TITLE: COLLECTION AND EXTRACTION OF RNA FROM NILE TILAPIA SKIN INFECTED WITH *Aeromonas hydrophila* FOR GENE EXPRESSION STUDIES

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ABSTRATC:

Nile Tilapia (*Oreochromis niloticus*) is considered one of the most produced in captive fish species in the world due to its rusticity characteristics. However, the super intensive culture environment provides the animal with stress either by the accommodation condition or by the quality of the aquatic environment, in addition to the expense of the fish that is often erroneously done causing injury to organs such as the skin, which is part of the innate immune response, and may contribute to opportunistic infections such as that caused by the bacterium *Aeromonas hydrophila*. Thus, this work aimed to develop a protocol for the collection and extraction of RNA from the skin of the Nile Tilapia infected with A. hydrophila for later use of the data in RNA-Seq analysis. Fish skin samples submitted to inoculation for 6 hours and 24 hours with *A. hydrophila* or 0.85% saline solution (control group) were initially collected with the removal of all scales on the left side of the animal with the aid of a scalpel the fish were then washed with sterile DEPC water at 4 ° C and removed from 1 cm^2 of skin, followed by two washes in sterile DEPC water at 4 ° C, followed by packing in cryogenic tubes and storage in ultrafreezer at -80 ° C. Total RNA extraction was performed using the combination of the Trizol (Invitrogen) and the RNeasy Mini Kit (Qiagen) method. The samples were analyzed for their purity, concentration and integrity in NanoDrop2000c spectrophotometer (Thermo Fisher Scientific), Qubit fluorimeter (Invitrogen), Bioanalyser (Agilent Technologies) and 1% agarose gel. The RNAs extracted from the animals inoculated or not with the bacterium had yields higher than 400 ng / μL and RNA (Integrity Number) RNA values higher than 7.0. This process of obtaining biological material from the skin with extraction of RNA can be limited by the composition and stiffness of the organ besides, it makes difficult the homogenization of the tissues and by the presence of RNAses. The established protocol was efficient and presented good RNA quality and yield, thus allowing the application of the samples in sequencing analysis, Seq RNA library construction and gene expression studies.

Keywords: Molecular biology, *Oreochromis niloticus*, Ribonucleic acid and RNA-Seq.

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