FAECAL GLUCOCORTICOID METABOLITES IN AFRICAN PENGUIN: BIOLOGICAL VALIDATION OF AN ENZYME IMMUNOASSAY

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Introduction
Captive animals are subject to a variety of physical, ecological, and social limitations. Physiological responses to stress can be used to evaluate their welfare. Exposure to stress usually results in an increased secretion of glucocorticoid hormones (GCs) from the adrenal cortex. The predominant avian GC is corticosterone (Fig. 1). The corticosterone plasmatic level is a biologically meaningful marker of stress levels in birds, and an accepted non-invasive method for the evaluation of avian adrenocortical activity is the measurement of Faecal Glucocorticoid Metabolites (FGM) (Goymann, 2005). A widely accepted immunoassay method to assess adrenocortical activity is a practical approach, based on the development of immunoassays that exploit so-called broad-selective antibodies, and the demonstration of the capability of these assays to reflect adrenocortical activity by a biological validation (Touma and Palme, 2005). Biological validation is performed using different stressors relevant to the animal (e.g., restraint, blood sampling, transportation), demonstrating that the technique can detect biologically meaningful changes in GCs levels.

The aim of this study was to validate a Competitive Enzyme Immunoassay (EIA) and determine the peak of secretion of FGM in African Penguin (Spheniscus demersus). Samples were collected from a colony in a zoological institution (Zoom Torino, Italy) (Fig. 2).

Materials and methods
To perform the biological validation, we used a known stressful event: capture and immobilization (Fig. 3). Faecal samples (n=22) were collected from three males and two females after the stressful event. Sample collection started immediately after the stressful event, and continued during the following 30 hours, except at night. Moreover, during an ordinary day without known stress, samples were collected (n=12). Sample frequency and numerosity depended on the individual. All samples were stored at -20°C immediately after collection and FGM were extracted with 5 ml of methanol:water (70:30, v/v). Samples were analysed by an expressly developed EIA based on the use of antibodies against corticosterone (for more details about EIA see Anfossi et al., 2014).

Results
The developed EIA, proved to be rapid (the test could be completed in 90 minutes), and broad-selective, as it cross-reacted with the major corticosteroids, thus allowing the detection of excreted FGM resulting from a biological stressor. Figure 4 shows a typical calibration curve obtained under optimized conditions.

Regarding the FGM levels after the stressful event, the results showed a trend, present in all animals, an example is shown in figure 7. We identified three time frames: from the stressful event to 5.5 hours after the stress, from 5.5 to 10 hours, and from 20 to 30 hours. There wasn’t samples collection from 10 to 20 hours after the stress as it was night. Results showed a statistically significant difference among the levels of FGM during the time frames (Kruskal-Wallis: y²=6.7246; df=2; p=0.034), and there was a statistically significant difference between the levels of FGM statistically different only between the first and second time frames (p=0.024) (fig. 8).

We identified a peak of secretion of FGM during the second time frame, in particular four penguins showed the peak between 7 and 10 hours after the stress. After the peak, the FGM levels gradually decreased.

Conclusions
The enzyme immunoassay developed in this study allowed the detection of an adrenocortical response to a biological stress (animal capture) in African Penguins. The assay demonstrated the difference between the values of FGM in absence of stress and after the stress and their increase and decrease. Despite individual variability, results from all five animals qualitatively agreed in suggesting a peak of FGM production between 7 and 10 hours after the stressful circumstance. This observation is in good agreement with results previously reported for other birds: 6-18 hours in Adélie Penguins (Pygoscelis adeliae) (Nakagawa et al., 2003) and 5.5-8 hours in Chicken (Gallus domesticus) (Dethier et al., 2003). The peak of secretion of FGM during the second time frame was shown to be accurate, precise and decidedly more rapid than previously reported radio and enzyme immunoassays intended for measuring FGM: the time needed to complete the analysis was 90 minutes, rather than overnight incubations.

The enzyme immunoassay can be suggested as a reliable tool to evaluate the effect of potential stressful circumstances that these animals may undergo in captivity, such as, visitor flow and excessive noise. By identifying stressful stimuli, efforts can be made to reduce their effect and prevent their occurrence, in order to improve the general welfare of captive animals.

REFERENCES

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