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Effect of fasting and feeding on growth, intestinal morphology and enteroendocrine cell density in *Rhamdia quelen* juveniles

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Abstract

This study was carried out to assess the effect of fasting and feeding on growth, intestinal morphology and density of cholecystokinin (CCK-) and neuropeptide Y (NPY-) immunoreactive cells in *Rhamdia quelen*. Fish were fed during 30 days with three commercial feeds containing different protein levels (T1 = 25%, T2 = 30% and T3 = 45%) while one group remained food deprived (T0). Our results show that the T3 group presented higher final mean weight and specific growth rate, while food-deprived group showed a significant weight loss. Histological analyses showed that the epithelial area of the intestine was significantly affected by fasting. Also, immunohistochemical analyses showed changes in enteroendocrine cells density, according to nutritional status. Cholecystokinin cell density was higher in T2 and T3 groups, while no differences in NPY cell density were observed between fed groups. Neuropeptide Y and CCK cell densities decreased in fasted group. Nevertheless, this group presented a higher NPY:CCK cell ratio (5:1) compared to fed groups (1–1.5:1), suggesting NPY acts as a peripheral orexigenic factor. These results show that the structure and endocrine functions of *R. quelen* intestine respond with a downregulation mechanism to endure long-term starvation.

KEYWORDS
cholecystokinin, immunohistochemistry, intestine, neuropeptide Y, neuropeptides, starvation

1 | INTRODUCTION

Several studies on fish nutrition were carried out in the last years, with particular attention to the mechanisms that regulate appetite (Narnaware & Peter, 2002; Rønnestad et al., 2017; Silverstein & Plysetskaya, 2000; Volkoff, 2016; Volkoff, Hoskins & Tuziak, 2010; Volkoff et al., 2005). The knowledge of actions and interactions of hormones and neurotransmitters that influence the energy balance and the control of body weight would optimize growth (Volkoff et al., 2010) and reduce production costs related to food (>50%) (Wong, Mo, Choi, Cheng & Man, 2016).

In fish, as in other vertebrates, the central nervous system controls food intake by integrating multiple neuronal and hormonal connections (Gorissen, Flik & Huising, 2006; Hoskins & Volkoff, 2012; Volkoff et al., 2005). In this complex multifactorial system, there are interactions between peripheral signals (nerve, endocrine and metabolic) mainly from the gastrointestinal tract (GIT) and central responses (hypothalamus) that allow perceiving the energy state of the body and regulating food intake (Rønnestad et al., 2017; Volkoff, 2006, 2016). The GIT and hypothalamus are considered the main production sites for appetite regulating neuropeptides (Hoskins & Volkoff, 2012; Rønnestad et al., 2017). In addition, most neuropeptides produced in the GIT and released in response to feeding affect motility, secretion, absorption, immunity and play a role in the control of food intake (Rønnestad et al., 2017; Volkoff, 2006). Cholecystokinin (CCK) and neuropeptide Y (NPY) are neuropeptides that

influence the digestive processes and play a role in the gut–brain control of food intake by anorexigenic and orexigenic signals respectively (Volkoff, 2006).

Cholecystokinin, after being secreted by the presence of food in the GIT, intervenes on digestive processes and acts as a peripheral signal of satiety (MacDonald & Volkoff, 2009; Murashita, Fukada, Ronnestad, Kurokawa & Masumoto, 2008; Peterson et al., 2012; Rubio, Sanchez-Vazquez & Madrid, 2008). Several studies determined that food intake is suppressed by intracerebroventricular (ICV), intraperitoneal (IP) (Himick & Peter, 1994; Volkoff, Eykelbosh & Peter, 2003) or oral administration of CCK (Rubio et al., 2008). In addition, CCK mRNA expression patterns decrease significantly during fasting (Koven & Schulte, 2012; MacDonald & Volkoff, 2009).

Neuropeptide Y is a potent, highly conserved, multifunctional peptide among vertebrates, including fish. It has an important role in feeding behaviour regulation, energy metabolism and digestive processes (MacDonald & Volkoff, 2009; Volkoff, 2006; Volkoff et al., 2005; Zhou et al., 2013). Intracerebroventricular, IP or oral administration of NPY stimulates food intake (Himick & Peter, 1994; Kiris, Kumlu & Dikel, 2007; Volkoff et al., 2003). Previous studies have shown that hypothalamic NPY mRNA expression may increase during fasting (Narnaware & Peter, 2001; Narnaware, Peyon, Lin & Peter, 2000; Silverstein, Breninger, Baskin & Plisetskaya, 1998), decrease during feeding (Narnaware & Peter, 2001) or be specifically influenced by macronutrients of the food (Narnaware & Peter, 2002).

Furthermore, it is known that during fasting or feeding the GIT may experience profound morphological and cellular changes (Buddington, Krogdhal & Bakke-McKellep, 1997; Krogdahl & Bakke-McKellep, 2005; Wang, Hung & Randall, 2006). Several studies showed that fasted fish present significant reduction in number of enterocytes, absorptive area (Emadi Shaibani, Mojazi Amiri & Khodabandeh, 2013) and changes in goblet cell density (Zeng et al., 2012). However, few studies evaluated the influence of different diets or starvation on the enteroendocrine cell density (Burkhardt-Holm & Holmgren, 1989; Pereira et al., 2017; Weng & Fang, 2011). In addition, in terms of stimulus or depression of feeding behaviour, the signalling between intestinal hormones and the brain is complex and still not fully understood in fish (Koven & Schulte, 2012). Possibly this is due to the wide structural, morphological and functional diversity of fish GIT, which responds dynamically to different foods or starvation periods (Buddington & Krogdahl, 2004; Hoskins & Volkoff, 2012; Wilson & Castro, 2010).

As the digestive system plays a fundamental role in fish growth, the present study assessed the effect of fasting and feeding with different diets on growth, intestinal morphology and number of CCK and NPY hormone-secreting cells in *Rhamdia quelen* (silver catfish) juveniles to obtain a better understanding of the enteroendocrine signals.

2 | MATERIALS AND METHODS

2.1 | Fish and culture conditions

The experiment was conducted in the Northeast Institute of Ichthyology (INICNE), School of Veterinary Sciences (Corrientes, Argentina). In all, 60 silver catfish (weight 1.86 ± 0.20 g) were placed in a 150 L tank, acclimated to the laboratory conditions for 10 days and fed with commercial food (Kilomax® 28% crude protein).

Water parameters were checked daily (temperature, pH and dissolved oxygen). These parameters remained stable throughout the acclimation and experimental periods. The water temperature was maintained at 24.2 ± 0.22°C, pH at 7.03 ± 0.02 and dissolved oxygen at 6.68 ± 0.4 mg/L.

The procedures adopted with the animals in this research were in accordance with the ethical principles of animal experimentation, and approved according to protocol N° 0001/14-2011-02827 by the Ethics and Biosafety Committee of School of Veterinary Sciences of the Northeast National University (UNNE) of Argentina.

2.2 | Diets and experimental design

After the acclimation period, fish were randomly divided into 60 5-L cages (1 fish/cage) distributed into four experimental groups, three of which were fed with commercial diet containing different protein levels: T1 = 25%, T2 = 30% and T3 = 45% crude protein, while T0 group was food deprived. The protein level used in the T3 group is the recommended level for this fish size, while the T1 group and T2 group levels are considered insufficient to maximize growth (Meyer & Fracalossi, 2004; Salhi, Bessonart, Chediak, Bellagamba & Carnevia, 2004). The experimental design resulted in 15 replicates for each treatment (n = 60). Groups that received the commercial diets were fed until apparent satiation once a day (06:00 p.m.) for 30 days. All cages were cleaned 30 min after feeding via siphoning to remove wastes (remains of food and faeces) and 25% of water volume was replaced.

2.3 | Sample collection and analytical methods

2.3.1 | Growth performance

The fish were weighed on days 0, 10, 20 and 30 of the experimental period. The growth performance was calculated as follows: weight (W) and specific growth rate (SGR) = 100 × (ln final weight − ln initial weight)/days of experiment.

2.4 | Histological and immunohistochemical procedures

At the end of the experimental period, fish were euthanized by an overdose of anaesthetic (benzocaine 100 ppm), followed by severance of the spinal cord. Samples of the anterior intestine were sectioned and preserved in Bouin's solution (12 hr) and embedded in paraffin wax. Microtome sections (1–3 μm thick) were collected on slides pretreated with silane (3-amino-propyltriethoxysilane; Sigma Chemical, St Louis, MO, USA), allowed to dry overnight and then dewaxed and hydrated. Samples were routinely stained with haematoxylin and eosin (H&E). Immunostaining was performed with

anti-cholecystokinin 8-Azide free (CCK, Abcam™ Labs, ab27441) and anti-NPY (Abcam™ Labs, ab30914) primary rabbit polyclonal antibodies.

For immunostaining, all incubations were performed at room temperature in a humid chamber, except the primary antibodies, and all washing procedures consisted of three successive 5 min immersions in 0.1 M phosphate-buffered saline (PBS; 8 mM Na_2HPO_4, 3 mM NaH_2PO_4, 150 mM NaCl). Endogenous peroxidase activity was inhibited through immersion in a peroxidase blocking solution (3% H_2O_2 in PBS) for 30 min and then rinsed with PBS. After that, sections were treated with 3% skim milk powder for 15 min to prevent non-specific antibody binding. After a second PBS rinse, tissue sections were incubated with CCK (1:250) and NPY (1:250) overnight in a humidified chamber at 4°C and then washed with PBS. Later, a 30 min incubation at room temperature with super enhancer (Super Sensitive™ Link Detection System, BioGenex, CA) and another 30 min incubation with polymer-HRP (Super Sensitive™ Label HRP Detection System, BioGenex, CA) label followed. Immunostaining was finally developed with Vector Vip (Vector Laboratories, CA), immersed in de-ionized water to stop the reaction, dehydrated and coverslipped. In each series of stained sections, positive and negative controls were included to assess the specificity of the assay. Negative control slides were sections in which the primary antibody was replaced by negative reagent control (normal mouse Ig in PBS).

Histological analyses were performed to evaluate the following morphological features of the anterior intestine: (i) epithelial area (EA) in mm^2, (ii) total number of endocrine cells (ECs) for each antibody, manually counted and (iii) NPY:CCK cell ratio. All measurements were performed using a Leica DM500 microscope, a Leica ICC50 digital camera and the morphometric analysis software LAS (Leica Application Suite, version 3.4.1).

These parameters were obtained from independent transverse sections of the anterior intestine of 15 fish per treatment and reported as average values with standard error from 500 μm of intestinal perimeter (Hall & Bellwood, 1995). This methodology standardizes the measure of the EA and provides useful information on the increase or decrease in the internal surface of the intestine and its relation with the ECs density, independently of intestine diameter.

2.5 | Statistical analysis

Results obtained from different variables were analysed according to alternative statistical models.

For histological and immunohistochemical data obtained from each fish at the end of the experiment, a one-way ANOVA was applied and the significance level was set at $p < .05$. When significant effects were detected, the means were compared using Tukey's test.

General linear mixed models were fitted to each of two response variables, weight and SGR, assuming a normal conditional distribution of the response. For weight, the linear predictor included the fixed effects of time (0, 10, 20 and 30 days), diet (T0, T1, T2 and T3 treatments) and their two-way interaction, in addition to the random effects of fish/cage to properly recognize experimental units for each of the factors. For SGR, the linear predictor included the fixed effects of time (0–10, 10–20 and 20–30 day intervalues), diet (T0, T1, T2 and T3 treatments) and their two-way interaction, in addition to the random effects of fish/cage to properly recognize experimental units for each of the factors. Model assumptions were evaluated using externally studentized residuals and were considered to be reasonably met. Variance components were estimated using restricted maximum likelihood. Kenward–Roger's method was used to estimate degrees of freedom and make the corresponding adjustments in estimation of standard errors. The model was fitted using the Infostat software (version 2016). Estimated least square means and corresponding estimated standard errors are presented. Relevant pairwise comparisons were conducted using Tukey–Kramer or Bonferroni adjustments, as appropriate in each case, to avoid inflation of Type I error rate due to multiple comparisons.

3 | RESULTS

3.1 | Growth performance

No mortality was observed throughout the 30-day feeding trial.

In fed groups, differences in growth during the study were observed. Differences in weight appeared on the 10th day of the experiment, being affected by the increase in the protein level of the diet. This trend remained until the end of the feeding period, where the highest weight was observed in fish-fed diets containing 45% protein (T3, $W = 3.97 \pm 0.21$ g), differing from fish-fed diets with 25% and 30% protein (T1 and T2, $W = 3.04 \pm 0.15$ and 3.12 ± 0.15 g respectively) ($p < .05$). Fasted fish presented weight loss, ending the experiment with the lowest values ($W = 1.68 \pm 0.11$ g) ($p < .05$) (Figure 1).

Similarly, at the end of the feeding period, the highest SGR was observed in the T3 group ($1.35 \pm 0.05\%$), whereas the T0 group presented negative values ($-0.71 \pm 0.08\%$) ($p < .05$) (Figure 1).

3.2 | Histological and immunohistochemical analysis

The morphology of the intestinal mucosa of all evaluated groups showed regular shape in the analysed intestinal portion. However, fasted fish showed the lowest EA value (0.05 ± 0.02 mm^2) ($p < .05$), while fish from the T3 group reached the highest value (0.18 ± 0.03 mm^2) ($p < .05$). Fish from the T1 and T2 groups did not present statistical differences between them ($p > .05$) (Figure 2).

Cholecystokinin- and NPY-immunoreactive (IR) cells were observed in the epithelium of the anterior intestine of fish from all experimental groups (Figure 3a). After 30 days of starvation, the enteroendocrine cell density was significantly affected by fasting, with the T0 group presenting the lowest mean values of CCK-IR cell density, while T3 was the group with the highest cell density ($p < .05$) (Figure 3b).

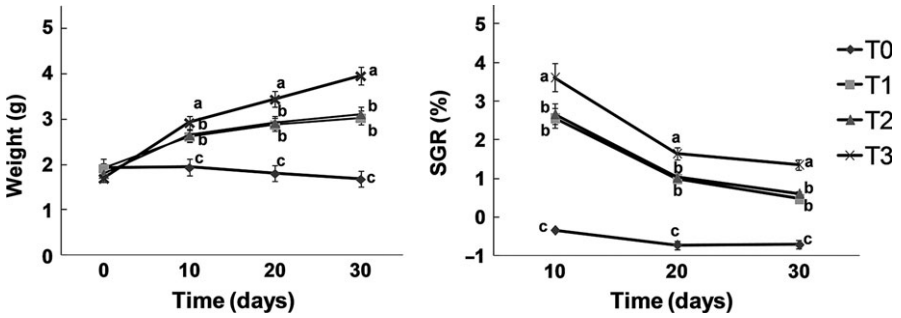

FIGURE 1 Growth of *Rhamdia quelen* juvenile fed with different diets or fasted for 30 days. Values are mean standard error of 15 replicates per feeding group. Points within the same day having different superscripts are significantly different ($p < .05$)

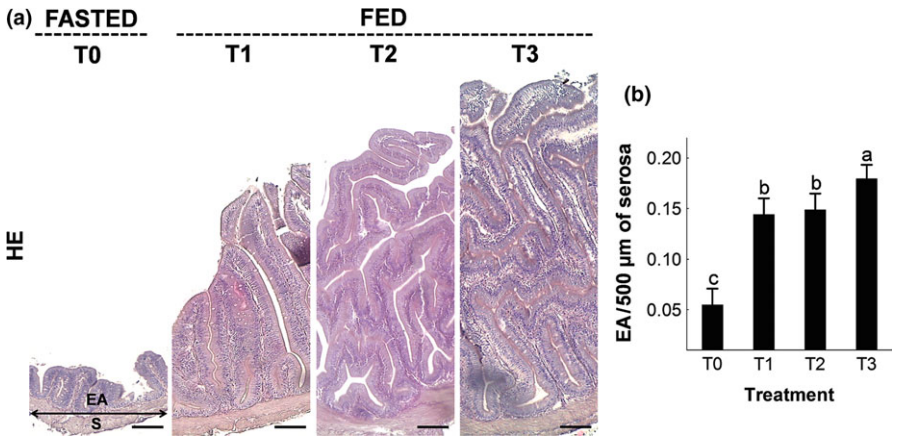

FIGURE 2 Cross-sectional anterior intestine corresponding to samples from *Rhamdia quelen* juvenile fasted or fed for 30 days. (a) Note the clear variation in epithelial area (EA) between fasting and fed groups. Scale bars = 100 μm. (b) Changes in the EA of *R. quelen* responding to fasting or feeding conditions. Different letters above the bars indicate significant differences between experimental groups ($p < .05$). S: serosa

Neuropeptide Y-IR cells showed a similar behaviour, where the T0 group presented lower values than the T2 and T3 groups ($p < .05$) (Figure 3b).

The highest NPY:CCK density cell ratio was found in the fasted group (5:1) ($p < .05$). However, no significant differences were found between fed groups, showing a ratio of 1.5:1 for T1 and 1:1 for T2 and T3 groups ($p > .05$) (Figure 3b).

4 | DISCUSSION

In aquaculture, an adequate feeding, considering both quantity and quality of food, is an indispensable factor to ensure healthy growth and development of fish (National Research Council, 2011). Our results showed that the T3 group obtained the highest weight values throughout the experiment. Considering that the protein of the diet is an essential factor for fish performance, the highest growth observed in the T3 group could be partially explained by the high dietary protein level (45%). In agreement with our results, previous studies in *R. quelen* juveniles showed that best growth is obtained with protein levels above 37% (dry basis) (Meyer & Fracalossi, 2004; Salhi et al., 2004). In addition, the lower growth in the T1 and T2 groups compared to the T3 group could be related to a low utilization of non-protein energy as recently demonstrated by Gominho-Rosa et al. (2015) and Moro, Camilo, Moraes and Fracalossi (2010) in *R. quelen* juveniles.

In the present study, significant weight loss and negative specific growth rates in fish kept in fasting for 30 days were observed (T0). Different studies determined that fish can endure long periods in fasting although suffering important body mass losses related to the duration of the fasting (Emadi Shaibani et al., 2013; Krogdahl & Bakke-McKellep, 2005; Ostaszewska, Korwin-Kossakowski & Wolnicki, 2006; Zeng et al., 2012). This fact, commonly observed in nature, is considered to be a survival process for many species of fish in situations of food deprivation (negative energy period), using different endocrine responses to prolong energy homeostasis (Silverstein & Plysetskaya, 2000; Wang et al., 2006; Won & Borski, 2013). During these particular metabolic states, it is known that ghrelin, NPY and growth hormone (GH) are involved in lipolytic catabolism to direct the energy of endogenous reserves to maintain basal metabolic processes instead of growth (Bergan, Kittilson & Sheridan, 2015; Moro et al., 2010; Rodrigues, Gominho-Rosa, Cargnin-Ferreira, de Francisco & Fracalossi, 2012; Won & Borski, 2013). This fact was particularly reported in *R. quelen* juveniles subjected to fasting, in

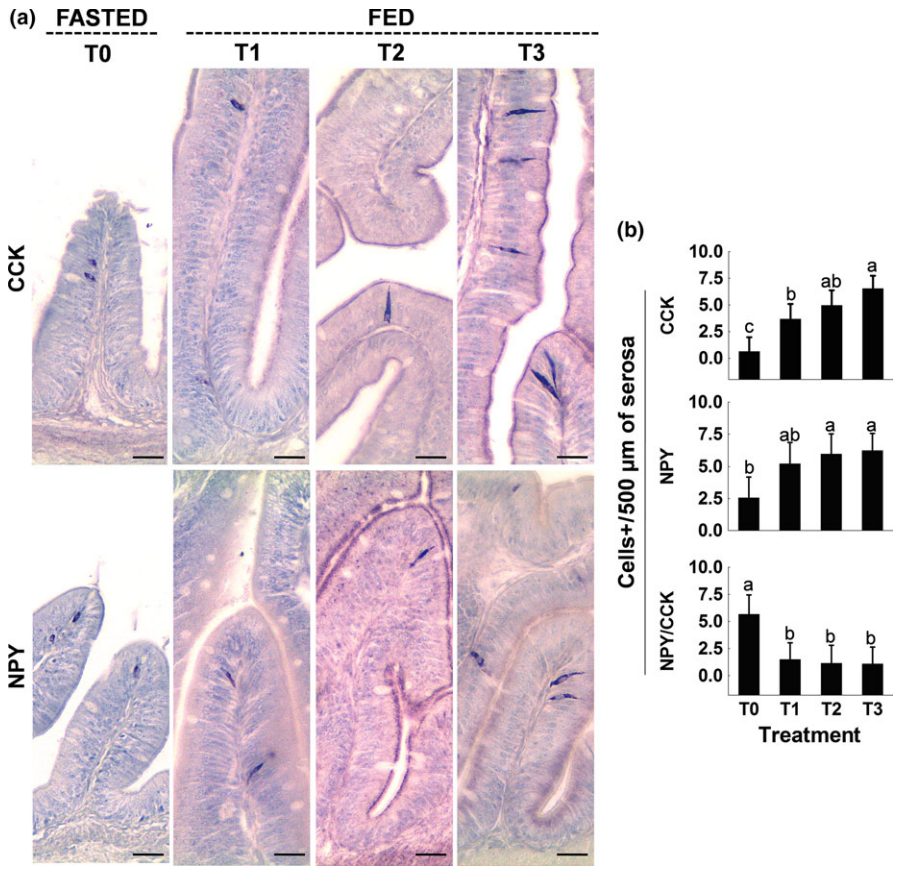

FIGURE 3 (a) Comparative photomicrographs of cholecystokinin (CCK)- and neuropeptide Y (NPY)-IR neuroendocrine cells in *Rhamdia quelen* juvenile fasted or fed for 30 days. Scale bars = 20 μm. (b) Mean number of neuroendocrine cells immunoreactive to each antiserum employed of 15 replicates per group. Different letters above the bars indicate significant differences between experimental groups ($p < .05$)

which a significant increase in GH expression and decrease in liver glycogen, glucose and triglycerides to maintain constant plasma glucose and amino acids levels was observed (Barcellos, Marqueze, Trapp, Quevedo & Ferreira, 2010; Menezes et al., 2015).

Our results showed that the histological characteristics of the anterior intestine of *R. quelen* juveniles were influenced by fasting or feeding. The lowest EA values observed in the anterior intestine of fish subjected to fasting (T0) probably are related to energy-saving mechanisms during starvation, considering that the GIT has a high resting metabolic rate (Buddington & Krogdahl, 2004; Hoskins & Volkoff, 2012; Wang et al., 2006). In this sense, different studies suggest that during fasting, the GIT is the main physiological system affected, showing rapid morphological changes, such as intestinal shortening, degeneration, atrophy and proteolysis of the intestinal mucosa, among others (Emadi Shaibani et al., 2013; Wang et al., 2006; Zeng et al., 2012). Likewise, we observed that *R. quelen* maintained for 30 days under fasting reduced up to 70% of the EA of the anterior intestine, accompanied by a reduction of 15% of body weight. This higher reduction of the EA, in relation to the body weight, would correspond to a decrease in the energy expenditure of the intestinal mucosa in absence of digestive processes, being also able to serve as a protein contribution to the body metabolism (Buddington et al., 1997; Jobling, 1994; Krogdahl & Bakke-McKellep, 2005; Wang et al., 2006). In fact, this observation is consistent with previous studies where it was demonstrated that fish under fasting for a short period of time or with an inappropriate nutrition reduce the intestinal mucosal area and enzymatic activity between 20% and 75% (Hayes & Volkoff, 2014; Krogdahl & Bakke-McKellep, 2005; Xu, Li, Tian, Jiang & Liu, 2016). Similar results were reported in fasted *Salmo trutta caspius*, in which a decrease in the number and height of epithelial cells was observed, resulting in the reduction of villi and EA (Emadi Shaibani et al., 2013). In fasted Atlantic salmon (*Salmo salar*), a reduction of the intestinal mucosa during the first 2 days of fasting was observed, with a marked mobilization of the intestinal protein resources for systemic use (Krogdahl & Bakke-McKellep, 2005).

In the present study, a higher number of CCK-IR cells per EA were observed in *R. quelen* juveniles fed with the diet containing the highest protein level (T3), while the lowest values corresponded to fasted group (T0). However, it was observed that fish fed with the lower protein level (T1) presented low density of CCK-IR cells. Similar to our results, fasted Nile tilapia (*Oreochromis niloticus*) presented lower density of CCK-8$^+$ cells compared to fed ones (Pereira et al., 2017) and high concentrations of fish meal in the diet have a more

potent effect on the stimulation of CCK synthesis and digestive enzymes secretion than other protein sources in yellowtail (*Seriola quinqueradiata*) Furutani, Masumoto and Fukada (2012). This demonstrates that the source or dietary protein level can positively influence CCK synthesis and promote digestive activities such as pancreatic activity, fluid secretion and intestinal motility (Gribble, 2012; Murashita et al., 2008). In previous studies, we observed that *R. quelen* presents a high density of CCK-IR cells in the anterior intestine, being considered as a primary organ for CCK synthesis (Hernández et al., 2012) and important for nutrient digestion and absorption (Hernández, Pérez Gianeselli & Domitrovic, 2009). This suggests that CCK would be one of the main factors involved in the regulation of digestive processes in *R. quelen* juveniles, being stimulated by the protein content of the food. On the other hand, studies in *Pseudopleuronectes americanus* (MacDonald & Volkoff, 2009), *Ctenopharyngodon idellus* (Feng et al., 2012), *Tautogolabrus adspersus* (Babichuk & Volkoff, 2013; Hayes & Volkoff, 2014) and *Megalobrama amblycephala* (Ji et al., 2015) showed that mRNA levels of CCK expression in the intestine decreased during fasting. Similarly, the lowest density of CCK-IR cells and the lowest EA were observed in fasting *R. quelen* juveniles, suggesting that the lack of direct stimulation of the food in the intestinal lumen might affect concomitantly both variables (Krogdahl & Bakke-McKellep, 2005; Murashita et al., 2008), to conserve energy (Babichuk & Volkoff, 2013). However, this may not be the pattern observed in all fish because in *Poecilia reticulata* and *Leuciscus idus melanotus* the enteroendocrine CCK cell density was not altered after 8 weeks of fasting (Burkhardt-Holm & Holmgren, 1989).

Neuropeptide Y and related peptides carry out multiple physiological actions in the organism by interacting with a system of receptors called *Y* (Cerda-Reverter & Larhammar, 2000). In particular, NPY is distributed in the fish brain and exerts a central role in the regulation of appetite through the *Y1* receptor signalling pathway (Matsuda, Sakashita, Yokobori & Azuma, 2012; Volkoff, 2016). Several studies have shown that NPY transcription in the brain increases during fasting and is depressed with feeding (Ji et al., 2015; Leibowitz, 1995; Narnaware & Peter, 2001, 2002). The GIT is the largest producer organ of peripheral NPY, with distribution on all layers. In mammals, it has a greater distribution in the neurons of the submucous and myenteric plexuses (Bär et al., 2014; Gomez, Englander & Greeley, 2012), whereas in fish the greater distribution is observed in the enteroendocrine cells of the first intestinal portions such as reported in pejerrey, *Odontesthes bonariensis* (Vigliano et al., 2011), *R. quelen* (Hernández et al., 2012), dorado, *Salminus brasiliensis* (Pereira et al., 2015), milkfish, *Chanos chanos* (Lin, Wang, Ou, Li & Wen, 2017) and Nile tilapia (*O. niloticus*) (Pereira et al., 2017). In the GIT, NPY exerts mainly inhibitory effects on secretion, intestinal motility and blood flow (Gomez et al., 2012; Lundberg, Terenius, Hokfelt & Goldstein, 1983; Shahbazi, Holmgren, Larhammar & Jensen, 2002; Uesaka, Yano, Sugimoto & Ando, 1996) and induces immune activation or suppression (Carpio, León, Acosta, Morales & Estrada, 2007; Farzi, Reichmann & Holzer, 2015). In addition, in mammals a gut–brain peripheral signalling mechanism has been established (Holzer, Reichmann & Farzi, 2012), whereas in fish the peripheral signalling has not been extensively studied yet. In this sense, in a fasting-refeeding trial performed in blunt snout bream (*M. amblycephala*), no differences in NPY intestinal expression were found, whereas the brain expression levels increased after fasting and decreased with refeeding (Ji et al., 2015). These authors suggest that central NPY would function as an orexigenic factor, whereas intestinal NPY would not act as a brain–gut peptide to stimulate appetite. Likewise, in *P. reticulata*, *L. idus melanotus* (Burkhardt-Holm & Holmgren, 1989) and *Epinephelus coioides* (Weng & Fang, 2011) under fasting treatment, no differences in NPY-IR cell density in the intestine were found, as well as in *R. quelen*-fed diets containing different percentages of *Aloysia triphylla* essential oil (Zeppenfeld et al., 2016). However, Pereira et al. (2017) observed that the number of NPY enteroendocrine cells was higher in 24 hr-fasted Nile tilapia (*O. niloticus*) compared to the fed group. In contrast, in our study, fish kept in fasting for 30 days presented lower density of NPY-IR cells. However, this group showed higher NPY/CCK cell ratio (approximately 5:1) than fed groups (1–1.5:1). These results suggest different possibilities. Firstly, if the differences reported between the different studies would be more related to differences between species or type and duration of the experiments, then intestinal NPY would not have a role as a peripheral appetite stimulatory factor, as proposed by Ji et al. (2015). Secondly, considering that the number of NPY-IR cells shows a differential behaviour than observed for CCK-IR cells, it could be speculated that NPY-IR cell number would not be affected during starvation, maintaining a relative high density to conserve the peripheral signal of appetite (Billington, Briggs, Grace & Levine, 1991; Burkhardt-Holm & Holmgren, 1989; Zhou et al., 2013). Besides, Silverstein et al. (1998) and Imai et al. (2007) proposed that during fasting, NPY and insulin would be involved in a strong negative association mechanism to control or decrease energy expenditure of the organism. In addition, evidence obtained from NPY pharmacological studies demonstrated an appetite stimulating effect with immersions of *Clarias gariepinus* larvae (Carpio et al., 2007), as well as oral administration of NPY in *O. niloticus* (Kiris et al., 2007). Considering that NPY can cross the blood–brain barrier by diffusion (Kastin & Akerstrom, 1999; Silverstein & Plysetskaya, 2000), the appetite stimulating action would be determined by the functional relationship with the stimulated NPY receptor rather than by the secretion site (central or peripheral) (Cerda-Reverter & Larhammar, 2000; Larhammar & Salaneck, 2004; Zhou et al., 2013). In this sense, it can be considered that the marked difference in the NPY/CCK cell rate found between the fasting group compared to the other groups (about 5:1 vs. 1–1.5:1) would be a consequence of an unequal differentiation process that occurred between the two cell types during the 30 days of fasting. During this period, the epithelium can be renewed more than once (Buddington et al., 1997), showing that CCK is significantly affected during fasting. On the other hand, NPY maintained a high relative density during the fasting period, evidencing the importance of this neuropeptide in a context of reduction of epithelial area and energy expenditure. Consequently, it is conceivable, but not conclusive, that the

differentiation of neuropeptides producing cells, related to food intake, would remain relatively high, in detriment of others cells related to satiety, contributing to fish response against starvation. According to Vigliano et al. (2011), a high number of NPY cells in the gut may indicate that the region would act as a primary source of peripheral signals to stimulate food intake in absence of food in this site. This indicates that regulation of fish feeding is the result of the integration of various endocrine responses, and fish-and-species-specific mechanisms of agonism and antagonism may exist (Gorissen et al., 2006; Volkoff, 2016), requiring more studies for a better understanding of NPY's role in gut–brain signalling mechanisms.

To summarize, our results show that fish fasted or fed with low protein levels were significantly affected on growth, intestinal morphology and endocrine functions, with a decrease in the CCK- and NPY-IR cell density.

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REFERENCES

Babichuk, N. A., & Volkoff, H. (2013). Changes in expression of appetite-regulating hormones in the cunner (*Tautogolabrus adspersus*) during short-term fasting and winter torpor. *Physiology & Behavior*, *120*, 54–63. https://doi.org/10.1016/j.physbeh.2013.06.022

Bär, F., Foh, B., Pagel, R., Schroder, T., Schlichting, H., Hirose, M., … Sina, C. (2014). Carboxypeptidase E modulates intestinal immune homeostasis and protects against experimental colitis in mice. *PLoS One*, *9*, 1–9. https://doi.org/10.1371/journal.pone.0102347

Barcellos, L. J. G., Marqueze, A., Trapp, M., Quevedo, R. M., & Ferreira, D. (2010). The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundiá *Rhamdia quelen*. *Aquaculture*, *300*, 231–236. https://doi.org/10.1016/j.aquaculture.2010.01.013

Bergan, H. E., Kittilson, J. D., & Sheridan, M. A. (2015). Nutritional state modulates growth hormone-stimulated lipolysis. *General and Comparative Endocrinology*, *217–218*, 1–9. https://doi.org/10.1016/j.ygcen.2015.04.017

Billington, C. J., Briggs, J. E., Grace, M., & Levine, A. S. (1991). Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. *American Journal of Physiology*, *260*, R321–R327. https://doi.org/10.1152/ajpregu.1991.260.2.R321

Buddington, R. K., & Krogdahl, A. (2004). Hormonal regulation of the fish gastrointestinal tract. *Comparative Biochemistry and Physiology, Part A*, *139*, 261–271. https://doi.org/10.1016/j.cbpb.2004.09.007

Buddington, R. K., Krogdhal, A., & Bakke-McKellep, A. M. (1997). The intestines of carnivorous fish: Structure and functions and the relations with diet. *Acta Physiologica Scandinavica Supplementum*, *638*, 67–80.

Burkhardt-Holm, P., & Holmgren, S. (1989). A comparative study of neuropeptides in the intestine of two stomachless teleosts (*Poecilia reticulata*, *Leuciscus idus melanotus*) under conditions of feeding and starvation. *Cell and Tissue Research*, *255*, 245–254.

Carpio, Y., León, K., Acosta, J., Morales, R., & Estrada, M. (2007). Recombinant tilapia Neuropeptide Y promotes growth and antioxidant defenses in African catfish (*Clarias gariepinus*) fry. *Aquaculture*, *272*, 649–655. https://doi.org/10.1016/j.aquaculture.2007.08.024

Cerda-Reverter, J. M., & Larhammar, D. (2000). Neuropeptide Y family of peptides: Structure, anatomical expression, function, and molecular evolution. *Biochemistry and Cell Biology*, *78*, 371–392. https://doi.org/10.1139/o00-004

Emadi Shaibani, M., Mojazi Amiri, B., & Khodabandeh, S. (2013). Starvation and refeeding effects on pyloric caeca structure of Caspian salmon (*Salmo trutta caspius*, Kessler 1877) juvenile. *Tissue and Cell*, *45*, 204–210. https://doi.org/10.1016/j.tice.2013.01.001

Farzi, A., Reichmann, F., & Holzer, P. (2015). The homeostatic role of neuropeptide Y in immune function and its impact on mood and behaviour. *Acta Physiologica*, *213*, 603–627. https://doi.org/10.1111/apha.12445

Feng, K., Zhang, G. R., Wei, K. J., Xiong, B. X., Liang, T., & Ping, H. C. (2012). Molecular characterization of cholecystokinin in grass carp (*Ctenopharyngodon idellus*): Cloning, localization, developmental profile, and effect of fasting and refeeding on expression in the brain and intestine. *Fish Physiology and Biochemistry*, *38*, 1825–1834. https://doi.org/10.1007/s10695-012-9679-0

Furutani, T., Masumoto, T., & Fukada, H. (2012). Response of cholecystokinin and digestive enzyme mRNA levels to various feed ingredients in yellowtail *Seriola quinqueradiata*. *Fisheries Science*, *78*, 1075–1082. https://doi.org/10.1007/s12562-012-0537-x

Gomez, G. A., Englander, E. W., & Greeley, G. H. (2012). Postpyloric gastrointestinal peptides. In L. R. Johnson (Ed.), *Physiology of the Gastrointestinal Tract* (5th edn, pp. 155–198). New York, NY: Elsevier Inc. https://doi.org/10.1016/b978-0-12-382026-6.00007-5

Gominho-Rosa, M., Rodrigues, A., Mattioni, B., de Francisco, A., Moraes, G., & Fracalossi, D. (2015). Comparison between the omnivorous jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) on the utilization of dietary starch sources: Digestibility, enzyme activity and starch microstructure. *Aquaculture*, *435*, 92–99. https://doi.org/10.1016/j.aquaculture.2014.09.035

Gorissen, M., Flik, G., & Huising, M. (2006). Peptides and proteins regulating food intake: A comparative view. *Animal Biology*, *56*, 447–473. https://doi.org/10.1163/157075606778967829

Gribble, F. M. (2012). The gut endocrine system as a coordinator of postprandial nutrient homoeostasis. *Proceedings of the Nutrition Society*, *71*, 456–462. https://doi.org/10.1017/s0029665112000705

Hall, K. C., & Bellwood, D. R. (1995). Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. *Journal of Fish Biology*, *47*, 438–454. https://doi.org/10.1111/j.1095-8649.1995.tb01913.x

Hayes, J., & Volkoff, H. (2014). Characterization of the endocrine, digestive and morphological adjustments of the intestine in response to food deprivation and torpor in cunner, *Tautogolabrus adspersus*. *Comparative Biochemistry and Physiology, Part A*, *170*, 46–59. https://doi.org/10.1016/j.cbpa.2014.01.014

Hernández, D. R., Pérez Gianeselli, M., & Domitrovic, H. A. (2009). Morphology, histology and histochemistry of the digestive system of South American catfish (*Rhamdia quelen*). *International Journal of Morphology*, *27*, 105–111. https://doi.org/10.4067/S0717-95022009000100019

Hernández, D. R., Vigliano, F. A., Sánchez, S., Bermúdez, R., Domitrovic, H. A., & Quiroga, M. I. (2012). Neuroendocrine system of the digestive tract in *Rhamdia quelen* juvenile: An immunohistochemical study. *Tissue and Cell*, *44*, 220–226. https://doi.org/10.1016/j.tice.2012.03.005

Himick, B. A., & Peter, R. E. (1994). CCK/gastrin-like immunoreactivity in brain and gut, and CCK suppression of feeding in goldfish. *American Journal of Physiology*, *267*, R841–R851. https://doi.org/10.1152/ajpregu.1994.267.3.R841

Holzer, P., Reichmann, F., & Farzi, A. (2012). Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut–brain axis. *Neuropeptides*, *46*, 261–274. https://doi.org/10.1016/j.npep.2012.08.005

Hoskins, L. J., & Volkoff, H. (2012). The comparative endocrinology of feeding in fish: Insights and challenges. *General and Comparative Endocrinology*, *176*, 327–335. https://doi.org/10.1016/j.ygcen.2011.12.025

Imai, Y., Patel, H. R., Hawkins, E. J., Doliba, N. M., Matschinsky, F. M., & Ahima, R. S. (2007). Insulin secretion is increased in pancreatic islets of neuropeptide Y-deficient mice. *Endocrinology*, *148*, 5716–5723. https://doi.org/10.1210/en.2007-0404

Ji, W., Ping, H. C., Wei, K. J., Zhang, G. R., Shi, Z. C., & Yang, R. B., . . . Wang, W. M. (2015). Ghrelin, neuropeptide Y (NPY) and cholecystokinin (CCK) in blunt snout bream (*Megalobrama amblycephala*): cDNA cloning, tissue distribution and mRNA expression changes responding to fasting and refeeding. *General and Comparative Endocrinology*, *223*, 108–119. https://doi.org/10.1016/j.ygcen.2015.08.009

Jobling, M. (1994). *Fish bioenergetics* (p. 309). London, UK: Chapman & Hall.

Kastin, A. J., & Akerstrom, V. (1999). Nonsaturable entry of neuropeptide Y into the brain. *American Journal of Physiology*, *276*, E479–E482.

Kiris, G. A., Kumlu, M., & Dikel, S. (2007). Stimulatory effects of neuropeptide Y on food intake and growth of *Oreochromis niloticus*. *Aquaculture*, *264*, 383–389. https://doi.org/10.1016/j.aquaculture.2006.12.004

Koven, W., & Schulte, P. (2012). The effect of fasting and refeeding on mRNA expression of PepT1 and gastrointestinal hormones regulating digestion and food intake in zebrafish (*Danio rerio*). *Fish Physiology and Biochemistry*, *38*, 1565–1575. https://doi.org/10.1007/s10695-012-9649-6

Krogdahl, A., & Bakke-McKellep, A. M. (2005). Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology*, *Part A*, *141*, 450–460. https://doi.org/10.1016/j.cbpb.2005.06.002

Larhammar, D., & Salaneck, E. (2004). Molecular evolution of NPY receptor subtypes. *Neuropeptides*, *38*, 141–151. https://doi.org/10.1016/j.npep.2004.06.002

Leibowitz, S. F. (1995). Brain peptides and obesity: Pharmacologic treatment. *Obesity Research*, *3*, 573S–585S. https://doi.org/10.1002/j.1550-8528.1995.tb00230.x

Lin, X., Wang, P., Ou, Y., Li, J., & Wen, J. (2017). An immunohistochemical study on endocrine cells in the neuroendocrine system of the digestive tract of milkfish *Chanos chanos* (Forsskal, 1775). *Aquaculture Research*, *48*, 1439–1449. https://doi.org/10.1111/are.12979

Lundberg, J. M., Terenius, L., Hokfelt, T., & Goldstein, M. (1983). High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. *Neuroscience Letters*, *42*, 167–172. https://doi.org/10.1016/0304-3940(83)90401-9

MacDonald, E., & Volkoff, H. (2009). Cloning, distribution and effects of season and nutritional status on the expression of neuropeptide Y (NPY), cocaine and amphetamine regulated transcript (CART) and cholecystokinin (CCK) in winter flounder (*Pseudopleuronectes americanus*). *Hormones and Behavior*, *56*, 58–65. https://doi.org/10.1016/j.yhbeh.2009.03.002

Matsuda, K., Sakashita, A., Yokobori, E., & Azuma, M. (2012). Neuroendocrine control of feeding behavior and psychomotor activity by neuropeptide Y in fish. *Neuropeptides*, *46*, 275–283. https://doi.org/10.1016/j.npep.2012.09.006

Menezes, C., Ruiz-Jarabo, I., Martos-Sitchab, J. A., Toni, C., Salbego, J., & Becker, A., . . . Baldisserotto, B. (2015). The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): A metabolic and endocrine approach. *Aquaculture*, *435*, 257–264. https://doi.org/10.1016/j.aquaculture.2014.09.044

Meyer, G., & Fracalossi, D. M. (2004). Protein requirement of jundiá fingerlings, *Rhamdia quelen*, at two dietary energy concentrations. *Aquaculture*, *240*, 331–343. https://doi.org/10.1016/j.aquaculture.2004.01.034

Moro, G. V., Camilo, R. Y., Moraes, G., & Fracalossi, D. M. (2010). Dietary non-protein energy sources: Growth, digestive enzyme activities and nutrient utilization by the jundiá catfish, *Rhamdia quelen*. *Aquaculture Research*, *41*, 394–400. https://doi.org/10.1111/j.1365-2109.2009.02352.x

Murashita, K., Fukada, H., Ronnestad, I., Kurokawa, T., & Masumoto, T. (2008). Nutrient control of release of pancreatic enzymes in yellowtail (*Seriola quinqueradiata*): Involvement of CCK and PY in the regulatory loop. *Comparative Biochemistry and Physiology*, *Part A*, *150*, 438–443. https://doi.org/10.1016/j.cbpa.2008.05.003

Narnaware, Y. K., & Peter, R. E. (2001). Effect of food deprivation and refeeding on NPY mRNA levels in goldfish. *Comparative Biochemistry and Physiology*, *129*, 633–637. https://doi.org/10.1016/s1096-4959(01)00359-1

Narnaware, Y. K., & Peter, R. E. (2002). Influence of diet composition on food intake and neuropeptide Y (NPY) gene expression in goldfish brain. *Regulatory Peptides*, *103*, 75–83. https://doi.org/10.1016/s0167-0115(01)00342-1

Narnaware, Y. K., Peyon, P. P., Lin, X. W., & Peter, R. E. (2000). Regulation of food intake by neuropeptide Y in goldfish. *American Journal of Physiology*, *279*, R1025–R1034. https://doi.org/10.1152/ajpregu.2000.279.3.R1025

National Research Council (2011). *Nutrient requirements of fish and shrimp* (p. 392). Washington, DC: The National Academies Press.

Ostaszewska, T., Korwin-Kossakowski, M., & Wolnicki, J. (2006). Morphological changes of digestive structures in starved tench *Tinca tinca* (L.) juveniles. *Aquaculture International*, *14*, 113–126. https://doi.org/10.1007/s10499-005-9016-z

Pereira, R. T., Costa, L. S., Oliveira, I. R., Araujo, J. C., Aerts, M., Vigliano, F. A., & Rosa, P. V. (2015). Relative distribution of gastrin-, CCK-8-, NPY- and CGRP-immunoreactive cells in the digestive tract of dorado (*Salminus brasiliensis*). *Tissue and Cell*, *47*, 123–136. https://doi.org/10.1016/j.tice.2015.01.009

Pereira, R. T., Rodriguez de Freitas, T., Cardozo de Oliveira, I. R., Costa, L. S., Vigliano, F. A., & Vieira Rosa, P. (2017). Endocrine cells producing peptide hormones in the intestine of Nile tilapia: Distribution and effects of feeding and fasting on the cell density. *Fish Physiology and Biochemistry*, *43*, 1399–1412.

Peterson, B. C., Waldbieser, G. C., Riley, L. G. Jr, Upton, K. R., Kobayashi, Y., & Small, B. C. (2012). Pre- and postprandial changes in orexigenic and anorexigenic factors in channel catfish (*Ictalurus punctatus*). *General and Comparative Endocrinology*, *176*, 231–239. https://doi.org/10.1016/j.ygcen.2012.01.022

Rodrigues, A. P. O., Gominho-Rosa, M. D. C., Cargnin-Ferreira, E., de Francisco, A., & Fracalossi, D. M. (2012). Different utilization of plant sources by the omnivores jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition*, *18*, 65–72. https://doi.org/10.1111/j.1365-2095.2011.00877.x

Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jönsson, E., & Volkoff, H. (2017). Appetite-controlling endocrine systems in teleosts. *Frontiers in Endocrinology*, *8*, 73. https://doi.org/10.3389/fendo.2017.00073

Rubio, V. C., Sanchez-Vazquez, F. J., & Madrid, J. A. (2008). Role of cholecystokinin and its antagonist proglumide on macronutrient selection in European sea bass *Dicentrarchus labrax*, L. *Physiology & Behavior*, *93*, 862–869. https://doi.org/10.1016/j.physbeh.2007.12.001

Salhi, M., Bessonart, M., Chediak, G., Bellagamba, M., & Carnevia, D. (2004). Growth, feed utilization and body composition of black catfish, *Rhamdia quelen*, fry fed diets containing different protein and energy levels. *Aquaculture*, *231*, 435–444. https://doi.org/10.1016/j.aquaculture.2003.08.006

Shahbazi, F., Holmgren, S., Larhammar, D., & Jensen, J. (2002). Neuropeptide Y effects on vasorelaxation and intestinal contraction in the Atlantic cod *Gadus morhua*. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, *282*, 1414–1421. https://doi.org/10.1152/ajpregu.00434.2001

Silverstein, J. T., Breninger, J., Baskin, D. G., & Plisetskaya, E. M. (1998). Neuropeptide Y-like gene expression in the salmon brain increases with fasting. *General and Comparative Endocrinology*, *110*, 157–165. https://doi.org/10.1006/gcen.1998.7058

Silverstein, J. T., & Plysetskaya, E. M. (2000). The effects of NPY and insulin on food intake regulation in fish. *American Zoologist*, *40*, 296–308.

Uesaka, T., Yano, K., Sugimoto, S., & Ando, M. (1996). Effects of neuropeptide Y on ion and water transport across the seawater eel intestine. *Zoological Science*, *13*, 341–346. https://doi.org/10.2108/zsj.13.341

Vigliano, F. A., Munoz, L., Hernández, D., Cerutti, P., Bermudez, R., & Quiroga, M. I. (2011). An immunohistochemical study of the gut neuroendocrine system in juvenile pejerrey *Odontesthes bonariensis* (Valenciennes). *Journal of Fish Biology*, *78*, 901–911. https://doi.org/10.1111/j.1095-8649.2011.02912.x

Volkoff, H. (2006). The role of neuropeptide Y, orexins, cocaine and amphetaminerelated transcript, cholecystokinin, amylin and leptin in the regulation of feeding in fish. *Comparative Biochemistry and Physiology*, *144*, 325–331. https://doi.org/10.1016/j.cbpa.2005.10.026

Volkoff, H. (2016). The neuroendocrine regulation of food intake in fish: A review of current knowledge. *Frontiers in Neuroscience*, *10*, 1–31. https://doi.org/10.3389/fnins.2016.00540

Volkoff, H., Canosa, L. F., Unniappan, S., Cerda-Reverter, J. M., Bernier, N. J., Kelly, S. P., & Peter, R. E. (2005). Neuropeptides and the control of food intake in fish. *General and Comparative Endocrinology*, *142*, 3–19. https://doi.org/10.1016/j.ygcen.2004.11.001

Volkoff, H., Eykelbosh, A. J., & Peter, R. E. (2003). Role of leptin in the control of feeding of goldfish *Carassius auratus*: Interactions with cholecystokinin, neuropeptide Y and orexin A, and modulation by fasting. *Brain Research*, *972*, 90–109. https://doi.org/10.1016/s0006-8993(03)02507-1

Volkoff, H., Hoskins, L. J., & Tuziak, S. M. (2010). Influence of intrinsic signals and environmental cues on the endocrine control of feeding in fish: Potential application in aquaculture. *General and Comparative Endocrinology*, *167*, 352–359. https://doi.org/10.1016/j.ygcen.2009.09.001

Wang, T., Hung, C. C., & Randall, D. J. (2006). The comparative physiology of food deprivation: From feast to famine. *Annual Review of Physiology*, *68*, 223–251. https://doi.org/10.1146/annurev.physiol.68.040104.105739

Weng, Y., & Fang, Y. (2011). Effects of starvation on the morphology, localization and distributional density of endocrine cells in the gastrointestinal tract of juvenile *Epinephelus coioides*. *Journal of Oceanography in Taiwan Strait*, *30*, 10–16.

Wilson, J. M., & Castro, L. F. C. (2010). Morphological diversity of the gastrointestinal tract in fishes. In: M. Grosell, A. P. Farrell & C. J. Brauner (Eds.), *Fish Physiology Volume 30. The multifunctional gut of fish* (pp. 1–55). San Diego, CA: Academic Press.

Won, E. T., & Borski, R. J. (2013). Endocrine regulation of compensatory growth in fish. *Frontiers in Endocrinology*, *4*, 1–13. https://doi.org/10.3389/fendo.2013.00074

Wong, M., Mo, W., Choi, W., Cheng, Z., & Man, Y. (2016). Recycle food wastes into high quality fish feeds for safe and quality fish production. *Environmental Pollution*, *219*, 631–638. https://doi.org/10.1016/j.envpol.2016.06.035

Xu, C., Li, X. F., Tian, H. Y., Jiang, G. Z., & Liu, W. B. (2016). Feeding rates affect growth, intestinal digestive and absorptive capabilities and endocrine functions of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish Physiology and Biochemistry*, *42*, 689–700. https://doi.org/10.1007/s10695-015-0169-z

Zeng, L. Q., Li, F. J., Li, X. M., Cao, Z. D., Fu, S. J., & Zhang, Y. G. (2012). The effects of starvation on digestive tract function and structure in juvenile southern catfish (*Silurus meridionalis Chen*). *Comparative Biochemistry and Physiology*, *Part A*, *162*, 200–211. https://doi.org/10.1016/j.cbpa.2012.02.022

Zeppenfeld, C. C., Hernández, D. R., Heinzmann, B. M., Santinón, J. J., Alves da Cunha, M., Schmidt, D., & Baldisserotto, B. (2016). Essential oil of *Aloysia triphylla* as feed additive promotes growth of silver catfish (*Rhamdia quelen*). *Aquaculture Nutrition*, *22*, 933–940. https://doi.org/10.1111/anu.12311

Zhou, Y., Liang, X. F., Yuan, X. C., Li, J., He, Y., & Fang, L., … Shen, D. (2013). Neuropeptide Y stimulates food intake and regulates metabolism in grass carp, *Ctenopharyngodon idellus*. *Aquaculture*, *380–383*, 52–61. https://doi.org/10.1016/j.aquaculture.2012.11.033

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