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Growth and gastrointestinal indices in Nile tilapia fed with different diets

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ABSTRACT. The adequate diet of Nile tilapia in their growth early stages is fundamental to the success of culture subsequent stages. The goal of the present work was to evaluate the Nile tilapia growth fed with different diets, and to morphometrically characterized the gastrointestinal tract of this species. The treatments consisted of three diets: (D_1) natural food, (D_2), commercial feed and (D_3) commercial feed + natural food. In the qualitative phytoplankton analysis, there was greater representation of the genus *Chlorella,* while zooplankton community analysis revealed greater number of *Brachionus* rotifer. Growth and survival in D_2 and D_3 did not reveal differences ($p > 0.05$), while in D_1, the results were significantly lower ($p < 0.05$). The gastro-somatic and entero-somatic indices, and the intestinal quotient revealed higher values when only natural food was available. Nile tilapia reached best zootechnical performance when commercial feed was included in the diet. The intake of natural and/or artificial food is related to the growth and development of the gastrointestinal indices, emphasizing that these indicators can be altered by the type of feeding.

Keywords: *Oreochromis*, diets, natural food, plankton, digestive tract.

Crescimento e índices gastrintestinais de tilápia do Nilo alimentadas com diferentes dietas

RESUMO. A dieta adequada de tilápia do Nilo em suas fases iniciais de crescimento é fundamental para o sucesso das etapas subsequentes do cultivo. O objetivo do presente trabalho foi avaliar o crescimento de tilápias do Nilo alimentadas com diferentes dietas e caracterizar morfometricamente o aparelho gastrintestinal da espécie. Os tratamentos constaram de três dietas: (D_1) alimento natural, (D_2) ração comercial e (D_3) ração comercial + alimento natural. Na análise qualitativa do fitoplâncton, observou-se maior representação do gênero *Chlorella* enquanto a análise da comunidade zooplanctônica revelou maior número de rotíferos do gênero *Brachionus.* O crescimento e a sobrevivência em D_2 e D_3 não apresentaram diferenças ($p > 0.05$), enquanto em D_1 os resultados foram significativamente inferiores ($p < 0.05$). Os índices gastrossomáticos, enterossomáticos e o quociente intestinal apresentaram, de uma maneira geral, valores superiores nos tratamentos que utilizaram alimento natural na dieta. Tilápia do Nilo alcançou melhor desempenho zootécnico quando a ração comercial foi incluída na dieta. A ingestão do alimento natural e/ou artificial está relacionada com o desenvolvimento dos índices gastrintestinais, reforçando que estes indicadores podem sofrer mudanças ocasionadas pelo tipo de alimentação.

Palavras-chave: *Oreochromis*, dietas, alimento natural, plâncton, aparelho digestivo.

Introduction

The activity of aquiculture has increased through a process of professionalization, where the producer is closer to the used management and inputs (FILHO et al., 2010). According to the Food and Agriculture Organization (FAO, 2009), fish farming business increases more than other animal production enterprise and Brazil now ranks sixth, among the world's leading producers of tilapia, accounting for 3.3% of total production.

Hatchery is of fundamental importance for raising quality animals, and proper nutrition plays an important role in ensuring the success of subsequent stages of farming. The use of artificial diets as the only source of food may not bring good results for most fish species in their early stages of development, and live food can bring more satisfactory effects (BOSCOLO et al., 2001). The microalgae contain high concentrations of soluble fiber and fatty acids of omega-3 series and can contribute positively for the aquatic organisms feeding (AZAZA et al., 2007). According to Faria et al. (2001) certain species confer a greater survival for post-larvae of Nile tilapia and can be used as a food alternative source. The tilapia shifts from visually feeding on zooplankton when juveniles to mostly filter feeding on phytoplankton when adults. When reared using an appropriate ration in intensive aquaculture systems, also consume algal-based detritus (GILLES et al., 2008). Lu et al. (2004) used the microalgae *S. platensis*, *Euglena gracilis* and *Chlorella vulgaris* as food supplementation during the post-larval period of Nile tilapia. The authors evaluated the rates of ingestion (IT) for each microalgae and concluded that *S. platensis* was the most efficient of the species tested, with ingestion rates of 80%.

However, the Nile tilapia, *Oreochromis niloticus*, does not vitally depend on this combination (MEURER et al., 2002). The meal ration can be utilized without damage on performance, survival, or sexual reversal efficiency, during sex reversion phase (BOMBARDELLI et al., 2004). Tachibana et al. (2004) observed that Nile tilapia fed with only commercial diet during sex reversal phase showed 92.86% survival. The body composition of fish is influenced by diet and, if it does not meet the species requirements or result in low intake of essential nutrients, can lead to visceral fat deposition (REIDEL et al., 2010).

Knowledge on the species biology used in fish farming, is a requirement for the proper development, as an understanding of its anatomy and physiology (ROTTA, 2003). In fish, the gastrointestinal system presents large and numerous adaptive variations, due to several feeding habits of the different species (RODRIGUES; MENIN, 2008). Among its components, the buccopharyngeal cavity has attracted great attention among researchers, as it is intrinsically related to the selection, capture, directing and preparation of the food to be swallowed (LOURES; LIMA, 2001). There is a close interdependence between nutrition, habitat, and the organization of the digestive tract, which is particularly apparent in the various adaptations and modifications (MEURER et al., 2002).

The aim of this study was to determine the growth of the Nile tilapia fed different diets, and to morphometrically characterize the gastrointestinal tract of this species.

Material and methods

The experiment lasted 100 days and was carried out at the Professor Raimundo Saraiva da Costa Fish Farming Station of the Fishing Engineering Department of the Agrarian Sciences Center, Federal University of Ceará. Three-hundred post-larvae (pl's) of Nile tilapia, was obtained at the above institution, with an average weight and length of 0.01 ± 0.01 g and 1.03 ± 0.01 cm, respectively.

The experimental design was completely randomized, with three treatments and five repetitions each. The treatments consisted of three diets: (D_1) natural food, (D_2), commercial feed and (D_3) commercial feed + natural food. In the first 40 days (first phase), the 20 fish were kept in outdoor polyethylene tanks (12L: 12D photoperiod) with a useful volume of 80 L and fed (treatments D_2 and D_3) with powder commercial ration (1.0 mm) containing 55% of crude protein. After this period, individuals were transferred to outdoor polyethylene reservoirs (12L: 12D photoperiod) containing 800 liters of water, where they were raised for a further 60 days (second phase) and fed (treatments D_2 and D_3) with an extruded commercial ration (1.7 mm) with 45% of crude protein.

Initially, treatments D_1 and D_3 received "green water" (with phyto- and zooplankton) from a pre-established farming of Nile tilapia in 3,000 L (3 x 1 x 1 m) concrete reservoirs and, after 20 days of experiment, the natural food had blossomed naturally in the farming units, whereas the fish in treatment D_2 were farmed in clear water with no natural food. To prevent proliferating plankton in this treatment, the sunlight was blocked of the boxes cultivation through polyethylene caps.

In treatments D_2 and D_3, the daily amount of commercial feed was adjusted to 10% of the stored biomass every 20 days. In all three treatments, the water was renewed on a weekly basis - 50% in the first phase of the experiment and 10% in the second phase. In relation to culture water, the physical and chemical parameters, dissolved oxygen, pH and temperature were measured daily, while total ammonia, nitrite, carbon dioxide, general and carbonate hardness were evaluated weekly. Fish were weighted every 20 days for performance analyses, according to the following formulas: weight gain (%) = (final weight - initial weight /initial weight) x 100; length gain (cm) = final length – initial length; and survival rate (%) = (final number of animals /initial number of animals) x 100, according to the method of Candido et al. (2006).

Quantification of natural food in the water of treatments D_1 and D_3 was performed using a linear regression from the strong correlation observed between the dry algal biomass (g L^{-1}) and the optical density of the reservoir water at a wavelength of 680 nm (DO_{680nm}). At the beginning of the experiment, green water was first filtered in 100 μm mesh plankton net to separate the macrozooplankton, and then in a 30 μm mesh net to obtain concentrated phytoplankton sample. From the concentrated sample, four serial dilutions were placed in a spectrophotometer for the DO_{680nm} reading. Each sample was then centrifuged at 3,000 x g for 5 minutes, washed twice with deionized water and centrifuged again. Subsequently, the samples were filtered through filter paper, previously weighed on a centesimal scale (0.01 g), oven dried at 105°C for 16 hours, and weighed again on a centesimal scale (TAKAGI et al., 2006). Finally, the linear correlation between DO_{680nm} of each sample and its respective dry biomass (g L^{-1}) was established to determine the linear regression equation (XU et al., 2006).

Qualitative analysis of live food was performed through three monthly samplings of water from the pre-established farming of tilapia, all performed at 10 o'clock in the morning. The water (50 L) of each sample was filtered through a 60 μm mesh plankton net, and the retained material was stored in a 400 mL glass container and fixed with 4% formaldehyde neutralized with sodium tetraborate (30 g L^{-1}). After this procedure, aliquots of sediments in the glass container were examined under a binocular microscope with phase contrast, and the organisms were identified to genus level with the aid of identification keys (BOLTOVSKOY, 1981; BICUDO; MENEZES, 2006).

At the end of the farming process, five individuals from each repetition were sacrificed by lethal anesthesia using menthol (500 mg L^{-1}) and necropsied. Stomachs and intestines were carefully dissected using surgical instruments, weighed on a centesimal scale and measured with a caliper to obtain the following parameters: gastro-somatic index (iGas) = (stomach weight/total weight of the individual)*100; entero-somatic index (iEns) = (intestine weight/total weight of the individual)*100 and intestinal quotient (Qi) = intestine length/total length of the individual, according to the method of Pereira and Mercante (2005).

Data were submitted to single-factor analysis of variance at 5% of statistical significance. When statistically significant difference was found, the averages were compared in pairs, by Tukey's test. In cases where the assumptions for the parametric tests were not reached, the Kruskal-Wallis and Dunn nonparametric tests were used to replace the parametric ones. All analyses were performed using the software BioEstat 4.0. An angular transformer (arcsine square root) was used to homogenize the variances of percentage values, but the figures were presented in their original form.

Results and discussion

The average physical and chemical parameters of the water in the treatments remained within the levels considered suitable for tilapia farming (VINATEA, 2004) and showed no significant difference ($p > 0.05$) (Table 1).

Table 1. Culture water physical and chemical parameters (mean ± standard error) of Nile tilapia fed different diets.

Physical and chemical parameters	Treatments		
	*D_1	**D_2	***D_3
Dissolved oxygen (mg L^{-1})	8.92 ± 0.30	7.50 ± 0.10	8.84 ± 0.12
pH	8.33 ± 0.35	7.32 ± 0.50	7.96 ± 0.33
Temperature (°C)	29.17 ± 0.10	28.67 ± 0.2	28.40 ± 0.20
Total ammonia (ppm)	0.50 ± 0.12	0.45 ± 0.21	0.50 ± 0.13
Nitrite (ppm)	< 0.3 ± 0.10	< 0.45 ± 0.14	< 0.25 ± 0.10
Carbon dioxide (mg L^{-1})	0.60 ± 0.71	0.53 ± 0.30	0.57 ± 0.32
General hardness	2.5 ± 0.30	2.5 ± 0.25	2.80 ± 0.21
Carbonate hardness	5.80 ± 0.10	4.00 ± 0.12	6.00 ± 0.20

*D_1 = Natural food; **D_2 = Commercial feed and ***D_3 = Commercial feed + natural food.

There is little information available about the importance of phytoplankton in the tilapia hatchery (LU et al., 2004). However, these fish are important primary consumers (BWANIKA et al., 2006; SEMYALO et al., 2011). According to Loures et al. (2001), tilapia has preference for artificial feed, followed by phytoplankton, which is constantly consumed. Fish performance (weight and length gain) in the treatments that included commercial feed showed no significant difference ($p > 0.05$), while in the treatment with only natural food, performance was significantly lower ($p < 0.05$) (Table 2, Figures 1 and 2). Survival rate in treatments D_2 and D_3 did not differ significantly, and were similar (90% on average) to the values of around 97.5% found by Meurer et al. (2003). Turker et al. (2003b) cultured tilapia in algal-rich water dominated by green algae (i.e., *Scenedesmus* and *Ankistrodesmus*) and cyanobacteria (i.e., *Microcystis* and *Merismopedia*) to determine filtration rates. The cell counts of phytoplankton in water filtered by tilapias indicated significant reduction in green algae and cyanobacteria. Lu et al. (2006) examined the effect of *Spirulina* on larval tilapia at different growing stages during the development. Tilapias could efficiently assimilate and utilize the ingested marine microalgae from the onset of exogenous feeding. The use of moist marine microalgae offered directly or encapsulated in copepods results in better growth of tilapia (MOREIRA et al., 2010). Moreira et al. (2011a), examining the final of sex reversal phase, found total average growth of 1.64 ± 0.10 g, when fed to tilapia *S. platensis*. The sex reversal phase of tilapia, supplemented with *S. platensis* can also be successfully carried out in water with high levels of salinity (MOREIRA et al., 2011b).

Table 2. Growth, survival rate and gastrointestinal indices (mean ± standard error) of Nile tilapia fed different diets.

Growth and gastrointestinal indices	Treatments		
	*D_1	**D_2	***D_3
Initial weight (g)	0.01±0.01	0.01±0.01	0.01±0.01
Weight gain (g)	15.23 ± 0.62^{a}	60.08 ± 0.92^{b}	72.52 ± 0.64^{b}
Initial length (cm)	1.03 ± 0.01	1.03 ± 0.01	1.03 ± 0.01
Length gain (cm)	14.05 ± 0.32^{a}	20.81 ± 0.16^{b}	24.65 ± 0.12^{b}
Survival rate (%)	25 ± 3.12^{a}	89 ± 6.74^{b}	91 ± 5.67^{b}
[1](iGas) Gastro-somatic indice (%)	3.09 ± 0.20^{a}	1.39 ± 0.21^{b}	2.45 ± 0.11^{a}
(iEns) Entero-somatic indice (%)	9.43 ± 0.42^{a}	4.75 ± 0.34^{b}	6.95 ± 0.48^{c}
(Qi) Intestinal quotient (%)	7.22 ± 0.10^{a}	4.80 ± 0.01^{b}	6.32 ± 0.41^{c}

*D_1 = Natural food; **D_2 = Commercial feed; ***D_3 = Commercial feed + natural food. Identical letters in the rows indicate no significant difference ($p < 0.05$). [1]Gastro-somatic indice (iGas) found for the three treatments did not show normal distribution or homoscedasticity, then the values were submitted to the Kruskal-Wallis and Dunn tests.

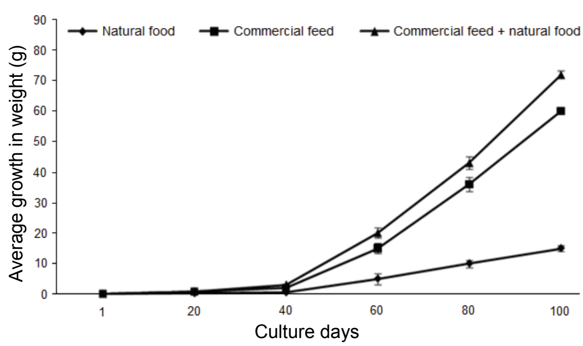

Figure 1. Average growth in weight (g) of Nile tilapia fed different diets.

Figure 2. Average growth in length (cm) of Nile tilapia fed different diets.

Water absorbance and natural food dry weight values showed a strong positive linear correlation ($r = 0.891$). Thus, monitoring the absorbance of water revealed a greater amount of natural food in treatment D_3, which may be due to the introduction of commercial feed, providing to this environment the essential nutrients for the development of natural food. In treatment D_2, the absorbance values were almost zero throughout the experiment. Probably this was due to water changes and isolation of the system from sunlight, hindering the development of phytoplankton. Qualitative analysis of phytoplankton showed the higher contribution of the genus *Chlorella*, followed by *Scenedesmus*, and in smaller amounts, *Staurastrum* (Figure 3). Turker et al. (2003a) demonstrated that water from Nile tilapia farming was dominated by phytoplankton, mainly *Scenedesmus* sp. Analysis of the zooplankton community (Figure 4) revealed a higher number of rotifer of the genus *Brachionus*, followed by the Calanoida, *Calanus* sp. In Nile tilapia reservoir water, the high amount of rotifers is due to the great diversity of phytoplankton (SUN et al., 2009). Corgosinho and Pinto-Coelho (2006) reported that Cladocera and Cyclopoida were the most abundant zooplanktonic groups in eutrophic stations of their study, while Calanoida were prevalent in more oligotrophic sites, in freshwater reservoirs. Uddin et al. (2009) investigated the growth of tilapia and freshwater prawn in a polyculture system, and identified 29 genera of algae and 9 genera of zooplankton from the pond water.

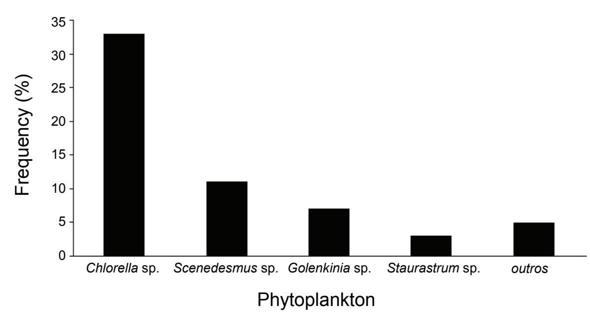

Figure 3. Frequency of occurrence (%) of the main phytoplankton organisms in the water from the farming of Nile tilapia fed different diets.

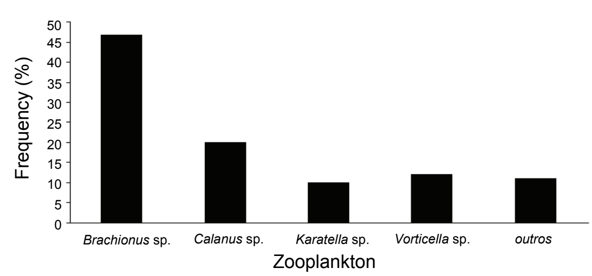

Figure 4. Frequency of occurrence (%) of the main zooplankton organisms in the water from the farming of Nile tilapia fed different diets.

Gastro-somatic (iGas) and entero-somatic (iEns) indices, and intestinal quotient (Qi) were higher in the treatments using natural food, compared with treatments using only commercial feed (Table 2). This fact is directly related to the use of natural food, which results in an increased contact surface of the stomach and intestine, enabling greater absorption of nutrients. The gastro-somatic index (iGas), being quantitative in nature, provides more accurate information about the feeding habit (HAHN; DELARIVA, 2003).

The iGas obtained from fish fed only natural food did not differ significantly from that found in animals fed with natural food and commercial feed. However, comparisons between D_1 and D_2 (commercial feed only) and D_2 and D_3 evidenced statistically significant difference ($p < 0.05$).

Single-factor analysis of variance pointed statistically significant differences in the entero-somatic indices (iEns) of the fish submitted to the three treatments ($p < 0.05$) and the Tukey's test showed that the iEns of fish fed only with natural food (D_1) was higher than that of the animals of treatment D_3 (natural food and commercial ration), which in turn, was higher than that of fish fed only with commercial ration (D_2).

With respect to the intestinal quotient (Qi), the single-factor analysis of variance also indicated a statistically significant difference ($p < 0.05$) among the three treatments, and the Tukey's test revealed that fish fed only with natural food (D_1) had a greater Qi than those of treatment D_3, which in turn, was higher than that of fish fed only with D_2. These results contrast with those found by Costa et al. (2008), in which the characterization of gastrointestinal indices showed no significant differences for common carp (*Cyprinus carpio*) fed with teosinte grass (*Euchlaena mexicana*) supplemented with commercial ration. In fish farming, to optimize feeding efficiency, it is necessary to integrate factors such as: physiological characteristics, feeding habits, nutritional requirements, chemical composition, and availability of nutrients in the ingredients selected in the preparation of a complete commercial ration (LANNA et al., 2004). Further studies will require an analysis of the anatomy of the organs of the digestive system of tilapia, through histological studies, as this will enable a better understanding of the impact of natural and artificial feeding on the physiology of the individuals.

Conclusion

Nile tilapia fed only with natural food does not reach commercial size in a short time, making it necessary to complement with commercial feed. The combination of both types of food results in significant weight gain and growth, and therefore, a higher survival rate. Intake of natural food and/or commercial ration is directly related to the development of gastrointestinal indices, highlighting that these indicators may undergo changes caused by the type of food.

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