Aquaculture 465 (2016) 152–157

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Daily self-feeding activity rhythms and dietary self-selection of pirarucu (*Arapaima gigas*)

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ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 31 August 2016
Accepted 2 September 2016
Available online 07 September 2016

Keywords:
Feeding behaviour
Feeding schedule
Food preference
Nutritional challenge
Nutritional wisdom

ABSTRACT

Daily feeding rhythms and the ability of pirarucu (*Arapaima gigas*) to compose a balanced diet through macronutrient self-selection were evaluated. Twelve fish (1573.3 ± 74.4 g) were distributed in six tanks of 250 l, two fish per tank. First, three experimental diets were prepared using an incomplete mixture of macronutrients (75%protein/25%carbohydrate-PC, 75%protein/25%fat-PF and 10% protein/45%fat/45%carbohydrate-PFC). These diets were provided to fish through a self-feeding system connected to a computer to record feeding activity. After this procedure, fish were challenged with a 50% protein dilution (diets: PC50 and PF50). The results showed that pirarucu exhibited a strict diurnal feeding pattern with 95.4% of daily feeding activity observed in the daytime. Fish selected 56.3% P, 24.2% C, 19.5% F, and consumed 150–151 kJ/kg BW/day of energy. After protein dilution, fish sustained energy intake by increasing the consumption of PC50 and PF50 to maintain the target protein intake of the previous stage. When fish were protein-restricted, they failed to sustain previous energy intake. These findings can be used to design feeding regimes and for formulating aquafeed for pirarucu.

Statement of relevance: The use of an automatic feeder system activated by the fish could allow the animal to feed at their preferred time and hence reduces waste and improve the intake. Studies that consider the fish preference as a guide in designing diets can provide data on mechanisms of nutrient intake regulation for the development of aquafeeds for new species with potential for aquaculture.

1. Introduction

The different fish species are not active both in the daytime and at night, but show daily behaviour rhythms (Sánchez et al., 2009). Some environmental events are unpredictable (e.g., weather changes), while others (e.g., daily light cycles) occur repeatedly because they are associated with periodical geophysical cycles, such as the Earth's rotation on its axis. Fish, as most animals, have biological rhythms synchronised to these dependable cycles. Thus they display feeding rhythms (López-Olmeda and Sánchez-Vázquez, 2010). The demand-feeding system has become a useful tool for assessing fish feeding rhythms and food preferences (Azzaydi et al., 1998; Montoya et al., 2012). Different researchers have developed various devices coupled to computers to conduct such research using electric sensors (Boujard et al., 1992), stretch sensors (Sánchez-Vázquez et al., 1994) and infrared photocells (Kitagawa et al., 2015). Over the years these systems have helped to design effective feeding protocols that avoid feed wastage and improve feed utilisation in fish (Cho, 1992; Fortes-Silva et al., 2011a).

Aquaculture fish display a wide variety of feeding behaviours compared to terrestrial livestock, are considered good experimental models to study nutrient intake regulation (Volkoff and Peter, 2006), and possess considerable plasticity for feeding rhythms (Sánchez-Vázquez et al., 1996). According to Simpson and Raubenheimer (2001), fish are able to regulate nutrient intake and defend a nutritional target. This ability is based on the "nutritional wisdom" observed in studies conducted with different carnivorous fish species, such as *Solea senegalensis* (Rubio et al., 2009), *Oncorhynchus mykiss* (Sánchez-vázquez et al., 1999) and *Dicentrarchus labrax* (Rubio et al., 2003), and omnivore fish like *Oreochromis niloticus* (Fortes-Silva and Sánchez-Vázquez, 2012) and *Carassius auratus* (Sánchez-Vázquez et al., 1998). Nutrient self-selection could be used to optimizes the diet composition of farmed fish (Fortes-Silva et al., 2016), and to enable the development of more efficient feeding schedules for fish species in aquaculture (Kitagawa et al., 2015). The studies that contemplate fish as a guide to design diets can provide data on mechanisms of nutrient intake regulation (Fortes-Silva et al., 2011b; Fortes-Silva and Sánchez-Vázquez, 2012), and on fish welfare according

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http://dx.doi.org/10.1016/j.aquaculture.2016.09.005
0044-8486/

to free food selection choices (Volpato et al., 2007, Kulczykowska and Sánchez-Vázquez, 2010).

Pirarucu (*Arapaima gigas*) is one of the largest freshwater fish species in the Amazon basin whose captive breeding is being increasingly exploited (Núñez et al., 2011). Pirarucu farming in the Brazilian Amazon is an important economic activity that has grown in the last few years (Malheiros et al., 2016). Its juveniles display high specific growth rates, and can reach a weight of over 10 kg in 1 year (Imbiriba, 2001). Pereira-Filho et al. (2003) obtained the best biomass growth for pirarucu on extruded diets (40% crude protein, 3.400 Kcal/kg diet). Young pirarucu (body weight between 11 g and 108 g) fed on extruded diets (45% crude protein) showed the best feed conversion of 0.8 (Cavero et al., 2003). When verifying the effect of four protein levels on pirarucu growth (32.7%, 39.3%, 43.4% and 48.6% crude protein), Ituassú et al. (2005) found that these levels have no effect on feed conversion and protein efficiency ratios, but the dietary protein level required for maximum growth was 48.6% for juveniles with 120.7 ± 3.5 g of weight. These results reveal that more nutritional studies are needed. Despite their great potential for aquaculture, there are no studies on this species' feeding behaviour and food preferences, which are key issues for successfully farming this fish species (Ono et al., 2008).

The present paper aimed to assess the daily feeding rhythms of pirarucu, and to evaluate not only its ability to self-compose a balanced diet (nutritional target) based on incomplete mixtures of macronutrients, but also its capacity to defend such a target following nutritional challenges (i.e. protein dilution and deprivation).

2. Material and methods

2.1. Animal housing

This research was conducted in the Fish Nutrition and Feeding Behavior Laboratory (AQUAUFRB-NEPA), Federal University of Recôncavo of Bahia (UFRB, Cruz das Almas, Bahia, Brazil). Twelve juvenile pirarucu, kindly provided by the AguaVale fish farm (Bahia, Brazil), with a body weight of 1573.3 ± 74.4 g (mean ± SEM) were distributed in six 250-l tanks (2 fish per tank). The system contained water recirculation, which comprised mechanical filters, biofilter ceramic rings and UV light (60 W). The experiment was conducted from May to July 2015. Light intensity was measured by a portable digital light meter probe with a photosensor (Luximeter, São Paulo, Brazil) placed above the tank both night and day. Fish received approximately 12 h of light and 12 h of darkness. The photoperiod was regarded as the beginning of the light period at 6 am, and received light intensity that started from 1 lx and gradually increased during the day to then return to the same 1 lx at the start of the evening period at 6 pm. Water parameters, such as temperature, pH, oxygen and ammonia, were measured daily, and the obtained values 29.0 ± 0.8 °C, 6.6 ± 0.5, 7.8 ± 0.6 mg/l and 5.0 ± 0.5 mg/l respectively, were considered appropriate for this species (Cavero et al., 2004). This research was in line with the ethics principles in Animal Experimentation of the Ethics Committee of the UFRB.

2.2. Experimental diets

Five diets were prepared with incomplete mixtures of macronutrients (Table 1). This protocol was performed according to the recommendations for carnivorous fish by Vivas et al. (2006). The experimental design was the same used by Aranda et al. (2001), in which fish were simultaneously provided with three feeds of two or three macronutrients (Table 1): protein/carbohydrate (PC: 55.40% + 18.50%, corresponding as total macronutrients: 75% P and 25% C), protein/fat (PF: 55.40% + 18.50%, corresponding to 75% P and 25% F) and protein/fat/carbohydrate (PFC: 7.40% + 33.25% + 33.25%, corresponding to 10% P, 45% C and 45% F). PFC contained 7.40% of protein because we were unable to obtain factory pellets with only carbohydrate and lipid as ingredients. Casein and gelatin (5:1) were used as protein sources, dextrin was the carbohydrate source, and a mixture of fish and soybean oil (3:1) was the fat source. Each diet was supplemented with an equal quantity of vitamins and minerals, sodium alginate as a binder, and cellulose as the filler. Ingredients were thoroughly mixed and moistened with 40% water in a blender (SAMMIC, MP3000) and then made into pellets by forcing the mixture through a meat press to be cut into approximately 4-mm length pellets. These pellets were dried and stored in a freezer (5 °C) until use.

Table 1
Composition of experimental paired diets.

Ingredients (g/100 g of diet)	PC	PF	PFC	PC50	PF50
Casein:gelatin (5:1)	55.40	55.40	7.40	27.70	27.70
Dextrin	18.50	0.00	33.25	9.30	0.00
Fish oil:soybean oil (3:1)	0.00	18.50	33.25	0.00	9.30
Vitamin and mineral mix[a]	2.00	2.00	2.00	2.00	2.00
CaCO3/CaPO4	4.00	4.00	4.00	4.00	4.00
Cellulose	15.00	15.00	15.00	51.90	51.90
BHT	0.50	0.50	0.50	0.50	0.50
Binder (sodium alginate)	4.60	4.60	4.60	4.60	4.60
Gross energy (kJ/g)[b]	16.20	20.30	20.20	8.10	10.20
Proximate analysis (%)					
Dry matter	92.29	92.73	92.60	92.26	92.62
Crude protein (N × 6.25%)	53.11	54.60	7.31	26.92	27.13
Crude fat	0.57	16.68	32.49	0.58	8.10
NFE	17.08	0.75	32.59	8.86	0.83
Ash	7.26	8.75	9.28	9.10	8.99

[a] Vitamins and minerals (mg/kg diet): Vit. A (min) 1,000,000 UI, Vit. D3 (min) 250,000 UI, Vit. E (min) 12,500 UI, Vit. K3 (min) 1250 mg, Vit. B1 (min) 1875 mg, Vit. B2 (min) 1875 mg, Vit. B6 (min) 1250 mg, Vit. B12 (min) 2500 mcg, Vit. C (min) 12.5 g, Pantothenic Acid (min) 5000 mg, Niacine (min) 10.0 g, Folic Acid (min) 625 mg, Biotine (min) 62.5 mg, Coline (min) 50 g, Copper (min) 625 mg, Iron (min) 6250 mg, Manganese (min) 1875 mg, Cobalt (min) 12.5 mg, Iodo (min) 62.5 mg, Zinc (min) 6250 mg, Selenium (min) 12.5 mg, Inositol (min) 12.5 g.

[b] Calculated from energy intake using the following energy coefficients: 23.6 kJ/g for protein; 38.9 kJ/g for fat; and 16.7 kJ/g for carbohydrate (Miglavs and Jobling, 1989).

The energy content of experimental diets was calculated by the following estimated metabolisable energy (ME) coefficients: 23.6 kJ/g for protein, 38.9 kJ/g for fat and 16.7 kJ/g for carbohydrates, which are the values found in the literature for carnivorous fish (Miglavs and Jobling, 1989). The proximate composition of diets was determined by standard methods of the Association of Official Analytical Chemists (AOAC, 2012): the following were determined: content moisture by drying for 24 h at 110 °C to constant weight; protein by the Kjeldahl method (N × 6.25); crude fat by diethyl ether extraction; ash by heating at 450 °C for 24 h; nitrogen-free extract (NFE) as the remainder of crude protein, crude fat and ash.

2.3. Experimental design

The purposes of Phase 1 were to evaluate the feeding activity by self-feeders system and also the intake target of macronutrients by pirarucu. Moreover, we assess whether the pattern of macronutrients intake keeps after challenge of diet dilution (Phases 2 and 3). Data collected of macronutrient intake target during the experimental phases were used for comparative purposes. The feeding activity pattern was measured only in the Phase 1 due dilution or restriction of macronutrients in the following phases that could lead to a bias in feeding activity. Initially fish were subjected to a 1-week acclimatisation period to adapt to the new experimental conditions, which included the assessment of fish's ability to trigger demand feeders. Three feeders were fitted (Igarapé, Barueri, Brazil feeders), and each contained one of the three different diets fed in each tank. Fish could trigger stretch sensors (placed 3 cm below the water surface), which were individually connected to their corresponding feeder. Each self-feeder was connected to a computer that used specific software (DIO98USB, University of Murcia, Spain) to record the animals' feeding activity.

For the diet self-selection trial (Phase 1), fish were had free access to the experimental diets (PC, PF and PFC) for 23 days. To evaluate feeding preferences, fish were provided with some challenges. On day 18, the