulose, hemicellulose, trypsin, and lectin inhibitor (Sarker et al., 2018), and their negative effect allelopathic compounds (Ammar et al., 2018), such as acid stearic acid or oleic acid (Sun et al., 2012), which make digestion difficult, especially when the fish is in the larval stage. However, the inclusion of *Isochrysis galbana* up to 4.5–5.0%, replacing fish oil, improved performance, and increased total fatty acids in juveniles of *Trachinotus ovatus*, starting from when it decreases. (He et al., 2018). However, in this experiment, the inclusion levels of *Isochrysis* sp. were higher than those used by He et al. (2018), which may indicate that the levels of inclusion were slightly high. Already for *N. oculata*, the inclusion of meal above 33% (Sarker et al., 2018) and integrally (Gbadamosi and Lupatsch, 2018) in the replacement of fish meal in tilapia juvenile feeding reduced performance due to the presence of factors antinutritional such as non-starch polysaccharides.

The addition of *Nannochloropsis* sp. (30 g kg-1) to the feed maintained the same resistance, degree of well-being, under saline water, which is an osmoregulatory response that varies according to each individual, genetic variability, and stage of development of the species (Fashin-Bomba and Busari, 2003) being size more important than age (Watanabe et al., 1985), confirming the performance results.

The inclusion of lyophilized *Nannochloropsis* sp. (30 g kg^{-1}) provided performance and resistance to saline stress similar to those obtained with the use of basal feed and high and stable survival values. Since survival is the main objective of the

producer of the juveniles, the inclusion of *Nannochloropsis* sp. (30 g kg^{-1}) in Nile tilapia larvae feed is indicated. Already, the inclusion of *Isochrysis* sp. in the feed should be better evaluated.

ACKNOWLEDGMENTS

To the Minas Gerais Research Funding Foundation (FAPEMIG - APQ-01530-16), Coordination for the Improvement of Higher Education Personnel – CAPES - (Financing Code 001), and to Banco do Nordeste (BNB - FUNDECI 2012/324) for financial support, and the Gustavo Eustáquio Brito Alvim de Melo for the priceless logistical support.

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Recebido em: 10/04/2022
Aprovado em: 15/05/2022
Publicado em: 19/05/2022