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ANIMAL SCIENCE

Growth and carbon turnover of *Piaractus mesopotamicus* Holmberg, 1887 (Osteicthyes: Characidae): contribution of extruded feed and natural food

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Abstract: *Piaractus mesopotamicus*, is a fish usually farmed in semi-intensive systems with access to natural food and supplementary feed. This study evaluates effects of feed allowance on the productive performance, carbon turnover and proportions of nutrient (carbon) contribution of feed and natural food for the growth of pacu. Juvenile fish were stocked in fiberglass tanks and fed to 100, 75, 50, 25, 0% apparent satiety (ApS), with a practical, extruded (C4 photosynthetic pathway) feed in a randomized design trial (n=3); plankton production for simulated semi-intensive farming system condition was induced by chemical fertilization. A control treatment was set up in tanks devoid of natural food. Data on muscle stable carbon isotope ratios were used to study carbon turnover using a relative growth-based model. Low variation of the $\delta^{13}C$ impaired fitting a turnover model curve for the 0 and 25 % ApS treatments. Fish of the 100% and 75% ApS treatments reached circa 95% and 82.85% of the carbon turnover, respectively. Extruded feed was the main nutrient source for the growth of pacu in the semi-intensive, simulated farming condition. The current study contributes to the knowledge of the relationship between feeding rates and carbon turnover rates in the pacu muscle.

Key words: mixing model, nutritional tracers, stable isotopes, trophic discrimination factor.

INTRODUCTION

Piaractus mesopotamicus (*Characiformes*: Characidae) is a Neotropical fish important for both fisheries and aquaculture purposes. Pacu is usually farmed in semi-intensive systems with access to natural food such as plankton, insects and detritus to take advantage of the omnivore feeding habit (Valladão et al. 2016). Quantitative or qualitative feed restrictions have been proven to increase the rate of utilization of natural food for some fish, that is, increased foraging activity to compensate feed restriction thus sustaining optimized growth rates (Bechara et al. 2005, Filbrun & Culver 2014).

Increasing foraging activity would require reduced quantities of feed per unit of fish biomass produced, thus reducing feeding costs (Bolivar et al. 2006). However, the optimization of feeding rates requires precise quantification of the effects of feed restriction (or allowance) on the proportional contribution of nutrients of feeds and natural food. The use of stable isotopes as tracers has been proven a powerful tool for the assessment of the nutritional contribution of natural food and feeds for the growth of fish and shrimps (Schroeder 1983, Jomori et al. 2008, Su et al. 2008, Asano et al. 2010, Filbrun & Culver 2014, Ferreira et al. 2020).

The use of stable isotope techniques is based upon the assumption that the fish reaches isotopic equilibrium with diets (natural food and artificial feed), therefore the characterization of the isotopic turnover is thus indispensable (Madigan et al. 2012, Xia et al. 2013). Carbon or nitrogen turnover rate is associated with growth rate of the fishes, and they are several model for their description including time based models and growth based models (Xia et al. 2013, Gamboa-Delgado 2022). In the same way, during the process of digestion and nutrient assimilation shifts in isotopic ratios between diet components (food and feeds) and the consumers (fishes) occur, these shifts are estimated as trophic discrimination factors (TDF), which are also important parameters in mixing models to estimate the contribution of feed and food to animals' diet (Bastos et al. 2017, Canseco et al. 2022). The aim of this study was to evaluate the effect of feed restriction (allowance) on the productive performance, the carbon turnover and the proportions of nutrient (carbon) contribution of feed and natural food for the growth of juvenile pacu, in semi-intensive farming conditions.

MATERIALS AND METHODS

Fish and facilities

Juvenile pacu (53,25 ± 0,30 g) were stocked in 18, 1500-L fiberglass tanks (15 fish per tank; 85% effective volume) and fed to 100, 75, 50, 25, 0% of apparent satiety (ApS) in two daily meals, with a commercial, extruded feed, green water condition, plankton production induced by chemical fertilization, in a randomized design trial (n=3). A control treatment was set up in tanks devoid of natural food, fish feeding on a formulated corn meal- and corn gluten meal-based (C4 photosynthetic pathway) the extruded diet, fed to stocked fish to apparent satiety two times a day (0830 and 1600). Overfeeding and feed wastes were carefully monitored and prevented, and the 100 % satiety feed biomass was adjusted weekly according to fish growth rate. Plankton abundance and growth in tanks of the control treatment was controlled by continuous water exchange by off-putting light incident as needed by partially covering the tanks with black, agricultural plastic sheeting. In the tanks with natural food the growth of plankton was fostered by initial inoculation with "green water" from a fish culture pond and continuous fertilization with dicalcium phosphate (1.6 mg L^{-1}). Treatments were assigned to tanks randomly and interspersion was confirmed. Dissolved oxygen, temperature, pH and turbidity was monitored twice a week (0800 and 1600) using a multiparameter Horiba U-50 probe, and Secchi disk visibility measured at the same time.

Feed and feed processing

A practical diet formulated to maximize $\delta^{13}C$ signal was formulated with corn meal, corn gluten and corn oil meal (C4 photosynthetic pathway) as major dietary feedstuffs (Table I). Dietary protein to digestible energy ratio (2.2 g CP MJ^{-1} DE) and amino acid contents followed recommendations of Bicudo et al. (2009, 2010). Feedstuffs were homogenized through a 1.0-mm sieve, mixed, moistened and extrusion-cooked (2.0 mm) in an experimental extruder (model PQ-30, Imbramaq, Ribeirão Preto, SP, Brazil). Processed diets were dried in a forced ventilation oven (50 °C; 24 h) and dried pellets were hermetically packed in plastic bags and stored at 4°C until use.

Table I. Composition of the "C4 pathway" experimental diet.

Feedstuff	Inclusion
	%
Corn grain	53.44
Corn gluten meal (61% CP)	36.80
Corn oil	1.00
Poultry by-product meal	3.17
L-lysine–HCL	1.38
$CaCO_3$	1.55
Cellulose	0.80
Calcium phosphate	0.84
BHT[a]	0.02
Mineral-Vitamin mix[b]	1.00
Nutrient content by analysis	
Dry matter	93.76
Crude Protein	31.93
Ether extract	5.43
Crude fiber	1.32
Ash	4.09
Gross energy (MJ kg^{-1})	19.12
Isotopic signal	unit
	‰
$\delta^{13}C$	-13.32
$\delta^{15}N$	3.15

[a] **Butyl-hydroxy-toluene.**
[b]**Premix Nutrifish Guabi (Campinas, SP, Brazil), enrichment per kg: Fe 1500 mg; Cu 1500 mg; Zn 12500 mg; I 375 mg; Mn 12500 mg; Se 87,5 mg; Co 125 mg; Vitamin A 2.500.000 IU; vit. D3 600.000 IU; vit. E 37.500 IU; vit. K 3750 mg; vit. C 50.000 mg; vit. B1 4.000 mg; vit. B2 4.000 mg; vit. B6 4.000 mg; vit. B12 4.000 mg; ác. Calcium Pantothenate 12.000 mg; Biotin 15 mg; Fólic acid 1.250 mg; Niacin 22.500 mg.**

Fish and plankton sampling

Juvenile pacu acquired were stocked in a fertilized tank to lower the isotopic signature ($\delta^{13}C$) in the tissues (initial $\delta^{13}C$ in the muscle = -18.29). At the beginning of the experiment all of fish o each tank were weighted and a sample of five individuals of the initial population were dissected and a composite pool of muscle sample were formed for isotope analysis, posteriorly at 23, 44, 65 and 99 days of the experiment, fish were weighted (at least 80% of the population of each experimental tank) and samples of muscle tissue were taken (one fish per tank). Muscle tissue was sampled by dissection of fish previously euthanized by anesthetic overdose (benzocaine; 500 mg L^{-1}) and samples were initially stored at -10°C and later lyophilized and analyzed for isotopic composition. Plankton samples were obtained the same day of sampling fish muscle by filtration of 50 L of water in each experimental tank in a plankton net with 20-µm mesh size (Cole 1983). Concentrated algal biomass was transferred to 50-mL Falconer tubes and kept at -10°C until lyophilization and analysis for isotopic composition. A pool of the three samples of each experimental tank for every sample time was analyzed for energy content by calorimetric pump (IKA Model C5003) and crude protein content (N*6.25) by the Kjeldahl method (Kjeldahl 1883).

Isotopic analysis

Isotopic analysis was performed at the Laboratory of Isotopic Ecology, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil – CENA-USP. Combustion of the samples was performed under a continuous flow of helium in an elemental analyzer (Carlo Erba, CHN – 1110), coupled with a Thermo Finnigan Delta Plus mass spectrometer; CO_2 and N_2 resulting from the combustion of samples were analyzed. The isotopic ratios for carbon and nitrogen are expressed as derivation in parts per thousand from the PDB limestone and from atmospheric air nitrogen international standards, respectively, by:

$$\delta X(‰) = \left(\frac{Rsample}{Rstandard} - 1\right)x1000$$

were: Rsample and Rstandard are the ratios ^{13}C:^{12}C or ^{15}N:^{14}N of the sample and the standard, respectively.

Data analysis and statistics

Productive performance daily weight gain (DWG) and apparent feed conversion ratio (FCR) were assessed as follows:

$$DWG\,(g) = \frac{W_f - W_i}{Days}$$

$$FCR = \frac{\mathrm{FI}}{W_f - W_i}$$

where: W_f is final body weight (g), W_i is initial body weight (g), and FI is feed intake (g). Between days 65 and 99 of the experimental period most fishes of the 0% treatment were lost and mortality was not evidenced in the ordinary management, therefore the productive performance was evaluated in two ways: initially the cumulative performance until day 65 including 0% treatment and posteriorly the cumulative performance until day 99 (end of experiment) excluding 0% treatment. The growth trajectories were modeled using appropriate linear or non-linear models according to the data trend shape of each treatment. Treatment effects were accessed by ANOVA followed by the Tukey test for mean separation. Statistical analysis was performed using the SAS software. ANOVA model assumptions (homoscedasticity and normal distribution of residuals) were assessed by Levene and Shapiro-Wilk test. Regression models' assumptions were also evaluated: homoscedasticity by inspection of residual plots and normality of residuals by Shapiro-Willk test. When influential data was detected (Cook distance) robust regression techniques were performed. The significance level for all tests was α = 0.05.

Variations in carbon stable isotope ratios were also modeled as a function of relative growth (Fry & Arnold 1982).

$$\delta_{WR} = \delta_f + (\delta_i + \delta_f)\,W_R^c$$

where δ_{WR} is the isotopic value at the relative body weight; δ_i and δ_f are initial and final carbon stable isotope ratios respectively; δ_i was fixed ($\delta_i{}^{13}C$ = -18.29) from the mean value of five samples from initial population; δ_f was also fixed ($\delta_f{}^{13}C$ = -14.07), given that preliminary analysis of the 75, 100 and control treatment showed this asymptotic isotopic value; W_R is the relative increase in body mass (weight) calculated as the final wet weight divided by the initial wet weight; *c* is the turnover rate constant as derived by iteration (minimizing the sum of the squared differences between measured and calculated data) using solver tool of Microsoft Excel 2010 ® (Maruyama et al. 2001, Jardine et al. 2004). In the model, if c = -1 growth is solely responsible for carbon turnover, whereas if c < -1, metabolic contribution to carbon turnover is in effect; the higher the negative values, the greater the contributions by metabolism. The amount of relative growth required to achieve a percent turnover of $\delta^{13}C$ was calculated as:

$$G_{\alpha/100} = e^{\ln(1-\frac{\alpha}{100})/c}$$

The growth-based half-life ($G_{0.5}$) is solved for α = 50% and represents the amount of growth needed for a 50% conversion between the initial and final isotopic values, and the half-life estimated with the growth-based model thus expressed as an *x*-fold mass increase (Buchheister & Latour 2010). The fractions of new tissue derived from growth (*D*g) and from metabolism (*D*m) were calculated at the midpoint between the old and new isotopic values.

$$D_g = 2(G_{0.5} - 1)/G_{0.5}$$

$$D_m = (2 - G_{0.5})/G_{0.5}$$

The number of days required to obtain the 50% conversion between the initial and final isotopic values were calculated from the fitted growth model for each treatment (Buchheister & Latour 2010). The percent of turnover reached by each treatment was obtained from the average W_R reached of each treatment. The delete-one Jackknife procedure was used to estimate uncertainties (standard error) of the turnover parameters (Harris 1998).

Proportions of carbon contribution from feed each source (processed feed and natural food) were estimated by Bayesian mixing models using the SIAR package (Parnell et al. 2010) performed in R v3.3.2, 2016 (R Development Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing, Vienna, Austria). Trophic discrimination factor (TDF) for feed were estimated from the δ_f (δ_f^{13}C = -14.07) and δ^{13}C of the feed (δ_f^{13}C‰ = -13.32), so TDF$_{feed}$ = -0.75 δ_f^{13}C. The TDF$_{food}$ was estimated from the mean of δ^{13}C in the fish muscle for 0% feeding rate treatment (δ^{13}C = -18.00± 0.25; μ±SD) and the δ^{13}C for the collected food for the same treatment (δ^{13}C = -20.52 ±3.07), so TDF$_{food}$ = 2.52±0.97 δ_f^{13}C. Standard deviation for TDF$_{food}$ was calculated according standard deviation calculation for the difference between two samples means (Steel & Torrie 1980).

The nitrogen trophic discrimination factor for feed was estimated from the mean of δ_f^{15}N for muscle of 100 fed rate without natural food treatment (δ_f^{15}N = 6.73 ±0.23) and δ^{15}N of the feed (δ_f^{15}N‰ = 3.15), so TDF$_{feed}$ = 3.59 δ_f^{15}N. The TDF$_{food}$ was estimated from the mean of δ^{15}N in the fish muscle for 0% feed rate treatment (δ^{15}N = 7.07 ± 0.42; mean ± s.d) and the δ^{15}N for the collected food for the same treatment (δ^{15}N = 0.88 ± 0.63), so TDF$_{food}$ = 6.19 ± 0.24 δ_f^{15}N.

Ethical note

The study was approved by the Ethics Committee of Animal Use of "Escola Superior de Agricultura Luiz de Queiroz" (protocols CEUA # 2014-01 and 2014-13) and was developed considering the national guidelines for care and use of animals.

RESULTS

Water quality and nutritional value of natural food

The water quality parameters registered on experimental tanks were appropriated for the specie development. The tanks of the 100 % fed rate without access to natural food showed lower temperature, dissolved oxygen and pH than tanks of the other treatments. As expected, the tanks of this treatment were characterized by null growth of plankton and low turbidity, as evidenced by transparent water (Table II).

At the first plankton sampling (day 23), the crude protein contents of "natural food" ranged

Table II. Water quality parameters on the experimental system. Values are means ± standard deviation.

Treatment	Temperature (°C)	Dissolved oxygen (mg L-1)	pH	Turbidity (NTU)	Secchi disk visibility (cm)
0 %	27.91 ± 2.62	10.47 ± 2.80	10.44 ± 2.21	149.79 ± 46.42	27.17 ± 3.92
25 %	27.91 ± 2.62	10.01 ± 3.10	9.99 ± 0.40	128.19 ± 36.55	31.89 ± 6.32
50 %	27.90 ± 2.54	9.48 ± 3.19	10.00 ± 0.35	156.61 ± 47.00	25.22 ± 4.13
75 %	27.99 ± 2.50	9.89 ± 3.45	9.93 ± 0.37	166.99 ± 64.44	25.42 ± 4.32
100 %	27.67 ± 2.61	9.81 ± 3.57	9.93 ± 0.55	155.75 ± 49.73	27.00 ± 5.74
Control*	26.51 ± 1.34	4.83 ± 1.06	8.99 ± 0.22	13.78 ± 21.94	-**

***Fish of control treatment was raised in tanks with water exchange without plankton presence. **Transparent water.**

on 22.76 % to 30.85 %, and tended towards decreasing on the subsequent samplings. However, the overall mean was similar for all treatments, ranging on 18.97 (75 % treatment) to 21.09 (25% treatment). Similarly, means of gross energy contents were similar among treatments, but tended to increase along time (Table III).

Productive performance

The productive performance of the fish without access to natural food and fed at apparent satiety did not differ from that of fish with access to natural food and fed at apparent satiety ($p<0.05$) (Table IV). Between days 65 and 99 of the experimental period most fishes of the 0% treatment were lost and mortality was not evidenced in the ordinary management, thus at the final biometry (day 99) only one fish per tank was found. Therefore, productive performance for this treatment could not be recorded. Also, between days 65 and 99 feed intake decreased noticeably in all treatments, therefore productive performance was slightly worse for that period. During the first 65 days, feed conversion ratio of fish of treatment 25% satiety was better than all other treatments ($p<0.05$).

Data on growth rate of fish with access to natural food until 65 days enabled estimating a linear regression curve of growth as function of apparent feed intake as expressed per unit of metabolic weight with an allometric exponent = 0.8. The estimations for feed intake of the fish without access to natural food neared same allometric exponent value, eliciting to infer that extruded feed was the principal nutrient source to support the growth of fish (Figure 1).

The growth curves for 100% of apparent satiety as fed rate treatments, both with and without natural food intake, was similar and showed a sigmoidal shape; a logistic model was thus fitted to recorded data. Growth rate for 25, 50 and 75% feeding rate treatments fitted exponential model curves. Data on growth rate of fish of 0% feeding rate treatments fitted a linear model curve (Figure 2).

Carbon turnover

It was not possible to fit a growth-based turnover model curve for treatments 0 and 25 % fed rate treatments, so a linear regression in time was preferred for modeling muscle $\delta_f^{13}C$ for these treatments. For all other treatments, the absolute value of the turnover rates increased as function of fed rate. Therefore, the proportion of metabolic contribution to carbon turnover increased and the growth-based half-life (x-fold increase) decreased as function of fed rate. The time to reach the half-life of carbon turnover decrease as function of fed rate, and the percent of reached carbon turnover increased as function

Table III. Crude protein and gross energy content of "natural food" collected on the experimental tanks (dry mater basis) of pacu fed at different fed rates (% of apparent satiety).

Day	Crude protein (%)						Gross energy (Mj kg^{-1})				
	0%*	25 %*	50 %*	75 %*	100 %*		0 %*	25 %*	50 %*	75 %*	100 %*
23	27.49	30.85	28.27	22.76	29.83		13.16	13.44	13.01	12.99	10.43
44	15.30	14.13	12.99	11.57	12.34		10.38	11.48	11.73	11.19	11.12
65	13.98	19.01	17.11	25.17	20.82		11.16	13.08	13.01	13.58	13.88
99	19.85	20.38	20.53	16.39	18.00		13.63	14.93	14.81	15.27	15.18
Mean	19.16	21.09	19.72	18.97	20.25		12.08	13.23	13.14	13.26	12.65

***Feeding rate (% of apparent satiety).**

of fed rate. The turnover parameters for both treatments with 100% of apparent satiety as fed rate was similar (Table V). Fishes of the 100% fed rates treatment reached circa 95% of the carbon turnover and the 75% treatment reached only 82.85% of the carbon turnover. The isotopic value of the natural food samples collected for the 50, 75 and 100% fed rates did not show significant variation over time; therefore, the mean values were considered (Figure 3).

The $\delta_f^{13}C$ values on the muscle of fish fed at 25% of apparent satiety increased with time; for fish at the 0% fed rate treatment (only natural food) the $\delta_f^{13}C$ also increased in time, but at inferior rate that the 25% treatment. The natural food for the 25% treatment decreased as function of the experiment time (p=0.16); for the 0% treatment the decrease of $\delta_f^{13}C$ values of the natural food over time was significant and it followed a quadratic trajectory, with a more pronounced decrease from day 60 forth (Fig. 4).

Changes in nitrogen isotopic signature over time

The $\delta^{15}N$ values in the muscle of fish fed at 100 % of satiety, both with and without natural food, showed low variability in time, mean values of 6.79 ‰ and 6.74 ‰, respectively. Similarly, the 75 and 50% feeding rates showed low variability over time, and the values were 6.86 ‰ and 6.98 ‰ respectively. For the 25 and 0% feeding rates the $\delta^{15}N$ values in the muscles increased over time (Fig. 5). The $\delta^{15}N$ values in the "natural food" were affected by sampling day, the treatment and their interaction (Apendix A).

Carbon contribution of feed and natural food to fish growth

For the 100% fed rate the Bayesian mixing model showed high density around the proportion 1 for the extruded feed, thus confirming that the carbon source the growth of juvenile pacu was the feed and the contribution of natural food

Table IV. Productive performance of juvenile pacu under different feeding rates (% of apparent satiety) in a "green water system". Values are means ± SE.

Period (days)	Feeding rate (%)	IBW	FBW	AFI	GR	AFC
0 - 65	0	53.62 ±0.39	48.98 ± 3.25 e	0	-0.07 ± 0.02 e	-
	25	52.95 ±0.08	59.24 ± 3.99 d	0.15 ± 0.02 d	0.10 ± 0.02 d	1.88 ± 0.13 a
	50	53.51 ±0.36	75.07 ± 3.25 c	0.33 ± 0.02 c	0.34 ± 0.02 c	1.00 ± 0.11 b
	75	53.13 ±0.73	79.61 ± 3.25 bc	0.45 ± 0.02 b	0.41 ± 0.06 bc	1.13 ± 0.11 b
	100	53.63 ±0.50	93.10 ± 3.25 ab	0.62 ± 0.02 a	0.61 ± 0.06 ab	1.05 ± 0.11 b
	Control*	52.89 ±0.13	100.75 ± 3.25 a	0.67 ± 0.02 a	0.74 ± 0.06 a	0.91 ± 0.11 b
0 - 99	25	52.95 ±0.08	64.34 ± 4.68 c	0.19 ± 0.05 d	0.12 ± 0.05 c	1.66 ± 0.10 a
	50	53.51 ±0.36	82.28 ± 5.73 bc	0.41 ± 0.04 c	0.29 ± 0.04 bc	1.42 ± 0.08 ab
	75	53.13 ±0.73	87.29 ± 4.68 ab	0.48 ± 0.04 bc	0.35 ± 0.04 ab	1.44 ± 0.08 ab
	100	53.63 ±0.50	96.54 ± 4.68 ab	0.66 ± 0.04 ab	0.43 ± 0.04 a	1.52 ± 0.08 ab
	Control*	52.89 ±0.13	105.65 ± 4.68 a	0.68 ± 0.04 a	0.53 ± 0.04 a	1.29 ± 0.08 b

***Apparent satiety without access to natural food. IBW: Initial body weight (g), FBW: Final body weight (g); Apparent feed intake (g fish^{-1} day^{-1}); GR: Growth rate (g fish^{-1} day^{-1}); AFC: Apparent feed conversion.**
Means followed by different letters represent statistically significant differences (Tukeys test; p≤0.05).

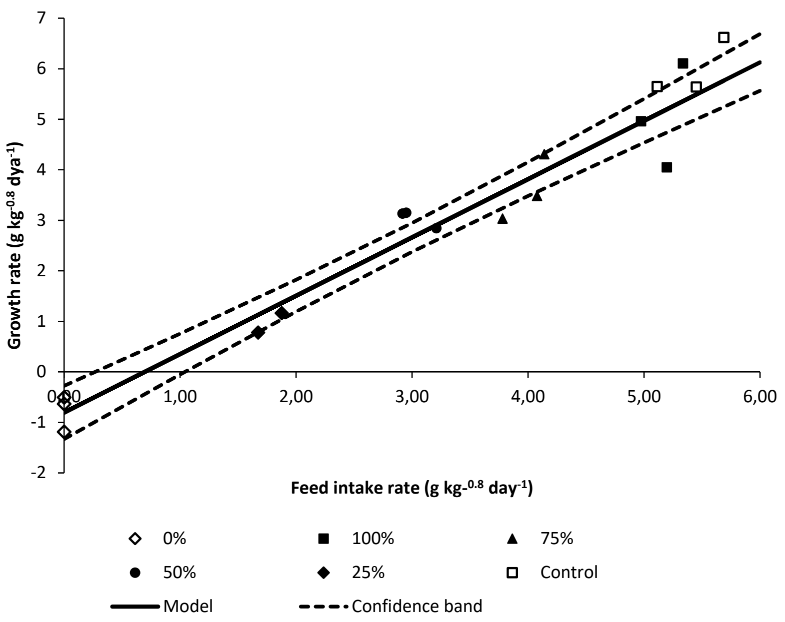

Figure 1. Linear regression of growth rate as a function of feed intake rate for pacu in a system with access to natural food (until 65 days). Data from without natural food treatment (Control) was not used to fit the model: $y = -0.804+1.1548x$. ($R^2 = 0.95$; $p < 0.05$). Dashed lines are estimates of the confidence band (95%). Data correspond to feeding level as percentage of apparent satiety.

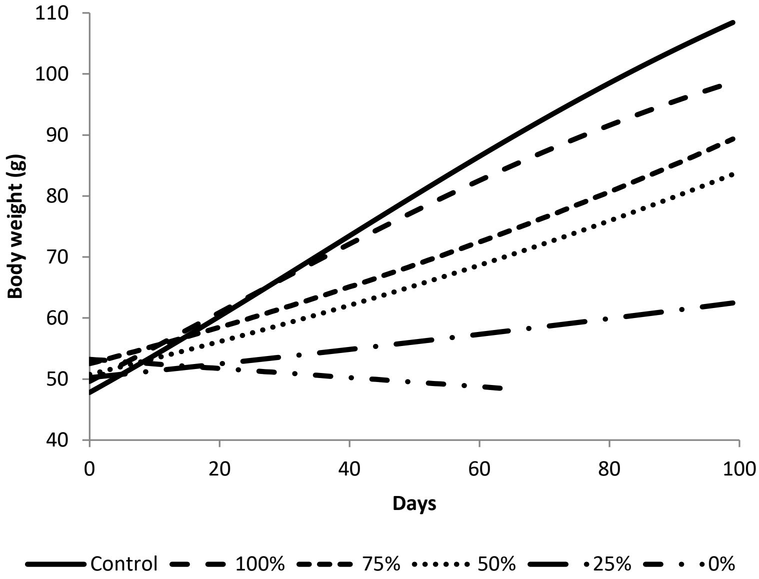

Figure 2. Effect of feed rates (% apparent satiety) on the growth curves of juvenile pacu on a system with access to natural food. Fitted models:
Control (100% Without natural food): $y = 142/(1+1.970*\exp^{(-0.0187*x)})$ ($R^2 = 0.86$; $p<0.05$)
100% satiety feeding rate: $y = 120/(1+1.423*\exp^{(-0.0193*x)})$ ($R^2 = 0.86$; $p<0.05$)
75% satiety feeding rate: $y = 52.52*\exp^{(0.0054*x)}$ ($R^2 = 0.86$; $p<0.05$)
50% satiety feeding rate: $y = 50.76*\exp^{(0.0050*x)}$ ($R^2 = 0.87$; $p<0.05$)
25% satiety feeding rate: $y = 50.24*\exp^{(0.0022*x)}$ ($R^2 = 0.66$;$p<0.05$)
0% satiety feeding rate: $y = 53.23-0.075*x$ ($R^2 = 0.52$; $p<0.05$).

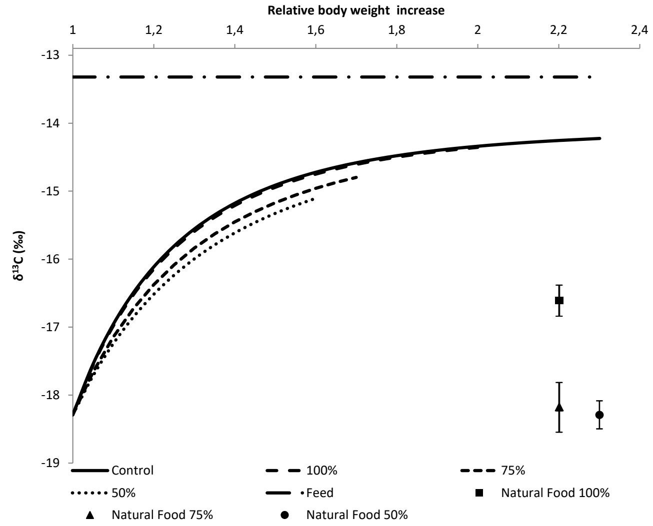

Figure 3. Effect of different feed rates (% of apparent satiety) on changes on $\delta_f{}^{13}C$ estimated by a turnover growth-based model for muscle of juvenile pacu. Control treatment was fed with experimental feed at apparent satiety. Values for the experimental feed ($\delta_f{}^{13}C$= -13.32) and the mean for the natural food of each treatment are also showed (100% $\delta_f{}^{13}C$= -16.61; 75% $\delta_f{}^{13}C$= -18.18; 50% $\delta_f{}^{13}C$= -18.29); error bars are standard errors.

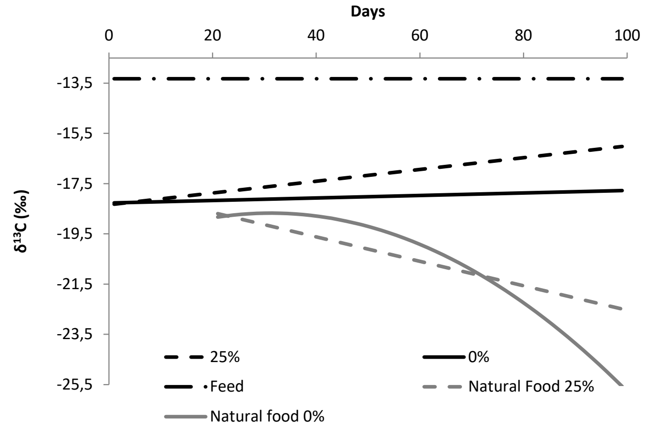

Figure 4. Effect of different feed rates (% of apparent satiety) on changes on $\delta^{13}C$ (‰) of the muscle of juvenile pacu as a function of extension of the feeding period.
25% satiety fed rate: $y = -18.34+0.0234*x$ (R^2 =0.79; $p<0.05$);
0% satiety fed rate: $y = -18.27+0.0050*x$ (R^2 =0.49: $p<0.05$)
Experimental feed: $y = -13.32$
Natural food at 25% fed rate: $y = -17.678-0.048*x$ (R^2 = 0.23; p = 0.16)
Natural food at 0% fed rate: $y= -20.14+0.0936*x-0.0015*x^2$ (R^2 = 0.81; $p<0.05$).

Table V. Carbon turnover estimates (± SE; delete-one jackknife procedure) of juvenile pacu under different feeding rates (% of apparent satiety) in a "green waters system".

Treatment	c	D_m	D_g	$G_{0.5}$	$G_{0.95}$	$G_{0.5}$ (Days)	Reached turnover (%)
50%	-2.99 ±0.47	0.59 ± 0.06	0.41 ± 0.06	1.26 ± 0.05	2.73 ± 0.14	46.40 ± 7.29	77.41 ± 5.51
75%	-3.31 ± 0.71	0.62 ± 0.07	0.38 ± 0.07	1.23 ± 0.06	2.47 ± 0.20	32.70 ± 6.60	82.85 ± 6.51
100%	-3.89 ± 1.05	0.67 ± 0.08	0.33 ± 0.08	1.20 ± 0.06	2.16 ± 0.39	16.90 ± 4.81	93.09 ± 4.73
Control*	-3.98 ± 0.71	0.68 ± 0.05	0.32 ± 0.05	1.19 ± 0.04	2.12 ± 0.27	14.70 ± 2.32	96.15 ± 2.08

*Apparent satiety without access to natural food. *c*, turnover constant; D_m, proportion of metabolic contribution to carbon turnover; D_g, proportion of growth contribution to carbon turnover; $G_{0.5}$, growth-based half-life (*x*-fold increase); $G_{0.95}$, amount of relative growth needed to reach 95% carbon turnover (*x*-fold increase); Time $G_{0.5}$ (Days), Days needs to reach $G_{0.5}$.

was negligible (Fig. 6). For all other treatments, as turnover was no reached, no model could be fitted, and therefore the isotopic value was not in equilibrium with the diet.

DISCUSSION

The analysis of productive performance and the isotopic carbon turnover showed that nutritional contribution of natural food to pacu growth was nearly negligible even at low feed rates; actually,

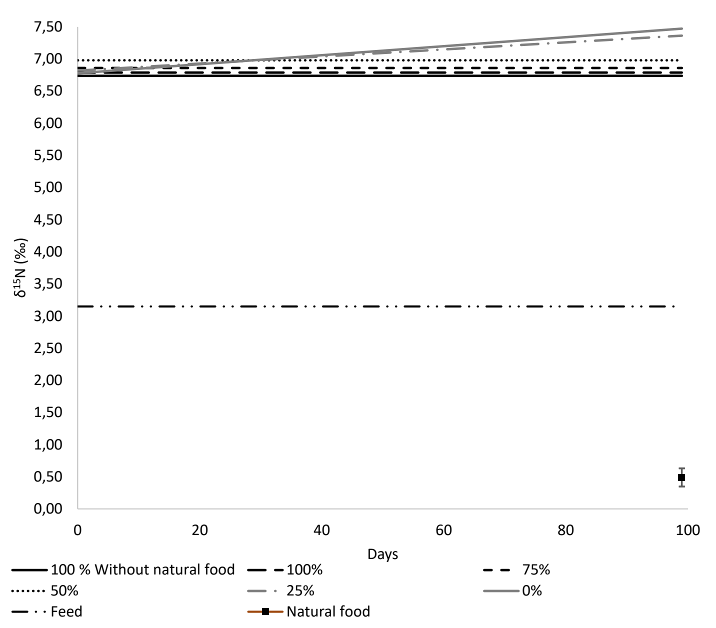

Figure 5. Effect of different feed rates (% of apparent satiety) on changes in $\delta^{15}N$ (‰) of the muscle of juvenile pacu as a function of extension of the feeding period. Error bars are standard errors. 100% satiety fed rate without natural food $y = 6.74$ 100% satiety fed rate: $y = 6.79$ 75% satiety fed rate: $y = 6.86$ 50% satiety fed rate: $y = 6.98$ 25% satiety fed rate: $y = 6.82+0.0055x$ ($R^2 = 0.43$; $p<0.05$) 0% satiety fed rate: $y = 6.78+0.0070x$ ($R^2 = 0.40$; $p<0.05$).

fish with no access to extruded feed showed loss of body weight. The fact that fish feeding only on natural food showed weight loss and increases on $\delta^{13}C$ and $\delta^{15}N$ on the muscle over time, even with decreases on $\delta^{13}C$ values on the collected food, it could be associated with energy mobilization and negative nitrogen balance, which is coherent with all bioenergetic models described for fish, which use protein as primary energy source (Millward 1989, Hobson et al. 1993, Hertz et al. 2015).

The dissection of some fish at the end of experimental period revealed that fish actual intake of natural food, however, the net energy and nutrient contents of natural food alone was not sufficient to support the maintenance and growth of fish. Pacu has been described as an omnivore fish (Urbinati & Gonçalves 2005), and in the larval stage natural food (plankton) is an important nutrient source to elicit satisfactory growth, being widely exploited in the hatchery of the species on fertilized ponds (Sipaúba-Tavares & Braga 1999, Jomori et al. 2003, Valladão et al. 2016). However, recorded data show that juvenile pacu already shows low capacity to utilize plankton. It is well documented that pacu undergoes an ontogenic diet shift already at juvenile stage, exploring macroscopic food sources such as fruits, seeds and flowers from the riparian dossel fallen in the surface of water bodies, as well as small freshwater crabs and other invertebrates (Wantzen et al. 2002). Fish of the *Piaractus* genera and other round Characins are also proven to play important role as seed disperser on flooded forests and plains (Galetti et al. 2008, Horn et al. 2011).

Values of crude protein and gross energy contents of natural food were lower that reported, for example, for gross energy – 19 to 27 MJ kg^{-1} – and crude protein – 18 to 46 % – for algae biomass harvested in culture reactors (Tibetts et al. 2015), and probably associated to low fat contents of natural food, given that lipid contents of freshwater algae vary to a great extent and those algae used for mass production in reactors are selected for higher lipid contents (Chisti 2007, Harun et al. 2010). Low crude protein and lipid contents could also be associated with high carbohydrate (cell wall) contents, of naturally lower digestibility to most fish (Lovell 1998). Therefore, low nutritional value and low

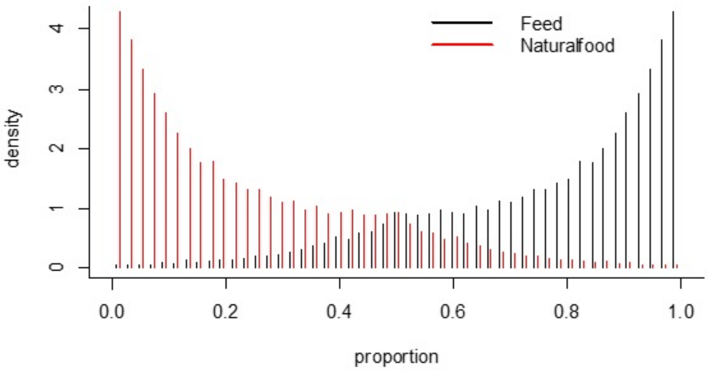

Figure 6. Proportional contributions of natural food and extruded feed to pacu growth estimated by isotopic analysis using the Bayesian mixing model.

digestibility of natural food could partially explain the low net contribution of natural food to fish growth on the current assay. On the other hand, considering that the Secchi disk visibility did not differ among treatments and ranged on 25.22 to 31.89 cm, which is close to recommended values for fish production in extensive and semi-intensive systems, that is, 25 to 30 cm (Carballo et al. 2008), available quantity of natural food seemed adequate in all treatments.

Although data from studies using stable isotopes to evaluate the contribution of feed and natural food for fish growth demonstrated significative contribution of plankton to the growth of juvenile fish. For instance, Mischke et al. (2019) demonstrated that the contribution of plankton as food in nursery ponds of channel and hybrid catfish ranged on 39% to 63 % hybrid catfish, ponds also supplied with feed ($\delta^{13}C$ = -23.3) at apparent satiety, fish growing from post-larval stage to 7.0 g in 53 days. The average contribution of phytoplankton and algae as food to juvenile Nile tilapia raised in ponds was 12 % and 25 %, respectively, for a weekly-based feed allowance of 23 % phytoplankton and 10 % algae (Narimbi et al. 2018). In a net cage production trial carried out in a reservoir in a semi-arid condition, northeast Brazil, the contribution of seston to the growth of Nile tilapia was 51.2 % (Ferreira et al. 2020). However, it shall be pointed out that the great contribution of natural food to tilapia growth is associated to the fact that the species predominant filter-feeding habit precedes its omnivorous behavior in farming conditions (Asano et al. 2010, Lu et al. 2004).

The relative growth to reach the 50% carbon turnover (half-life) decreased as function of feeding rate, from 1.26 for treatment 50% apparent satiety to 1.19 for apparent satiety. the contribution of growth to carbon turnover decreases as function of fed rate as well. Working with grass carp *Ctenopharyngodon idella*, Xia et al. (2013) recorded a turnover constant for carbon of -2.44, that is, a slower rate than in the current study, even considering the treatment 50 % apparent satiety. Consequently, the relative growth for reached the 50% of carbon turnover ($G_{0.5}$ = 1.35) was higher than the current study. Similarly, the amount of relative growth needed to reach 95% carbon turnover was higher in the reference study ($G_{0.95}$ = 3.68) than the 2.73 recorded in this study for the 50 % apparent satiety treatment. Therefore, the current results reiterate the sensibility of D_m, D_g, $G_{0.5}$, $G_{0.95}$, to changes of turnover constant of the growth turnover model.

In regard to carbon the TDF_{feed} =-0.75 and TDF_{food} =2.52 ± 0.97. Considering that δ^{13}Cfood = -20.52 ±3.07 and δ^{13}Cfeed = -13.32, a negative association between δ^{13}Cdiet and TDF was detected. Previously, Caut et al. (2009) working on a compilation of data from several studies, reported the same relationship and the equation TDF-diet = -0.2488*δ^{13}C-diet -3.477 suggested performing the estimation of TDF-diet from muscle samples instead would elicit using a single discrimination factor for carbon. Using such equation as suggested, the predictions were TDF_{feed} =-0.17 and TDF_{food} =1.6. Therefore, even though the model would underestimate TDF_{food} and overestimate TDF_{feed}, it could be considered a useful alternative when TDF cannot be measured along a given experimental period.

According with the performance and isotopic analysis, extruded feed was the primary nutrient source for the growth of pacu in tanks, either with or without access to natural food. For fishes fed above 50 % of apparent satiety, the proportion of metabolic contribution to carbon turnover increased and the growth-based half-life (x-fold increase) decreased as function of fed rate. Similarly, the time to reach the half-life of carbon turnover decrease as function of fed rate and the percent of reached carbon turnover increased as a function of the fed rate. Fishes fed only with natural food showed weight loss and increase in $\delta^{13}C$ and $\delta^{15}N$ on the muscle over time, even with decrease in $\delta^{13}C$ values on the collected food, suggesting energy mobilization from muscle tissue and negative nitrogen balance. Likewise, the use of mixing models allowed demonstrating that in the simulated farming condition the contribution of natural food (plankton) to the growth of the fish was low. The parameterization of the growth-based carbon turnover model showed the sensitivity of the carbon turnover rate to the growth rate associated with variations in the extruded feed allowance. The current study contributes to the knowledge of the relationship between feeding rates and carbon turnover rates in the pacu muscle, and to the knowledge of trophic discrimination factors for nitrogen and carbon for natural food and feed in the species. These contributions are valuable for future evaluations about the incorporation of nutrients and the contribution to pacu growth of alternative feedstuffs.

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Appendix A. Carbon-to-nitrogen ratio and isotope values of natural food at different fed rates (% of apparent satiety) of fishes and sampling times.

Day	Treatment	C (%)	N (%)	C/N	δ^{13}C (‰)	δ^{15}N (‰)
23	0	25.37 a	4.08 a	6.28 a	-18.83 ab	0.39 a
	25	29.54 a	5.06 a	6.26 a	-18.74 a	0.61 a
	50	25.69 a	4.84 a	5.48 a	-18.56 a	0.77a
	75	31.25 a	4.80 a	6.55 a	-17.46 a	0.11 a
	100	30.55 a	4.29 a	7.08 a	-17.13 a	-0.28 a
44	0	19.03 b	1.77 a	10.74 a	-18.81 a	1.27 a
	25	26.22 a	2.20 a	11.97 a	-19.92 a	0.68 a
	50	23.95 a	1.89 a	12.89 a	-18.23 a	0.92 a
	75	29.41 a	2.19 a	14.09 a	-18.85 a	0.77 a
	100	28.11 a	2.46 a	11.48 a	-16.27 a	0.89 a
65	0	20.86 b	3.36 a	6.80 b	-20.46 a	1.53 a
	25	31.51 a	2.13 a	14.47 a	-21.28 a	-1.04 b
	50	26.95 a	2.81 a	9.75 a	-17.73 a	0.73 ab
	75	30.48 a	3.47 a	9.67 a	-18.77 a	0.87 ab
	100	27.76 a	3.06 a	9.23 a	-16.86 a	0.99 ab
99	0	19.20 b	1.60 b	11.42 a	-25.48 b	0.38 a
	25	32.55 a	2.44 a	13.50 a	-23.00 ab	-0.48 a
	50	29.25 a	3.27 a	8.96 a	-18.63 ab	-0.34 a
	75	32.14 a	2.97 a	10.94 a	-17.64 a	0.19 a
	100	32.39 a	2.91 a	11.37 a	-16.19 a	0.88 a
	Pool means					
	0	20.97 B	2.72 A	8.81 B	-20.89 A	0.90 A
	25	29.94 A	2.98 A	11.57 A	-20.73 A	-0.09 B
	50	26.46 AB	3.21 A	9.27 B	-18.29 A	0.52 A
	75	30.82 A	3.36 A	10.31 AB	-18.18 A	0.48 AB
	100	29.70 A	3.18 A	9.79 AB	-16.61 A	0.62 A
23		28.48 A	4.62 A	6.33 C	-18.14 A	0.32 AB
44		25.34 A	2.10 B	12.24 A	-18.42 A	0.91 A
65		27.50 AB	2.98 B	10.00 B	-19.02 AB	0.61 AB
99		28.99 A	2.65 B	11.23 AB	-20.19 B	0.12 B
	Treatment	0.011	0.434	0.031	0.046	0.014
P-value	Day	0.001	<0.0001	<0.0001	0.003	0.048
	Interaction	0.458	0.625	0.089	0.004	0.011

Means followed by the same lowercase letter do not differ between treatments for each day by the Tukey-Kramer test ($p>0.05$). Pool means for each main effect (treatment or day) followed by the same capital letter do not differ by the Tukey-Kramer test ($p>0.05$)

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