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Purification and assay of *Arapaima gigas* vitellogenin: Potential use for sex determination

by

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ABSTRACT. - *Arapaima gigas* is an ancient, air-breathing, giant fish of Amazonian rivers. Due to its economic importance, the development of aquaculture for this species is currently a good alternative to counteract the decline of wild populations. Reproduction in captivity relies on the constitution of male and female pairs, which occur naturally in large ponds. But, as sex determination of *A. gigas* is not possible by morphological criteria it is very difficult to optimize reproduction success and fry production in each pond. This situation prompted us to develop a sexing methodology based on the detection of female specific plasma Vitellogenin (Vtg) using an Enzyme Immuno-Assay (EIA). Vitellogenin purification was performed by electro-elution after polyacrilamide gel electrophoresis (PAGE) from plasma of 17β-Estradiol treated *A. gigas* juveniles. Two different Vtg molecules were isolated, (Vtg_1 and Vtg_2) with 184 and 112 kDa apparent molecular masses repectively. Both antibodies, produced in rabbits, reacted with both vitellogenins in a competitive assay or by direct Vtg coating. For sexing of the fish, we finally chose the method of direct coating of serial plasma dilutions.

Key words. - *Arapaima gigas* - Sex determination - Vitellogenin, EIA - Amazon.

Introduction

Due to its economic importance, the development of aquaculture for *A. gigas* is currently a good alternative to counteract the decline of wild populations. From observations in captivity, natural reproduction occurs only once a year. Improving reproduction performance and fry production in captivity requires sex determination of broodstock and the constitution of mating couples. As no distinctive external sex characteristics are evident in this species, we planned to determine sex through plasma Vtg detection in maturing females.

Material and Methods

Vtg purification was performed by electro-elution after polyacrilamide gel electrophoresis (PAGE) of plasma from 17beta-Estradiol treated *A. gigas* juveniles. The Vtg preparations obtained were used as an antigen to obtain specific polyclonal antiserums in rabbits. Antibody specificity and affinity were tested by Enzyme Immuno-Assay methodology (EIA), either by competitive assay or by direct Vtg coating on 96-well plates.

Results and discussion

Using SDS-PAGE, the Estradiol treatment induced two major bands (Vtg_1 and Vtg_2), with 184 and 112 kDa apparent molecular masses respectively (Fig. 1A), which were absent in the control plasma. These bands correspond to 2 different vitellogenin molecules, since they also migrate as two separate bands under SDS-PAGE in reducing conditions (with β-mercapto-ethanol). The electro-eluted Vtg preparations were used as a coating and standard antigens in order to set up a specific EIA for each Vtg. This EIA allowed us to quantify plasma Vtg in different plasma samples with a sensitivity of around 10 $ng.mL^{-1}$ (Fig. 1B).

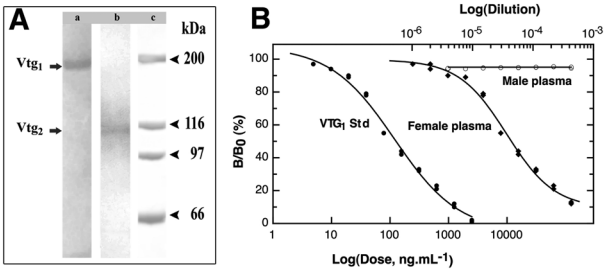

Figure 1. - **A**: SDS-PAGE of electro-eluted *A. gigas* Vitellogenins. a: Vtg_1, b : Vtg_2; c: Molecular Mass Markers. **B**: Standard curve of *A. gigas* Vtg_1 and male and female plasma dilutions. Coating, 400 $ng.mL^{-1}$; antibody dilution, 1:40,000; incubation time, 2 hours at 37°C.

Conclusions

We set up a Vtg EIA for *A. gigas*, which can detect very low Vtg concentrations in the plasma. As Vtg is a female specific molecule in normal rearing conditions, it may be possible to sex the 3 to 5 year-old fish since this age corresponds to the reported age of first maturation in captivity. At this age Vtg levels in females are higher than the assay sensitivity (10 $ng.mL^{-1}$), allowing sex determination by direct coating of plasma serial dilutions.

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