Report of Student Research Assistance Scheme

Project title: **The immune system as an indicator of toxicity of pesticides in *Caiman latirostris.***

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Introduction

Agricultural activities associated mainly to soybean cultures affect natural environment, including wildlife, by habitat destruction and the extensive use of agrochemicals. The effect of agrochemicals widely used in Argentina on components of the innate and acquired immune *C. latirostris* were evaluated.

According to the methodology described in the proposal, we use hatchlings of *C. latirostris* belonging to wild nests, collected in the Managed Nature Reserve "El Fisco" (30° 11'26 "S, 61° 0'27"O; Santa Fe, Arg.), Natural protected area (Law 12.930, 2008), under the "Proyecto Yacaré" (PY) ranching program (Fig 1). Animals were exposed to pesticides by voluntary immersion (Fig 2).

Before and after of study of pesticides exposure, the caimans were weighted (OHAUS® Compact scale CS200, precision 0.1 g) and measured in total length (TL) and snout-vent length (SVL) (tape measure, precision 0.5 cm) and then they were distributed in 6 treatments: a negative control (NC), vehicle control (VC), 2 concentrations of endosulfan (END1 y END2, END2>END1) and 2 concentrations of cypermethrin (CIP 1 y CIP 2, CIP2>CIP1).



Fig 1. Egg collection of *C. latirostris*



Fig 2. Exposition hatchlings *C. latirostris* to pesticides for immersion.

Immediately after exposure, all animals were bleeding. Whole blood samples were obtained from the spinal vein (Olson, 1977) with heparinized disposable syringes (Fig. 3) and then were applied different assays: Total leukocyte count, differential leukocyte count, and determination of natural antibodies.


Fig 3. Obtaining blood samples from the spinal vein.

Total leukocyte count (TWBC): An aliquot of blood was taken and diluted with 0.6% NaCl (Lewis et al., 2008), in a 1: 200 dilution and added in a Neubauer chamber. The samples were observed with an optical microscope (450x) using a Newbahuer chamber. Results were expressed as number of cells per cubic millimeter of blood.

Differential leukocyte count (DWLC): two smears were made per animal. Blood was fixed with ethanol for 10 min and then be stained with May Grunwald solution - Giemsa. Then they were observed under an optical microscope (1000x). The amount of the subtypes of white blood cells (heterophils, basophils, eosinophils, lymphocytes and monocytes) were expressed each 100 cells analyzed and expressed as percentage.

Determination of natural antibodies (Nab): Determination of NAbs titres (dilution factor -1) was carried out in triplicate, using a hemagglutination assay described by Matson et al. (2005), with modifications. The assay is carried out in 96-well bottom microplates.

Twenty-five µl of RRBC and 25 µl of 0.01 M phosphate buffered saline (PBS) were added in 12 consecutives columns. Caiman plasma was added only in the columns 1. Using a dilutor (fixed volume) or pipette, 25 µl of the contents in the column 1 were added to the contents of the column 2 wells through column 11 (serial dilution 1:2) and 25 µl of the column 11 were discarded. The column number 12 was negative control (PBS + RRBC). Each plate is then sealed with Parafilm and protected of the light during 90 min.

Determination of complement hemolysis test system (CS): To characterize the *C. latirostris* SC, the SRBC (Sheep red blood cells) were obtained from heparinized whole blood drawn from merino sheep (*Ovis aries*).

Sheep blood was centrifuged at 450 g for 20 min and the supernatant was discarded, performing repeated washings with a phosphate buffered saline solution (PBS, pH 7.4) until the supernatant was clarified. After the final wash, a solution of 2% (v/v) SBRC in PBS was prepared.

Yacaré plasma was incubated with SRBC for 30 minutes, then the mixed was centrifuged at 2500 x. Subsequently, 300 ul of supernatant were taken and transferred to a microplate for measuring the optical density in a microplate reader at 540 nm (Multiskan RC Labsystem, Helsinki, Finland). The result obtained was considered as the maximum hemolysis (MH).

Results

No differences between groups for both TWBC and growth measures were found. In the case of DWBC, there were differences in heterophils between NC vs CIP1 ($p= 0.013$), in lymphocytes between NC vs CIP1 ($p= 0.029$) and in NC vs END1 ($p= 0.018$) and monocytes between NC vs CIP1 ($p= 0.009$) and NC vs END1 ($p= 0.001$).

The determination of natural antibodies showed significant differences in the animals exposed to CIP2 and END2 compared with NC ($p<0.05$).

Similar results we have found in CS activity based on SRBC hemolysis, where an effect of the pesticides was observed, the group of animals exposed to CIP2 and END2 showed a lower CS activity than animals from the NC ($p<0.05$).

The immune system (IS) is an excellent indicator of the health of an organism (Burns et al., 1996). In this sense, the exposure of animals to certain pesticides, whether acute or chronic, could affect this defense mechanism, being an indicator of toxicity of great sensitivity. The pesticides exposure in controlled conditions showed a decreased in some immune parameters and we supposed that values affect the functionality.

The observed effects may be indicative of a general capacity reduced caimans exposed in natural environments, to respond to infectious agents. In this case, it would be highly important to study how is immune parameters behavior when they are challenged.

References

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