Report of CSG Student Research Assistance Scheme Grant

I am writing to inform the destination of the funds that I have received from CSG Student Research Assistance Scheme Grant. My proposal included the isolation and characterization of antimicrobial peptides (AMPs) expressed in caiman leukocytes. Based on my goals proposed I bled seven wild *Caiman latirostris* in the north of Santa Fe province, Argentina. Then, extracted the white cells from blood and kept refrigerated based in Merchant et al. (2006). Basically, it consist of keep settle and separate many times the top layers where are the leukocytes, approximately once per hour, during eight to ten hours. These extracts were centrifuged and cells pellets resuspended in saline solution.

Taking into account all of care with the samples, I travelled to United States with white cells samples to work with my advisor, Dr. Mark Merchant, at the Department of Chemistry at McNeese State University. In his lab, the leukocytes were disrupted and this homogenate were treated in different steps to obtain the caiman AMPs. The final leukocyte extract were exposed to different bacteria cultures during 48 hours to evaluate the antibacterial activity from the caiman AMPs. Unfortunately, we did not show any antibacterial activity when the extracts was exposed to the bacterial cultures studied.

I brought to US not only caiman latirostris white cell extracted but also serum from *Caiman latirostris and Caiman yacare*. I took advantage that I had been in US and make many assays with this serum.

One of the assays was to detect the activity of caiman Phospholipase A_2 (PLA_2). This enzyme act cleaving fatty acids from the *sn*-2 position of membrane phospholipids. There are two kinds of these enzymes of PLA_2, intracellular PLA_2 has been shown to be important for the generation of arachidonic acid, which leads to the synthesis of a wide range of eicosanoid paracrine hormones; and secretory PLA_2 in the serum, that has been reported to exhibit important immune activities. For the determination of the effects of serum on the activity of PLA_2 activity, different amounts of caiman serum were incubated with BODIPY-labeled *E. coli* bacteria in assay buffer. After incubation period, the reaction was stopped by the addition stop buffer (EGTA +tris-HCl, pH 8.0). The reactions were centrifuged and the clear supernatants were removed to plastic cuvettes. The fluorescent intensity was measured in a fluorometer.

Another assay was to detect and characterize the *Caiman latirostris* and *Caiman yacare* dipeptidyl peptidase IV (DPPIV). Dipeptidyl peptidase IV (DPPIV) is a well-characterized protease that plays an important role as physiological regulator of a number of peptides that serve as biochemical messengers within the immune system. Plasma DPPIV activity was characterized with respect to temperature, kinetics and concentration dependence. These results revealed that plasma DPPIV activities were different between these two crocodilian species and could be one of the reasons for the differences in susceptibility to infection in them**.** To characterize *C. latirostris* and *C. yacare* DPPIV activity, we used the assay adapted from American alligator plasma and whole blood by Merchant et al., (2009). This assay is based on the effects of DPPIV on the hydrolysis of Ala-Pro-AFC. Assay buffer to initiate the reaction (Tris HCl, ph 7.4) and stop buffer to finished it (Tris HCl, ph 7.4 and EDTA), were used.

And the last assay made with Caiman serum was collect caiman lectin from a glucosamine column followed by elution on a reversed phase column. Lectin proteins collected from Caiman was also separated for further analysis and comparison to lectin proteins collected from the American alligator. The 1D SDS-PAGE gel was stained with glycoprotein stain and 2 major bands at 37 and 75 kDa was observed from the Caiman lectin sample. These bands appear at similar masses to the protein bands that were separated for the alligator lectin. However, the mass at approximately 160 kDa that appeared from the alligator lectin sample is absent in the caiman sample (fig 1).

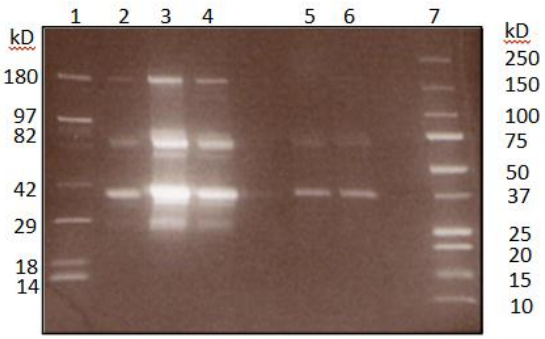


Figure 1. Coomassie staining of alligator and caiman lectin proteins separated on a 4-20% Tris-HCl gradient gel that was eluted from a mannose affinity column. Lane 1, glycoprotein molecular weight standards; lane 2, overflow from lane 3; lanes 3, and 4, lectin protein from alligator serum eluted from mannose column; lanes 5 and 6 lectin protein from caiman and lane 7 protein molecular weight standards.

Actually, a manuscript with one of these assays is under review in a peer review journal (Animal Biology) and another manuscript is in process. Probably lastone will be ready to submit at the end of this year.

Part of the cost of these assays was taken from SRAS, the rest of the reagents, equipment, and other materials were provided by University of Mc Neese (Lake Charles, LU, US) and Proyecto Yacaré (Santa Fe, Argentina). Details are showed below:

Details	U$S
Transport cost to take samples	300
Disposable materials: syringes, needles, tips, tubes, eppendorf, GUANTES, petri dishes, cuvettes, pipettes, microplates.	398
Chemicals: anticoagulants (EDTA, heparine), saline solution (ClNa), buffers (TRIS, PBS), nutrient broth, Clorhidric acid,	400
Columns	130
Enzymes Substrates	150
Total	1378*

*Extra money was provided by Dr. Mark Merchant.

We suspected that caiman leukocyte extracts will display antimicrobial properties, and that these activities are most likely due to the presence of cationic antimicrobial peptides. When we detected that is not that I suspected, we use the rest of the grant to make another assays as I explained above.



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