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Immunization of Hybrid Surubim (*Pseudoplatystoma corruscans* x *P. fasciatum*) Against Motile *Aeromonas hydrophila* Septicemia

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ABSTRACT

The aim of this study was to evaluate the immune response of hybrid surubim vaccinated by intraperitoneal injection and by immersion against Aeromonas hydrophila. *Vaccine was prepared with formalin-inactivated* A. hydrophila. *Forty eight fishes (47±9g) were distributed in 12 tanks (4 fish / tank) in the following treatments (4 tanks / treatment): unvaccinated fish, vaccinated intraperitoneally and vaccinated by immersion. After 21 days, intraperitoneally vaccinated fishes showed the highest values of total protein and immunoglobulins, agglutination titer and serum antimicrobial activity. Lysozyme concentration in the serum was higher in the fishes vaccinated by immersion than unvaccinated fishes. Intraperitoneal vaccination induced the highest immune response and could be used to improve the fish resistance against motile* Aeromonas.

Key words: *Pseudoplatystoma*, *Aeromonas hydrophila*, vaccine, immunology

INTRODUCTION

The hybrid surubim from pintado (*Pseudoplatystoma corruscans* Spix, Agassiz 1829) and cachara (*P. fasciatum* Linnaeus 1766), cultured in Brazil is one of the largest freshwater catfish in South America, presenting high commercial value (Godinho et al. 2007). Disease outbreaks in fish farms during the winter are responsible for mortality up to 80% in surubim production (Campos 2004). Silva et al. (2012) isolated *Aeromonas hydrophila* from the dead cultured hybrid surubins in an outbreak of fish mortality at a fish farm located in the State of Mato Grosso do Sul (Brazil) and confirmed that this strain was the cause of motile *Aeromonas* septicemia in surubim. To control the bacterial diseases, antibiotics are largely used. However, inappropriate use can cause development of resistant bacterial strains and environmental pollution (Klaenhammer and Kullen 1999). Thus, the development of alternative, as probiotics and vaccines, has been shown to be a promising tool against the fishes bacterial diseases (Shoemaker et al. 2010, Barbosa et al. 2011). The aim of this study was to evaluate the immune responses of

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hybrid surubim vaccinated by intraperitoneal injection (i.p.) and by immersion against *A. hydrophila*.

The *Aeromonas hydrophila* strain (228-08 CPQBA DRM) used in this experiment was isolated from the dead hybrid surubim (Silva et al. 2012). The *A. hydrophila* strain was grown in the BHI medium at 28°C for 24 h and then inactivated with 0.5% formalin and incubated for 24 h at 28°C. After that, the strain was centrifuged at 1400 *g* for 30 min at 4°C. The supernatant was discarded and the bacterial pellet was re-suspended in PBS to get a concentration of $2x10^8$ CFU mL^{-1}, according to the bacterial growth curve (bacterial concentration × absorbance) previously performed (Silva et al. 2012). Vaccine concentrations were chosen according to the concentrations used in scientific studies with freshwater fish (Silva et al. 2009, Klesius et al. 1999, Evans et al. 2004, Santos et al. 2005, Shoemaker et al. 2006). In the experiment, 48 fishes (47±9 g) were distributed in 12 tanks of 100 L in a closed recirculation system with heating. The treatments consisted of unvaccinated fishes, fishes vaccinated by injection i.p. (0.01 mL g^{-1} of fish) with solution containing 10^8 CFU mL^{-1} and fishes vaccinated by immersion bath (30 minutes) in the solution containing 10^7 CFU mL^{-1}. Daily measurements were taken for the pH, temperature, dissolved oxygen, ammonia and nitrite. Fishes were fed three times a day, at 3% of the biomass daily. During the acclimatization and after the vaccination of the fishes the salinity was kept at 1-3‰ by adding NaCl (not iodized) to maintain the animals comfort (Beux and Zaniboni-Filho 2007). After 21 days, four fishes per tank were anesthetized (benzocaine 0.1g L^{-1}) and the blood was collected by puncturing the caudal vein using 3-mL syringe (21G) without anticoagulant. The blood was allowed to clot for 1 h at 25°C, pooled from four animals in the same tank, and subsequently centrifuged at 1400 *g* for 10 min to obtain the serum. The serum was stored frozen at -20ºC until analysis. Agglutination titer and serum antimicrobial activity against *A. hydrophila* was analyzed in the microplates by serial dilutions (Silva et al. 2009). Agglutination titer was expressed by last serum dilution that showed their respective activities. The serum antimicrobial activity was expressed by lower serum protein concentration (mg mL^{-1}) that inhibited the pathogenic bacteria. Serum lysozyme was analyzed adding 10 µL of serum with 200µL of *Micrococcus lysodeikticus* cell suspension in the microplates and the initial absorbance and the absorbance after 20 minutes at 35ºC were measured at 492nm. The absorbance decrease rate was converted to lysozyme concentration (µg ml^{-1}) using a standard curve from the hen egg white lysozyme (adapted from Sankaran and Gurnani 1972). The total serum protein was measured by the method of Bradford (1976). To measured the concentration of total immunoglobulin, 100 mL of serum was mixed with 100 mL of 12% polyethylene glycol (PEG, 10,000 MW; Sigma Chemical, St. Louis, MO, USA). The samples were incubated for 2 h to precipitate the immunoglobulin molecules, which were then removed by centrifugation at 5000 *g* for 10 min (4 °C). The concentration of total immunoglobulin was calculated subtracting the serum protein treated with 12% polyethyleneglycol solutions of the total serum protein (adapted from Amar et al. 2000). Data were analyzed for homoscedasticity by Bartllet test ($p<0.05$), in cases of homogeneity of variances, the data were submitted to ANOVA ($p<0.05$), and if necessary, significant differences were determined by SNK test ($p<0.05$).

During the experiment, the pH was 6.68±0.21, dissolved oxygen 5.9±0.5 mg L^{-1}, total ammonia 1.21±0.7 mg L^{-1} nitrite 0.24±0.21 mg mL^{-1} and temperature 26.8±0.6ºC. The fishes vaccinated by different routes showed higher total protein and total immunoglobulin compared to the unvaccinated fishes ($p<0.02$). The fish vaccinated by i.p. showed the highest values ($p<0.01$) of agglutination titer and only the serum of these fishes showed antimicrobial activity against *A. hydrophila*. The lysozyme concentration in the serum of the fishes vaccinated by the immersion showed the highest values when compared to non-vaccinated ($p<0.02$) (Table 1).

Table 1- Immunological parameters (mean ± standard deviation) in the hybrid surubim (*Pseudoplatystoma corruscans* x *P. fasciatum*) vaccinated against *Aeromonas hydrophila*.

Treatments	Agglutination titer	Antimicrobial activity (mg.mL^{-1} protein)	Lysozyme (µg·mL^{-1})	Total protein (mg·mL^{-1})	Total immunoglobulin (mg·mL^{-1})
Unvaccinated	1:1600^{b}	NI	7.03±0.81^{b}	25.43±1.05^{b}	3.59±0.88^{b}
i.p. injection	1:3400^{a}	7.0±1,9	8.27±0.42^{ab}	32.17±096^{a}	12.22±3.57^{a}
Immersion	1:1600^{b}	NI	9.55±0.85^{a}	32.80±4.41^{a}	11.43±3.90^{a}

NI – No Inhibited.

An increase in the total protein of vaccinated fishes could have been caused by the increment of total serum immunoglobulins. The increase of these parameters together with the increased agglutination titer of i.p. vaccinated fishes indicated an enhancement in the specific humoral immune response for the vaccinated fishes (Kaattari and Piganelli 1996). Nile tilapia (*Oreochromis niloticus*) immunized with the polyvalent vaccine showed increased agglutination titer of the serum against *A. hydrophila*, *Pseudomonas aeroginosa* and *Enterococcus durans* (Silva et al. 2009, Bailone et al. 2010). Tu et al. (2010) also observed increased immunoglobulin in the serum of in Nile tilapia against *A. hydrophila* between fourth and seventh week post immunization. Serum antimicrobial activity of the teleost fish is due to several types of proteins and enzymes such as lysozyme, transferrin, antimicrobial peptides and anti-proteases. An increase in the serum antimicrobial activity indicates enhancement in the humoral innate immune response of the fishes (Ellis 1999). Like this study, serum antimicrobial activity was higher in the vaccinated tilapia (Silva et al. 2009). Enhancement in the lysozyme was also observed in European sturgeon (*Huso huso*) i.p. vaccinated against *A. hydrophila* (Khoshbavar-Rostami et al., 2007). The efficiency of the vaccine to stimulate the fish defense system is also associated to different routes of administration, each one with advantages and disadvantages (Santos et al. 2005). Turbot (*Scophthalmus maximus*) and eel (*Anguilla anguilla*) showed satisfactory results only after i.p. vaccination compared to the immersion bath against *A. salmonicida* and *Vibrio vulnificus*, respectively (Santos et al. 2005, Esteve-Gassent et al. 2004). Both the studies showed that i.p. vaccination was more effective at increasing the RPS (*relative percent survival*) and the immunoglobulin levels as compared with the bath immersion. Increased antimicrobial activity observed in this study in i.p. vaccinated fishes was in agreement with the findings of Silva et al. (2009) in tilapia.

The results demonstrated that the vaccinated surubim showed significant enhancement in the immune parameters evaluated with the i.p. route providing the better immune stimulation. However, more studies are needed to demonstrate the vaccine efficiency after the bacterial challenge.

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