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Gastrointestinal tract pH measurement in juveniles *Pacu Piaractus mesopotamicus* (Characiformes: Characidae)

WELLITON GONÇALVES-FRANÇA[1], TÂNIA C.PONTES[1], FABRÍCIO MARTINS-DUTRA[1], FRANK LIEBERT[2], EDUARDO L. CUPERTINO-BALLESTER[1] & LEANDRO PORTZ[1]*

[1] Universidade Federal do Paraná, Setor Palotina. Programa de Pós-graduação em Aquicultura e Desenvolvimento Sustentável, Departamento de Zootecnia, Rua Pioneiro, 2153, Jardim Dallas, 85950-000, Palotina, Paraná, Brasil.
[2] Georg-August-University Goettingen, Institute for Animal Physiology and Animal Nutrition, Goettingen, Germany.
**Corresponding author*: lportz@ufpr.br

Abstract. The study evaluated pH differences along the digestive tract of the *Piaractus mesopotamicus*. The higher pH variation occurs in the stomach region, where it occurs to break down of the food through the gastric enzymes, which requires acidic action to perform such function.

Keywords: aquaculture, nutrition, physiology, tropical fish.

Resumo. Medição do pH do trato gastrointestinal em juvenis de *Piaractus mesopotamicus* (Characiformes: Characidae). O estudo avaliou as diferenças de pH ao longo do trato digestivo do *Piaractus mesopotamicus*. A maior variação do pH ocorre na região do estômago, onde ocorre a quebra do alimento através das enzimas gástricas, o que requer ação ácida para realizar essa função.

Palavras-chave: aquicultura, nutrição, fisiologia, peixes tropicais.

The pacu (*Piaractus mesopotamicus*) is an omnivorous fish with a strong tendency to herbivory, feeding on organic detritus, plants and seeds. The species is highly appreciated due to the high quality of meat, rusticity, fast growth and easy adaptation to production systems (Stech et al. 2010). The species also can tolerate poor water quality and is resistant to pathogens (Abimorad & Carneiro, 2004). The studies using pacu (Abimorad & Carneiro, 2007; Bicudo et al. 2009) were carried out with the objective of maximizing the production potential of this species, among these, studies that refer to nutritional aspects has gained prominence.

The morphological and physiological characteristics of the fish are defined by species-specific feeding habits, influencing the stomach pH, morphology and intestinal enzymatic activity (Cyrino et al. 2010). The gastrointestinal system of omnivores exhibit an adaptive variation due to different eating habits. The alimentary canal begins at the mouth and ends in the anal opening, it may be divided into three parts, head intestine (pharyngeal buccal cavity), foregut and hindgut (Moreira et al. 2001). The passage of food through the digestive tract of fish can be altered by various factors such as the physical and chemical characteristics of the feed, the abiotic factors of water that can influence the metabolism and amount of food consumed (Possompes et al. 1975, Usmani & Jafri 2002, Dias-Koberstein et al. 2005). Therefore, the study between the co-relations of the diet and the characteristics of the digestive tract has gained prominence in different zoological groups, being indispensable for the manipulation and formulation of balanced diets (Seixas Filho et al. 2000).

According to Chakrabarti et al. (1995), the intestinal tract of the fish is in an evolutionary stage in which most regions can produce all the enzymes necessary to the digestive process. The activity of the enzymes is affected by the intestinal pH, in some cases; the pH change can affect 50% of the activity of an enzyme (Deguara et al. 2003). Several studies have been carried out with the objective of knowing the intestinal morphometry of the fish and the time of gastrointestinal transit (Montagne et al. 2003; Fabregat et al. 2011; Dias-Koberstein et al. 2005; Rodrigues et al. 2010), but there is little information relating the intestinal pH during fasting and the changes that occur after feeding. Stomach and intestinal pH undergoes variations according to feeding conditions or fasting. The reduction of pH in the stomach and feed depends largely on the buffering capacity of the feed ingredients (Freitag 2007). Thus, the objective of this study was to evaluate the difference in pH along the digestive tract of pacu (*P. mesopotamicus*) to support future nutrition studies on the species.

The experiment was conducted with 24 juvenile of *P. mesopotamicus* with an average weight of 125.0 ± 5.7 g and a total length of 17.2 ± 2.3 cm. Juveniles were also separated into six net pen, totaling four fish per experimental unit. The fishes were maintained under natural photoperiod.

For the evaluation of gastric and intestinal pH, six different times were used, the first evaluation was performed with the animals starved, with the fish fasted for 24 hours prior to study initiation. After checking the pH in fasting, fishes were feed with a commercial diet containing 28% CP and 3000 Kcal/kg-1. The pH was measured before feeding, during feeding and at four equal intervals of two hours (2, 4, 6 and 8 hours after delivery of food).

In each evaluation, fishes were euthanized by an overdose of eugenol 400 mg.L-1 according to the methodology proposed by Rotili et al. (2012) (methodology approved by the committee of ethics in the use of animals – CEUA-UFPR) then they were carefully eviscerated in order to maintain the gastrointestinal tract intact, the stomach was then separated from the intestine, after that the intestine was divided into three equal portions (Fig. 1).

Figure 1. Gastrointestinal system of pacu: A - Stomach; B - Anterior intestine; C - Medium intestine; D - Posterior intestine.

The pH of each gastrointestinal tract sections was measured individually in triplicates directly in the tissue, using a Metrohm 632 pH meter, with a pH probe InLab® Micro.

For the statistical analysis, the data were subjected to normality verification through the Kolmogorov-smirnov & Liliefors test and to homogeneity through Levene's test. When those two requirements were accomplished, ANOVA was applied followed by Tukey's test ($\alpha = 0.05$).

The variation of pH in the gastrointestinal tract of pacu was more evident in the stomach than in the other intestinal portions evaluated (Fig. 2).

The pH of the stomach showed statistical differences ($p<0.05$), with a range of 1.47 to 5.12 during the evaluation, the lowest value observed in the stomach was 1.18 before feeding. The portions of the anterior intestine and medium intestine showed no significant changes ($p<0.05$) in any evaluation with pH means ranging from 7.09 to 8.13. The third portion of the posterior intestine showed statistical differences ($p<0.05$) among the times (Fig. 2). An increase in pH was observed after feeding, from 7.65 to 8.07 decreasing after 4 hours until the end of the evaluations, which showed pH of 7.22.

It was observed that after the fish were fed the stomach pH increased significantly, thus suggesting the influence of the feed buffering power over the gastric pH (Maier & Tulles 1984). This feed buffering capacity was observed in other studies as Deguara et al. (2003) using *Sparus aurata*, with pH average decreasing of 5.50 to 2.50 in the stomach portion and values of 6.95 to 7.90 in the anterior and posterior portions. Yufera et al. (2012) described for juvenile *Diplodus sargus*, the stomach pH range variation of 7.00 to 3.00 for approximately 8 hours after feeding. Remote studies reported fast pH reduction in the stomach of some fish species like *Lepomis macrochirus* (Norms et al. 1973), *Oreochromis niloticus* (Moriarty 1973) and *Oreochromis mossambicus* (Maier & Tullis 1984). The low stomach pH emulsifies the lipids, denature proteins and carbohydrates, and facilitates the action of digestive enzymes, causing the release of free amino acids present in the food (Bowen 1980, NRC 2011).

Chakrabarti et al. (1995) observed in a broad study assessing digestive enzymes of 11 freshwater fish species, where the pH was measured with satiated fish presented variations among 5.32 to 7.02 for the stomach, from 5.74 to 8.02 for anterior portion of 5.60 to 8.10 for the medium portion and 5.41 to 7.89 for posterior portion, similar pH values were observed in this study when the fishes were fed.

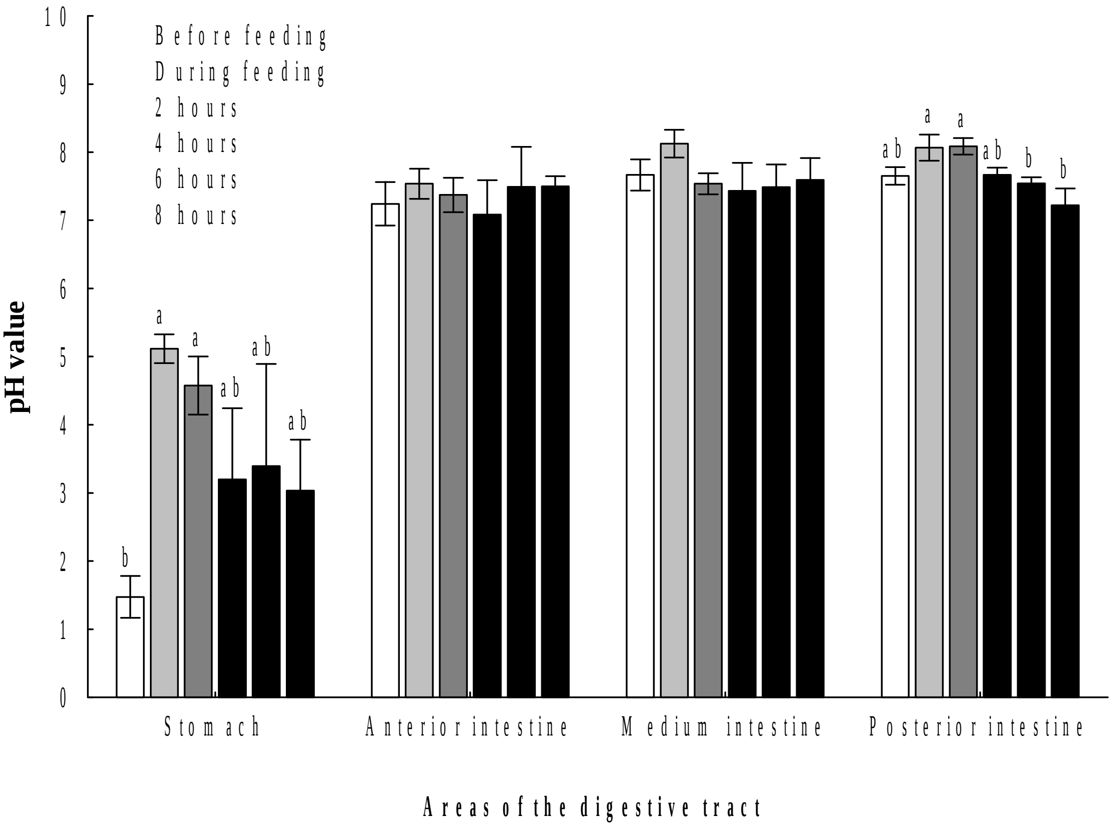

Figure 2. Comparison of pH on the digestive tract pacu *P. mesopotamicus* over time.

The pH changes in the stomach can be explained by normal digestive processes, so the decrease in pH occurs as digestive enzymes are secreted in response to the food present in the stomach (Deguara et al. 2003). According to Prosser (1973) and Guyton (1981) food in the stomach is known to stimulate gastric acid secretion, a mixture of acid and enzyme, justifying the changes in the stomach pH values observed in this study.

According to Wilson et al. (2002), Grosell et al. (2005) and Taylor & Grosell (2006) food intake in the anterior intestine can increase the pH values by neutralization of the gastric acid stimulated by bolus enters in the anterior intestine due to release of pancreatic juice secretion, reducing the activity of pepsin and placing close to neutral pH (7.00 to 7.50), suitable for the action of other digestive enzymes located in the intestine.

Furthermore, the amount of feed consumed in a meal can change the velocity of the bolus as the pH variation. Also according to the food habits of the animals, the feed rate, the diet composition and the time after the meal the pH of the stomach may oscillate of 1.00 to 6.00 (Pérez-Jiménez et al. 2009).

Through this study, it was possible to trace pH variation in the gastrointestinal tract of *P. mesopotamicus* at different times. Therefore, we can conclude that the pH has a greater variation in the stomach, especially at the moment of feeding where the pH tends to become less acid due to food intake. This was already expected since the low amount of gastric enzyme tends to be neutralized by the feed which presents pH close to the neutral, requesting greater release of gastric enzymes in the stomach to break down and digest the alimentary particles, as it is observed with the increase of the pH two hours after the feeding. The study also served to provide data for other works that will use enzymes that require specific pH to act.

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