

## The use of *Arthrospira platensis* in rearing Nile tilapia (*Oreochromis niloticus*) in salt water<sup>1</sup>

### Uso da *Arthrospira platensis* na alevinagem da tilápia do Nilo (*Oreochromis niloticus*) em água salgada

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**ABSTRACT** - Nile tilapia (*Oreochromis niloticus*) is a *euryhaline* species which, in marine systems, can provide animal protein where fresh water is scarce. As a result, adequate water management to maintain sustainability is essential, and the use of a salt water recirculating system would be an advantage at the present time. In addition, *Arthrospira platensis* is used in the diet of aquatic organisms, as it is rich in protein. The aim of this study, therefore, was to evaluate the use of *A. platensis* as a food supplement in the performance of Nile tilapia in a salt water recirculating system. The experimental model included two groups, with four replications. Each crop had a duration of 45 days (control and test), giving a total of 90 days, with the control being offered commercial feed, and the test crop commercial feed supplemented with 20% *A. platensis* meal. Water quality results showed that total ammonia (NH<sub>3</sub>) and phosphate (PO<sub>4</sub><sup>3-</sup>) levels remained high during cultivation. In zootechnical performance, specific growth rate and survival were higher in the control; on the other hand, for the percent composition of the filleted fish, the treatment supplemented with *A. platensis* had a higher level of protein. It is therefore possible to cultivate Nile tilapia in salt water at a salinity of 35 ppt.

**Key words:** Microalgae. Pisciculture. Recirculation. Salinity.

**RESUMO** - A tilápia do Nilo (*Oreochromis niloticus*) é uma espécie eurialiana e em sistemas marinhos é capaz de fornecer proteína animal onde a água doce é escassa. Com isso, torna-se imprescindível o gerenciamento adequado da água para manutenção da sustentabilidade, e o uso do sistema de recirculação de água salgada seria vantajoso para o atual momento. Além disso, a *Arthrospira platensis* é utilizada nas dietas de organismos aquáticos, pois é rica em proteínas. Portanto, o objetivo do trabalho foi avaliar a utilização da *A. platensis* como suplemento alimentar no desempenho da tilápia do Nilo em sistema de recirculação de água salgada. O modelo experimental teve dois grupos, contendo quatro repetições. Cada cultivo teve duração de 45 dias (controle e teste), totalizando 90 dias, sendo para o controle ofertada ração comercial, e para o teste ração comercial com 20% de farinha de *A. platensis*. Os resultados de qualidade de água mostraram que amônia total (NH<sub>3</sub>) e fosfato (PO<sub>4</sub><sup>3-</sup>) permaneceram elevados durante o cultivo. No desempenho zootécnico, a taxa de crescimento específico e sobrevivência foram maiores para o controle. Em contrapartida, na composição centesimal do filé de peixe, o tratamento que recebeu a suplementação com *A. platensis* apresentou um maior nível de proteína. Portanto, é possível cultivar tilápia do Nilo em água salgada com salinidade de 35 ppt.

**Palavras-chave:** Microalga. Piscicultura. Recirculação. Salinidade.

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## INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a robust species, with rapid growth, easy breeding in captivity and good acceptance on the consumer market. In addition, it can adapt to high salinities, tolerating variations in the range of 0-35 ppt, and is considered a *euryhaline* species (BASUKI; REJEKI, 2015; SÁ, 2012). It is known that *euryhaline* fish in brackish or marine water systems are able to provide animal protein in places where fresh water is scarce (FRIDMAN; BRON; RANA, 2012a).

Research shows that the availability of fresh water is decreasing, making proper water management essential (FRIDMAN; BRON; RANA, 2012b). It is therefore necessary to seek diversification in aquaculture practices through the introduction of new species or the adaptation of existing technologies, such as growing tilapia in salt water and/or employing models that would reduce the use of new water. One solution for easing these problems would be a system of recirculation (FRIDMAN; BRON; RANA, 2012b; OLIVEIRA; SANTOS, 2015).

The main characteristic of a recirculating system is the reuse of water. It is considered a viable technology for the intensive cultivation of different species, maximising production and using less water when compared to other types of systems (RIJN, 2013; SÁNCHEZ; MATSUMOTO, 2012).

According to Brito and Silva (2014), the cultivation of Nile tilapia employing water recirculation is considered promising, with the success of the system being due to modern technology, the rational use of water and economic sustainability. In addition, to give better animal performance, several materials have been tested as an alternative source of protein, including microalgae (ABDULRAHMAN, 2014).

Microalgae are microscopic unicellular beings with numerous applications, one of which is to increase the value of food products and animal feed due to their chemical composition (PRIYADARSHANI; RATH, 2012). Among the microalgae, *Arthrospira platensis* has a high concentration of nutrients, with 60% digestible protein containing all the essential amino acids, and rich in B complex vitamins, minerals and carotenoids, among others (CAPELLI; CYSEWSKI, 2010), and can be used as a food supplement in cultivating fish.

When using alternative foods, such as *A. platensis*, as a substitute source of animal protein in fish feed, the final product may be economically advantageous due to a reduction in feeding costs, since commercial diets used in intensive fish farming account for 50 to 70% of the costs of production. However, zootechnical performance and body composition should be considered in evaluating the

final product (JESUS *et al.*, 2011; KUBITZA; CYRINO; ONO, 1998).

As such, research was carried with *A. platensis* as a supplement in the diet of Nile tilapia, giving an advantage in immunostimulatory effects (MAHMOUD *et al.*, 2018) and in zootechnical performance (MOREIRA; MARTINS; FARIAS, 2011).

The aim of this study was to evaluate the use of *A. platensis* as a dietary supplement in fingerlings of Nile tilapia in a salt water recirculating system.

## MATERIAL AND METHODS

The experiment was carried out at the Indoor Marine Cultivation Laboratory of the Centre for Applied Biotechnology in Aquaculture (CEBIAQUA), Department of Fisheries Engineering (DEP) at the Centre for Agricultural Sciences (CCA) of the Federal University of Ceará (UFC).

The species used in the present study were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under number A7704B7.

Reversed fingerlings of Nile tilapia with an average weight of  $1.0 \pm 0.02$  g were obtained from the Aquaculture Station at UFC and placed in four aquariums (25 fish/aquarium) for period of acclimatisation that lasted six days. During this period, the animals were fed three times a day on commercial feed containing 40% crude protein (CP), at 08:00, 11:00 and 14:00.

Initially, dechlorinated fresh water was placed in the aquariums. The salinity was subsequently adjusted with sea water, increasing by 5 ppt a day until reaching 30 ppt. The fish were then transferred to the salt water recirculating system (salinity 35 ppt). This procedure was carried out with both the Control and Test groups, since they were performed at different times.

The seawater that supplied the recirculating system came from the beach at Abreulândia (COFECO) in Fortaleza, Ceará. The indoor marine recirculating cultivation system contained four 100 L polyethylene tanks, a 60 L biological filter and a 500 L supply tank. The four tanks had a controlled water supply, driven by gravity, which previously passed through an ultraviolet light filter to eliminate micro-organisms. The tank supplying the system contained a skimmer that removed unwanted organic waste. The effluent from the tanks was drained by gravity through a biological filter, where a submerged pump forced the water to circulate through the system.

The biological filter contained a thick woollen screen, spongy material and porous limestone rocks, among other materials, to facilitate the growth of microorganisms that decompose the organic matter and increase the reduction of nitrogenous compounds.

The fish were stocked in the circular tanks at a density of 10 fish tank<sup>-1</sup> under constant aeration. The water was not exchanged during cultivation, but simply replaced in order to maintain the level of the biological filter due to evaporation and maintaining the salinity at 35 ppt.

The experimental design was completely randomised, with one control group and one test group, and a total of four replications per group. The fish in the Control group received commercial feed containing 32% CP, while the Test group received commercial feed containing 28% CP, supplemented with *A. platensis meal* at 20% of the dry biomass. The fish were fed twice a day, at 10:00 and 14:00, at a rate of 20% live weight per day.

Biometry was carried out every 11 days with the aid of a digital balance (0.01 g) and pachymeter to monitor the weight and growth of the fish. Both the Control and the Test crop had a duration of 45 days, giving a total of 90 days.

The four culture tanks were siphoned daily. The water containing organic matter was filtered and reused by the system, as the organic matter was retained by a 60 µm mesh. The supply tank and skimmer were washed daily to keep the water clean and organic free.

Dry *A. platensis* meal was obtained from an outdoor recirculating monoculture, located at the Aquaculture Station, DEP/UFC. The biomass was filtered through a 60 µm mesh, washed in running water to remove the salt, and then dried in an oven at 60 °C. The contents were then powdered in an industrial blender, ready for the feed preparation process.

The commercial feed was also powdered in an industrial blender; it was then mixed with the *A. platensis* meal together with colourless and unflavoured gelatine (5%) to serve as a binder. The dry ingredients and the binder were mixed gradually in order to homogenise and form a paste.

The paste was placed in a press to form pellets, which were then left in an oven at 60 °C for 36 h. After drying, these were stored in a freezer at -20 °C. The same procedure was carried out with the Control feed containing 32% CP, without the addition of the *A. platensis* meal.

The water quality variables were measured weekly. The dissolved oxygen (mg L<sup>-1</sup>) was measured with the aid of a HANNA HI9146 probe, and the temperature and salinity with a HANNA HI9828 probe. The pH was measured

with a benchtop pH meter. The nitrogenous compounds, ammonia (NH<sub>3</sub>), nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>), as well as the reactive phosphorus (PO<sub>4</sub><sup>3-</sup>) were measured using a HACH DR 2700 spectrophotometer, following the manufacturer's methodology and instructions on the device.

Values for the weight and length of the cultivated fish were determined from biometrics, and allowed the zootechnical performance indices to be calculated: Weight gain - WG (g); Mean growth - MG (cm); Survival - S (%); Specific Growth Rate - SGR (%); Food Conversion Factor - FCF (g feed.g fish<sup>-1</sup>) and Feed Efficiency - FE (%), according to the equations below:

$$WG = W_{mf} - W_{mi} \quad (1)$$

where:

WG = weight gain (g);

W<sub>mf</sub> = final mean weight (g);

W<sub>mi</sub> = initial mean weight (g).

$$MG = G_{mf} - G_{mi} \quad (2)$$

where:

CM = mean growth (cm);

C<sub>mf</sub> = final mean growth (cm);

C<sub>mi</sub> = initial mean growth (cm).

$$S = N_f \times 100/N_i \quad (3)$$

where:

S = survival rate (%);

N<sub>f</sub> = final number of fish;

N<sub>i</sub> = initial number of fish.

$$SGR = \frac{\ln P_f - \ln P_i}{T} \times 100 \quad (4)$$

where:

SGR = specific growth rate (%);

Ln = neperian logarithm e;

P<sub>f</sub> = final mean weight (g);

P<sub>i</sub> = inicial mean weight (g);

T = time of cultivation (days).

$$FCF = QR/EG \quad (5)$$

where:

FCA = feed conversion factor (g feed.g fish<sup>-1</sup>);

QR = quantity of feed consumed (g);

GB = gain in biomass (g).

$$FA = WG \times 100 / QR \quad (6)$$

where:

FA = feed efficiency (%);

WG = weight gain (g);

QR = quantity of feed consumed (g).

To analyse the percent composition, 10 fish with a mean weight of  $9.16 \pm 0.02$  g and a mean length of  $7.71 \pm 0.04$  cm were filleted at the end of each treatment. The fillets were vacuum packed and stored at  $-18^{\circ}\text{C}$  until the respective day of each analysis. The samples were analysed as per the Association of Official Analytical Chemists (2000) for moisture, lipids, proteins and ash. The analyses were carried out at the Laboratory for Fish Technology (LATEPE), DEP, UFC.

For a statistical analysis of the data, the BioEstat 5.0 software (2012) and Excel 2013 (Microsoft Corp.) were used. The parameters of water quality, zootechnical performance and percent composition of the fillet and feed were submitted to single factor variance analysis (ANOVA), and the mean values  $\pm$  standard deviation (SD) were determined for the four replications, comparing their mean values by Tukey's test when the difference between them was significant at a level of 5%.

## RESULTS AND DISCUSSION

For the percent composition of the feed, there was no statistically significant difference for crude protein or ash content between the treatments ( $p > 0.05$ ). Whereas for the moisture content and lipids, there was a statistically significant difference between the Control and the Test ( $p < 0.05$ ), as can be seen in Table 1.

In recent years, one of the research topics for nutrition in aquaculture has focused on fish diets, with the aim of finding alternative sources of protein to reduce

costs and a possible improvement in the nutritional value of the fish (JAFARI *et al.*, 2014).

It is known that lipids are considered the main source of energy when formulating feed and can act as a substitute for the protein that comes from the diet, so it is interesting to have an adequate level of lipids in the feed offered to the cultivated organisms.

Values for protein found in the feed were very close, and one explanation might be that supplementation was insufficient, or that there may even have been a problem during preparation of the test feed, such as loss of mass during pellet production. It is worth pointing out that feed for aquaculture is rich in protein, generally containing 26 to 50% CP (SA, 2012). In intensive fish farming, feed accounts for 50 to 70% of production costs (KUBITZA; CYRINO; ONO, 1998), showing the need to formulate diets with cheaper ingredients that have the same effect on fish growth. One alternative that has been used is the addition of *A. platensis* (Gomes *et al.*, 2012): it is easily digested, since its cell wall is composed of mucopolysaccharides (simple sugars and proteins) (BEZERRA *et al.*, 2010), which afford better and faster use of the protein by the cultivated organism.

The water quality variables, ammonia ( $\text{NH}_3$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), showed no statistically significant difference ( $p > 0.05$ ), while a statistically significant difference ( $p < 0.05$ ) was found for temperature ( $^{\circ}\text{C}$ ), salinity (ppt), oxygen ( $\text{mg L}^{-1}$ ), pH and phosphate ( $\text{PO}_4^{3-}$ ). This can be seen in Table 2.

It is known that *A. platensis* has a high protein content (COLLA *et al.*, 2007), a fact that may have contributed to ammonia levels remaining high, where the range considered ideal is less than  $0.05 \text{ mg L}^{-1}$ . As such, the fish do not fully utilise the feed they consume, with whatever is not digested entering the water in the form of faeces, which decompose, releasing ammonia into the water (SÁ, 2012).

**Table 1** - Percent composition of the feed used in the cultivation of juvenile Nile tilapia (*Oreochromis niloticus*) in a salt water recirculating system

VARIABLE	TREATMENT	
	Control	T-20%
Moisture (%)	$4.44 \pm 0.11$ a	$5.75 \pm 0.22$ b
Lipids (%)	$6.04 \pm 0.03$ a	$4.12 \pm 0.06$ b
Crude Protein (%)	$31.23 \pm 0.05$ a	$32.60 \pm 0.97$ a
Ash (%)	$10.94 \pm 0.04$ a	$10.73 \pm 0.22$ a

Values represent mean values  $\pm$  standard deviation. Different letters on the same line show a significant statistical difference ( $p < 0.05$ ); the same letters on a line indicate the absence of any significant difference between mean values ( $p > 0.05$ )

**Table 2** - Water quality variables in the cultivation of juvenile Nile tilapia (*Oreochromis niloticus*) in a salt water recirculating system

VARIABLE	TREATMENT	
	Control	T-20%
Temperature (°C)	27.31 ± 0.26 a	27.01 ± 0.17 b
Salinity (ppt)	33.30 ± 0.77 a	35.03 ± 0.14 b
Oxygen (mg L <sup>-1</sup> )	5.58 ± 0.06 a	5.10 ± 0.05 b
pH	8.16 ± 0.13 a	7.76 ± 0.29 b
Ammonia - NH <sub>3</sub> (mg L <sup>-1</sup> )	0.18 ± 0.09 a	0.27 ± 0.10 a
Nitrite - NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.04 ± 0.04 a	0.05 ± 0.03 a
Nitrate - NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	10.40 ± 1.27 a	13.53 ± 1.60 a
Phosphate - PO <sub>4</sub> <sup>3-</sup> (mg L <sup>-1</sup> )	6.49 ± 0.77 a	8.53 ± 0.45 b

Values represent mean values ± standard deviation. Different letters on the same line show a significant statistical difference (p<0.05); the same letters on a line indicate the absence of any significant difference between mean values (p>0.05)

Nitrite remained within the ideal range, i.e. below 1 mg L<sup>-1</sup>. The values obtained for nitrate are probably related to the nitrogenous compounds (ammonia and nitrite), which are transformed into nitrate by means of biological processes carried out by the autotrophic chemosynthetic aerobic bacteria. While ammonia and nitrite are toxic to animals, nitrate is practically non-toxic (SÁ, 2012).

The values for oxygen and pH remained within acceptable limits, i.e. above 4 mg L<sup>-1</sup> and ranging from 6.5 to 9.0 respectively. These parameters are considered important in the field of aquaculture: within these ranges, organisms find the ideal conditions for adequate development (SÁ, 2012).

In a study by Basuki and Rejeki (2015), they suggest that the ideal salinity range for cultivating Nile tilapia is 15 ppt (where they perform well), being able to tolerate up to 30 ppt. However, the present study found

that Nile tilapia grow in up to 35 ppt, showing good performance and a high survival rate.

The phosphate remained high in both the Control and the Test. This is due to the principal source of phosphate for the organisms originating in the artificial diet, i.e. in the feed. This was higher in the Test group, since the microalgae are known to contain the carbohydrate, meso-inositol phosphate, which has the characteristic of an excellent source of phosphate (OLIVEIRA *et al.*, 2013).

The variables of zootechnical performance did not present a statistically significant difference (p>0.05), except for specific growth rate (SGR) and survival, as can be seen in Table 3. In general, the Nile tilapia performed well when cultivated in salt water.

The SGR showed a statistical difference (p<0.05) associated with several factors, including ontogenetic variation, or even adaptation to the culture environment, and may show different results for individuals of the same

**Table 3** - Variables of zootechnical performance in the cultivation of juvenile Nile tilapia (*Oreochromis niloticus*) in a salt water recirculating system

VARIABLE	TREATMENT	
	Control	T-20%
Mean final weight (g)	10.27 ± 2.89 a	8.05 ± 1.12 a
Weight gain (g)	9.21 ± 2.71 a	6.98 ± 1.11 a
Mean growth (cm)	3.79 ± 0.41 a	3.73 ± 0.28 a
Survival (%)	100 ± 0.00 a	90 ± 8.17 b
SGR (%)	5.12 ± 0.31 a	4.57 ± 0.32 b
FCF (g g <sup>-1</sup> )	4.99 ± 0.36 a	5.89 ± 1.07 a
Feed Efficiency (%)	44.22 ± 22.54 a	24.85 ± 4.88 a

Values represent mean values ± standard deviation. Different letters on the same line show a significant statistical difference (p<0.05); the same letters on a line indicate the absence of any significant difference between mean values (p>0.05)

species (GONÇALVES JUNIOR; ALMEIDA; SOUZA-FILHO, 2007).

Ibrahem, Mohamed and Ibrahim (2013), evaluated the potential of *A. platensis* on the growth of Nile tilapia, and found that it improved the SGR of the cultivated organisms.

Survival showed a statistically significant difference, with a lower result for the Test group. This difference can be attributed to the lipid content of the test feed, since a diet deficient in essential fatty acids reduces fish performance, generally after a lengthy period (GATLIN, 2010).

In an experiment that used the microalgae *A. platensis* as a dietary supplement during the sexual reversal of Nile tilapia, there was no statistically significant difference in survival (MOREIRA *et al.*, 2010).

In the percent composition of Nile tilapia fillets, the variables moisture and ash did not present a statistically significant difference ( $p>0.05$ ), whereas a difference was seen for lipids and crude protein ( $p<0.05$ ), as shown in Table 4.

**Table 4** - Percent composition of juvenile fillets of Nile tilapia (*Oreochromis niloticus*) in a salt water recirculating system

VARIABLE	TREATMENT	
	Control	T-20%
Moisture (%)	79.89 ± 1.80 a	78.50 ± 0.43 a
Lipids (%)	3.49 ± 0.36 a	2.35 ± 0.07 b
Crude Protein (%)	14.97 ± 1.44 a	17.77 ± 0.47 b
Ash (%)	1.31 ± 0.02 a	1.13 ± 0.11 a

Values represent mean values ± standard deviation. Different letters on the same line show a significant statistical difference ( $p<0.05$ ); the same letters on a line indicate the absence of any significant difference between mean values ( $p>0.05$ )

Knowing the percent composition or chemical composition of feed is an essential factor in dietary decision-making, especially in relation to nutritional criteria. The chemical composition of fish varies greatly, and depends on several factors, such as species, environment, time of year, quantity and quality of consumed food, stage of sexual maturation, age and body part under analysis (LIMA; MUJICA; LIMA, 2012; SOUZA *et al.*, 2015).

Fish muscle may contain 64 to 90% moisture, 8 to 23% protein, 0.5 to 25% fat, and 1 to 2% ash. It is important to point out that lipids vary according to age, biological status, type of feed, nutritional status of the fish and water temperature (FOOD INGREDIENTS BRASIL, 2009).

Fish protein is highly digestible and rich in methionine and lysine, considered essential amino acids, which are not synthesised by the human body, and whose intake is fundamental in a diet (FOOD INGREDIENTS BRASIL, 2009).

Nile tilapia is high in protein and low in fat (SALES; MAIA, 2012). This study, which added *A. platensis* to the diet of Nile tilapia, provided evidence that the microalgae is able to increase the protein content of the fish while maintaining a low fat content. There was no statistically significant difference between the control and test feed in relation to CP content, which leads to the conclusion that the fish were able to make good use of the protein in the microalgae.

Teimoure, Amirkolaie and Yeganeh (2013) carried out a study including *A. platensis* as a food additive in the diet of rainbow trout, where it proved to be efficient in raising protein levels in the muscle of the species, emphasising yet again the benefit of including this microalgae in the diet of aquatic organisms.

## CONCLUSION

The feed supplemented with the microalgae *A. platensis* increased the protein levels of the fillets of Nile tilapia fingerlings, as well as maintaining good zootechnical performance for the fingerlings when cultivated in a salt water recirculating system at a salinity of 35 ppt.

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