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Stable isotopes of C and N as dietary indicators of Nile tilapia *(Oreochromis niloticus)* cultivated in net cages in a tropical reservoir

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ABSTRACT

The relative contribution of natural feed sources and commercial diets to the nutrition of Nile tilapia (*Oreochromis nilloticus*) was evaluated in a net cage production carried out in a reservoir in the semi-arid northeast region of Brazil. The present study tested the hypothesis that tilapia uses natural sources of feed for growth, even when confined in cages and artificial feed resources are available. The fish and various feed sources (commercial feed, seston and periphyton) were sampled and the water quality was monitored during the cultivation, which was carried out for 120 days. The fish were fed three times daily with two commercial feeds of 40 and 32 % crude protein, both of which used fish meal as a protein source. Stable isotopes analyses were carried out by measuring the ^{13}C and ^{15}N of fish muscle tissue and the feed items using the MixSIAR mixing model to determine the relative contribution of the nutrient sources to fish growth. The tilapia showed adequate weight gains with the total contributions of the feed items: periphyton (14.4 %), seston (51.2 %) and commercial feed (34.4 %) throughout the 120 days of culture. It can be concluded that the natural feed sources of seston and periphyton contributed more to the growth of the Nile tilapia than the commercial feed when cultivated in net cages, changing the notion that the commercial feed contributes predominantly to the intensive farming of this species in cages. The results of the present study suggest a more sustainable feed management in the farming of Nile tilapia in net cages.

1. Introduction

Knowledge of the feed management and adequate nutrition of commercial fish species is fundamental in understanding the ecological aspects and the economics of their cultivation. Evaluation of the relationship between the diet and fish growth can improve production while optimizing the use of artificial feed. Excessive use of feed leads to inadequate water quality and economic losses. Furthermore, high feed input in intensive aquaculture systems has a negative impact on the environment when considering the accumulation of nutrients as unconsumed feed and fish feces and their release through effluents (e.g., Montanhini Neto and Ostrensky, 2015; Moura et al., 2016). The nutrient loads in effluents from fish farming depend on the quality of the commercial feed and on the availability of natural food sources in the aquatic environment. An abundance of natural food sources in aquaculture production systems can allow the sparing of commercial feed input (e.g., Garcia et al., 2016, 2017; David et al., 2018), ultimately lowering the nutrient loads released into the environment.

The excessive use of artificial feed tends to increase the presence of periphyton and planktonic organisms in seston. These organisms use the nutrients from feed leftovers and wastes from fish farming in net cages. Thus, it is essential to utilize different food resources for more sustainable management, as described for some semi-intensive aquaculture systems (e.g., Pucher et al., 2014a, 2015). Semi-intensive management of filter feeders such as silver carp, bighead carp and tilapia that includes high quality fertilization has been shown to improve the natural food base (Spataru, 1977; Turker et al., 2003; Pucher et al., 2014a, 2015; Pucher and Focken, 2016). The contribution of natural food sources to

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fish growth has been evaluated using stable isotopes in semi-intensive aquaculture (Pucher et al., 2014b; Pucher and Focken, 2016), intensive aquaculture (Burford et al., 2002) and biofloc systems (Avnimelech, and Kochba 2009).

Stable isotopes analyses have been used to estimate the contributions of artificial and natural feed sources to the production of various fish species (e.g., Li et al., 2013; Gondwe et al., 2012; Rao et al., 2015; Nachev et al., 2017). Stable carbon and nitrogen isotopes are the most common markers for carrying out tilapia nutrition studies (e.g., Schroeder, 1983; Focken, 2008; Kelly and Martinez Del Rio, 2010). Stable isotopes analyses have shown how the tilapia obtains nutrients from the natural environment (e.g., Rao et al., 2015) and in artificial rearing systems (e.g., Zuanon et al., 2007; Gondwe et al., 2012). This species has omnivorous and planktivorous feeding habits (Attayde and Menezes, 2008; Attayde et al., 2010) and readily consumes commercial feed, phytoplankton, and other natural feed sources (e.g., Bhujel, 2014; Ogello et al., 2014). However, only a few analyses have used mixed models to understand the simultaneous assimilation of several feed sources available in the natural or culture environment (e.g., Campbell et al., 2005; Kadye and Booth, 2012; Phillips et al., 2014; Rao et al., 2015; de Moraes and Henry-Silva, 2018). The use of these models permits the inclusion and utilization of distinct discrimination factors for each food item consumed in a given period of time and during a specific stage of the fish life cycle. This information advances the understanding of food ecology for species produced in inland aquaculture systems. Thus, the objective of the present study was to determine the relative contribution of commercial feed and natural feed items (seston and periphyton) to the growth of Nile tilapia (*Oreochromis niloticus*) cultivated in net cages in a reservoir of northeastern Brazil using a Bayesian mixing model (MixSIAR).

2. Materials and methods

2.1. Experimental design

Nile tilapia (*O. niloticus*) were cultivated for a period of 120 days in net cages (20 mm mesh size) with a stocking density of 150 fish m^{-3} (720 fish/cage) in the Santa Cruz reservoir (5°45′18,0″ S 37° 49′ 11,4″ W), which is in the semi-arid region of northeastern Brazil. Each net cage had dimensions of 2 m width x2 m length x1.2 m depth and the bottom of the cage was not in contact with the sediment. The fish were fed with a pelleted commercial feed (40 % crude protein) three times daily for phase I and with a different feed (32 % crude protein) for phases II and III of the grow-out, of which both feeds had fish meal as a protein source. According to the fish farmers, the daily feed rate during the grow-out started at 6% of fish biomass and was gradually reduced by 1% each month. The production system consisted of 120 net cages that have an annual production of approximately 33 tonnes of Nile tilapia (Moura et al., 2016).

Samples of tilapia muscle tissue and feed (commercial feed, seston and periphyton) were collected between August and November of 2013. The first samples were taken 30 days after the beginning of the cultivation (average fish weight =7.3 g - Phase I); the second set of samples were at 75 days (average fish weight =36.1 g - Phase II) and the third set at 120 days (average fish weight =158.7 g - Phase III). The stable isotopes analyses of $\delta^{13}C$ and $\delta^{15}N$ were carried out using the following samples: (A) 15 dorsal muscle tissue samples of Nile tilapia (six samples in phases I and II and three samples in phase III) obtained after harvesting and processing; (B) 15 samples of each commercial feed (six samples in phases I and II and three samples in phase III) offered for each culture phase; (C) 15 samples of periphyton (six samples in phases I and II and three samples in phase III) obtained by scraping the screens of the net cages; (D) six samples of seston (two samples in each phase) obtained by filtering one liter of water from the environment where the farming activities were carried out.

Samples of fish muscle tissue and commercial feed were dried in an oven at a temperature of 55−60 °C for 72 h. Periphyton samples were dried at a temperature of 50−55 °C for 36 h. The quartz filters used to filter water samples with the seston were dried at a temperature of 55−60 °C for 48 h. Muscle tissue, feed and periphyton samples were ground to a fine powder using a mortar and pistil. Filters with seston were scraped after the water samples were filtered. All samples were then weighed using an analytical balance and stored in tin capsules. Stable isotope analyses of ^{13}C and ^{15}N were carried out by UC Davis Stable Isotope Facility, Department of Plant Sciences, California University - USA.

2.2. MixSIAR mixing model

The present study used a MixSIAR bayesian mixing model (Stock and Semmens, 2013) with the software R (version 3.2.2). Crude data of $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios of samples of fish and all feed sources were used in the MixSIAR. The isotopic ratios of the $\delta^{13}C$ and $\delta^{15}N$ were obtained based on the natural abundance of the isotopic ratios of C and N (Eq. 1) (Post, 2002; Leal et al., 2008; Philippsen and Benedito, 2013).

$$\delta(X)=[(R_{sample}/R_{standard})-1]*1000 \quad (1)$$

Where: X (‰) is the value of abundance for ^{13}C or of ^{15}N, and R_{sample} is the ratio between ^{13}C:^{12}C or ^{14}N:^{15}N, and the $R_{standard}$ is derived from the standard of each element.

The discrimination rates were calculated between analyzed specimens and different feed sources. The isotopic discrimination factor (Martinelli et al., 2009; Philippsen and Benedito, 2013) was then calculated for each feed item using the following equation:

$$\Delta X= \delta X_{muscle\ tissue} - \delta X_{food} \quad (2)$$

Where: ΔX is the discrimination rate obtained between tissue samples and between each feed item. $\delta X_{muscle\ tissue}$ is the value of abundance for ^{13}C or of ^{15}N for each muscle tissue and δX_{food} is the value of abundance for ^{13}C or of ^{15}N for each natural and artificial food item available for the fishes.

The data were submitted to the mixing model, applying the Monte Carlo Markov Chain (MCMC) simulation method. Subsequently, a simulation process was developed based on *a posteriori* distributions of the variables analyzed in the present study, using the Markov chains. This model allowed the use of distinct discrimination rates for each feed item and determined the relative contribution rates of these items individually based on the isotopic values of the fish and feed sources. The convergence in the Markov chains was analyzed using a diagnostic test described in Gelman -Rubin (1992, 2014). After the modeling, graphs were generated and the results were shown in the R console. The means, standard deviations, and quantiles for the contents of the diet were saved in the item “Summary Statistics.txt” and tests of convergence diagnostics saved in the item “diagnostics.txt”.

2.3. Statistical analysis

The results of the C and N isotope ratios for the feed sources and fish and the relative contributions of the feed sources were analyzed using the software R (version 3.2.2). When conditions of normality and homogeneity were met, data were subjected to a one-way ANOVA and Tukey’s *a posteriori* test. Data that showed indaquate normality or homogeneity were subjected to the Kruskal-Wallis non-parametric test and the multiple comparisons test (two-tailed).The data of the isotopic ratios and the relative contributions of the feed items were analyzed separately according to the different stages of the grow-out phase to verify the relative importance of each feed item in the fish diet for each stage of the grow-out.

Table 1
Mean values (± standard deviation) of the water quality variables during the three sampling periods.

Water Quality Variables	30 days (Phase I)	75 days (Phase II)	120 days (Phase III)
Electrical conductivity (mS/cm)	0.3370 ± 0.0010	0.3285 ± 0.0015	0.3045 ± 0.0075
Hydrogen ionic potential (pH)	8.08 ± 0.07	7.49 ± 0.29	8.00 ± 0.04
Total dissolved solids (ppm)	0.219 ± 0.001	0.214 ± 0.002	0.198 ± 0.005
Dissolved oxygen (mg/L)	8.36 ± 0.60	10.35 ± 0.15	9.20 ± 0.20
Temperature (°C)	28.53 ± 0.17	29.40 ± 0.20	28.65 ± 0.15
Transparency (m)	3.75 ± 0.75	3.05 ± 0.05	3.5 ± 0.4
Turbidity (NTU)	2.90 ± 0.10	1.80 ± 0.20	5.50 ± 0.50
Current (m/s)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Ammonia (mg/L)	0.072 ± 0.006	0.093 ± 0.026	0.011 ± 0.004
Nitrite (mg/L)	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Nitrate (mg/L)	0.250 ± 0.008	0.270 ± 0.009	0.295 ± 0.005
Total nitrogen (mg/L)	0.345 ± 0.045	0.462 ± 0.031	0.513 ± 0.044
Total phosphorus (mg/L)	0.024 ± 0.002	0.014 ± 0.002	0.061 ± 0.029
Chlorophyll *a* (μg/L)	5.073 ± 0.801	3.872 ± 1.736	2.270 ± 0.401

2.4. Water quality variables

The following water quality variables were analyzed during the grow-out: electrical conductivity (EC), pH, total dissolved solids (TDS), dissolved oxygen (DO), temperature (T), transparency using the Secchi disk (Transp.), turbidity and velocity of water current (Current). With the exception of transparency, all water quality variables were measured using a multi-parameter probe. Nitrite ($N\text{-}NO_2$), nitrate ($N\text{-}NO_3$) and ammonia (NH_3) concentrations were determined according to methodologies described in Mackereth et al. (1978). Total nitrogen (N_{total}) and total phosphorus (P_{total}) were determined using methods described in Koroleff (1976) and Golterman et al. (1978), respectively. Chlorophyll *a* (μg/L) was extracted with 90 % acetone and values were obtained using spectrophotometry (Arar, 1997) (Table 1).

3. Results

The results of the $\delta^{13}C$ isotope ratios showed mean values of -25.22 ± 1.02 ‰ for the seston and -19.58 ± 1.20 ‰ for the commercial feed. The periphyton had a higher value of $\delta^{13}C$ (-16.57 ± 0.71 ‰), being more enriched at ^{13}C, while in the fish musculature the mean value was -18.11 ± 1.02 ‰. For the $\delta^{15}N$ ratios, the mean values were 7.38 ± 0.69 ‰ for seston and 6.82 ± 0.49 ‰ for fish. Mean values for the commercial feed and periphyton were 4.70 ± 0.49 ‰ and 4.25 ± 1.23 ‰, respectively (Table 2). Variations were observed in the isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ for the fish in relation to the assimilation of each food item. This characteristic was verified by the discrimination rates of $\Delta^{13}C$ and $\Delta^{15}N$ in different phases of the grow-out. The fish showed mean rates of $\Delta^{13}C$ and $\Delta^{15}N$, of 1.48 ± 1.45 ‰ and 2.12 ± 0.62 ‰ for commercial feed, 7.05 ± 1.07 ‰ and -0.48 ± 0.39 ‰ for seston, and -1.54 ± 1.51 ‰ and 2.57 ± 1.02 ‰ for the periphyton, respectively (Table 2).

The Gelman-Rubin convergence test showed a scale reduction factor (R) for the 60 variables analyzed (individual and period relative contribution rates for food items: commercial feed, seston and periphyton) with lower values since the diagnosis of Gelman-Rubin should be <1.05. Among the 60 variables analyzed (0 > 1.01;0 > 1.05 and 0 > 1.1), none presented any value higher than the lowest estimated factor of 1.01 for this test (Annex 1). The relative contribution of available feed items throughout the cultivation period indicated that the periphyton contributed 14.4 %; seston with 51.2 % and the commercial feed with 34.4 %, showing greater general contributions from the natural feed sources (65.6 % seston + periphyton).

The seston and the commercial feed were predominant in the feeding of Nile tilapia during the grow-out. The periphyton presented significantly lower contribution values. The three food items showed significant differences in their contribution percentages in the last two stages of the cultivation, with the contributions of seston (58.6 % - Phase II and 51.8 % - Phase III) significantly higher than contributions of the commercial feed (30.6 % - Phase II and 31.2 % - Phase III) and the periphyton (10.8 % - Phase II and 17 % - Phase III). No significant differences were shown in Phase I between the contribution percentages of the commercial feed (41.5 %) and the seston (43.2 %), and both of which were significantly higher than the periphyton (15.3 %) (Fig. 1).

Table 2
Mean values (± standard deviation) of the isotopic concentrations of ^{13}C and ^{15}N of the samples.

Samples	Isotopic composition of the samples			
Fish	δ ^{13}C (‰)	δ ^{15}N (‰)	Δ ^{13}C (‰)	Δ^{15}N (‰)
30 days (Phase I)	−18.61 ± 1.32^{a}	6.37 ± 0.46^{b}	———	———
75 days (Phase II)	−17.42 ± 0.46^{a}	7.03 ± 0.12^{a}	———	———
120 days (Phase III)	−18.47 ± 0.08^{a}	7.30 ± 0.10^{a}	———	———
Seston	δ ^{13}C (‰)	δ ^{15}N (‰)	Δ ^{13}C (‰)	Δ^{15}N (‰)
30 days (Phase I)	−26.26 ± 0.80^{a}	6.66 ± 0.60^{a}	7.65 ± 0.70	−0.29 ± 0.42
75 days (Phase II)	−25.04 ± 0.28^{a}	7.71 ± 0.29^{a}	7.62 ± 0.67	−0.68 ± 0.21
120 days (Phase III)	−24.36 ± 0.90^{a}	7.77 ± 0.59^{a}	5.88 ± 0.70	−0.46 ± 0.46
Commercial Feed	δ ^{13}C (‰)	δ ^{15}N (‰)	Δ ^{13}C (‰)	Δ^{15}N (‰)
30 days (Phase I)	−19.51 ± 1.26^{ab}	4.61 ± 0.37^{a}	0.90 ± 0.41	1.75 ± 0.55
75 days (Phase II)	−20.34 ± 0.49^{b}	4.54 ± 0.55^{a}	2.92 ± 0.86	2.48 ± 0.63
120 days (Phase III)	−18.22 ± 0.98^{a}	5.20 ± 0.33^{a}	−0.25 ± 0.90	2.10 ± 0.38
Periphyton	δ ^{13}C (‰)	δ ^{15}N (‰)	Δ ^{13}C (‰)	Δ^{15}N (‰)
30 days (Phase I)	−16.73 ± 1.02^{a}	3.54 ± 0.67^{a}	−1.88 ± 2.32	2.83 ± 0.60
75 days (Phase II)	−16.37 ± 0.52^{a}	4.31 ± 1.47^{a}	−1.05 ± 0.73	2.71 ± 1.43
120 days (Phase III)	−16.65 ± 0.24^{a}	5.56 ± 0.16^{a}	−1.83 ± 0.25	1.75 ± 0.11

δ (‰) is the value of the abundance for ^{13}C or of ^{15}N, and Δ (‰) is the discrimination rate obtained between the fish muscle tissue samples and between the samples of the feed sources.
Different letters for the isotopic concentrations of the fish and of each feed item indicate significant differences between phases of the grow-out, for the carbon in the feed items by the Tukey test (<0.05) and for the nitrogen in the samples of fish tissue by the multiple comparisons test (two-tailed) (<0.05).

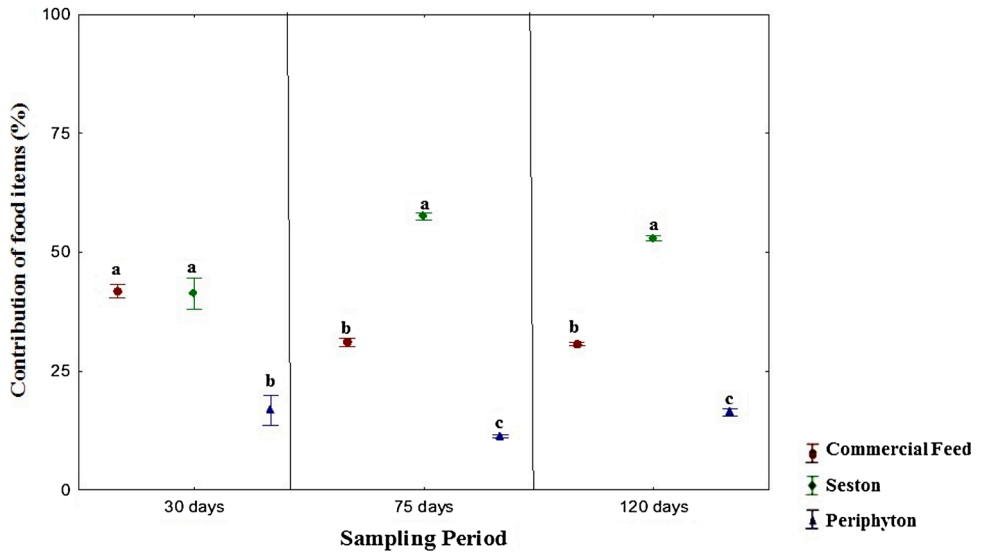

Fig. 1. Mean values (±SD) of the contributions of the various feed items for the three sampling periods. Different letters for each period between feed items indicate significant differences according to the Tukey test (<0.05).

4. Discussion

Nile tilapia is an omnivorous and planktivorous species (Attayde and Menezes, 2008; Attayde et al., 2010) that primarily consumes phytoplankton, zooplankton, periphyton and suspended detritus (e.g., Bhujel, 2014; Ogello et al., 2014). In artificial rearing systems, this species also consumes commercial and artisanal feeds made with alternative ingredients (e.g., Bhujel, 2014; Ogello et al., 2014). In the present study, the seston and the commercial feed were the main food items that contributed to Nile tilapia growth, with a greater contribution from the seston. The higher consumption of this feed source may have been influenced by the accumulation of nutrients from unconsumed feed and fish excrement, which stimulate the plankton community near the net cages. Nevertheless, the accumulation of nutrients from commercial feed causes eutrophication and increases the availability of natural food sources (Flickinger et al., 2019, 2020a; Flickinger et al., 2020b). The growth and abundance of plankton depend on bioavailable phosphorus, of which the total phosphorus of the water and the seston increased in the final stage of the grow-out. It is noteworthy that seston is composed mostly of plankton (Richardson et al., 2009; Caraballo et al., 2011).

Recent studies have described potential benefits of using alternative ingredients in feeds and exploiting the natural feed sources to supplement the nutrition of tilapia in different cultivation systems (e.g., Gondwe et al., 2012; Ng and Romano, 2013; Montanhini Neto and Ostrensky, 2015). Other studies have shown that the use of physical structures (substrates) to develop the periphyton community may increase the availability of natural feed (periphyton) for fish in experimental production systems and in natural environments (e.g., Huchette et al., 2000; Azim et al., 2003; Zorzal-Almeida and Fernandes, 2014; Abwao et al., 2014; Garcia et al., 2016; Garcia et al., 2017). Rao et al. (2015) analyzed the natural feed sources assimilated by wild specimens of Nile tilapia, which were free-swimming and had access to substrates, and concluded that the average contribution rates of the periphyton and seston to fish growth were 26 % and 20 %, respectively. The reduced contribution of the periphyton (14.4 %) to fish cultivated in the Santa Cruz reservoir was perhaps related to its low availability, since the tilapia were confined and only had access to periphyton that developed in net cages.

Research using stable isotopes has verified the assimilation of plankton by tilapia. Britton et al. (2009) observed that *O. niloticus baringoensis* had $\delta^{13}C$ values that reflected a strong dependence on basal plankton resources with an isotopic change related to the increasing size of the animals, indicating that the smaller fish fed directly on the phytoplankton while the larger specimens consumed zooplankton. Rao et al. (2015) also concluded that small fish were more dependent on periphyton, phytoplankton (seston) and detritus. In the initial phase of the grow-out in the present study, the smaller individuals of Nile tilapia fed on the seston, commercial feed and the periphyton in the net cages. The same individuals assimilated these food items throughout the cultivation, with predominance of seston toward the end of the grow-out. Thus, semi-intensive aquaculture systems with the Nile tilapia benefit from natural feed sources and the use of commercial feeds, enabling a better feed conversion and greater profitability of their production in net cages. In pond polycultures that include the Nile tilapia, the semi-intensive management strategy showed a higher net oxygen production, less turbidity, better light penetration, a greater phototrophic layer and greater abundance of phytoplankton when compared to the traditional management strategy. In general, the described semi-intensive management strategy was shown to improve the natural food base for fish that feed on seston, such as the tilapia (Pucher et al., 2014a).

Pucher et al. (2014b) used ^{15}N as a tracer to evaluate the flow of nitrogen in food webs of sub-tropical aquaculture ponds subjected to different management strategies and reported that nitrogen fixed in suspended particles was significantly higher with the described semi-intensive management of the ponds when compared to the traditional management. Seston (1–15 μm) was the dominant pelagic resource in all ponds throughout the culture. The semi-intensive management was shown to have significantly higher biomass in the planktonic fractions of seston (1–15 μm) and small microplankton (15–60 μm). Nitrogen and seston in the present study showed patterns that are comparable to the prior study, suggesting that eutrophication increases the availability and contribution of seston to tilapia growth.

Moura et al. (2016) evaluated the sustainability of farming Nile tilapia in net cages in the Santa Cruz reservoir and found that 21 % of the nitrogen and 17 % of the phosphorus from feed were recovered as harvested fish biomass while the rest of the nutrients were lost to the environment, which led to a eutrophication potential of 8.6 kg of phosphorus per ton of fish produced. Hence, most nutrients in the commercial feed are unavailable for assimilation by Nile tilapia in this type of cultivation system. This is perhaps due to the consumption of natural feed (periphyton and seston) by Nile tilapia in the Santa Cruz reservoir.

The mass of seston needed to sustain the production described in the

present study was analyzed based on the annual production of 33 tonnes of tilapia in the Santa Cruz reservoir (Moura et al., 2016), considering that half of the fish diet came from seston. Considering that the 33 tonnes of tilapia mass are equivalent to approximately 8.2 tonnes of dry mass (Dantas and Attayde, 2007), approximately 4.1 tonnes of seston would be needed to maintain this annual production of tilapia. Thus, assuming that half of the dry mass of the seston is made of carbon, 2.0625 tonnes of seston carbon (2062 kg C/year or 5.65 kg C/day of seston) would be needed to sustain the annual production of 33 tonnes of tilapia in net cages in the Santa Cruz reservoir. The net primary production of phytoplankton (NPP) at the site of the net cages was 28.2 mgC/m^3/h (Gross Primary Production of 58.6 mgC/m^3/h and respiration of 30.4 mgC/m^3/h), that is, NPP of 0.000677 kg C/m^3/day (Dantas, 2016; de Oliveira, 2015). These results show that the demand for seston by the tilapia is 5.65 kg C/day and the production would require a volume of 8345 m^3 per day to flow through the tanks in a 100 % conversion scenario. However, when considering a realistic ecological conversion of 10 %, the volume of water that should flow through the net cages would then be 83,456 m^3/day. Thus, considering that the volume of Santa Cruz is 599,712,000 m^3, 83,456 m^3/day would correspond to 0.014 % of the total volume of the reservoir passing through the net cages each day to maintain the production of 33 tonnes of tilapia.

The results of the present study showed the importance of planning the feed management for the intensive farming of Nile tilapia in net cages, adjusting the use of feed according to the availability of natural food sources. Natural feed, such as seston, can contribute to the growth of the target species while minimizing the use of commercial feed and as a consequence, improves the economic feasibility and environmental sustainability of the cultivation. A reduction in commercial feed in fish farming may decrease nutrient loads in the effluents, thereby minimizing impacts on the water quality of surrounding aquatic environments (e.g., Montanhini Neto and Ostrensky, 2015; Moura et al., 2016). Nevertheless, the present study suggests that natural feed sources contribute more than the commercial feed to the growth of Nile tilapia farmed in net cages, contrasting the conventional feed management of using artificial feed as the main nutrient source for this species in intensive systems.

Declaration of competing interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

CRediT authorship contribution statement

Cyntia Rafaela Ferreira de Moraes: Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **José Luiz de Attayde:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft. **Gustavo Gonzaga Henry-Silva:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A

Fig. A1

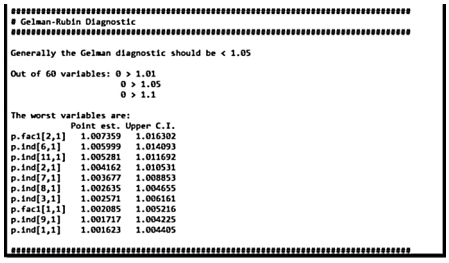

Fig. A1. Gelman-Rubin diagnostic of 60 variables tested in MixSIAR, in software R – version 3.2.2. Generally the Gelman diagnostic should be <1.05. Out of 60 variables: 0 > 1.01, 0 > 1.05, 0 > 1.1. Point est = Potential Scale Reduction Factor- PRSF; Upper C.I = default confidence level of 95 % for the upper confidence limit.

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