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ORIGINAL ARTICLE

Feeding frequency affects feed intake and growth in juvenile pirarucu (*Arapaima gigas*)

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ABSTRACT

Pirarucu is one of the main fish species for the development of aquaculture in the Amazon. In this study, the optimal feeding frequency for juvenile pirarucu was assessed based on growth and feed efficiency. Juvenile pirarucu weighing *ca.* 80 g were fed once, twice, three or four times daily until apparent satiation for 63 days. Fish fed three or four times per day presented higher growth, feed intake, and fat body content than those fed once or twice per day. There was no significant difference for feed conversion ratio, and protein and energy retention rates among treatments, suggesting that increased feeding frequency did not affect feed utilization efficiency. Mean feed intake per meal was higher when fish were fed once per day, possibly causing hyperphagic behavior. Results suggest that feeding three times per day was sufficient to secure adequate feeding and growth of juvenile pirarucu.

KEYWORDS: feed management, feed utilization, feeding practice, Osteoglossiformes

Frequência alimentar afeta a ingestão alimentar e o crescimento de juvenis de pirarucu (*Arapaima gigas*)

RESUMO

O pirarucu é uma das principais espécies de peixe para o desenvolvimento da aquicultura na região amazônica. Neste trabalho foi avaliada a frequência de alimentação ideal para juvenis de pirarucu com base em crescimento e eficiência alimentar. Juvenis de aproximadamente 80 g foram alimentados uma, duas, três ou quatro vezes ao dia até a saciedade aparente durante 63 dias. Maior crescimento, ingestão alimentar e gordura corporal foram observados nos peixes alimentados três ou quatro vezes ao dia em comparação com aqueles alimentados uma ou duas vezes ao dia. Não houve diferença significativa para as taxas de conversão alimentar, retenção proteica e retenção energética entre os tratamentos, indicando que o aumento da frequência de alimentação não resultou em prejuízo da eficiência de utilização do alimento. O consumo médio de alimento por refeição foi maior nos peixes alimentados apenas uma vez ao dia, que provavelmente desenvolveram comportamento hiperfágico. Os resultados sugerem que três refeições diárias foram suficientes para garantir o consumo de alimento e crescimento adequados nessa fase.

PALAVRAS-CHAVE: manejo alimentar, utilização do alimento, prática de alimentação, Osteoglossiformes

INTRODUCTION

Pirarucu (*Arapaima gigas*) is a fish species endemic to the Amazon River basin of high economic and social importance for the region (Núñez *et al.* 2011; Pereira-Filho and Roubach 2010). It is one of the world's largest freshwater fish species, achieving up to 200 kg in nature (Saint-Paul 1986). The decline of natural stocks, and some characteristics of the species, such as obligatory air breathing, rusticity, and high growth rate (up to 10 kg in the first year), resulted in the development of pirarucu farming in recent years, and the growing interest from investors in this economic sector (Lima *et al.* 2015; IBGE 2016). The high consumer demand and approval rate for pirarucu owes to its mildly flavored, light-colored boneless meat, making pirarucu one of the main fish species for the development of aquaculture in the Amazon region (Lima *et al.* 2015).

Considering the carnivorous feeding habit of the species and that protein is the most expensive nutrient in the diet, it is fundamental to develop feeding practices that secure adequate feeding to maximize growth and feed efficiency with reduced environmental impact from extensive to intensive rearing systems

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(Lee *et al.* 2000; Wu *et al.* 2015). In this context, feeding frequency is a major component in feed management in aquaculture, as it may affect food ingestion, digestion and absorption, and, consequently, production yield and costs related to food and labor costs (Silva *et al.* 2007; NRC 2011; Baloi *et al.* 2016). It is also important to avoid overfeeding as it can impair water quality through the waste of non-consumed feed (Lee *et al.* 2000).

The optimal feeding frequency depends on the species feeding behavior and gastric capacity, which makes it species-specific (Riche *et al.* 2004; Muntaziana *et al.* 2017). For some fish species, high feeding frequencies can increase the foraging and aggressive behavior, and, consequently, energy expenditure, impairing growth and feed efficiency (Muntaziana *et al.* 2017). In other species, low feeding frequencies may restrict food ingestion and reduce the amount of available energy for fish growth (Al-Khafaji *et al.* 2017). Feeding frequency also varies with developmental stage (Lee *et al.* 2000; Booth *et al.* 2008) and rearing conditions, such as water temperature (Wang *et al.* 2009) and availability of natural food items (Biswas *et al.* 2006). Although no relation is usually observed between photoperiod and feeding frequency (Zolfaghari *et al.* 2011; Veras *et al.* 2016), higher feeding frequencies, together with longer photoperiod, improved growth of Australian snappers, *Pagrus auratus* possibly by synchronizing locomotor and feeding activity rhythms (Tucker *et al.* 2006).

In Brazil, pirarucu farming is mostly carried out in earthen ponds in two-stage systems (Lima *et al.* 2015). The first stage starts with fish of *ca.* 10 g weight until they reach 500-1,000 g, and are transferred to the second stage until they reach 10-12 kg, which is the market size of farmed pirarucu (Lima *et al.* 2015). Daily feed intake and feeding behavior have been studied with fish in the first stage (Crescêncio *et al.* 2005; Mattos *et al.* 2016; Mattos *et al.* 2017; Lima *et al.* 2018). However, feeding frequency has been studied only for juvenile pirarucu of 1.0 to 1.7 kg (Gandra *et al.* 2007), and no information is available on the feeding frequency of smaller fish. The objective of this study was, therefore, to determine the optimal feeding frequency for juvenile pirarucu of 80 g in terms of growth and feed efficiency.

MATERIAL AND METHODS

Fish handling

Juvenile pirarucu of 13.9 ± 5.0 g were purchased from a local commercial fish farm and acclimated to laboratory conditions for 42 days. Fish were fed a commercial extruded feed designed for carnivorous fish species (45% crude protein; 2-4 mm; Laguna, Socil, Descalvado, SP) four times per day until apparent satiation. Following acclimation, 160 fish were weighed and measured individually (80.3 ± 16.4 g individual mean weight) and randomly distributed in sixteen 300-L circular tanks at a density of 10 fish per tank supplied with continuous water flow (4.5 L min^{-1}). Water was supplied from an artesian well and filtered through a sand filter and a charcoal filter. Four feeding frequencies were evaluated (once, twice, three and four times per day) at a completely randomized design with four replicates. Photoperiod (approximately 12L:12D) and water temperature were kept under natural conditions. Throughout the 63 days of experiment duration, water temperature, dissolved oxygen concentration, pH, and toxic ammonia were at 26.1 ± 0.9 °C, 5.6 ± 0.4 mg L^{-1}, 7.0 ± 0.1 and < 0.05 mg L^{-1}, respectively.

Fish were fed by hand until apparent satiation. We used a mix of two commercial feeds for carnivorous fish species (2-4 mm and 4-6 mm; Laguna, Socil, Descalvado, SP). Large pellet feed (4-6 mm) gradually replaced small pellet feed (2-4 mm) during the experiment. A sample of the feed mix was taken weekly to form the composite sample for proximate composition analysis (41.8% crude protein, 18.4 MJ kg^{-1} crude energy, 6.67% ether extract, 12.2% ash, 17.6% neutral detergent fiber and 94% dry matter). During the acclimation time, fish exhibited better feed consumption during the afternoon. Therefore, fish were fed during the day at: 1600 h (once per day), 1100 h and 1600 h (twice per day), 1100 h, 1400 h and 1600 h (three times per day), and 0840 h, 1100 h, 1400 h and 1600 h (four times per day). Feed was gradually supplied to fish, to minimize waste. Each meal lasted about 15 minutes, to ensure the fish reached apparent satiation. Approximately 10 minutes after each meal, any uneaten feed was collected from the water surface with a net, dried in forced-air oven at 55 °C and weighed. Daily feed intake per meal was calculated by subtracting the uneaten feed (in the water and in the container) from that in the pre-weighed feed container. Tanks were syphoned daily to remove excess feces.

Growth parameters

Fish were group-weighed every two weeks to monitor growth. At the end of the experiment, fish were fasted for 24 h and individual body weight and total length were measured. The following performance indexes were assessed:

- Weight gain (g) = final weight - initial weight;

- Specific growth rate (% day^{-1}) = 100 x [(ln final weight - ln initial weight)/feeding period];

- Coefficient of variation (%) = (standard deviation/mean) x 100;

- Daily feed intake (% body weight day^{-1}) = [total feed intake (as fed basis)/(final biomass + initial biomass/2)]/feeding period x 100;

- Survival (%) = 100 x (final number of fish/initial number of fish);

- Feed conversion ratio = total feed intake (as fed basis)/ weight gain.

Body composition analyses

Eight fish were sampled at the beginning of the experiment for the initial body composition analysis. At the end of the experiment, two fish per tank were sampled for final body composition analysis. Feed and fish samples were analyzed according to the methods described by AOAC (1990) for dry matter (method 930.15), ash (942.05), crude protein (Nx6.25; 988.05) and ether extract (petroleum ether, 920.39). Crude energy was measured by calorimetry and neutral detergent fiber by the method of Van Soest *et al.* (1991). Apparent protein and energy retention rates were calculated according to the formula:

Apparent protein/energy retention rate (%) = [(final biomass x final body protein/energy) - (initial biomass x initial body protein/energy)]/total protein/energy intake x 100.

Statistical analysis

Data was submitted to analysis of variance and difference between paired means was detected by a Tukey test (P < 0.05). When the premises of residue homoscedasticity and normality were not met, data was transformed (Box and Cox 1964) or submitted to non-parametric tests of Mann-Whitney-Wilcoxon or Kruskal-Wallis.

Ethics and legal aspects

The study complied with the Brazilian guidelines for the care and use of animals for scientific and educational purposes (CONCEA - CEUA protocol 07/2017), and with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado - AA4F2B0).

RESULTS

Survival varied between 95 and 100%, with no significant difference between treatments. Final weight, weight gain, final length and specific growth rate were significantly higher in juvenile pirarucu fed three and four times per day than in those fed once or twice per day (Table 1). Feed conversion ratio, and protein and energy retention rates did not differ between treatments (Table 1). The coefficients of variation for final weight and total length were not significantly different for the feeding frequencies tested (Table 1).

Fish fed three or four times per day presented significantly higher daily feed intake than those fed once or twice per day (Table 1). On the other hand, mean feed intake per meal was higher in fish fed once per day, intermediate in fish fed twice or three times per day, and lower in those fed four times per day (Figure 1). For fish fed more than one meal per day, feed intake did not differ between feeding times (Figure 1). In fish body composition, fat content followed the same pattern of daily feed intake showing higher levels for fish fed three and four times per day in comparison to those fed once or twice per day (Table 2).

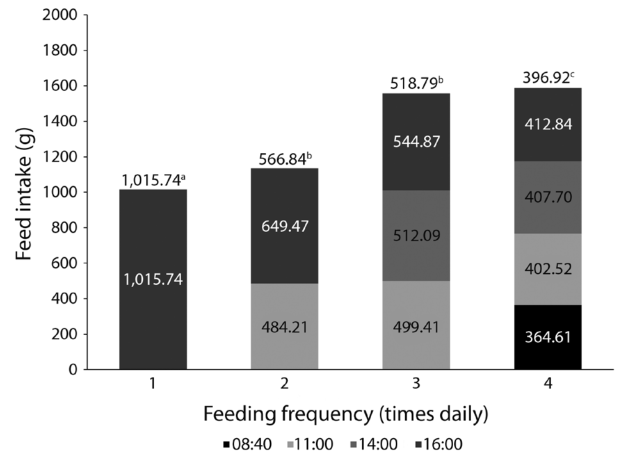

Figure 1. Total (within bars) and mean (top of bars) feed intake per meal for each feeding frequency tested for juvenile pirarucu, *Arapaima gigas* (80 g average initial weight), during 63 days. There were no significant differences in total feed intake among meals for fish fed twice per day (Mann-Whitney-Wilcoxon, P > 0.05), nor for fish fed three or four times per day (Kruskal-Wallis, P > 0.05). Different superscript letters indicate significant differences among treatments (Kruskal-Wallis, P < 0.05).

Table 1. Growth performance of juvenile pirarucu, *Arapaima gigas* (80 g average initial weight) fed for 63 days to apparent satiation at four feeding frequencies.

	Feeding frequency (times per day)			
	1	2	3	4
Survival[1] (%)	97.5 ± 5.0	100	95.0 ± 5.8	100
Initial weight (g)	79.9 ± 1.6	80.1 ± 1.2	81.1 ± 0.9	80.1 ± 0.9
Final weight (g)	203.4 ± 5.9[b]	219.5 ± 19.7[b]	264.9 ± 13.0[a]	263.6 ± 14.0[a]
CV final weight (%)	22.9 ± 7.2	21.6 ± 8.6	20.2 ± 4.3	14.4 ± 1.5
Weight gain (g)	123.5 ± 5.5[b]	139.4 ± 19.9[b]	183.7 ± 13.5[a]	183.5 ± 14.3[a]
Initial length (cm)	22.5 ± 0.2	22.6 ± 0.1	22.6 ± 0.2	22.6 ± 0.1
Final length (cm)	30.8 ± 0.3[b]	31.7 ± 1.1[b]	33.6 ± 0.2[a]	33.6 ± 0.6[a]
CV final length[2] (%)	7.2 ± 2.9	7.0 ± 3.5	5.9 ± 0.9	4.4 ± 0.5
SGR (% day^{-1})	1.5 ± 0.0[b]	1.6 ± 0.2[b]	1.9 ± 0.1[a]	1. 9 ± 0.1[a]
Daily feed intake (% day^{-1})	1.3 ± 0.0[b]	1.3 ± 0.1[b]	1.5 ± 0.1[a]	1.5 ± 0.1[a]
Feed conversion ratio	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.0
Protein retention rate (%)	35.1 ± 4.4	38.5 ± 3.5	35.2 ± 2.4	34.9 ± 3.8
Energy retention rate (%)	22.1 ± 1.4	24.1 ± 2.5	22.0 ± 1.6	22.5 ± 1.0

Different superscript letters in the same row indicate significant differences among treatments (Tukey test, P < 0.05).
[1]Kruskal-Wallis test (P > 0.05).
[2]Data transformed (Box and Cox 1964) to achieve homoscedasticity.

Table 2. Body composition (%, wet basis) of juvenile pirarucu, *Arapaima gigas*, fed to apparent satiation at four feeding frequencies for 63 days.

	Feeding frequency (times daily)			
	1	2	3	4
Moisture	80.4 ± 0.5	80.2 ± 0.6	79.6 ± 0.5	80.3 ± 0.7
Crude protein	13.4 ± 0.5	13.6 ± 0.4	13.7 ± 0.5	13.2 ± 0.9
Lipid	1.7 ± 0.1[b]	1.8 ± 0.1[b]	2.1 ± 0.1[a]	2.1 ± 0.1[a]
Ash	3.4 ± 0.2	3.3 ± 0.1	3.6 ± 0.2	3.4 ± 0.2

Different superscript letters in the same row indicate significant difference (Tukey test, P < 0.05).

DISCUSSION

In the present study, feeding frequency significantly affected growth of juvenile pirarucu, whereas feed utilization efficiency was not affected. Feeding fish three and four times per day resulted in increased feed intake, with a positive effect on growth. Similar results have been reported for juvenile hybrid sunfish (♀ *Lepomis cyanellus* x ♂ *L. macrochirus*), yellowtail flounder (*Limanda ferruginea*) and Atlantic halibut (*Hippoglossus hippoglossus*) with feed intake as the main limiting factor of growth when fed at different frequencies (Wang *et al.* 1998; Dwyer *et al.* 2002; Schnaittacher *et al.* 2005). In juvenile dolly varder char (*Salvelinus malma*) increased weight gain as a function of increased feed intake was also related to an immune system enhancement (Guo *et al.* 2018). Yet, in gibel carp (*Carassius auratus gibelio*) higher growth with increased feeding frequency was associated with higher feed utilization efficiency rather than increased feed intake (Zhou *et al.* 2003).

Although daily feed intake was higher in fish fed three and four times per day, mean feed intake per meal was 1.8 to 2.6 times higher in fish fed once per day. Fish fed once per day may have developed hyperphagic behavior, which is commonly observed in fish submitted to food deprivation or low feeding frequency (Grayton and Beamish 1977; Chatakondi and Yant 2001; Zhu *et al.* 2004). Such behavior was not observed in our fish fed twice per day, which had mean feed intake per meal similar to that of fish fed three times per day. The proportionality observed for feed intake in the distinct meals of fish fed three and four times per day may have resulted from a balanced feed supply and gastric emptying rate (Dwyer *et al.* 2002; Riche *et al.* 2004). Although the same has been observed for fish fed twice per day, the amount of feed supplied once or twice per day was not sufficient for pirarucu to ingest the amount needed during the growth stage evaluated in the present study, despite the size and elasticity of its stomach (Rodrigues and Cargnin-Ferreira 2017), resulting in lower performance.

Juvenile pirarucu of 1 kg fed once and twice per day, daily or every other day, presented higher weight gain, feed intake and feed conversion ratio with two daily meals (Gandra *et al.* 2007). However, these authors did not evaluate higher feeding frequencies, so it is uncertain if, in that stage, pirarucu could benefit from more than two meals per day, as observed in our study. Crescêncio *et al.* (2005) tested two meals during the day, two meals at night, and four meals during the day and night, to assess the influence of feeding time on the growth performance of 318-g juvenile pirarucu. Higher growth and feed intake were observed in fish fed four times during the day and night, which possibly resulted from the number of meals and feeding to satiation rather than the time of feeding itself, as pirarucu feeds mostly during the day (Mattos *et al.* 2016; 2017). Contrary to Crescêncio *et al.* (2005) and Gandra *et al.* (2007), we did not observe an association between increased feeding frequency and an increase in feed conversion ratio, so that, even when fish were fed four times per day, the shorter interval between meals did not affect the feed utilization efficiency.

The relatively low specific growth rate observed in our study (1.5 – 1.9% day^{-1}) may be related to the temperature of the water in the experimental tanks. The optimum temperature for pirarucu farming ranges from 28 to 30 °C (Ono and Khedi 2013), so that the lower mean water temperature during the experimental period (26 °C) possibly had a negative influence on fish growth. Other studies on growth of pirarucu in the same weight range and water temperatures ranging from 24.7 to 28.7 °C found similar specific growth rates (Ituassú *et al.* 2005; Andrade *et al.* 2007; Del Risco *et al.* 2008). Interestingly, these studies achieved final stocking densities ranging from 3.53 to 10.2 kg m^{-3}. Therefore, the high stocking density at the end of our study (*ca.* 8 kg m^{-3}) probably did not influence specific growth rates, corroborating other studies that showed that high stocking densities can be used in pirarucu production [Cavero *et al.* (2003) (28 kg m^{-3}), Oliveira *et al.* (2003) (26 kg m^{-3}) and Ono and Khedi (2013) (66 kg m^{-3})].

Increased body fat deposition with increased feeding frequency has also been reported for other fish species, such as the olive flounder (*Paralichthys olivaceus*), golden pompano (*Trachinotus ovatus*), Brazilian sardinella (*Sardinella brasiliensis*) and Dolly Varden char (Lee and Pham 2010; Wu *et al.* 2015; Baloi *et al.* 2016; Guo *et al.* 2018). In the case of pirarucu and olive flounder, body fat increase can be directly related to higher feed intake, which may have favored body fat deposition. On the other hand, in striped snakehead (*Channa striatus*) body fat deposition was reduced with increased feeding frequency, which may have been due to higher aggressiveness and active behavior during feeding, and consequent higher energy expenditure (Muntaziana *et al.* 2017).

The non-significant difference in the coefficients of variation of final weight and length suggests that the variation in feed intake, as a function of feeding frequency, did not affect the social hierarchy of juvenile pirarucu (Wang *et al.* 1998; Dwyer *et al.* 2002; Baloi *et al.* 2016). Feeding to apparent satiation may have influenced this result, as the dispute for food is one of the factors responsible for increased coefficients of variation of weight and length in fish (Volpato and Fernandes 1994). However, field validation of these results is needed, as agonistic behavior may be enhanced in intensive farming conditions (Costa-Bomfim *et al.* 2014). Additionally, pirarucu of up to 900 g can feed on natural food available in the rearing pond, even when fed commercial feeds (Lima *et al.* 2018), so that feeding frequency may be affected at this initial stage (Biswas *et al.* 2006), further supporting the need for field validation.

CONCLUSIONS

In the present study, juvenile pirarucu of average 80 g body weight presented higher growth and feed intake when fed commercial feed three and four times per day, without negatively affecting feed utilization efficiency. As results for fish fed three and four meals per day were not significantly different, it is suggested that feeding three times per day is sufficient for the development stage studied. Validation of the results in field conditions of commercial aquaculture, such as ponds or cages, is recommended.

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