Comparative Biochemistry and Physiology, Part A 203 (2017) 241–254

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Appetite regulating factors in pacu (*Piaractus mesopotamicus*): Tissue distribution and effects of food quantity and quality on gene expression

Hélène Volkoff [a,b,*], Rafael Esatevan Sabioni [c], Luiz Lehmann Coutinho [d], José Eurico Possebon Cyrino [c]

[a] Department of Biology, Memorial University of Newfoundland, St. John's, NL A1B3X9, Canada
[b] Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL A1B3X9, Canada
[c] Departamento de Zootecnia, Setor de Piscicultura, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 13418-900 Piracicaba, SP, Brazil
[d] Departamento de Zootecnia, Laboratório de Biotecnologia Animal, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 13418-900 Piracicaba, SP, Brazil

ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form 22 September 2016
Accepted 27 September 2016
Available online 04 October 2016

Keywords:
Pacu
Feeding
Fasting
Hormone
Expression
Plant-based diets
Soy protein concentrate

ABSTRACT

The pacu *Piaractus mesopotamicus* is an omnivorous fish considered a promising species for aquaculture. Little is known about the endocrine regulation of feeding in this species. In this study, transcripts for orexin, cocaine and amphetamine regulated transcript (CART), cholecystokinin (CCK) and leptin were isolated in pacu. Orexin, CCK and leptin have widespread mRNA distributions in brain and periphery, CART is limited to the brain. To examine the role of these peptides in the regulation of feeding and energy status, mRNA expression levels were compared between fed and fasted fish and around feeding time. Both orexin and CART brain expressions were affected by fasting and displayed periprandial changes, suggesting a role in both short- and long-term regulation of feeding. CCK intestinal expression decreased in fasted fish and displayed periprandial changes, suggesting CCK acts as a peripheral satiety factor. Leptin was not affected by fasting but displayed periprandial changes, suggesting a role as a short-term regulator. To examine if these peptides are affected by diet, brain and gut expressions were assessed in fish fed with different diets containing soy protein concentrate. Food intake, weight gain and expressions of orexin, CART, CCK and leptin were little affected by replacement of fish protein with soy protein, suggesting that pacu is able to tolerate and grow well with a diet rich in plant material. Overall, our results suggest that orexin, CART, CCK and leptin are involved in the physiology of feeding of pacu and that their expressions are little affected by plant-based diets.

1. Introduction

In fish (Hoskins and Volkoff, 2012a; Volkoff et al., 2005), as in mammals (Crespo et al., 2014; Suzuki et al., 2010), the regulation of food intake is achieved by appetite-regulating factors produced in the brain and peripheral organs. Appetite stimulators (or orexigenic factors) include orexins and inhibitors (or anorexigenic factors) include cocaine and amphetamine regulated transcript (CART), cholecystokinin (CCK) and leptin and ghrelin (Parker and Bloom, 2012; Volkoff et al., 2010).

Orexins have been identified in several fish species, including goldfish *Carassius auratus* (Hoskins et al., 2008), zebrafish *Danio rerio* (Novak et al., 2005), Atlantic cod *Gadus morhua* (Xu and Volkoff, 2007) and in fish from the Characiforme order, i.e. cavefish *Astyanax mexicanus* (Wall and Volkoff, 2013), red-bellied piranha *Pygocentrus nattereri* (Volkoff, 2014) and pirapitinga *Piaractus brachypomus* (Volkoff, 2015a). Similar to mammals (Fernø et al., 2015), orexins have been shown to act as appetite stimulators in fish, including goldfish (Facciolo et al., 2011; Hoskins et al., 2008; Matsuda et al., 2011; Nakamachi et al., 2006; Volkoff et al., 1999), cavefish (Penney and Volkoff, 2014), and zebrafish (Yokobori et al., 2011).

Multiple genes encoding CART variants have been identified in fish, including goldfish (Volkoff and Peter, 2001), common carp *Cyprinus carpio* (Wan et al., 2012), zebrafish (Nishio et al., 2012), Atlantic salmon *Salmo salar* (Valen et al., 2011), channel catfish *Ictalurus punctatus* (Kobayashi et al., 2008) and sole *Solea senegalensis* (Bonacic et al., 2015), as well as three Characins, i.e. cavefish (Wall and Volkoff, 2013), red-bellied piranha (Volkoff, 2014) and pirapitinga (Volkoff, 2015a). In several fish [e.g. goldfish (Volkoff and Peter, 2000), channel catfish (Kobayashi et al., 2008) and medaka *Oryzias latipes* (Murashita and Kurokawa, 2011)], CART acts as an anorexigenic factor.

In goldfish (Peyon et al., 1998), blunt snout bream *Megalobrama amblycephala* (Ji et al., 2015), channel catfish (Schroeter et al., 2015), Ya fish *Schizothorax prenanti* (Yuan et al., 2014), Atlantic cod (Tillner et al., 2013), as well as cavefish (Wall and Volkoff, 2013), and red-bellied piranha (Volkoff, 2014), CCK has been shown to act as an

* Corresponding author at: Department of Biology, Memorial University of Newfoundland, 232 Elizabeth Avenue, St. John's, NL A1B3X9, Canada.
E-mail address: hvolkoff@mun.ca (H. Volkoff).

http://dx.doi.org/10.1016/j.cbpa.2016.09.022
1095-6433/

anorexigenic/satiety factor and/or to induce the release of digestive enzymes.

In fish, leptin appears to act as an anorexigenic factor [reviewed in (Londraville et al., 2014) and (Gorissen and Flik, 2014)] and its expression and plasma levels are affected by nutritional status [e.g. in mandarin fish *Siniperca chuatsi* (Yuan et al., 2016), red-bellied piranha (Volkoff, 2015b), blunt snout bream (Xu et al., 2016), white-clouds mountain minnow *Tanichthys albonubes* (Chen et al., 2016), zebrafish (Gorissen and Flik, 2014), rainbow trout, *Oncorhynchus mykiss* (Salmeron et al., 2014), Arctic charr *Salvelinus alpinus* (Jorgensen et al., 2013), and fine flounder, *Paralichthys adspersus* (Fuentes et al., 2013)].

The pacu *Piaractus mesopotamicus* (Holmberg, 1887) belongs to the Serrasalmidae family (order Characiformes), which includes other "pacus" (e.g. pirapitinga and tambaqui, *Colossoma macropomum*) and piranhas (e.g. red-bellied piranha) (Orti et al., 2008; Ribeiro et al., 2014). It is an omnivorous (mainly herbivorous) fish considered a promising species for aquaculture due to its ease to handle, its rapid growth, its omnivore feeding habits making it adaptable to processed, low-cost feed, and its high-quality, richly flavored meat (Abimorad et al., 2007; Bicudo et al., 2010; Dairiki et al., 2010; de Oliveira et al., 2016; Jomori et al., 2005; Mesa-Granda and Botero-Aguirre, 2007; Queiroz et al., 2005).

Despite their economical importance and several studies on their growth and feeding habits, very few studies have examined the endocrine physiology of pacus, in particular with regards to the control of feeding. One study shows that intraperitoneal injections of recombinant human growth hormone to cross-bred tambacu fish (male pacu X female tambaqui cross) does not affect food intake although GH-induced increases in body weight are observed (Macari et al., 1994). Appetite-related proteins such as glucagon, insulin and somatostatin (de Lima et al., 1999) and gonadotropin releasing hormones (GnRHs) (Powell et al., 1997) have been isolated by HPLC in pacu, and a few transcripts for appetite regulators have recently been characterized for pirapitinga (Volkoff, 2015a) and for red-bellied piranha (Volkoff, 2014, 2015a).

Aquaculture feeds usually contain high levels of fishmeal, which are costly and endanger stocks of small species used for their production (Olsen and Hasan, 2012). Consequently, replacing fishmeal with plant protein sources might be crucial to developing sustainable aquaculture practices (Hardy, 2010; Naylor et al., 2000). Although all fish require overall similar amounts of dietary protein, herbivorous/omnivorous fish (such as pacu) might be better fitted to adapt to plant-based proteins than carnivorous fish (Naylor et al., 2000). Soybean products (in particular soybean protein concentrate, SPC) represent a good alternate source of protein as it is widely available and reasonably priced, and also contains relatively low levels of anti-nutrients, which might hinder nutrient assimilation (Gatlin et al., 2007; Hardy, 2010; NRC, 2011). Soybean has been one of the most studied alternative protein to fish meal [e.g. flounders (Li et al., 2015; Mamauag et al., 2011; Song et al., 2014; Ward et al., 2016), Atlantic halibut, *Hippoglossus hippoglossus* (Murray et al., 2010), Nile tilapia (Trosvik et al., 2013), red drum *Sciaenops ocellatus* and shortfin corvina *Cynoscion parvipinnis* (Minjarez-Osorio et al., 2016), red sea bream *Pagrus major* (Kader et al., 2012), giant grouper *Epinephelus lanceolatus* (Garcia-Ortega et al., 2016)]. However, there are very few published studies examining how plant-based diets might affect feeding and feeding-related hormones in fish [e.g. Atlantic cod (Tuziak et al., 2014), cobia *Rachycentron canadum* (Nguyen et al., 2013), salmon (Hevroy et al., 2008)]. Previous studies on juvenile pacu indicate that high dietary levels of soybean meal have little effect on weight gain and food intake (Stech and Carneiro, 2015) but there is no available data on the effects of SPC or food composition on appetite-related hormones in this species.

The aim of this study was to characterize appetite-related hormones (CART, orexin, CCK and leptin) in pacu by isolating cDNAs encoding for these peptides and examining their tissue distribution. To examine the possible role of these peptides in the regulation of feeding and energy status, brain and intestine mRNA expression levels of these peptides were compared between fed and fasted fish and at various time points around feeding time (periprandial changes). Moreover, to examine if these peptides are affected by diet, their brain and gut expression was assessed in fish fed with different diets with varying inclusions of plant (SPC) material.

2. Material and methods

2.1. Animals and experimental protocols

All animal experiments were set up in the Laboratório de Nutrição de Peixes of the Departamento de Zootecnia of the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) of the Universidade de São Paulo (USP) in Piracicaba (São Paulo, SP, Brazil, 22° 42′ 25″ S, 47° 38′ 29″ W). All experiments were carried out following protocols authorized by the ESALQ/USP ethics committee in accordance with the guidelines of the ethical principles in animal experimentation, adopted by the Colégio Brasileiro de Experimentação (COBEA).

2.1.1. Fasting experiment

Juvenile pacus [62.4 ± 2.4 g, TL (total length) 14.8 ± 0.3 cm] obtained from a local commercial supplier were randomly stocked in six indoor, 300-L polyethylene tanks (20 fish per tank), with water from a recirculation system and continuous aeration, a temperature of 26 °C, natural photoperiod (13 h light: 11 h dark, natural light from the outside). Sex could not be determined as these fish were juvenile [sexual maturity occurs in fish with total length exceeding 50 cm (Costa and Mateus, 2009)] and had indistinguishable gonads.

Fish were fed once daily (between 11:00 and 12:00) with a commercial diet (Mix Fish 40%, Agromix, Jaboticabal, SP, Brazil) till apparent satiation. A feeding frequency of one feeding a day was chosen, as this feeding regimen has been shown to be sufficient to induce growth in pacu (Dieterich et al., 2013) and made sampling at or around a single feeding time more accurate than if several feeding times had been used. Fish were acclimated under these standard conditions for two weeks before the start of the experiments.

After the acclimation period, three tanks continued to receive food and three tanks were fasted for seven days. Although pacus have previously been shown to be able to sustain longer fasting periods [e.g. 4 to 6 weeks (Gimbo et al., 2015; Souza et al., 2003)], a 7-day fasting period was chosen as it has been shown to induce changes in the expression of appetite regulators in other fish, without causing starvation (depletion of the fish reserves) (Bar, 2014). On the day of sample collection, the fed fish groups were fed at the regular feeding time and sampled 30 min post-feeding [to allow the presence of food in the stomach; digestion has not yet occurred as gastric evacuation times are long (Dias-Koberstein et al., 2005; Honorato et al., 2014)]. Four fish were then randomly sampled from all tanks (n = 12 fish per group). Fish were killed by overdose of alcoholic solution of benzocaine (250 mg/l), followed by spinal section, measured and weighed, and their brains and intestine sampled.

2.1.2. Periprandial study

Ninety-six juvenile pacus (67.2 ± 2.6 g, TL 15.1 ± 0.2 cm) were randomly stocked in 10 indoor, 300-L polyethylene tanks (9–10 fish per tank), with water at 26 °C from a recirculation system and continuous aeration, and under natural photoperiod (12 h light: 12 h dark). Fish were fed once daily (12:00) with a commercial diet (Mix Fish 40%, Agromix, Jaboticabal, SP, Brazil) till apparent satiation.

Fish were acclimated to these standard conditions for two weeks before the start of the experiment. On the day of the experiment, 4–5 fish were sampled from two random tanks one hour before feeding time (n = 8–10 per group, sampled from 2 different tanks). At feeding time, four tanks were fed and four tanks were not; 4–5 fish per tank were then sampled from two fed tanks and two unfed tanks at feeding