

Dietary protein levels in *Piaractus brachypomus* submitted to extremely acidic or alkaline pH

Níveis de proteína na dieta de *Piaractus brachypomus* submetidos a pH extremamente ácidos ou alcalinos

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ABSTRACT

The objective this study was to evaluate the effects of dietary protein levels in pirapitinga, *Piaractus brachypomus*, submitted to extremely acidic or alkaline pH. Juveniles were fed for 20 days with three diets with different crude protein (CP) levels (25.3, 32.4 and 40.0%) and then separated in five groups (n=10, three replicates each) which were kept in 60 L aquaria and exposed to pH 3.0, 3.5, 7.0, 10, or 10.5. Fish were removed from aquaria when they showed loss of swimming balance, and then blood was collected and plasma separated for measurement of Na⁺, Cl⁻ and K⁺ levels. The increase of dietary protein levels (up to 40.0% CP) provided some protection for pirapitinga at pH 3.5 or 10.0 because the time to lose equilibrium increased after acute exposure, but was not effective for compensating ion loss at very acidic (Na⁺ and Cl⁻) and alkaline (Cl⁻) pH.

Key words: pirapitinga, fish, ionoregulation, extreme pH.

RESUMO

O objetivo deste estudo foi avaliar o efeito dos níveis de proteína na dieta de pirapitinga, *Piaractus brachypomus*, submetidas a pH extremamente ácido ou alcalino. Os juvenis foram alimentados durante 20 dias com três dietas com diferentes níveis de proteína bruta (PB) (25,3; 32,4 e 40,0%) e, em seguida, foram separados em cinco grupos (n=10, três repetições cada), os quais foram colocados em aquários de 60L e expostos aos pH 3,0; 3,5; 7,0; 10 ou 10,5. Os peixes foram removidos dos aquários quando apresentaram perda de equilíbrio natatório, o sangue foi então coletado e o plasma separado para determinação dos níveis de Na⁺, Cl⁻ e K⁺. O aumento dos níveis de proteína na dieta (até 40% PB) forneceu alguma proteção para pirapitingas em pH 3,5 ou 10,0, porque o tempo para perder o equilíbrio natatório aumentou após a exposição aguda, mas não foi efetivo para compensar a perda de íons em pH muito ácido (Na⁺ e Cl⁻) ou alcalino (Cl⁻).

Palavras-chave: pirapitinga, peixe, ionorregulação, pH extremos.

INTRODUCTION

Fish exposed to acidic pH presented net Na⁺, K⁺ and Cl⁻ effluxes, with consequent decrease of ion plasma levels (D'CRUZ & WOOD, 1998; PARRA & BALDISSEROTTO, 2007). Exposure of rainbow trout *Oncorhynchus mykiss* to alkaline waters also reduced branchial uptake and plasma Na⁺ and Cl⁻ levels, with no effect on branchial diffusive efflux (YESAKI & IWAMA, 1992; WILKIE et al., 1996), and silver catfish *Rhamdia quelen* presented higher net Na⁺ loss (COPATTI et al., 2011). The ion loss in fish exposed to acidic and alkaline pH can be compensated by ion dietary uptake because when fish are fed adequately this ionoregulatory disturbance is minimized (DOCKRAY et al., 1996; D'CRUZ & WOOD, 1998; COPATTI et al., 2011).

The pirapitinga, *Piaractus brachypomus*, is a native fish from the Amazon and Orinoco river basins (Latin America) and can be found in acidic waters (VÁSQUEZ-TORRES, 2005). This species is a rustic, fast-growing species, prized for its meat and offering excellent conditions for culture (MELARD et al., 1993). It has considerable economic importance on a commercial scale in Colombia, Brazil, Peru, Venezuela and Central America (VÁSQUEZ-TORRES et al., 2002). Pirapitinga is an omnivorous species and thus its diet includes leaves, fruits, tiny

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fish, and small crustaceans. Nutritional protein requirements for pirapitinga should be approximately 32%, lipid 4 to 6% and carbohydrates above 36% to achieve the best performance (VÁSQUEZ-TORRES, 2005; VÁSQUEZ-TORRES et al., 2011).

Dietary salt supplementation is very important to maintain ion homeostasis in fish exposed to low pH (D'CRUZ & WOOD, 1998; COPATTI et al., 2011). However, to our knowledge, there are no studies regarding dietary protein levels and ionoregulation in fish exposed to acidic and alkaline pH. In addition, no studies related to ionoregulation of pirapitinga exposed to extreme pH were performed. Therefore, the objective of this study was to evaluate the effects of dietary protein levels on ionoregulation and time to loose equilibrium in pirapitinga submitted to extremely acidic or alkaline pH.

MATERIAL AND METHODS

Piaractus brachypomus juveniles were obtained from the Institute of Aquaculture at the Universidad de los Llanos, in Villavicencio, Colombia. The fish were kept in nine 200L tanks (n=20 per tank) in a closed system consisting of four biofilters in series, continuous flow of 2L min⁻¹ tank⁻¹. The water quality was maintained in: oxygen close to saturation (7.9±0.2mg L⁻¹), temperature 25.2±1.2°C, pH 6.5±0.3 and total ammonia levels <0.02mg L⁻¹. These parameters were recorded weekly using a multiparameter probe Orion 5 Star (Thermo Electron Corporation) and total ammonia levels were measured with Ammonium test (Merck® Spectroquant 1.14752.0001).

One hundred eighty fish were separated in three treatments (n=60 each) and fed for 20 days with formulated experimental diets with different crude protein (CP) levels (25.3, 32.4 or 40.0%) (Table 1). Juveniles were fed twice a day to apparent satiety and the uneaten food as well as other residues and feces were siphoned out 30min after feeding.

At the end of feeding period, each treatment was subdivided in five groups of five fish each (10.4±0.4cm and 9.7±1.2g), that were kept in 60L aquaria with 6.4±0.4mg L⁻¹ dissolved oxygen levels and temperature of 25.2±0.2°C. Each group was exposed to a different pH (3.0, 3.5, 7.0, 10, or 10.5, in duplicate). The pH was adjusted by adding either H₂SO₄ or NaOH (1M). In all treatments juveniles were maintained under observation and were not fed throughout exposure to the experimental pH.

Fish were collected from the aquaria when they showed loss of equilibrium and absence of opercular movements or at the end of the experiment

Table 1 - Diet composition used for *Piaractus brachypomus* juveniles in the experimental period.

Ingredients	----- Crude protein (%) -----		
	25.3	32.4	40.0
Soybean meal	30.0	30.0	30.0
Wheat bran	23.4	25.0	21.4
Rice meal	15.0	14.2	15.0
Whole soybean	1.0	0.0	1.0
Manioc flour	12.0	12.0	1.0
Whole wheat	13.5	3.1	1.0
Corn gluten	2.0	10.2	27.7
Meat meal	1.0	1.2	1.0
Fish meal	1.0	1.0	1.0
Blood meal	1.0	3.1	0.8
Vitamin mix ¹	0.1	0.1	0.1
Mineral mix ²	0.035	0.035	0.035
Proximate analysis	----- % in dry matter -----		
Dry matter	94.5	93.7	93.4
Crude protein	25.3	32.4	40.0
Digestible protein	22.0	30.0	37.0
Gross energy (cal g ⁻¹)	4455	4647	4796
Crude fat	2.8	3.3	4.2
Ash	5.7	5.8	5.5

¹Rovimix Vitamins® Lab. Roche S.A.: Vit A 8.0*10⁶UI, Vit D3, 1.8*10⁶UI, Vit E 66.66g, Vit B1 6.66g, Vit B2 13.33g, Vit B6 6.66g, Ca pantothenate 33.33g, Biotin 533.3mg, folic acid 2.66g, ascorbic acid 400.0g, nicotinic acid 100.0g, Vit B12 20.0mg, Vit K3 6.66g, vehicle csp 1.0kg.

²Premix microminerals® Lab. Roche S.A.: composition for 100g: magnesium 1.0, zinc 16.0, iron 4.0, copper 1.0, iodine 0.5, selenium 0.05, cobalt 0.01.

(8h of exposure), the blood was rapidly collected from the caudal vein with heparinized syringes and centrifuged at 2,000rpm for 5min to separate the plasma. After sampling, all fish were euthanized by section of the spinal cord. All procedures were conducted according to rules of the Brazilian Council of Animal Experimentation Control (CONCEA). Plasma samples were stored at -20°C until use. Ion plasma levels were analyzed as described by BOLNER & BALDISSEROTTO (2007).

Data are reported here as mean ± SEM (N). The homogeneity of variances between groups was tested with the Levene test. Comparisons of plasma ion levels between different treatments were made by a two-way ANOVA (pH X CP) and a Tukey test. All tests were performed with the software Statistica 7.0 (1997; StatSoft Inc., Tulsa, OK, USA). The linear relationships between dietary protein levels and time to lose equilibrium at the different pH were calculated with Sigma Plot 11.0 software (Systat Software Inc., San

Jose, CA, USA). The minimum significance level was set at $P < 0.05$.

RESULTS

The increase of the dietary protein level provided to pirapitinga increased proportionally to the time to lose equilibrium when exposed to pH 3.5 or 10.0, but decreased with the time to lose equilibrium after exposure to pH 3.0 and did not affect the time to lose equilibrium after exposure to pH 10.5 (Figure 1). The relationships between time to lose equilibrium (y – in min) and dietary crude protein levels (x – in %) are expressed by pH 3.0 $y = 455.88 - 7.85x$ ($r^2 = 0.939$), pH 3.5 $y = 228.66 + 6.38x$ ($r^2 = 0.980$) and pH 10.0 $y = 304.42 + 2.72x$ ($r^2 = 0.999$).

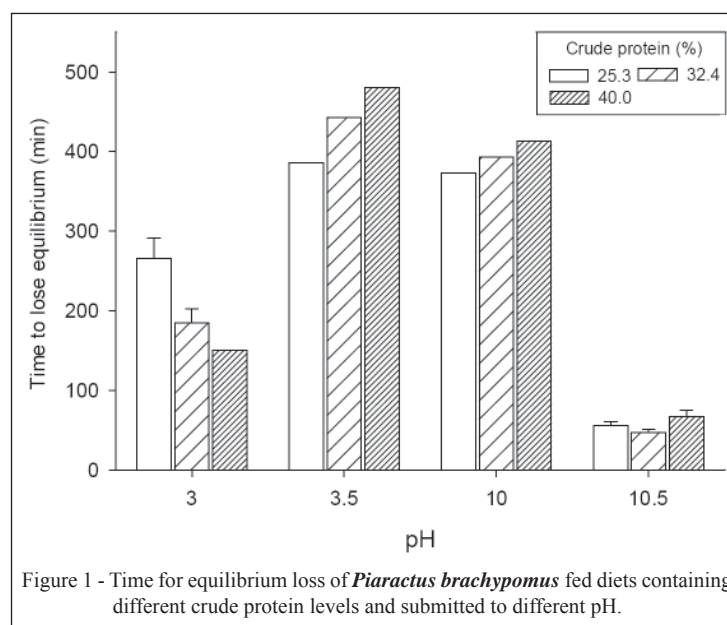
Irrespective of experimental diet, juveniles exposed to acidic water (3.0 or 3.5) showed plasma Na^+ levels significantly lower than those exposed to pH 7. However, only juveniles fed the higher dietary protein levels (32.4 and 40.0%) and transferred to pH 10.0 presented significantly higher plasma Na^+ levels in relation to those kept at pH 7.0 and fed the same experimental diets. Exposure to pH 10.5 did not change plasma Na^+ levels. At pH 3.0 and 3.5 the juveniles fed with 32.4% CP had significantly lower and higher plasma Na^+ levels, respectively, compared to those fed with 25.3 and 40.0% CP at these same pH. Juveniles fed 32.4% CP and transferred to pH 10.0 showed plasma Na^+ levels significantly higher than those fed with 25.3% CP (Figure 2A).

Juveniles fed with all experimental diets and transferred to both acidic and alkaline pH showed significantly lower plasma Cl^- levels than those maintained at pH 7.0 (Figure 2B). Juveniles exposed to acidic (3.0 and 3.5) and alkaline (10.0) pH presented significantly higher plasma K^+ levels than those maintained at pH 7.0 and 10.5 irrespective of experimental diets. The plasma K^+ levels of the juveniles exposed to pH 3.0 and fed with the diet containing 32.4% CP were significantly lower than those fed with 25.3% CP at the same pH (Figure 2C).

DISCUSSION

The increase of dietary protein levels increased the time for pirapitinga to lose equilibrium at pH 3.5 or 10.0. This result indicates that a higher dietary protein level could be tested to improve survival and/or growth of this species at less acidic or alkaline waters. However, as the diet with higher protein level also presented around 7% higher gross energy, it is not possible to exclude that dietary energy may also contribute to this variation. In agreement with the present study, rainbow trout fed with 39% digestible protein (energy content 16.3Mj kg^{-1}) presented higher growth and food conversion efficiency than those fed with 23% digestible protein (energy content 9.8Mj kg^{-1}) when maintained at pH 5.2 (D'CRUZ & WOOD, 1998).

Pirapitinga juveniles submitted to acute acidic exposure (pH 3.0 or 3.5) presented Na^+ and Cl^- loss in comparison with fish exposed to neutral



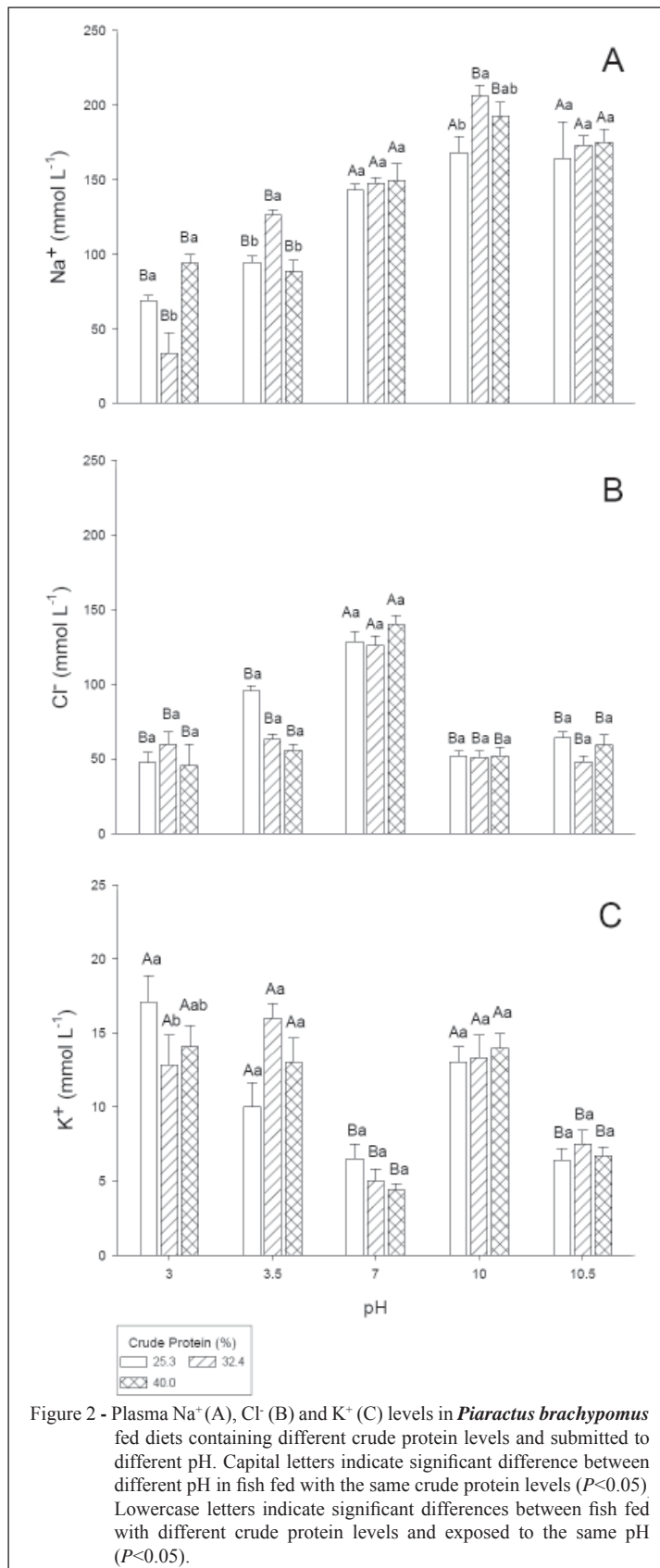


Figure 2 - Plasma Na⁺ (A), Cl⁻ (B) and K⁺ (C) levels in *Piaraactus brachyomus* fed diets containing different crude protein levels and submitted to different pH. Capital letters indicate significant difference between different pH in fish fed with the same crude protein levels ($P < 0.05$). Lowercase letters indicate significant differences between fish fed with different crude protein levels and exposed to the same pH ($P < 0.05$).

pH. The same was described for other species exposed to acidic pH: common carp *Cyprinus carpio* (pH 4.0) (CHEZHIAN et al., 2011), pupfish *Cyprinodon variegatus variegatus* (BRIX et al., 2013) zebrafish *Danio rerio* (KWONG & PERRY, 2013) and silver catfish (pH 4.0 and 5.5) (ZAIONS & BALDISSEROTTO, 2000; COPATTI et al., 2011). In acidic waters the uptake of Na⁺ and Cl⁻ is probably inhibited due to a competition of H⁺ with Na⁺ transport sites (PARRA & BALDISSEROTTO, 2007). In addition, high H⁺ disrupt the tight junctions of gill epithelia causing greater ionic loss by paracellular route (KUMAI et al., 2011; KWONG & PERRY, 2013), and the net H⁺ excretion by the H⁺-ATPase observed in neutral waters is reduced (BRIX et al., 2013). Higher plasma K⁺ levels after exposure to acidic pH (pH 5.0) was also observed in common carp (MATHAN et al., 2010). These authors supposed that this elevation is due to intracellular K⁺ release from muscles as H⁺ enters.

In alkaline waters, ion loss probably occur due to an inhibition of branchial Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchangers (PARRA & BALDISSEROTTO, 2007), which would explain the lower plasma Cl⁻ levels in pirapitinga at pH 10.0 and 10.5. Ions loss after exposure to alkaline waters also occurred in silver catfish at pH 9.4-10.0 (ZAIONS & BALDISSEROTTO, 2000), *O. mykiss* at pH 9.0-10.5 (YESAKI & IWAMA, 1992; McGEER & EDDY, 1998) and *Oncorhynchus clarki henshawi* at 10.0-10.5 (WILKIE et al., 1994). However, exposure of pirapitinga to alkaline pH did not change or increased (at pH 10.0 in fish fed the higher dietary protein levels) plasma Na⁺ levels. Apparently alkaline water has a different effect on Na⁺ and Cl⁻ transporters. Similar effects on Na⁺ and Cl⁻ plasma levels were observed in silver catfish exposed to pH 9.0 for 24h (BOLNER & BALDISSEROTTO, 2007).

The increase of dietary protein (and energy) levels did not improve the ionoregulatory response of pirapitinga against acidic or alkaline pH exposure. Higher dietary energy and protein level also did not improve the ionoregulatory response in rainbow trout exposed to pH 5.2 (D'CRUZ & WOOD, 1998).

In conclusion, the increase of dietary protein levels (with a possible contribution of dietary energy) increased the time for pirapitinga to lose equilibrium at pH 3.5 or 10.0, but was not effective for compensating ion loss at very acidic (Na⁺ and Cl⁻) and alkaline (Cl⁻) pH.

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