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Title: **An Enzyme-Linked Immunosorbent Assay Is Not Effective for Sampling Blood Plasma Insulin Concentrations in Red Pacu, *Piaractus brachypomus* and Black Pacu, *Colossoma macropomum***

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Abstract: Culture of Red Facu (RP), *Piaractus brachypomus* and Black Facu (BP), *Colossoma macropomum* is increasing due to increasing demand from human populations and declining supply caused by depletion of wild fish so practical diet formulations need to be developed for pacu. Insulin assays are a valuable tool in assessing carbohydrate utilization in fish for diet development. Therefore, we conducted procedures to validate an Enzyme-Linked Immunos osorbent Assay (ELISA) for detection of plasma insulin concentrations in RP and BP.
Red and black pacu were fed a commercial catfish diet containing approximately 40010 soluble carbohydrates (32% protein, 6% fat). Both species were then bled and plasma was used for validation of the assay. An ELISA was conducted using the Food and Drug Administration's Center for Veterinary Medicine validation of analytical procedures methodology. The results from this assay validation study indicate that an ELISA insulin kit was not suitable for experimental detection of blood plasma insulin concentrations in RP and BP. Ahnost no insulin (0.34 to 0.48 ng mL^{-1}, for red pacu; 0.40 to 0.67 ng mL^{-1}, for black pacu) was detected in unknown blood plasma samples from the fish. This indicated that the mammalian insulin antibodies are more derived or that the molecular structure of the insulin variants produced by pacu are not capable of being bound by the antibodies in the ELISA assay. The accuracy (mean recovery of spiked samples was 56.0010 for RP and 68.6% for BP), linearity (R^2 = 0.0011 for RP and R^2 = 0.1822 for BP), precision (mean recovery of serial dilutions was 212.8% for RP and 209.2% for BP) and reproducibility of the data were poor.

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