Zootechnical performance and fillet quality of Colossoma macropomum fed with a diet supplemented with Spirulina platensis / Desempenho zootécnico e qualidade do filé de Colossoma macropomum alimentados com uma ração suplementada com Spirulina platensis

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ABSTRACT
The objective of this work was to verify the zootechnical development and the quality of Colossoma macropomum fillet fed with diets containing different levels of inclusion of Spirulina platensis. The research was divided in two phases. In the first phase, with 60 days, a stocking density of 40 fish.m\(^{-2}\) was adopted, while in the second phase, with 105 days, 20 fish.m\(^{-2}\) was used. The animals were fed four times a day with commercial feed with the addition of 20% Spirulina (T1), 40% Spirulina (T2) and, as a control (C), commercial feed containing 50% crude protein (CP) was used. At the end of the first phase, no significant difference was observed in survival, or in the final biomass. Otherwise, the highest final average weight was observed in the control group (15.18 ± 0.57 g), being significantly higher than those obtained in T1 (12.00 ± 1.40 g) and T2 (12.09 ± 1.22 g). In the second stage, there were also no significant differences among treatments in terms of survival and final biomass. But again, the highest final average weight was observed in the control, with 84.86 ± 8.44 g, significantly higher than those found in T1 (75.87 ± 6.00 g) and T2 (70.68 ± 6.16 g). When analyzing the quality of fillet, those obtained from fish fed 20% Spirulina (T2) were significantly higher than the others, in terms of protein (17.78 ± 0.04%), lipid (1.23 ± 0.04%) and energy (82.13 ± 0.18 kcal.100 g\(^{-1}\)) levels.

Keywords: Nutrition. Microalgae. Spirulina.

RESUMO
O objetivo deste trabalho foi verificar o desenvolvimento zootécnico e a qualidade do filé de Colossoma macropomum alimentados com ração que continha diferentes níveis de inclusão de Spirulina platensis. A pesquisa foi dividida em duas fases. Na primeira fase, com duração de 60 dias, foi adotada uma densidade de estocagem de 40 peixes.m\(^{-2}\), enquanto na segunda fase, com 105 dias, foi usada uma densidade de estocagem de 20 peixes.m\(^{-2}\). A alimentação dos animais foi feita quatro vezes ao dia com ração comercial com a adição de 20% de Spirulina (T1), 40% de Spirulina (T2) e, como controle (C), foi utilizada ração comercial contendo 50% de proteína bruta (PB). Ao termino da primeira fase, não foi observada diferença significativa na sobrevivência nem na biomassa final, enquanto o maior peso médio final foi observado no grupo controle (15.18 ± 0.57 g), sendo significativamente superior aos obtidos no T1 (12.00 ± 1.40 g) e T2 (12.09 ± 1.22 g). Na segunda etapa também não se observou diferenças significativas entre os tratamentos quanto à sobrevivência e a biomassa final. Quanto ao peso médio final o maior valor foi, mais uma vez, observado no controle, com 84.86 ± 8.44 g, significativamente superior aos encontrados em T1 (75.87 ± 6.00 g) e T2 (70.68 ± 6.16 g). Ao se analisar a qualidade do
filé, os obtidos dos peixes que se alimentaram da ração contendo 20% de Spirulina (T2) foram significativamente superiores aos demais quanto aos níveis proteico (17,78 ± 0,04%), lipídico (1,23 ± 0,04%) e energético (82,13 ± 0,18 kcal.100 g-1).

Palavras-chave: Nutrição. Microalga. Spirulina.

1 INTRODUCTION

*Colossoma macropomum* is an endemic species in the follow rivers: Amazonas, Orinoco, Beni-Mamoré-Guaporé, Paraná-Paraguai-Pantanal and La Plata (GOMES et al., 2010) and it is the most produced native fish in brazilian aquaculture, with more than 102,554 tons (IBGE, 2018). This species is very well adapted to fishculture, due to a lot of factors: (a) it is too rustic (resistant to the handling); (b) it can be grewed in a high stocking density and in a low concentrations of dissolved oxygen in the water; (c) it accepts extruded and pelleted feeds; (d) it makes a better use of animal protein; (e) its breeding technique is completely dominated; (f) it is indicated for extensive, semi-intensive, intensive and super-intensive fish farming; and (g) it can be grown in dams, lakes, streams, supply channels, tanks, ponds and cage (SILVA et al., 2007; SILVA et al., 2013).

The cyanobacterium *Spirulina platensis*, also described as *Arthrospira platensis*, is a photosynthetic microorganism originating from Chad lakes in Africa and Texcoco in Central America, being morphologically characterized by helical filaments (VONSHAK, 1997). It is a microorganism rich in a lot of compounds, such as proteins, lipids, vitamins, phenolic compounds, carotenoids and phycocyanin (COLLA et al., 2007; MENDIOLA et al., 2007; CHIA-HUNG et al., 2014; THANH-SANG; DAI-HUNG; SE-KWON, 2015), that may have antioxidant, antimicrobial, anticancer activities, among others (AMBROSI et al., 2008; GRAWISH, 2008; MATSUDO et al., 2009; PARISI et al., 2009; CHOI et al., 2013; AMAL et al., 2013).

For many decades, *S. platensis* has been used as a source of human healthy food and can be used as a nutritional supplement, because it has antioxidant agents. In human food, *S. platensis* can be incorporated into cereals, fruit bars and beverages, thus helping to fight chronic diseases that are directly related to free radicals (BERMEJO et al., 2008; ANWER et al., 2013).

Due to its high nutritional value, having all essential amino acids, B complex vitamins and fatty acids (especially linolenic acid), this cyanophycea is an excellent supplement in the diet of both terrestrial and aquatic animals, helping them in their health and well-being (HOLMAN; MALAU-ADULI, 2013).

The main ingredients used to formulate feeds to aquatic animals nutrition are usually fish meal and fish oil, because they are sources of excellent protein levelswith a high palability. In addition, they have a balanced form of essential amino acids and fatty acids, as well as high rates of digestive energy. However, the fastaquaculture growth has increasing the demand of these inputs, resulting in
rising prices and a lot of críticas about the environmental dimension of sustainability offish meal and oil production from fishery activity. Thus, it is necessary to use alternative protein sources, increasing the efficiency of aquatic organisms nutrition, which, together with technological and genetic advances, will enable the growth of this activity (NAYLOR et al., 2000; HARDY, 2006).

The biomass of S. platensis has been used to improve growth rates and to increase the fish and crustaceans immunity, being an excellent option for replacing the fish meal used in diets for shrimp and fish and showing good results not only ingrowing, but also in larviculture (LU et al., 2006; MOREIRA et al., 2011a).

In fish nutrition, S. platensis has been used to replace fish meal in fish feed, allowing better growth rates and non-specific immune response (KIM et al., 2013). This seaweed also proved to be effective when used as a food additive, resulting in the improvement of Oncorhynchus mykiss fillet pigmentation (being a substitute for synthetic astaxanthin, since it becomes a natural source of carotenoids). At this way, it increases the quality and acceptability of the product in the market and makes it more attractive to the consumer (TEIMOURI et al., 2013).

The use of Spirulina platensis during larviculture of Oreocrhomis niloticus (sexual reversion period) resulted in the improvement of post-larval performance and survival. This cyanophyte has also been shown to be effective on O. Niloticus healthy, due to promotes benefits to protect tissues, acting as an antioxidante. Furthermore, it has an effective use in fish grown, when in stressful or immunosuppressive conditions (MOREIRA et al., 2011b; IBRAHEM; IBRAHIM, 2014).

The objectives of this work were to cultivate C. macropomum fingerlings fed with fish feed enriched with S. Platensis; to determine the effects of its use in the biochemical composition of the fillet; and to monitor the zootechnical parameters performance and the concentrations of ammonia, nitrates, nitrites in this water.

2 MATERIALS AND METHODS

The experiment was developed at Prof. Dr. Raimundo Saraiva da Costa Aquaculture Station from Fisheries Engineering Department - Federal University of Ceará (Fortaleza, Ceará, Brazil). The fingerlings of C. Macropomum were of the same offspring orginated from Rodolpho Von Ihering Aquaculture Research Center of National Department of Living with Droughts (DNOCS), in Pentecoste (Ceará, Brazil). They were acclimated for 7 days, stored in twelve hapas with a volume of 1.0 m³ and installed in a 48 m³ tank. S. platensis was acquired at Poy Algae Fish Company, also located in Pentecoste (Ceará, Brazil). The chemical composition was determined at Industrial Technology Center of Ceará (NUTEC), in Fortaleza (Ceará, Brazil).
The experiment lasted 165 days and was divided in two phases. The first one, with 60 days, used a stocking density of 40 fish.m⁻³, while the second one, with 105 days, used a stocking density of 20 fish.m⁻³, and was called as the control group (C), in which the animals were fed with commercial fish feed containing 50% crude protein (CP). The treatment 1 (T1) consisted of providing a diet with 28% CP, supplemented with 20% of *S. platensis* flour, while in treatment 2 (T2), the fingerlings were fed the same diet (28% CP), but supplemented with 40% of the microalgae flour.

An extruded commercial diet containing 28% CP was used as base to prepare the diets supplemented with *S. Platensis*. Initially, the extracts of this feed were disintegrated in an industrial blender and after this, they were sieved to separate the dust from larger particles. Subsequently, the powdered feed was mixed with 20% *S. platensis* for T1 and with 40%, for T2 and a homogenization was done manually. As a binder, it was used 1% of a colorless and tasteless gelatin powder, dissolved in preheated water. The feed used in the control group had the same processing of T1 and T2. The wet rations were manually pressed, resulting in pellets compatible with the animals mouth’s size. Then, the fish feeded were dried in a greenhouse with air recirculation at 60°C for 24 hours and packed in plastic containers kept at -20°C for later use and to determinate its chemical composition (Table 1).

The biometries were performed biweekly to determine the zootechnical performance indexes: average weight, survival, weight gain, average daily weight gain, biomass and feed conversion. During the first phase of the experiment, five biometries were done, with the first one at the beginning (day 0) and the last one with 60 days. In the second phase, the initial biometry (day 0) and biweekly biometries were done, ending after 105 days. All the fish were captured, kept in containers containing 10 L of water with 8 g.L⁻¹ of salt and weighed (GOMES et al., 2003).

At the end of this period, the specimens were harvested, placed in containers and killed by hypothermia. After this, the fish went through the process of scaling, eviscerating and filleting. To remove the fillets, a cut was made in the muscle in the longitudinal direction, along the entire spine of the cranial portion, until the end of the caudal peduncle, performed from the back towards the animal's belly. Subsequently, the frozen fillets (-20°C) were transported in a thermal container to NUTEC to make the analysis of chemical composition. The analyzes were performed in duplicate, according to the recommendations of Adolfo Lutz Institute (IAL, 2007). The humidity was determined by the oven drying method at a temperature of 105 °C, until reaching a constant weight. To determine the ether extract, the Soxhlet method was utilized, using hexane as extracting solvent. The fillets protein determination was done by micro-Kjeldahl method (N × 6.25), while the mineral matter determination was done after incineration in a muffle at 550 °C and subsequent weighing.
Dissolved oxygen and temperature were monitored weekly with a digital oximeter at 8:00 am and 6:00 pm, while the pH was also monitored weekly at 12:00 using a pH meter. The levels of ammonia, nitrite and nitrate were determined biweekly by spectrophotometry.

The data of zootechnical performance, final weight, final total length and weight gain were submitted to a variance analysis with a single factor (ANOVA) and when a significant difference was observed, the averages were submitted to the Tukey test for averages of Biostat program 5.0. All analyzes were performed with 5% statistical significance.

3 RESULTS

During the cultivation period, the average temperature was 29.1 ± 2.2 ºC, while pH showed an average value of 7.8 ± 0.8. Dissolved oxygen (DO) had an average value of 7.5 ± 1.6 mg.L\(^{-1}\). For nitrogen compounds: ammonia, nitrite and nitrate, the average values were 0.330 ± 0.060 mg.L\(^{-1}\), 0.038 ± 0.013 mg.L\(^{-1}\) and 1.400 ± 1.001 mg.L\(^{-1}\), respectively.

The values of the growth parameters for the first phase are shown in Table 2, while Table 3 presents the values for the second phase. Finally, the chemical composition of the fish muscle is shown in Table 4.

4 DISCUSSION

*Colossoma macropomum* is a tropical fish and, according to Gomes *et al.* (2010), the average water temperature in the rivers where this species occurs varies between 25ºC and 40 ºC. The same temperature range was kept in this experiment and no mortality had occurred by this parameter. This species is endemic in Amazon River, which has rivers with acidic waters, like Negro River, which is rich in humic acids, (PH ranges from ≤ 5.5 to 6.0) or Solimões River (Neutral PH = 7.0). However, in lakes formed by Amazon River, PH can become alkaline, with values around 8.0 (DARWICH *et al.*, 2005; QUEIROZ *et al.*, 2009), similar to that found in the present study.

In general, it is recommended that, in fish farming, DO concentration can be kept above 4 mg.L\(^{-1}\) (BOYD, TUCKER, 1998). In the present experiment, it was possible to keep DO levels around 7.5 ± 1.6 mg.L\(^{-1}\). Brito et al. (2014) had studied the limnological variables of a lake connected to Negro and Solimões Rivers and found DO concentration in the water surface ranged from 4.4 ± 0.7 to 2.7 ± 0.5 mg.L\(^{-1}\), during the dry and low water periods, respectively.

Boyd and Tucker (1998) recommend that, in fish farming, the ammonia concentration should not exceed 0.5 mg.L\(^{-1}\), and in the present study, the ammonia levels of both the first and second phase were below this limit. Similarly, the values observed in the present work for nitrite and nitrate were below the lethal levels observed to tropical fish (Ferreira da Costa *et al.*, 2004; Oliveira *et al.*, 2007).
In both phases, survival did not show any significant difference between treatments. The values obtained are in accordance to the literature. Silva et al. (2007) cultivated *C. macropomum* with an average weight of 2.67 ± 1.13g in a net tank at a density of 80 fish.m⁻³ for 45 days and obtained a survival rate between 97.92 ± 1, 91 to 87.92 ± 20.93%.

According to Lovell (1998), fish feed must contain between 25% and 50% of protein and a higher concentrations of protein must be offered during hatching. In the present experiment, the protein content of the control group was 54%, higher than diets used in treatments T1 and T2 (Table 2), which may justify the greater weight gain of the fish from the control group.

Signor et al. (2010) investigated the protein and energy levels that maximized the growth of *Piarractus mesopotamicus* grown in 5m³ cages. For this, they fed the fish (average initial weight of 293.38 ± 5.67 g) with formulated diets containing energy values of 3,250 and 3,500 kcal.kg⁻¹ and three different protein levels (25, 30 and 35% CP). At the end of the experiment, it was found that a diet containing 25% CP and 3,250 kcal.kg⁻¹ of digestible energy met the nutritional requirements of this species under this study conditions.

Rodrigues (2014) reports a great lack of information regarding the nutrition of *C. macropomum* at intermediate and final stages of cultivation and also about the ideal nutrition for broodstock and larvae. He also said that there is a concentration of nutritional studies with this fish with weights between 1.0g and 100.0g.

The use of alternative ingredients is quite effective in formulations to this species, due to its omnivorous eating habit. Campeche et al. (2014) elaborated pelleted diets, substituting the bran of *Zea mays* for the bran of *Syagrus coronata* (co-product of oil extraction) in the hatching of *C. macropomum* with initial average weight of 3.18 ± 0.5g. At the end of 50 days cultivation, animals with an average weight of 32.95 ± 1.33 g were obtained in the treatment where the *Z. mays* bran was replaced by 33.33% of *S. coronata* bran.

Fingerlings of *Oreochromis sp.* with an initial average weight of 1.0g, fed with a diet containing 20% *Spirulina maxima* (diet with 28.32% CP and 3,750 kcal.kg⁻¹) for 90 days, presented a weight gain of 36.43 g, resulting in an average daily weight gain of 0.4 g (RINCÓN et al., 2012), higher than 0.18 g.dia⁻¹ obtained in this work for *C. macropomum* fed with diets containing higher protein contents.

Teimouri et al. (2013) evaluated the effects of *Spirulina platensis* as an alternative ingredient to fish meal in diets for *Oncorhynchus mykiss*. These researchers formulated diets with increasing levels (2.5%, 5.0%, 7.5% and 10%) of substituting fishmeal for this cyanophyte. Juveniles with an initial average weight of 100 g, after 70 days of cultivation, showed statistically significant differences in final weights, which ranged between 217.7 and 235.8 g in treatments with 5% and 10%
of *S. platensis* in the diets, respectively. In addition, animals fed with diets containing 10% *S. platensis* showed daily weight gain of 1.87 g.

Different protein levels in the diet can influence the final biomass, as stated by De Almeida *et al.* (2011). These authors found significant statistical differences in biomass gain when the animals were fed with diets containing 35% CP (551 g) and 20.5% CP (316 g). However, in the present experiment, the final biomass of the fish showed no statistically significant difference between the control group and the treatments that used *Spirulina* in the feed.

No significant difference was observed for feed conversion in the second phase, which is much higher than that observed in the first phase. Pelleted diets result in worse rates of zootechnical performance, when compared to extruded diets, due to the rapid sinking of the pellets, reducing the capture time of the animals and resulting in food waste (FURUYA *et al*., 1998; SIGNOR *et al*., 2011).

According to Gonçalves (2009), the edible part of the fish can also be called carcass or clean body. It is the part with no guts and scales, and the fillet is composed of fish musculature, which is the most noble part of the fish. In general, the humidity values tend to have close values, if the animals belong to the same species and under the same cultivation conditions. According to Arbeláez-Rojas *et al.* (2002), the amount of water present in the fillet of *C. macropomum* can vary if they are produced in different cultivation systems, which did not occur in this experiment.

Vidal-Júnior *et al.* (1998) cultivated *C. macropomum* weighing between 30 g and 250 g and observed that the increase in protein level in diets had linearly reduced the protein efficiency and the productive value of the protein. In addition, the deposition of protein in the carcass had a reduction when the protein level of the feed was high, similarly to that observed in this work. Furthermore, when the protein level in fish feed was increased from 33.6 to 36.1% of CP, with the increase of *Spirulina* from 20% to 40%, the protein content was also reduced in the fish fillets. Thus, the animals fed with a diet containing 20% *Spirulina* and 33.6% CP had the highest levels of protein, ether extract and energy in the fillets.

Pereira-Júnior *et al.* (2013) evaluated the centesimal composition of *C. macropomum* when they were fed with diets containing *Leucaena leucocephala* leaf flour. For this purpose, isoproieteic (32% CP) and isocaloric (3,500 kcal) diets were developed with increasing levels of *L. leucocephala* flour. At the end of the experiment, which lasted 60 days, analyzes of *C. macropomum* muscle showed a protein value of 13% in the control diet (did not contain *L. leucocephala*) and 12.2% in the diet contained 24% *L. leucocephala*. These values are lower than those found in this study, which presented protein values close to 17%.

The inclusion of 5% *Spirulina* in diet offered to *Clarias gariepinus* resulted in a fillet containing 33.0% protein and 34.1% fat, while fish fed with 3% *Spirulina* had a statistically different
value for protein content (31%), but statistically similar for the amount of fat that was 33.8% (PROMYA; CHITMANAT, 2011).

5 CONCLUSIONS

These results make possible to believe in the viability of using *S. platensis* as an ingredient to feed *C. macropomum*. However, additional studies must be developed to determine the optimal level of inclusion and/or substitution, the evaluation of fish feed production costs and also about fillet efficiency and quality.

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| Parameter | Feed          | S. platensis | Control | T1 (20% Spirulina) | T2 (40% Spirulina) |
|-----------|---------------|--------------|---------|--------------------|--------------------|
| Protein (%) | 47.6 ± 0.1 | 54.1 ± 0.1  | 33.6 ± 0.3  | 36.1 ± 0.2         |
| Ethereal extract (%) | 1.0 ± 0.1 | 7.1 ± 0.1  | 3.01± 0.1  | 3.1 ± 0.2         |
| Ash (%) | 16.2 ± 0.3 | 11.9 ± 0.1  | 9.9 ± 0.1  | 9.7 ± 0.1         |
| Humidity (%) | 10.3 ± 0.1 | 6.2 ± 0.1  | 12.2 ± 0.1  | 12.1 ± 0.1         |
| Energia (kcal.kg⁻¹) | 2,990.0 ± 12.4 | 3,632.6 ± 1.4  | 3,303.0 ± 0.8  | 3,281.0 ± 15.4    |

Table 2 Survival, initial and final weights, weight gain, daily weight gain (DWG), initial biomass, final biomass of tambaqui fingerlings cultured for 60 days with fish feed supplemented with *S. platensis* in stocking density of 40 fish.m³

| Parameter | Feed          | Control | T1 (20% Spirulina) | T2 (40% Spirulina) |
|-----------|---------------|---------|--------------------|--------------------|
| Survival (%) | 92.5 ± 7.6 | 87.5 ± 11.9  | 84.4 ± 10.9         |
| Initial weight (g) | 0.9 ± 0.2 | 0.9 ± 0.2  | 0.9 ± 0.2 | |
| Final weight (g) | 15.2 ± 0.6ᵃ | 12.0 ± 1.4ᵇ | 12.1 ± 1.2ᵇ |
| Weight gain (g) | 14.3 ± 0.6ᵃ | 11.1 ± 1.5ᵇ | 11.1 ± 1.2ᵇ |
| DWG (g.day⁻¹) | 0.24 ± 0.08 | 0.18 ± 0.05  | 0.18 ± 0.06         |
| Initial biomass (g) | 36.8 ± 2.3 | 28.3 ± 2.3  | 37.7 ± 0.6        |
| Final biomass (g) | 532.0 ± 120.4 | 418.2 ± 95.9  | 377.8 ± 86.2    |
| FCR | 1.6 ± 0.4 | 1.6 ± 0.1  | 1.6 ± 0.2         |

Data are averages ± S.D. of three replicate cages. For each row, averages with different letters as superscripts are significantly different (*P*<0.05)
Table 3 Survival, initial and final weights, weight gain, daily weight gain (DWG), initial biomass, final biomass of tambaqui fingerlings cultured for 60 days with fish feed supplemented with *S. platensis* in stocking density of 20 fish.m$^3$

| Parameter          | Control                  | T1 (20% Spirulina) | T2 (40% Spirulina) |
|--------------------|--------------------------|-------------------|--------------------|
| Survival (%)       | 91.7 ± 5.8               | 80.0 ± 17.8       | 86.7 ± 10.4        |
| Initial weight (g) | 15.2 ± 3.1               | 11.6 ± 2.2        | 12.1 ± 2.2         |
| Final weight (g)   | 84.9 ± 8.4               | 75.8 ± 6.0        | 70.7 ± 6.2         |
| Weight gain (g)    | 70.1 ± 8.4               | 59.1 ± 8.9        | 58.7 ± 5.5         |
| DWG (g.day$^{-1}$) | 0.7 ± 0.1                | 0.6 ± 0.2         | 0.5 ± 0.3          |
| Initial biomass (g)| 532.0 ± 120.4            | 418.2 ± 95.9      | 377.8 ± 86.2       |
| Final biomass (g)  | 1,334.5 ± 112.0          | 1,121.3 ± 191.4   | 1,099.4 ± 117.1    |
| FCR                | 3.1 ± 0.8                | 3.5 ± 1.0         | 3.9 ± 1.2          |

Data are averages ± S.D. of three replicate cages. For each row, averages with different letters as superscripts are significantly different ($P<0.05$)

**Tabela 4** Chemical composition of fish muscle after experimental period

| Parameter          | Control                  | T1 (20% Spirulina) | T2 (40% Spirulina) |
|--------------------|--------------------------|-------------------|--------------------|
| Protein (%)        | 17.2 ± 0.1               | 17.8 ± 0.1        | 17.1 ± 0.1         |
| Ethereal extract (%)| 1.1 ± 0.1                | 1.2 ± 0.1         | 1.1 ± 0.1          |
| Ash (%)            | 1.1 ± 0.2                | 1.1 ± 0.1         | 1.1 ± 0.1          |
| Humidity (%)       | 80.6 ± 0.1               | 79.8 ± 0.1        | 80.6 ± 0.1         |
| Energy (kcal.kg$^{-1}$) | 78.4 ± 0.2               | 82.1 ± 0.1        | 78.4 ± 0.5         |

Data are averages ± S.D. of three replicate cages. For each row averages with different letters as superscripts are significantly different ($P<0.05$)

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