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FEEDING FREQUENCY IN REARING JUVENILES OF SURUVI *Steindachneridion scriptum**

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ABSTRACT

The present study aimed to determine the ideal feeding frequency of feeding for suruvi juvenile. A study was conducted over 60 days to determine the effects of feeding frequency on growth metrics, body composition, and digestive enzyme activity of juvenile suruvi (initial mean weight: 60.19 ± 10.67 g). The experimental units were 1.0 m^3 circular tanks, stocked with 94 fish, connected to a recirculating aquaculture system. The feed was offered during the dark phase of the 12:12 photoperiod with the following treatments in triplicate: FF1 = once a day at 20:00h; FF2 = twice a day at 20:00h and 06:00h; and FF3 = three times a day at 20:00h, 01:00h, and 06:00h. The fish fed a commercial carnivorous fish feed comprised of 42% crude protein, 11% lipids, 20% carbohydrates, and 4,380 kcal kg^{-1} of crude energy in 6.0 mm pellets. At the end of the feeding, no significant differences were observed in the growth variables, body composition of proteins and lipids, and alkaline proteases and lipase activity ($p>0.05$) between treatments. However, there were significant differences in amylase activity ($p<0.05$). Our findings demonstrated that suruvi juveniles could be sustainably fed only once a day.

Keywords: proximate chemical composition; nutrient utilization; digestive enzymes; feed conversion.

FREQUÊNCIA ALIMENTAR NA CRIAÇÃO DE JUVENIS DE SURUVI *Steindachneridion scriptum*

RESUMO

O presente estudo teve como objetivo determinar a frequência de alimentação para juvenis de suruvi. Foi realizado um estudo durante 60 dias para determinar os efeitos da frequência de alimentação nas medidas de crescimento, composição corporal e atividade enzimática digestiva do juvenil de suruvi (peso médio inicial: 60,19 ± 10,67 g). As unidades experimentais foram tanques circulares de 1,0 m^3, estocados com 94 peixes, conectados a um sistema de recirculação. A alimentação foi oferecida durante a fase escura do fotoperíodo (12L:12D), sendo fornecida com os seguintes tratamentos em triplicata: FA1 = uma vez ao dia às 20:00h; FA2 = duas vezes ao dia às 20:00h e 06:00h; e FA3 = três vezes ao dia às 20:00h, 01:00h e 06:00h. Os peixes foram alimentados por uma ração comercial para peixes carnívoros, composta por 42% de proteína bruta, 11% de lipídios, 20% de carboidratos e 4.380 kcal kg^{-1} de energia bruta em peletes de 6,0 mm. Não foram observadas diferenças significativas nas variáveis de crescimento, composição corporal de proteínas e lipídios e proteases alcalinas e atividade de lipase ($p>0,05$) entre os tratamentos. No entanto, houve diferenças significativas na atividade da amilase ($p<0,05$). Nossos resultados demonstraram que os juvenis de suruvi podem ser alimentados de forma sustentável apenas uma vez ao dia.

Palavras-chave: Composição química corporal; utilização de nutrientes; enzimas digestivas; conversão alimentar.

INTRODUCTION

The population of suruvi *Steindachneridion scriptum,* a carnivorous catfish native to the south of Brazil, shows reduction due to anthropic pressures in the upper Uruguay River basin (Beux and Zaniboni-Filho, 2008), such as the implantation of dams and the fishing. Thus, aquaculture emerged as a viable alternative to enable the repopulation of the species in the wild.

In aquaculture, the species exhibits docile behavior during handling, high rates of survival, tolerance to low temperatures (Zaniboni-Filho et al., 2008), and constant

growth between the sexes during breeding (Maghelly et al., 2014). Studies on the cultivation of *S. scriptum* in cages revealed that the species has excellent performance for breeding in this production system (Zaniboni-Filho et al., 2010).

Despite presenting those favorable characteristics, studies related to *S. scriptum* are scarce, and essential technical and scientific are lacking to evaluate the real potential of this species in fish farming, among them the feeding frequency and feeding times, which are important factors that promote species growth (Ng et al., 2000; Sousa et al., 2012).

The effects of feeding frequency on the growth of Brazilian native catfish have been analyzed for the surubim *Pseudoplatystoma corruscans* (Bogiane et al., 2018), silver catfish *Rhamdia quelen* (Canton et al., 2007), and pacamã *Lophiosilurus alexandri* (Silva et al., 2014). These effects were also analyzed for non-native catfish bred in Brazil, such as the African catfish *Clarias gariepinus* (Aderolu et al., 2010), channel catfish *Ictalurus punctatus* (Jarboe and Grant, 1996), and Asian catfish *Pangasius hypophthalmus* (Hung et al., 2001).

The efficiency of feed nutrient absorption during feeding may be related to the interval between meals and animal feeding habits (Baldisserotto, 2002) and depends on the concentration of digestive enzymes present in the digestive tract (Tengjaroenkul et al., 2000). Therefore, fish can improve the ability to digest food due to the activity of the enzymes responsible for nutrient digestion (Furne et al., 2005), but in a condition of reduced feed offering and long breaks between meals, digestives enzymes activities increase to compensate for the lack of food (Thongprajukaew et al., 2017).

In carnivorous fish like suruvi, feed transit in the gut is slower, and thus high feeding frequencies within short time intervals can increase feed waste, reduce digestion and nutrient uptake, and consequently lead to low protein and lipid retention (Baldisserotto, 2002; Carneiro and Mikos, 2005; Wang et al., 2009; Bogiani et al., 2018).

Regarding *S. scriptum*, only two studies were conducted related to feeding management, one that identified that the highest feed intake occurs during the dark period (Zaniboni-Filho et al., 2008) and the other that determined the adequate photoperiod to be used during larviculture (Schütz et al., 2008).

In this way, the present study aimed to determine the ideal feeding frequency for *S. scriptum* juvenile by analyzing their effect on growth, body composition, and digestive enzymes activity.

MATERIAL AND METHODS

Study location

Experiments were carried out at the Laboratory of Biology and Cultivation of Freshwater Fish (LAPAD - 48°50'15"S, 27°92'50"W, Aquaculture Department, Federal University of Santa Catarina) for 60 days.

Biological material and experimental procedures

We used juvenile suruvi *S. scriptum* (60.19 ± 10.67 g; 18.26 ± 1.01 cm) that were descendants from the induced reproduction of first-generation fish of the upper Uruguay River basin. Juveniles were stocked and acclimated for 20 days in 9 circular tanks with 1.0 m^3 (94 fisk per tank). Each of them were coupled to a water recirculation system, equipped with constant aeration and mechanical and biological filtration, with a flow rate of 720 mL min^{-1} and photoperiod adjusted to 12:12h (light: dark), with lights turned on at 07:00h.

During the acclimation and experimentation, the water temperature (25.5 ± 0.6°C), the dissolved oxygen concentration (7.42 ± 0.33 mg L^{-1}), the pH (7.17 ± 0.20), the electrical conductivity (6.13 ± 0.26 mS cm^{-1}), and salinity (3.30 ± 0.14 ppt) were measured daily with a YSI Professional Plus multiparameter. Total ammonia (0.30 ± 0.36 mg L^{-1}) and nitrite (0.02 ± 0.12 mg L^{-1}) concentrations were determined once a week with an AT 10P photo colorimeter. Until the experiment beginning, fish were fed commercial feed once a day to satiation during the night on under dim light. The Animal Use Ethics Committee of the Federal University of Santa Catarina approved the experimental protocols used (Protocol CEUA 25166290818).

Experimental design and feed management

Feeding frequency for *S. scriptum* juveniles was tested using a completely randomized experimental design, with treatments that consisted of three feeding frequencies (FF), applied with three replications: FF1 = once a day at 20:00h; FF2 = twice a day at 20:00h, and 06:00h; and FF3 = three times a day at 20:00h, 01:00h, and 06:00h.

The feed was offered until apparent satiety during the dark phase of the photoperiod since the species consume more food during the night or under dim light when grown in a photoperiod of 12:12h (Zaniboni-Filho et al., 2008). The completion of feeding management in the absence of light was adjusted during acclimatization and was enough to observe the feeding activity of the fish and do not disturb them. Feed offered was weighed before and after feeding the fish to calculate feed consumption at each time.

The fish fed a commercial carnivorous fish feed (GuabiTech®, São Paulo, Brazil), which according to the manufacturer, comprised 42% crude protein, 11% lipids, 20% carbohydrates, and 4,380 kJ kg^{-1} of crude energy in 6 mm pellets.

Zootechnical performance

Biometrics were performed at the beginning of the experiment and every 30 days when fish (n = 94) were measured in an analytical balance (0.01 g) and an ichthyometer (0.01 cm) to analyze their zootechnical performance. Fish were previously fasted for 24h and anesthetized with 50 mg L^{-1} of Eugenol® (Escama Forte, Botucatu, Brazil) for the biometrical procedure.

The following variables were calculated from biometric data and feed intake quantification:

Survival (%) = *100* × (*final number of individuals/initial number of individuals*)

Total weight gain (*g*) = *final weight – initial weight*

Coefficient of variation (%) = *100* × (*standard deviation/average fish weight*)

Specific growth rate (% *day*$^{-1}$) = *100* × [(*ln average final weight – ln average initial weight*)/*experiment days*]

Feed conversion = *total feed intake* (*g*)/*biomass gain* (*g*)

Daily dietary intake (% *day*$^{-1}$) = *100* × (*total dietary intake/feeding days*)

Protein and lipid analysis

The protein and lipid content were analyzed in each experimental unit from fish fasted for 24h before sampling. Three samples per experimental unit were collected at the beginning and end of the experiment from fish euthanized with an overdose (100 mg L^{-1}) of the anesthetic Eugenol, according to ethical standards (CONCEA, 2013). The samples were stored at -18ºC until further analysis and thawed before the laboratory procedures.

The crude protein content was obtained using the Kjeldahl macro method, and the lipid content was analyzed by acid hydrolysis, followed by Soxhlet extraction (AOAC, 1995).

The following rates were also calculated: protein retention rate (%) = 100 × [(final average weight × final body protein) – (initial average weight × initial body protein)/protein consumed] and lipid retention rate (%) = 100 × [(final mean weight × final body lipid) – (initial mean weight × initial body lipid)/lipid consumed].

Digestive enzyme activity

Samples used to analyze the digestive enzyme activity were collected at the beginning (n = 6) and the end (n = 6; two from each experimental unit) of the experiment from fish euthanized with an overdose (100 mg.L^{-1}) of the anesthetic eugenol (CONCEA, 2013). The samples were dried with a paper towel, weighed, wrapped in aluminum foil, frozen in liquid nitrogen, and stored in the freezer at -18ºC. These samples were used to determine alkaline and acid proteases, amylase, and lipase activities.

After thawing, the entire gastrointestinal tract of each fish was dissected, removed, weighed, fragmented on ice, and then homogenized in ice-cold ultrapure water (1:6 ratio, w:v) through a van Potter Elvehjen Tissue Homogenizer (Thomas Scientific, Swedesboro, USA) for 2.5 min (5 shakes of 30 seconds, with at approximately 5 minutes intervals for cooling). Homogenates were then transferred to Eppendorf® tubes and centrifuged at 20817 × *g* (Eppendorf centrifugal, model 5804 R) for 15 minutes at 4°C.

The supernatants were then transferred to Eppendorf® tubes, which were identified and used as an enzymatic extract for quantifying soluble proteins and digestive enzyme activities. The crude extract samples were kept on crushed ice throughout the quantitation procedure.

The concentration of soluble protein in the crude extract was determined by Bradford's (1976) method and bovine serum albumin (Sigma-Aldrich Corporation®, Missouri, United States) as the standard. All enzymatic assays were incubated at 25°C, and the absorbances of the formed products were read using a microplate reader (Spectramax, Plus-384, Molecular Devices®, California, USA).

The total alkaline protease activity was quantified by 1.0% azocasein hydrolysis (Sigma-Aldrich Corporation®, Missouri, United States) using the methods described by Garcia-Carreño et al. (1997), and was expressed as specific activity (Δ absorbance$_{366\ nm}$ min^{-1} mL^{-1} mg^{-1} protein).

Acid protease activity, expressed as specific activity (µmol tyrosine min^{-1} mL^{-1} mg^{-1} protein, was determined by hydrolysis of bovine hemoglobin 2.0% (Sigma-Aldrich Corporation®, Missouri, USA) following the methodology recommended by Anson (1938) and described by Vega-Orellana et al. (2006) using the L-tyrosine standard curve.

Amylase activity was determined by 1.0% starch hydrolysis (Merck KGaA®, Darmstadt, Germany) based on the method of Rick and Stegbauer (1974) and described by Baloi et al. (2017). The quantification of the products of amylase activity was estimated by 3.5-dinitrosalicylic acid reagent, according to Miller (1959), with maltose as the standard curve and expressed as specific activity (µmol maltose min^{-1} mL^{-1} mg^{-1} protein).

Lipase activity was determined by hydrolysis of the synthetic substrate 4-nitrophenylmiristate (0.4 mM) according to the methodology described by Sæle et al. (2010) and expressed as specific activity (nmol 4-nitrophenol produced min^{-1} mL^{-1} mg^{-1} protein) using 2.2 mM^{-1} as the extinction coefficient for 4-nitrophenol.

Statistical analysis

The analysis of variance, followed by Tukey's test when necessary, was applied to the zootechnical performance, body composition, and digestive enzyme activity variables. The homoscedasticity requirements of variance and normality were evaluated by Levene and Shapiro-Wilk tests, respectively. A significance level of 5.0% was used in all analyses.

RESULTS

Zootechnical performance and feed intake

The zootechnical variables did not differ ($p>0.05$) among the feeding frequencies (Table 1). Fish presented similar weight patterns in all tanks and heterogeneous biomass.

Fish fed once a day consumed more feed at the first meal at 20:00h than those fed at 01:00h and 06:00h for the other treatments. Concerning the last meal, the fish fed twice a day consumed more feed than those fed three times a day. However, there was no significant difference in the mean dietary intake between treatments (Figure 1).

Table 1. Zootechnical variables (mean ± standard deviation) of *Steindachneridion scriptum* juveniles subjected to different feeding frequencies (FF1 = feed offered once a day – 20:00h; FF2 = feed offered twice a day – 20:00h and 06:00h; FF3 = feed offered three times a day – 20:00h, 01:00h and 06:00h) for 60 days.

Variables	Feeding schedule		
	FF1	FF2	FF3
Survival (%)	100.0±0.0	99.6±0.6	99.3±0.6
Biomass gain (g)	1,161.7±383.4	1,627.2±604.5	1,098.3±650.5
Individual biomass gain (g)	12.36±4.08	17.31±6.43	11.68±6.92
Coefficient of variation (%)	22.8±0.7	23.3±2.1	25.9±3.5
Specific growth rate (%)	0.3±0.1	0.4±0.1	0.3±0.2
Daily consumption of ration (%)	1.5±0.1	1.6±0.1	1.7±0.1
Food conversion	4.9±1.5	4.1±1.7	4.2±0.6

Note: Different feeding frequencies were compared through ANOVA and values are expressed as mean ± SD; Main effects were compared by Tukey's test (α = 0.05).

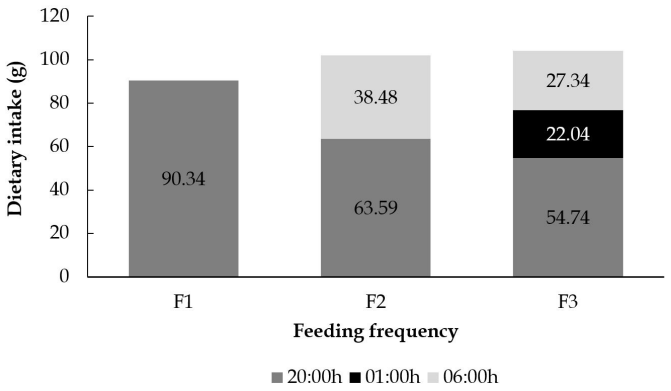

Figure 1. Average dietary intake (g) for each treatment during each feeding schedule for *Steindachneridion scriptum* juveniles subjected to different feeding frequencies (FF1 = feed offered once a day – 20:00h; FF2 = feed offered twice a day – 20:00h and 06:00h; FF3 = feed offered three times a day – 20:00h, 01:00h and 06:00h) for 60 days.

Body composition

The body content of protein, lipid, and moisture did not differ significantly ($p>0.05$) among fish fed once, twice, and three times a day. The moisture content was constant among treatments (Table 2).

Digestive enzyme activity

There were no significant differences among the specific activities of enzymes, except for amylase ($p<0.05$). Fish fed twice a day had the highest specific activity of amylase (Table 3).

DISCUSSION

The suruvi juveniles were highly resilient to the present study's management conditions, with animals' survival rate in all treatments being higher than more than 99.0%. These findings were consistent with those of previous studies for suruvi (Zaniboni-Filho et al., 2008) and other native catfish (Fracalossi et al., 2004; Bittencourt et al., 2018; Bogiani et al., 2018).

Despite feeding suruvi juveniles in the period recommended in a previous study (Zaniboni-Filho et al., 2008), food intake was low when compared to other juvenile native catfish grown in intensive systems (Piedras et al., 2004; Scorvo-Filho et al., 2008).

However, the species should not be disregarded for aquaculture, since Zaniboni-Filho et al. (2010) showed that *S. scriptum* present excellent performance in cage cultivation. In this way, the results obtained for feed consumption, feed conversion, and growth suggest that it would be necessary to carry out additional studies with the species related to fish feed formulation and to management strategies that could improve suruvi performance in an aquaculture recirculation system.

Although the coefficients of variation of the treatments did not show a significant difference, fish presenting different sizes were found in the experimental units. The larger fish may have exhibited dominant behavior and suppressed the growth of subordinate fish, leading to heterogeneity increase among fish of the same treatment, and being the leading cause for the absence of significant differences for most zootechnical variables.

Heterogeneity may occur due to the territorial behavior of the animals (Vaz-Serrano et al., 2011), stocking density (Wocher et al., 2011), photoperiod, and feeding frequency (Kestemont et al., 2003). This heterogeneity can cause social stress in farmed fish and, consequently, affect the food consumption of smaller fish, which increases the size variation of the stock and compromises the feed conversion (Dou et al., 2004; Zhang et al., 2018). The differences in zootechnical performance between heterogeneous and homogeneous lots have already been recorded for sea bass *Centropomus parallelus* (Corrêa and Cerqueira, 2007).

Thus it is essential to conduct biometric screenings during suruvi breeding to separate the animals into size classes for more homogeneous and efficient fish breeding and to improve the zootechnical indices of farmed animals (Ozorio et al., 2004; Campos, 2010; Davis, 2015). However, this procedure is not feasible in research experiments since they are framed in the rigid structure of the experimental design.

Table 2. Body composition (g/100 g wet weight) (mean ± standard deviation) of *Steindachneridion scriptum* juveniles subjected to different daily feeding frequencies (FF1 = feed offered once a day – 20:00h; FF2 = feed offered twice a day – 20:00h and 06:00h; FF3 = feed offered three times a day – 20:00h, 01:00h and 06:00h) for 60 days.

Feeding schedule	Protein (%)	Lipids (%)	Moisture (%)	Protein retention rate (%)	Lipids retention rate (%)
Initial sampling*	19.5	1.7	78.6	-	-
FF1	16.1±0.1	10.4±1.9	69.9±1.5	0.04±3.1	111.9±33.3
FF2	16.4±0.2	7.3±2.4	71.6±3.2	4.04±2.9	68.9±17.0
FF3	16.6±0.3	9.0±1.3	70.3±1.9	0.48±4.5	82.7±21.5

* n = 1; 100 g fish/repetition. Note: Different body composition were compared through ANOVA and values are expressed as mean ± SD. Main effects were compared by Tukey's test (α = 0.05).

Table 3. Specific activity (n = 6, mean ± standard error) of *Steindachneridion scriptum* juvenile digestive enzymes submitted to different feeding frequencies (FF1 = feed offered once a day – 20:00h; FF2 = feed offered twice a day – 20:00h and 06:00h; FF3 = feed offered three times a day – 20:00h, 01:00h and 06:00h) for 60 days.

	Alkaline Protease [1]	Acid Protease [2]	Lipase [3]	Amylase [4]*
Initial sampling	0.009±0.004	0.746±0.216	1.101±0.175	0.586±0.226
FF1	0.014±0.005	1.364±0.074	1.178±0.233	0.582±0.087[a]
FF2	0.005±0.001	1.551±0.116	1.156±0.082	0.826±0.179[b]
FF3	0.011±0.004	1.506±0.048	1.765±0.328	0.577±0.314[a]

[1] Specific activity: Δ absorbance$_{366\ nm}$ min^{-1} mL^{-1} mg^{-1} protein [2] Specific activity: µmol tyrosine min^{-1} mL^{-1} mg^{-1} protein [3] Specific activity (mU/mg protein): nmol 4-nitrophenol min^{-1} mL^{-1} mg^{-1}protein [4] Specific activity: µmol maltose min^{-1} mL^{-1} mg^{-1} protein *Note: Specific activity of enzymes were compared through ANOVA and values are expressed as mean ± SD; different letters in the column indicate significant differences. Main effects were compared by Tukey's test (α = 0.05).

The present study's feed conversion findings corroborate those of Zaniboni-Filho et al. (2008), who submitted suruvi juveniles to different photoperiods and temperatures in a recirculation system and reported that most animals showed conversions between 3.0 and 4.0 until the 60th day. Feed conversion studies on feeding frequency with young forms of other catfish revealed that this zootechnical index might vary among species and fish sizes (Hossain et al., 2001; Carneiro and Mikos, 2005; Aderolu et al., 2010; Fan et al., 2017; Bogiani et al., 2018).

Because growth was not significantly enhanced by the increase in feeding frequency, feeding suruvi juveniles only once was sufficient for their growth. An increase in the number of feeds would require more labor and raise feed costs (Wang et al., 2009). The average daily feed intake revealed that the highest feeding activity occurred at the first meal (20:00h).

Regarding the centesimal composition, the body protein content of suruvi juveniles in the present study was consistent with previous findings for the same species and other catfish (i.e., 15–19%) (Martino et al., 2005; Reidel et al., 2010; Zaniboni-Filho et al., 2015). Their fat content can classify fish as lean (<5.0% fat) or fat (>5.0% fat) (Penfield and Campbell, 1990), and fish body fat is related to the composition of the diet (Johansen and Jobling, 1998). At the end of this study, the suruvi juveniles that were fed 11.0% lipid diets were classified as fatty fish, although Zaniboni-Filho et al. (2015) quantified values <5.0% in suruvi juveniles fed an 8.9% lipid ration.

Suruvi feeding habits (Nuñer and Zaniboni-Filho, 2012) may be related to the species' proteolytic activity. Although carnivorous fish show limited amylase production, higher activity was found in suruvi juveniles fed twice a day with a diet containing 20% carbohydrates. Higher starch contents (36%) were shown to impair amylase induction in *P. corruscans* (Lundstedt et al., 2004).

However, as seen in suruvi juveniles, other carnivorous fish, such as *P. corruscans* (Seixas-Filho et al., 2000; Lundstedt et al., 2004) and *Xiphister atropurpureus* (Chan et al., 2004) showed an ability to optimize carbohydrate digestion, that is, high amylase activity. Buddington et al. (1987) previously observed that fish could adjust the digestive enzyme production profile according to the composition of their diet. This plasticity concerning amylase may present a favorable characteristic that improves the breeding success of suruvi since fishes would be better able to digest carbohydrates in the diet.

CONCLUSION

Since feeding frequency did not significantly affect zootechnical variables, suruvi juveniles should be fed once a day.

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