

Influences of behaviour and physiology on body mass gain in the woylie (*Bettongia penicillata ogilbyi*) post-translocation

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Appendix S1. Enzyme immunoassay process

A commercial human corticosterone ELISA kit (K014-H1: Arbor Assays®, Michigan, USA) was used for the analysis of faecal corticosterone concentrations following faecal steroid extraction. Corticosterone is identical across all species (Hill *et al.* 1991) so it was expected that the human kit would be able to measure corticosterone from woylies; however, to confirm this, validation for woylie faecal samples was carried out. The validation process involved examination of the parallelism and fitted regression between the human corticosterone standard curve and a serially-diluted woylie faecal extract curve (Fig. S1). There was no significant difference in the faecal extract curve between the two species ($p > 0.05$). The sensitivity of the assay was 18.6 pg/mL, the limit of detection was 16.9 pg/mL and the intra-assay precision was 3.9 % (COV).

The faecal steroid extraction was based on the DetectX™ Steroid Solid Extraction Protocol. Each animal's total faecal sample was pre-blended to obtain an homogenous 0.2 g sub-sample. Each sub-sample was mixed with 1 ml of 80 % methanol per 0.1 g of faecal solid. The samples were then placed in an overhead shaker for 30 min before centrifugation at 3,000 rpm for 15 min. The supernatant (2 ml) was evaporated to dryness under nitrogen, and then dissolved with 100 µL of 100 % ethanol and 400 µL of the assay buffer provided in the kit. An additional 500 µL of assay buffer was then added to each tube to ensure that the ethanol content was below 5 %. Samples were then covered with parafilm and refrigerated overnight.

Before starting the assay, kits and extracted samples were removed from the refrigerator for 30 min to bring them up to room temperature. The DetectX™ Assay Protocol was then followed. Firstly, the wash buffer concentrate was diluted to 1:20 by adding 30 mL of the concentrate to 570 mL of distilled water. For standard preparation, 450 µL of assay buffer was pipetted into tube number 1 and 250 µL into tubes numbered 2 to 8. Then 50 µL of the corticosterone solution was added to tube no. 1 and the mixture was vortexed. A total of 250 µL of the corticosterone solution was pipetted from tube no. 1 and added to tube no. 2 and vortexed. This was repeated for tubes numbered 3 to 8. Then 50 µL of samples or standards were pipetted into each of the 96 wells in each plate. Plate 1 had eight wells of standards, a 5-stage serial dilution and 64 wells with faecal extract samples, all with duplicates. Plate 2 had eight wells of standards, 68 wells for samples, four wells for maximum binding, four wells for non-specific binding, plus duplicates. To start the assay, 75 µL of assay buffer was pipetted into the non-specific binding wells and 50 µL was pipetted into the maximum binding (zero standard) wells. Then 25 µL of the corticosterone conjugate was added to each well (except for the non-specific binding wells), followed by 25 µL of the corticosterone antibody. Plates were then placed in a mini-plate shaker for 1 hour, aspirated, then washed four times with 300 µL of wash buffer. A total of 100 µL of tetramethylbenzidine substrate was then added to each well and plates were incubated at room temperature for 30 min. Stop Solution (50 µL) was then added to each well and the optical density was read at 450 nm on a Bio-Rad iMark Microplate Reader. The corticosterone concentration (pg/mL) was then calculated using the Microplate Manager software package (BioRad, California, USA).

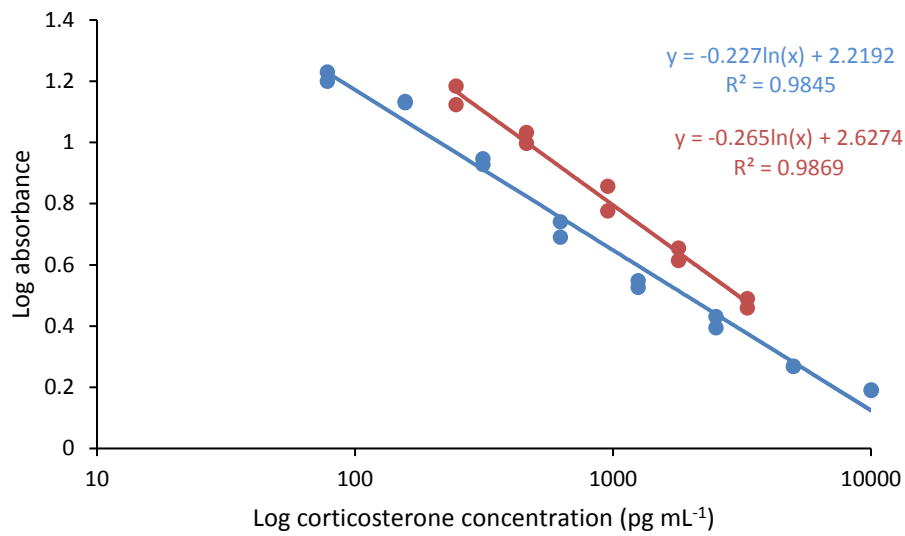


Fig. S1. Corticosterone parallelism validation curve for the human standard curve (lower line) and the woylie faecal extract curve (upper line).

Reference

Hill, R. A., Makin, H., Kirk, D., and Murphy, G. (1991) 'Dictionary of steroids.' (CRC Press: London.)