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Growth of silver catfish (*Rhamdia quelen*) exposed to acidic pH at different humic acid levels

Crescimento de jundiás (*Rhamdia quelen*) expostos ao pH ácido em diferentes níveis de ácido húmico

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ABSTRACT

*Humic substances are organic compounds that possess high molecular weight and are formed by the decomposition of plant materials. Humic substances comprise humic acids, fulvic acids and humin. Humic acids (HA) have shown to be able to provide some protection to biological membranes of fish in water with low pH, replacing the protective action of Ca^{2+}. Thus, this studyaimed to observe growth of silver catfish (**Rhamdia quelen**) juveniles exposed to pHs 5.5 and 6.5 and to different levels of HA: 0, 10, 25 and 50mg L^{-1}. Results showed that, irrespective of pH, HA was highly detrimental to silver catfish growth, since the higher the concentration of HA, the lower the weight gain and feed intake, resulting in lower biomass and lower specific growth rate of juveniles. Hence, HA is extremely damaging to the performance of silver catfish juveniles in the tested concentrations.*

Key words: *Humic substance, water pH, fish growth, organic carbon.*

RESUMO

*Substâncias húmicas são compostos orgânicos que possuem elevado peso molecular e são formadas pela decomposição da matéria orgânica de plantas. As substâncias húmicas compreendem ácidos húmicos, ácidos fúlvicos e humina. Os ácidos húmicos (AH) têm mostrado serem capazes de proporcionar proteção parcial das membranas biológicas de peixes em águas com baixo pH, substituindo a ação protetora do Ca^{2+}. Assim, este estudo teve como objetivo observar o crescimento de juvenis de jundiá (**Rhamdia quelen**) expostos a pH's 5,5 e 6,5 com diferentes níveis de AH: 0, 10, 25 e 50mg L^{-1}. Os resultados demostram que, independentemente do pH, o AH foi altamente prejudicial para o crescimento do jundiá, uma vez que, quanto maior era a concentração de AH, menor era o ganho de peso e a ingestão de alimentos, resultando em menor biomassa e taxa de crescimento específico inferior dos juvenis. Assim, os AH são extremamente prejudiciais para o desempenho de juvenis de jundiá nas concentrações testadas.*

Palavras-chave: *Substância húmica, pH da água, crescimento, carbono orgânico.*

INTRODUCTION

The organic compounds of humic substances result from decomposition of dead plants and animals and contain acidic functional groups, besides being important regulators of biogeochemical processes, such as global nutrients and carbon cycle (WOOD et al., 2003; MATSUO & VAL, 2007). Black waters of the Amazon region are known for their physicochemical characteristics, relatively uncommon to average global values, with particular respect to the acidic pH parameters (it can reach 3.0 in some situations) and low ionic concentration. In these dark waters, humic substance corresponds from 60 to 90% of the dissolved organic carbon (MATSUO & VAL, 2007).

In the ion poor blackwater rivers of Amazon, the humic substances may have a direct

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action on the gills, exerting a protective effect at low pH and, being able to replace any protective property that Ca^{2+} could possess (WOOD et al., 2003). Fish kept in natural black waters, rich in humic substances, show a lower ion efflux after exposure to low pH levels than fish maintained in water without humic substance, suggesting that it reduce gill permeability, preventing ion loss by diffusion (GONZALES et al., 2002; 2005; WOOD et al., 2003). Furthermore, humic substances facilitate the secondary repair of lesions induced by stress in fish (MEINELT et al., 2008).

The increased ion efflux in acidic water promotes a greater energetic expenditure so that fish can adapt to this condition. This demand diverts the amount of energy otherwise destined for growth to the osmoregulatory mechanisms (SOENGAS et al., 2007). Juveniles of silver catfish ***Rhamdia quelen*** maintained at pH 5.5 presented lower growth than those kept at pH 7.0-7.5 (COPATTI et al., 2005; 2011b), probably due to a decrease in food consumption. The optimum pH range for growth of this species is 6.0-8.0 (COPATTI et al., 2011a). A study with green swordtail (***Xiphophorus helleri***) has been the only to report some beneficial effect on fish growth for humic substances (MEINELT et al., 2004). Humic substances apparently have a protective effect on osmoregulation in some fish species exposed to acidic waters (GONZALES et al., 2002; 2005; WOOD et al., 2003), but not in silver catfish (COSTA et al., 2015). Due to the relevant commercial importance of silver catfish in the south of Brazil, this study aimed to evaluate the effect of different concentrations of humic acid (HA) (a fraction of humic substance) on the species performance when exposed to different pHs.

MATERIALS AND METHODS

Experimental design

Silver catfish juveniles were obtained from a commercial fish farm near Santa Maria city, southern Brazil. They were acclimated for a minimum of 4 days in 250L tanks with continuously aerated fresh water (minimum dissolved oxygen 6.00mg L^{-1}), devoid of HA, at controlled temperature of 21±0.5ºC. They were fed to satiation once a day with commercial concentrate for juveniles with 42% extruded crude protein (Alisul/Supra - São Leopoldo, RS, Brazil).

For the growth experiment, 480 silver catfish juveniles (3±1g) were maintained for 40 days in 40L polyethylene boxes, at a density of 2.60g L^{-1}. To evaluate the effect of the combination pH + HA on growth, the pHs 5.5 and 6.5 (control) were combined with 0, 10, 25 and 50mg L^{-1} HA, in triplicate. These HA concentrations were chosen because they are within the range observed in the water of the Rio Negro Basin (KÜCHLER et al., 2000). The pH 5.5 decreases growth (COPATTI et al., 2011b) and pH 6.5 is within the optimum range (COPATTI et al., 2011a). Animals were fed daily to satiation at 9 am with the same commercial feed provided in the acclimation period. Food remained, as well as residues and feces, were removed 30min after feeding, followed by an average 20% replacement of tank water previously prepared with the adequate pH and HA concentration. Anesthetized juveniles with 50µL L^{-1} eugenol (Odontofarma, Porto Alegre, Brazil) (CUNHA et al., 2010) were weighed and measured at 20 and at the end of the experiment (40 days) so that specific growth rate (SGR), feed intake and biomass could be estimated according to LIMA et al. (2011).

Water parameters

Levels of dissolved oxygen and temperature were measured daily with Orion 810 oxygen meter (Thermo Electro Corporation, Waltham, Al, USA). Water samples were collected every 2-3 days to verify total ammonia nitrogen (TAN) levels by the method of EATON et al. (2005). Un-ionized ammonia (NH_3) levels were calculated according to COLT (2002). Levels of Ca^{2+}, Na^+ and K^+ were determined with photometer Micronal B286 and Cl^- as outlined in ZALL et al. (1956). Nitrite was analyzed by spectrophotometry (BOYD & TUCKER 1992).

Humic acid and pH

Synthetic HA used in the trials (CAT: H1 0.675-2 Aldrich® - humic acid sodium salt) corresponded to 44% of dissolved organic carbon (DOC), according to MATSUO & VAL (2007). In this experiment, 50mg L^{-1} HA contained the nominal concentration of 20mg C^{-1} of DOC. Water pHs 5.5 and 6.5 were verified three times a day with pH meter DMPH-2 (Digimed, São Paulo, Brazil) and adjusted with sulfuric acid 1M when necessary.

Statistical analysis

Homogeneity of variances between treatments was assessed via Levene test and the comparison between pH was performed with two-way ANOVA (pH x HA) and Tukey test (Statistica software

7.0). Relationship between levels of HA within each pH and growth parameters (regression analysis) were performed with Sigma Plot 11.0 software. Minimum significance level was set at $P \leq 0.05$.

RESULTS

During the experimental period, the overall waterborne levels of Na^+, Cl^-, K^+ and Ca^{2+} were 4.02±0.95, 6.14±0.75, 0.04±0.01 and 0.021±0.005mg L^{-1}, respectively. Parameters of water quality were: temperature (20-22°C), dissolved oxygen levels 6.17±0.55mg L^{-1}, hardness 26.3±5.1mg $CaCO_3$ L^{-1}. Nitrite levels were kept at 0.362±0.037mg L^{-1} and non-ionized ammonia at 0.055±0.00073mg L^{-1}.

At 40 days fish maintained at pH 5.5 without HA showed significantly lower values for all measured variables compared to pH 6.5 ($P<0.05$), but there was no significant effect of pH when HA was present ($P>0.05$). Statistical analysis (ANOVA, corrected for the mean of the observations, where DF numerator and denominator are regression and residuals, respectively) revealed a significant effect of increasing levels of HA proportionally decreasing weight at pH 6.5 [$F(1,2)=31.11$, $P=0.031$] and pH 5.5 [$F(1,2)=159.84$, $P=0.0062$], length at pH 6.5 [$F(1,2)=621.95$, $P=0.028$] and pH 5.5 [$F(1,2)=181.01$, $P=0.05$], feed intake at pH 6.5 [$F(1,2)=367.36$, $P=0.037$] and pH 5.5 [$F(1,2)=20.99$, $P=0.04$], biomass at pH 6.5 [$F(1,2)=11486.32$, $P=0.006$] and pH 5.5 [$F(1,2)=300.33$, $P=0.04$], SGR at pH 6.5 [$F(1,2)=39.38$, $P=0.024$] and pH 5.5 [$F(1,2)=528.83$, $P=0.002$] (Figure 1).

DISCUSSION

Results obtained for Na^+, ammonia and nitrite were within the limits reported earlier as appropriate for silver catfish growth (LIMA et al., 2011; MIRON et al., 2011). Periodic exchange of 20% of the water volume in the experimental tanks was probably responsible for maintaining these parameters at suitable levels.

Exposure to acidic water increases efflux of ions through the gill epithelium and survival in an acidic environment appears to be primarily related to the ability to prevent ion loss (BALDISSEROTTO, 2011). Search for homeostasis at acidic pH apparently interferes with the energy demand in silver catfish, which can cause a delay in development (COPATTI et al., 2005; 2011b). In agreement with the above-mentioned, the present study demonstrated that the development of silver catfish juveniles in water without HA was considerably lower at pH 5.5 than at pH 6.5 after 40 days. These results are also consistent with previous findings: silver catfish exposed to pH 5.5 showed lower growth compared to pH 7.0-7.5 (COPATTI et al., 2005; 2011b); and no significant effect of pH was observed in the 6.0-8.0 range (COPATTI et al., 2011a).

Humic substances may be stimulant and protector of paracellular junctions of the gill epithelium, thus preventing ion efflux in some fish from the natural acidic black waters of Rio Negro (GONZALEZ et al., 2002; 2005; WOOD et al., 2003). This protective action could spare energy to be used in fish growth, explaining the greater development of green sword tail exposed to 180mg L^{-1} humic substance compared to control (0mg L^{-1} humic substance) (MEINELT et al., 2004). However, COSTA et al. (2015) demonstrated that HA does not protect silver catfish against acidic pH because increased mortality and Cl^- loss occurs at pH 4.0. Results of the current study are in agreement with the deleterious effect of HA in silver catfish, since the increasing HA concentration shows a negative relationship with parameters evaluated, namely weight, feed intake and standard growth rate, irrespective of the pH tested.

The initial hypothesis that humic substances would serve as stimulant and protector of paracellular junctions of the gill epithelium, thus preventing ion efflux and sparing energy to be used in fish growth, was not corroborated by results obtained in this study. Deleterious effects of HA were more evident at pH 6.5 than at pH 5.5. Acidification of humic substance molecules positively charged reduces the load and increase lipophilicity, thereby increasing toxicity (PETERSEN Jr & PERSSON 1987). Internally, humic substances can migrate to organs or organelles and cause the most diverse biological responses, such as stress and lipid peroxidation (MEINELT et al., 2008).

The present zootechnical evaluation did not support the protective effect previously observed for humic substances against ion regulatory disturbances induced by low pH in fish (STEINBERG et al., 2006; MATSUO & VAL, 2007). Poor development of silver catfish exposed to HA was probably a result of the increased energy demand to maintain homeostasis.

CONCLUSION

Water pH 5.5 impairs silver catfish growth compared to pH 6.5 in water without HA. Humic

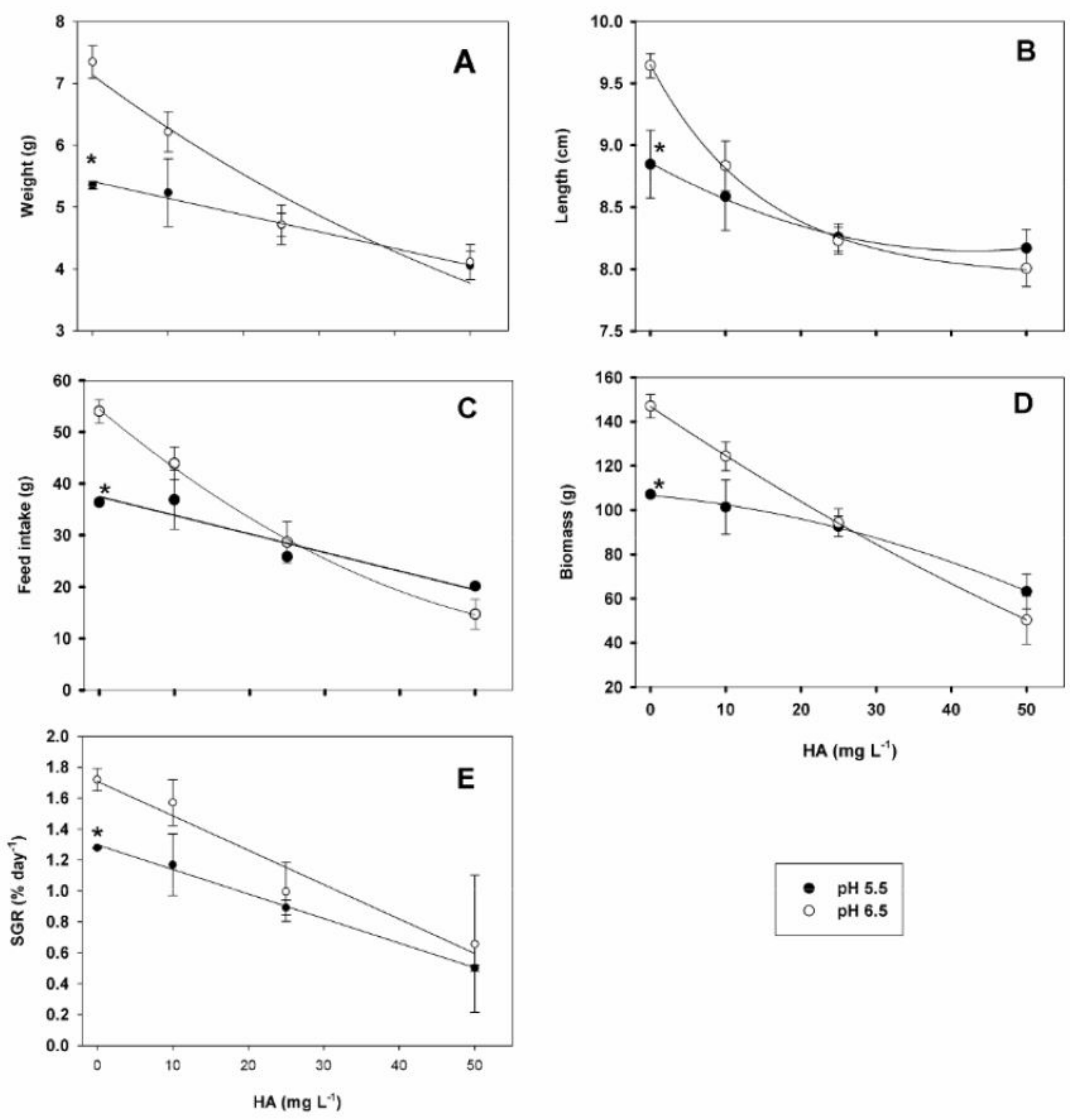

Figure 1 - Performance of silver catfish juveniles after 40 days of exposure to different levels of humic acid (HA) associated with pH 5.5 and 6.5. The curves can be represented by the following equations: (A) pH 6.5 $y=7.13e^{(-0.013x)}$ ($r^2=0.9396$), pH 5.5 $y=5.41-0.027x$ ($r^2=0.9876$), (B) pH 6.5 $y=7.93+1.71e^{(-0.0668x)}$ ($r^2=0.9992$), pH 5.5 $y=8.86-0.033x+0.0004x^2$ ($r^2=0.9972$), (C) pH 6.5 $y=54.41-1.22x+0.0085x^2$ ($r^2=0.9986$), pH 5.5 $y=37.49-0.36x$ ($r^2=0.9130$), (D) pH 6.5 $y= 146.82-2.29x+0.0072x^2$ ($r^2=1.0$), pH 5.5 $y=106.62-0.31x-0.0112x^2$ ($r^2=0.9983$), (E) pH 6.5 $y=1.71-0.0223x$ ($r^2=0.9517$), pH 5.5 $y=1.298-0.0159x$ ($r^2=0.9962$), where x = level of HA (mg L^{-1}) and y = weight (g) (A) length (cm) (B), feed intake (g) (C), biomass (g) (D), specific growth rate - SGR (% day^{-1}) (E).
*Significantly different from the group exposed to pH 6.5 at the same level of HA ($P<0.05$).

acid displays a detrimental effect on the development of silver catfish juveniles, i.e., the higher the concentration of HA, the greater the yield deficit of silver catfish, irrespective of pH tested.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study has been approved by Ethics Commitee (CEUA) of the Universidade Federal de Santa Maria (UFSM) (protocol number – 128/2010 (2)).

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