

**Development and validation of enzyme-linked immunosorbent assay (ELISA)  
vitellogenin in *Lates calcarifer*.**

ABSTRACT

A study was conducted to develop and validate a competitive enzyme-linked immunosorbent assay (ELISA) for detection of plasmatic vtg in Asian sea bass, *Lates calcarifer*. Highly specific polyclonal antibodies against purified vtg (antigen) were employed for quantifying the concentration of plasma vtg. The working ranges of the assay were 31.2 to 1000 ng mL<sup>-1</sup> with the sensitivity of 6.9 ng mL<sup>-1</sup>. Antigen concentration of 250 ng mL<sup>-1</sup> and antibody dilution of 1:1000 were selected as a workable ELISA after several preliminary test. The ELISA demonstrated precision with intra- and inter-assay Coefficient of Variations (CVs) at 90, 80 and 50% of binding were less than 8.4 (n = 9) and 12.1% (n = 5), respectively. Serial plasma dilutions from natural vitellogenic females and E2 treated were paralleled to the vtg standard curve (purified vtg) as analyzed by ANCOVA (p < 0.05). No cross-reaction was observed in analyses of male's plasma, indicating non-specific binding. The assay was validated by measuring plasma vtg levels in matured females and males (n = 5) obtained during the reproductive season in captive condition. Female's plasma vtg ranges from 0.9 to 1.54 mg mL<sup>-1</sup>, while no vtg was detected in males plasma. Our results indicated that vtg levels proposed as an indicator for maturing female Asian sea bass, *L. calcarifer* as well as in female species from genus *Lates*.

**Keyword:** Development and Validation; ELISA; Vitellogenin; *Lates calcarifer*.