RESEARCH ARTICLE

Food restriction in Nile tilapia juveniles (*Oreochromis niloticus*)

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Abstract

Aim of study: To evaluate if dietary restriction influence muscle and intestinal morphology as well as the production performance of juvenile Nile tilapia (*Oreochromis niloticus*).

Area of study: State University of Western Paraná (Universidade Estadual do Oeste do Paraná – Unioeste), Toledo Campus, Brazil.

Material and methods: The experimental design was completely randomized with four treatments and five replicates. The treatments were: 7:0 (fed daily; control); 6:1 (fed 6 consecutive days followed by 1 day of fasting); 5:2 (fed 5 consecutive days followed by 2 days of fasting); and 1:1 (fed 1 day followed by 1 day of fasting). A commercial ration with 33.70% crude protein and 4.60% lipid was used, and fish were fed four times daily until apparent satiety for 60 days.

Main results: The following parameters were found to have differences among treatments ($p<0.05$): final weight, weight gain, final length, hepatosomatic index, visceral fat, intestinal quotient, protein efficiency rate, protein retention, ash, and frequency of muscle fiber diameters within the classes of <20 μm and between 20 and 50 μm. The intestinal villi height and hepatic glycogen content did not differ ($p>0.05$) among treatments. The cost of food and partial net revenue were higher in the 7:0 treatment compared to the experimental treatments.

Research highlights: Dietary restriction for Nile tilapia in the juvenile phase negatively influences productive performance, centesimal composition, and muscle growth, which demonstrates that this practice is economically unfeasible for commercial production.

Additional key words: dietary regime; feed cost evaluation; skeletal muscle fiber; zootechnical performance

Abbreviations used: CF (condition factor); FC (feed conversion); FEC (cost of feeding); FING (cost fingerlings); FL (final length); FW (final weight); GR (gross revenue); IQ (intestinal quotient); L (labor); PER (protein efficiency rate); PNR (partial net revenue); PR (protein retention); R$ (real – Brazilian currency); SUR (survival); VF (visceral fat); WG (weight gain)

Introduction

The Brazilian territory is endowed with a wide coastal area and abundant aquatic natural resources. Among more than forty freshwater species with potential for aquaculture (Godinho, 2007), tilapia is the most produced in the country (PeixeBR, 2019) This species stands out because of its rapid growth, robustness, good adaptation to the climate and environment, resistance to diseases, and easy adaptation to commercial rations, which enables the elaboration of low cost and high nutritive value diets (Pereira *et al.*, 2017; El-Sayed, 2019).

As with other containment systems, the costs of balanced feed accounts for a significant portion of total production costs (Koch *et al.*, 2014). Food restriction strategies may be an alternative to reduce food costs in fish production; however, in detriment to deprivation, physiological changes in fish vary and may be influenced by factors such as climatic seasonality during cultivation, water quality parameters, the species' stage of development, and nutritional "status". These possible physiological changes may cause distinct metabolic effects associated with the fasting period biologically regulated by the species in question (Bastrop *et al.*, 1991).

In fish, the physiological variation among species, stage of development at the onset of food deprivation, age of sexual maturity, and the refeeding model exert great importance in compensatory responses, which may occur in different ways. In partial compensation, animals cannot reach the same size of others not submitted to food restriction but may present improved feed conversion. At total compensation, fish that suffer some type of food restriction reach the same size at the same time as those fed continuously. Non-compensation is characterized by not presenting compensatory responses in the refeeding period after the end of the food restriction period (Ali *et al.*, 2003).

Studies on food restriction have been carried out to determine the production strategies without harming the development of animals but improving diet utilization, maintaining good water quality, reducing ration waste and production and manpower costs, and obtaining final high-quality products for consumers.

This study evaluated different feeding strategies in Nile tilapia juveniles (*Oreochromis niloticus*) analyzing parameters of animal performance as well as morphometry of muscle fibers, intestinal villi, and partial budget costs.

Material and methods

Animals and experimental design

The experiment was carried out at the Experimental Greenhouse of the Aquaculture Management Group (GEMAq) at the State University of Western Paraná, Unioeste, Toledo Campus (PR, Brazil) and was approved by the Committee of Ethics in Animal Experimentation of the same Institution under protocol number 41/15. A total of 160 animals with a mean total length and weight of 3.23 ± 0.07 g and 5.70 ± 0.37 cm, respectively, were distributed in 20 plastic mesh hapas. These mesh hapas had 0.15 m³ of culture volume each and were arranged in a concrete tank with constant aeration.

The experimental design was completely randomized in four treatments and five replicates. The treatments consisted of 7:0 - fed daily; 6:1 - fed six consecutive days followed by one day of food restriction; 5:2 - fed five consecutive days followed by two days of food restriction; and 1:1 - fed one day followed by one day of food restriction.

Experimental diet and food management

The experimental diet was composed of in pellets of 2 mm in diameter and 33.70% of crude protein and 4.60% of lipids (Table 1). Fish were fed four times a day to apparent satiety (8 and 11 am, and 2 and 5 pm) for 60 days.

The feed was ground during the first 20 days of the experiment in order to reduce the size of pellets and facilitate ingestion by young fish with reduced mouth size.

Water quality

Water parameters such as pH (7.15 ± 0.11), dissolved oxygen (5.38 ± 1.49 mg L⁻¹), and conductivity (86.83 ±

Table 1. Assurance levels of commercial feed extruded used on experiment

Nutrients[1]	Assurance levels (per kg)
Calcium (min)	15.00 g
Calcium (max)	30.00 g
Ether extract (min)	60.00 g
Phosphorus (min)	9.00 mg
Fibrous matter (max)	80.00 g
Mineral matter (max)	160.00 g
Crude protein (min)	320.00 g
Moisture (max)	125.00 g
Folic acid (min)	0.20 mg
Nicotinic acid (min)	12.00 mg
Pantothenic acid (min)	7.50 mg
Biotin (min)	0.11 mg
Coline (min)	2.80 mg
Copper (min)	5.00 mg
Iron (min)	50.00 mg
Iodine (min)	1.00 mg
Manganese (min)	21.00 mg
Selenium (min)	0.10 mg
Zinc (min)	75.00 mg
Vitamin A (min)	7.00 UI
Vitamin B1 (min)	1.50 mg
Vitamin B12 (min)	0.02 mcg
Vitamin B2 (min)	2.10 mg
Vitamin B6 (min)	2.70 mg
Vitamin D3 (min)	800.00 UI
Vitamin E (min)	40 UI
Vitamin K3 (min)	0.40 mg
Vitamin C	145.00 mg
Analyzed values (%)[2]	
Moisture	15.11
Crude protein	33.70
Lipids	4.60
Mineral matter	10.33

[1]Values provided by the manufacturer. [2]Valued determined at the GEMAQ Quality Control Laboratory at the State University of Western Paraná, Unioeste.

1.32 µS cm⁻¹) were measured weekly through portable digital potentiometers; the water temperature (20.04 ± 2.68 ºC) was measured twice a day, in the morning and afternoon.

Data collection

Prior to termination of the experiment, the fish were fasted for 24 hours to ensure gastrointestinal tracts were empty. They were subsequently anesthetized with benzocaine at 75 mg L^{-1} (Gomes *et al.*, 2001) for the measurements of individual weight (g) and length (cm). Three fish from each experimental unit were euthanized with 250 mg L^{-1} of benzocaine (Gomes *et al.*, 2001) for the removal of visceral fat and liver.

The evaluated productive performance data were presented as: mean final weight (FW, in g); average final length (FL, in cm); weight gain (WG, final body weight – initial body weight); survival (SUR, 100*(final number of fish/ initial number of fish)); feed conversion (FC, consumed diet/weight gain); hepatosomatic index (HI, 100*(liver weight, g/final body weight, g)); visceral fat (VF, 100*(visceral fat weight, g/final body weight, g)); intestinal quotient (IQ, intestine length/final fish length); condition factor (CF, 100*(final body weight, g/final length³, cm)); protein efficiency rate (PER, 100*(weight gain, g/protein consumption, g)) and protein retention (PR, (((final body weight* final carcass crude protein) – (initial body weight*initial carcass crude protein))/protein consumption.

Chemical analysis

The proximate composition analysis was conducted in three whole fish from each experimental unit and included viscera, head, and fin. The samples were pre-dried in a forced ventilation oven (Solab, SL-102, Piracicaba, São Paulo, Brazil) at 55 ºC for 72 h. Moisture was determined in samples that were pre-dried in an owen (Quimis, Q-317D243, Diadema, São Paulo, Brasil) at 105 ºC for 8 h; crude protein was determined by the Kjeldhal method (Tecnal, MA-036, Piracicaba, São Paulo, Brazil) with sample digestion, distillation, and titration (N × 6.25); lipid composition was determined using a Soxhlet extractor and ether as the solvent (Tecnal, TE-044, Piracicaba, São Paulo, Brazil); and the determination of ash content determined after combustion in a muffle furnace (Tecnal, 2000B, Belo Horizonte, Minas Gerais, Brazil) with a temperature of 550 ºC for a period of 6 h, according to the AOAC (1995).

Muscle fiber morphometry

The muscle fiber morphometry was evaluated in samples from three fish from each replicate ("*n*" sampling of

15 fish per treatment); these fish were euthanized with 250 mg L^{-1} of benzocaine (Gomes *et al.*, 2001), and a sample of the right dorsal white muscle was removed above the lateral line with the aid of a scalpel. Samples were fixed in 10% buffered formaldehyde for 24 h and transferred to 70% ethanol, dehydrated in an increasing ethanol series, diaphanized in xylol, and included in histological paraffin. Cross-sectional histological semi-seriate sections (6 µm) obtained using a microtome (Microm HM 340 E, ThermoScientific, Germany) were stained with hematoxylin-eosin (HE).

The histological sections were analyzed using an optical microscope (P1 *Olympus* BX 50, Manila, Philippines) coupled with an Olympus camera (PMC 35 B Berlin, Germany) using the 40X objective to capture the fields of observation. The smallest diameter of 200 muscle fibers per animal (3,000 fibers per treatment) was determined in random fields of the histological slide using the ImagePro-Plus version 4.5 image analysis system. These measurements were grouped into classes of diameters (<20, 20-50, and>50 µm) to evaluate the contribution of each treatment to hyperplasia and hypertrophy on muscle growth (Almeida *et al.*, 2008).

Intestine morphology

The intestinal morphology was evaluated in ~ 4-cm long portions of the midgut collected from two fish euthanized with 150 mg L^{-1} benzocaine (Gomes *et al.*, 2001) from each experimental unit, totalizing an "*n*" sampling of ten fish per treatment. The samples were fixed in aqueous *Bouin* (Behmer *et al.*, 1976) for 4 h and transferred to 70% ethanol. Subsequently, they were dehydrated in increasing ethanol series, diaphanized in xylol, and included in histological paraffin. Cross-sectional semi-seriate 7 µm thick sections obtained in a rotating microtome (Microm HM 340 E, ThermoScientific, Germany) were stained with hematoxylin-eosin.

These histological sections were analyzed in an optical microscope (P1 *Olympus* BX 50, Manila, Philippines) coupled to an Olympus camera (PMC 35 B, Berlin, Germany) using the 20X objective to capture the fields of observation. The height of all villi (VH) around the intestinal lumen was measured using the ImagePro-Plus version 4.5 image analysis system; images were enlarged with a 2x zoom lens.

Liver histology

The quantification of hepatic glycogen was conducted in liver samples from two fish in each replicate, totaling an "*n*" sampling of 10 fish per treatment. The samples were fixed in aqueous *Bouin* solution (Behmer

et al., 1976) for 24 h and subsequently transferred to 70% ethanol, dehydrated in an increasing ethanol series, diaphanized in xylol, and included in histological paraffin. The cross-sectional semi-seriate 5 μm thick histological sections obtained with the aid of a microtome (Microm HM 340 E, ThermoScientific, Germany) were subjected to Schiff's Periodic Acid + Hematoxylin staining (Beçak & Paulete, 1976).

The quantification of glycogen in liver samples was performed using images captured in an optical microscope (P1 *Olympus* BX 50, Manila, Philippines) coupled to an Olympus camera (PMC 35 B, Berlin, Germany) and used a 40X objective to capture 15 images per fish, totaling 150 images per treatment. The standardized useful area was 20,914.72 μm². These measurements were performed using the Image Pro-Plus version 4.5 image analysis system aiming at the quantification of the field percentage occupied by glycogen.

Partial budget analysis

The partial feed budget analysis was performed with the purpose of determining production costs considering only the studied phase (juvenile). The costs considered in the economic evaluation were: labor (L - including overtime), purchase of fingerlings (FING), and feeding costs (FEC). The percentage of profit, calculated as the Gross Revenue (GR), was only based on the selling price of juveniles taking into account the survival rate. The values used in the calculations corresponded to actual values practiced in 2015.

Labor and overtime: The employee's salary was calculated (in real, R$, Brazilian currency) on the basis of the minimum wage (R$1032,02) for professionals employed in fishing activities in the State of Paraná, Brazil (DOE, 2015) added with 43% of income charges. A total of 240 monthly working hours on normal days (Monday to Saturday until noon) was used as the baseline, which corresponded to an estimated hourly pay of R$4.30. Overtime hours worked on Saturday afternoons were calculated with an increase of 50% over the regular pay (R$6.45) and on Sundays with a 100% increase (R$8.60).

- Cost of fingerlings: The purchase price of fingerlings was stipulated based on the price in the Western region of Paraná, Brazil (R$100.00/thousand).
- Feeding cost: The feeding cost was quoted at R$1.76 per kilogram of feed taking into account the feed conversion in each restriction level.
- Gross Revenue (GR): The sales value of juveniles was stipulated based on the price in the region (R$350.00/thousand) for 30 g fish. Therefore, the sales prices of juveniles were calculated in proportion to the final average weight of fish in each treatment, and survival was calculated as percentages in different dietary restrictions.

The following formula was used to calculate the Partial Net Revenue (PNR - considering only the cost of fingerlings, labor, and feeding):

$$PNR = GR - (FING + L + FEC).$$

For an expanded analysis, the values were extrapolated to an area of 5-ha of water surface and density of 40 juveniles m-2 over a period of 60 days.

Statistical analysis

The data of productive performance, proximal composition, morphometry of muscle fibers and intestinal villi were submitted to analysis of model assumptions; homogeinity by the Levene's test, normality by the Shapiro-Wilk's test and the independence of residue through graphical interpretation. According the assumptions, the data were submitted to the analysis of variance (ANOVA). The Tukey's test was applied at 5% significance level when significant differences were observed ($p<0.05$); the Statistic 7.1 software was used (Statsoft, 2005). Descriptive statistics were used in the economic analysis.

Results

Performance and carcass proximal composition

Fish fed daily (7:0) presented significantly higher ($p<0.01$) FW, WG and FL, than those fed five consecutive days followed by two days of food restriction (5:2) and those fed one day followed by one day of food restriction (1:1). However, fish fed daily showed similarly ($p>0.05$) results on these parameters to those fed 6 consecutive days followed by one day of food restriction (6:1) (Table 2).

Fish in the 6:1 treatment presented significantly higher HI compared to those in the 7:0 treatment. Fish in the 5:2 treatment showed high indexes ($p<0.05$) of VF, which differed only from those in the 1:1 treatment. Fish in the 1:1 treatment showed superior results ($p<0.05$) in PER and PR (Fig. 1), and IQ when compared to those in the 7:0 and 5:2 treatments. The SUR and FC rates were not influenced by the different levels of food restriction (Table 2).

The proximal composition analyses in whole fish showed differences in moisture, lipid, and ash ($p<0.05$) among the different levels of food restriction. The results for moisture and lipids were similar between fish in the 7:0 and 6:1 treatments; low values of moisture and high of lipids were observed. The opposite was observed in fish in the 5:2 and 1:1 treatment. Fish in the 7:0 treatment presented the lowest mineral matter levels compared to those in the 6:1 and 1:1 treatments. Crude protein was not influenced by the different dietary restrictions (Table 3).

Table 2. Productive performance of Nile tilapia juveniles (*Oreochromis niloticus*) fed different dietary restrictions.

Variables[1]	Treatments[2]				*p*-value	CV (%)
	7:0	**6:1**	**5:2**	**1:1**		
IW (g)	3.20	3.25	3.25	3.25	0.68	2.09
FW (g)	27.80a	24.82ab	22.49bc	20.50c	<0.01	14.17
WG (g)	24.60a	21.57ab	19.25bc	17.26c	<0.01	16.47
FL (cm)	10.81a	10.23ab	9.93b	9.69b	<0.01	5.31
HSI (%)	7.32b	9.41a	8.76ab	9.24ab	0.04	15.47
VF (%)	3.79ab	3.85a	2.80ab	2.25b	0.02	33.64
IQ	5.01b	5.86ab	5.20b	6.11a	<0.01	11.64
SUR (%)	95.00	90.00	95.00	97.50	0.68	10.06
FC	0.86	0.97	0.90	0.71	0.08	19.27
CF	2.20	2.32	2.29	2.25	0.19	5.31
PER	46.58b	44.10b	45.08b	54.01a	<0.01	10.56
PR	56.30b	51.96b	55.39b	71.11a	<0.01	17.67

[1]IW = Initial weight; FW = Final weight; WG = Weight gain; FL = Final length; HSI = Hepatosomatic index; VF = Visceral fat; IQ = Intestinal quotient; SUR = Survival; FC = Food conversion; CF = Condition factor; PER = Protein efficiency rate; PR = Protein retention. [2]7:0 - fed daily; 6:1 - fed six consecutive days followed by one day of food restriction; 5:2 - fed five consecutive days followed by two days of food restriction; and 1:1 - fed one day followed by one day of food restriction. Averages in the same row followed by different letters indicate statistical difference by the Tukey's test. CV (%) = coefficient of variation.

Morphometry of intestinal villi height, hepatic glycogen values, and frequency of muscle fibers

The height of intestinal villi and concentration of glycogen in the liver were similar among all dietary restrictions (Table 4).

The different dietary restrictions influenced (*p*<0.05) the frequency of muscle fibers within the fiber diameter classes defined as<20 μm and from 20 to 50 μm. The highest frequency of fibers in the<20 μm class was observed in the 1:1 and 7:0 treatments; the lowest frequency of fibers in the 20 to 50 μm class was observed in the 1:1 treatment (Table 5).

No effect (*p*>0.05) from the dietary regimens was observed on the frequency of muscle fibers with diameters in the>50 μm class (Table 5).

Partial feed budget analysis

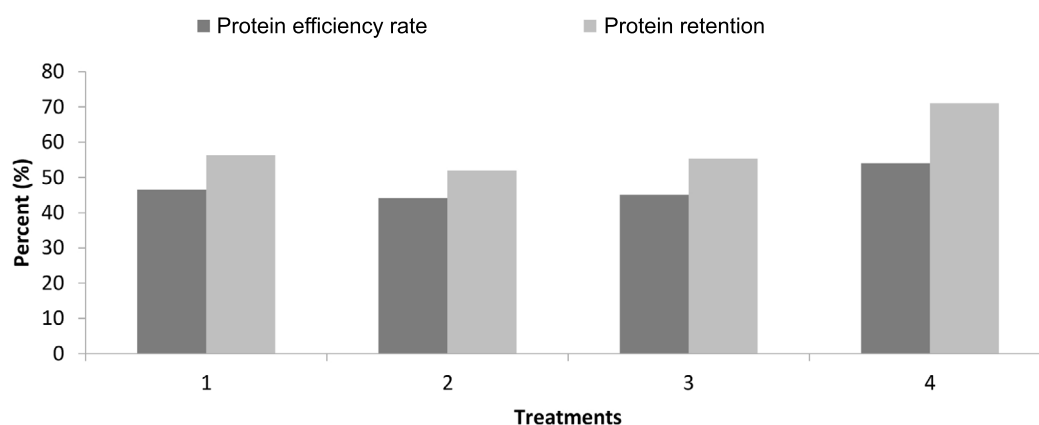The partial feed budget was influenced by the different dietary restrictions; the most economically viable

Figure 1. Protein efficiency rate and protein retention of Nile tilapia juveniles (*Oreochromis niloticus*) fed different dietary restrictions.

Table 3. Body composition of Nile tilapia juveniles (*Oreochromis niloticus*) fed different dietary restrictions. Data are presented as a percentage.

Variables[1]	Treatments[2]				*p*-value	CV (%)
	7:0	6:1	5:2	1:1		
Moisture	69.32b	69.38b	71.28a	71.85a	<0.01	1.92
Protein	15.62	15.92	15.69	16.32	0.38	4.31
Lipid	11.32a	11.07a	9.79b	9.01b	<0.01	10.95
Ash	3.51b	3.78a	3.66ab	3.80a	<0.01	4.07

[1] Treatments: see Table 2. CV (%) = coefficient of variation.

Table 4. Height of intestinal villi and hepatic glycogen values of Nile tilapia juveniles (*Oreochromis niloticus*) fed different dietary restrictions.

Variables[1]	Treatments[2]				*p*-value	CV (%)
	7:0	6:1	5:2	1:1		
Villi height (μm)	176.35	175.5	178.35	141.64	0.46	22.00
Liver glycogen (%)[2]	9.42	9.75	9.26	9.56	0.83	2.20

Tukey's test (*p*>0.05). CV = coefficient of variation. [1] Treatments: see Table 2. [2] Liver glycogen estimate based on histological staining.

Table 5. Frequency of distribution of muscle fibers in three classes of muscle diameters (<20 μm, among 20 and 50 μm, and>50 μm) in Nile tilapia juveniles (*Oreochromis niloticus*) fed different dietary restrictions.

Diameter classes	Treatments[1]				*p*-value	CV (%)
	7:0	6:1	5:2	1:1		
<20 μm	47.29ab	41.31b	41.99b	60.36a	0.02	39.01
20-50 μm	52.12ab	58.08a	57.39a	39.58b	0.02	34.30
>50 μm	1.86	1.43	1.88	0.41	0.49	76.88

[1] Treatments: see Table 2. Averages in the same row followed by different letters indicate statistical difference by the Tukey's test. CV (%) = coefficient of variation.

treatment was 7:0 and the least viable was 1:1. Labor costs were 16.66% lower in the 5:2 treatment than in the 7:0. The 1:1 treatment saved 38.58% in cost compared to the 7:0 treatment. However, the profit margin of the 7:0 treatment was higher than in other treatments with a greater range of difference when compared to the 1:1 treatment (35.97%) (Table 6).

Discussion

The values of water quality parameters were within those recommended for Nile tilapia (El-Sayed, 2019), with the exception of temperature, which remained below the temperature considered optimal for the species. However, although below the optimum temperature for development all fish were kept in the same system, the results expressed in this study reflect the effects provided by the applied feeding treatments under the reported environmental conditions.

In this study, fish in the 6:1 treatment showed similar growth to those continuously fed (7:0), suggesting a possible compensation. However, fish in the treatments with extended restriction (5:2 and 1:1), showed a partial response, *i.e.*, they failed to achieve the growth observed in those continuously fed. Probably there was a limited cellular protein synthesis that contributed to growth reduction in these fishes.

Rosauer *et al.* (2009) reported similar results in "walleye" fingerlings, *Sander vitreus*, demonstrating total compensation in final weight in fish submitted to five feeding days followed by two days of food restriction, and only partial compensation in fish fed three consecutive days followed by four days of food restriction.

Table 6. Partial budget of Nile tilapia juvenile (*Oreochromis niloticus*) fed different dietary restrictions for 60 days.

Variables (R$)	Treatments[1]			
	7:0	**6:1**	**5:2**	**1:1**
Labor	7,083.78	6,225.14	5,903.16	6,493.46
Purchase of fingerlings	200,000.00	200,000.00	200,000.00	200,000.00
Feed (ration)	79,954.10	76,270.86	67,691.92	49,101.29
Survival (unit)	1,900,000	1,800,000	1,900,000	1,950,000
Gross revenue	616,227.00	521,226.00	498,522.00	466,381.15
Partial net revenue	329,189.12	238,730.00	224,926.92	210,786.44

Based on two workers responsible for 5-ha of water surface with a density of 40 juveniles m^{-2} for a period of 60 days. [1] Treatments: see Table 2. R$ = real, Brazilian currency

The ability of organisms to recover from nutritional deficiencies arising from long periods of food restriction in the early stages of development may be compromised, mainly by protein degradation, when adequate levels of nutrition are restored (Metcalfe & Monaghan, 2001). Although growth compensation responses were not evaluated in the present study, the food restriction imposed on fish may have influenced recovery because the study was carried out in the juvenile phase, which presents high growth rate.

The percentage of VF in the 7:0, 6:1, and 5:2 treatments was higher than that in fish submitted to more intense food restriction (1:1). Probably under these conditions, growth is limited due to the mobilization of energy reserves, such as lipids and even amino acids, for the maintenance of vital processes. Fish undergoing food restriction can use fat from visceral content for metabolic maintenance during food deprivation (Cook *et al.*, 2000; Souza *et al.*, 2002), which justifies the low VF content in fish in the 1:1 treatment.

The fish intestines showed longer length in the 1:1 treatment compared to other treatments suggesting an adaptation that would allow food to stay longer inside the gastrointestinal tract. This could consequently maximize digestion and absorption and allow maximum extraction of nutrients from food that is converted into growth (Mihelakakis *et al.*, 2002; Eroldoğan *et al.*, 2004), and this fact can be demonstrated by the higher values in the PER and PR of the animals in the 1:1 treatment. This would support the fish's adaptation to new food conditions during the refeeding period. Although the IQ values showed differences among treatments, they are close to those observed by Buddington *et al.* (1987) in *Tilapia rendalli* (5,80). According to Ali *et al.* (2003) the compensatory growth may be an internal adjustment mechanism for animal to adapt to often dramatically varied environment.

The FC remained similar among the different food restriction treatments, probably because the food was supplied until apparent satiation, which led these values to remain unchanged. The same behavior of no differences in fish submitted to small dietary restrictions, only in the treatment with three consecutive days of restriction, was demonstrated by Palma *et al.* (2010) in Nile tilapia juveniles and Abdel-Hakim *et al.* (2009) in hybrid tilapia juveniles.

The food restriction did not significantly affect SUR. The same behavior of high survival rates was demonstrated by Arauco & Costa (2012) in juvenile tilapias, and by Nebo (2011) in treatments of food restriction periods of less than 20 days. This indicates that the fishes were well above basal metabolic needs as the fish grew hence survival.

The fact that the PER was higher in 1:1 treatment (more severe restriction) is in accordance with the works of Sevgili *et al.* (2012), Gong *et al.* (2017) and Xu *et al.* (2019) for *Oncorhynchus mykiss, Ctenopharyngodon idellus* and *Megalobrama ablycephala*, respectively. This may indicate a delay in the growth of Nile tilapia juveniles; however, a beneficial effect on the use of dietary nutrients, mainly lipids and proteins (Sanchez-Muros *et al.*, 1996), as also verified in the PR by animals of this same treatment, above 70%, helps to explain the larger intestine size of the fish in this treatment.

The proximal composition analysis in whole fish shows a reduction in the amount of lipids with increased periods of food restriction; the inverse effect occurred in relation to moisture. Part of lipids in food restriction conditions is probably used as an energy source for the maintenance of vital processes and the structure and function of cell membranes (Weatherley & Gill, 1987). Souza *et al.* (2002) reported similar results in pacu, *Piaractus mesopotamicus*, submitted to alternating cycles of food restriction and refeeding.

The feed restriction promotes a decrease in body lipids levels but not influence body protein. It can be explained because when the fish did not receive feed, lipids reserve

was used instead of protein. So, body protein was spared as observed as well by Souza *et al.* (2002). Abdel-Hakim *et al.* (2009) observed an increase in the mineral matter in hybrid tilapia according to the length of the food restriction period, similar to what was observed in the present study. These results may be influenced by several factors, such as fish age and food availability and the environment in which fish are grown (Shearer, 1994; Contreras-Guzmán, 2002).

According to Takashima & Hibiya (1995) and Wang *et al.* (2009), longer intestine villi resulted in an increased capacity of nutrient absorption due to an increase in the contact surface. In the present study, no differences in villi height ($p>0.05$) were observed among fish under the different dietary treatments, suggesting that the imposed food restriction did not affect villi height, which according to Arruda *et al.* (2008) could result in lower than normal nutrient absorption. Although the villi lengths have not been altered, the IQ was higher in fish under 1:1 treatments, suggesting an adaptation to this imposed condition, to meet their basal metabolism. The villi height observed in our study is slightly lower than that observed by Carvalho *et al.* (2011) who evaluated the intestinal morphometry of Nile tilapia in the juvenile phase and found mean villi height of 188.07 μm in the control diet, which may be related to the size of the animals.

Some fish species have the ability to preserve hepatic glycogen stocks by mobilizing large amounts of lipids or body proteins (Sheridan & Mommsen, 1991). In the present study, it was possible to observe a reduction in lipid content, starting with the carcass and visceral fat, which is similar to what is described by Favero *et al.* (2020) in pacus (*Piaractus mesopotamicus*). In addition, the hepatic glycogen reserve is rapidly restored after refeeding (Black & Love, 1986; Blasco *et al.*, 1992). Because tilapia juveniles remained fasting for 24 hours prior to euthanasia, all were fed on the same day, which contributes to their similar content in hepatic glycogen. However, Won & Borski (2013) emphasized that when fish are fasting, a demand for endogenous energy occurs with the consequence of a reduction in growth. We observed this fact in fish in the 5:2 and 1:1 treatments because of the demand to use fat and proteins for gluconeogenesis.

Considering the studied dietary restrictions, the morphometric analysis of muscle fibers was characterized by the growth occurred as the result of hyperplasia and the beginning of hypertrophy during the experimental period. Fibers with diameters < 20 μm indicate the occurrence of intense hyperplasia while fibers with diameters between 20 and 50 μm indicate the end of hyperplasia with the onset of hypertrophy, and fibers with diameters > 50 μm indicate hypertrophy (Valente *et al.*, 1999; Rowlerson & Veggetti, 2001).

The highest frequency of fibers with diameters < 20 μm was observed in fish in the 1:1 and 7:0 treatments,

which characterizes intense hyperplasia (Valente *et al.*, 1999). This hyperplasia is of the mosaic type, it is characteristic of fish in the juvenile period (Johnston & Hall, 2004), which was the period analyzed in this study, and very important for commercial aquaculture species including tilapia (Rowlerson & Veggetti, 2001). In mosaic hyperplasia, new muscle fibers are formed from the fusion and differentiation between satellite cells using differentiated fibers as support. Therefore, larger fibers were observed surrounded by newly formed fibers of small diameters in the studied histological sections (Rowlerson & Veggetti, 2001). It is possible that fish in the 6:1 and 5:2 treatments rapidly moved to the muscle fiber hypertrophy period while fish in the 1:1 treatment had a more pronounced period of hyperplasia. However, because we observed a large number of fibers with small diameters (> 60%), refeeding may have influenced the fiber re-growth process in this treatment.

The frequency of fish in the class with fibers with diameters between 20 and 50 μm was smaller in fish submitted to the 1:1 treatment than that of fish in other treatments; however, this did not differ in those fed daily (7:0). During fasting conditions, refeeding probably promotes a reversal in the processes of mobilization of body reserves to supply catabolism; diet will again favor growth only when this condition is satisfied (Hagen *et al.*, 2009).

The low frequency of fibers with diameters > 50 μm observed in all treatments is in agreement with that proposed by Almeida *et al.* (2008) when evaluating the frequency of muscle fibers in pacu, during the juvenile phase, and Neu *et al.* (2016) when evaluating the frequency of muscle fibers in Nile tilapia during the juvenile phase. This low frequency is related to the growth phase in which the animal was evaluated.

Regarding the partial budget, the partial cost of production and partial profit margins were influenced by the different food restriction treatments; however, in the present study, all treatments received the same type of ration, which indicates that these differences were due to production management, and consequently, to parameters of productive performance.

Labor and food costs were higher in the treatment with daily feeding (7:0). However, the calculated net partial revenue was 27.48% higher in the 7:0 treatment than in the treatment with feeding restricted one day a week (6:1). The treatment with every other day food restriction (1:1) showed 38.59% less in food cost. However, the calculated net partial revenue was 35.97% lower than that in the 7:0 treatment.

Souza *et al.* (2003) extrapolated their results to one hectare of the water surface in a study with pacu, and found higher values for gross revenue and partial net revenue in fish fed daily compared to those submitted to alternating cycles of food restriction. These results are similar

to those observed in the current study and demonstrate that the decrease in the amount of food supplied decreases costs, and therefore, the net partial revenue also decreases. Thus, based on this information, the profitability is higher when fish are fed daily.

In conclusion, food restriction in the juvenile stage of Nile tilapia (*Oreochromis niloticus*) negatively influences productive performance, proximal composition, and muscle growth. Hence, this practice discouraged for commercial production.

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