4-11-2011

Developing New Methods to Quantify Stress in Wildlife Using Liquid Chromatography Tandem Mass Spectrometry

Brandon Harper
Idaho Science Talent Expansion Program (Idaho STEP)

Shin Pu
Department of Biological Science, Boise State University

Jennifer Forbey
Department of Biological Sciences, Boise State University

Developing New Methods to Quantify Stress in Wildlife Using Liquid Chromatography Tandem Mass Spectrometry

Abstract

Stress levels in wildlife species are an accurate indicator of an animal's well-being and can reflect decreases in habitat quality. Stress levels can be measured by the presence of the stress response hormones cortisol, cortisone, and corticosterone. Analysis of these stress hormones in fecal samples has been widely used because feces can be easily obtained and non-invasively collected in the field. Methods of detecting stress levels from fecal samples of wildlife species are currently limited to enzyme immunoassay testing. This method uses antibodies to bind to target stress hormones. However, immunoassay testing can be time consuming and very expensive[2]. We propose that Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) offers a new method to quantify levels of the stress hormones from fecal samples that is less expensive and time consuming than traditional immunoassays[1]. As part of the Idaho Science Talent Expansion Program (STEP), we are developing a simple, accurate, and relatively inexpensive method to detect stress hormones in fecal samples from free-ranging pygmy rabbits (Brachylagus idahoensis) and sage grouse (Centrocercus urophasianus) using LC-MS/MS.

Developing New Methods to Quantify Stress in Wildlife
using Liquid Chromatography Tandem Mass Spectrometry

BRANDON HARPER[1], Shin Pu[2], and Jennifer Forbey[2]
[1]Idaho Science Talent Expansion Program (Idaho STEP)
[2]Boise State University, Department of Biological Science, Boise, ID

Background

Stress levels in wildlife species are an accurate indicator of an animal's well-being and can reflect decreases in habitat quality. Stress levels can be measured by the presence of the stress response hormones cortisol, cortisone, and corticosterone. Analysis of these stress hormones in fecal samples has been widely used because feces can be easily obtained and non-invasively collected in the field. Methods of detecting stress levels from fecal samples of wildlife species are currently limited to enzyme immunoassay testing. This method uses antibodies to bind to target stress hormones. However, immunoassay testing can be time consuming and very expensive[2]. We propose that Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) offers a new method to quantify levels of the stress hormones from fecal samples that is less expensive and time consuming than traditional immunoassays[1]. As part of the Idaho Science Talent Expansion Program (STEP), we are developing a simple, accurate, and relatively inexpensive method to detect stress hormones in fecal samples from free-ranging pygmy rabbits (Brachylagus idahoensis) and sage grouse (Centrocercus urophasianus) using LC-MS/MS.

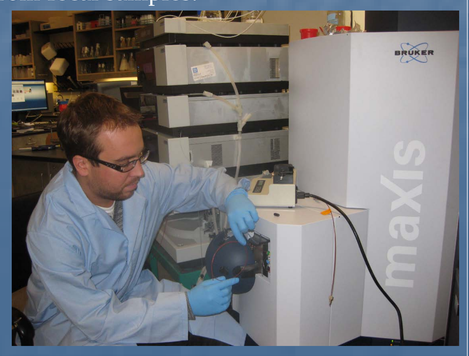
Objective

To develop a quick and accurate chemical method for effectively detecting and quantifying stress hormone levels in wildlife species.

Initial Method Development

We used Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) to quantify levels of the stress hormones Cortisol, Cortisone, and Corticosterone from fecal samples.

Sample Preparation
o Dried, ground, and spiked fecal samples with known concentrations of stress hormones (Range: 0.1 – 50 µg/mL)
Extraction
o Extracted spiked fecal samples with 80% Ethanol
Detection
o Analyzed extract by LC –MS/MS



Results

o All three stress hormones were accurately detected by LC-MS/MS.
(See Figures 1-4)

o Percent recovery of stress hormones in spiked fecal samples of pygmy rabbits was low
(See Table 1)

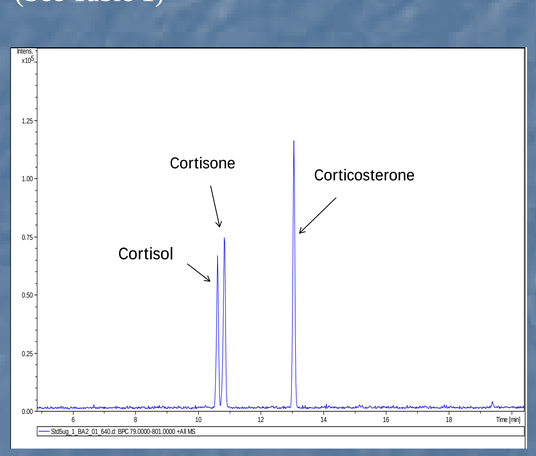


Figure 4: Stress Hormone Chromatogram

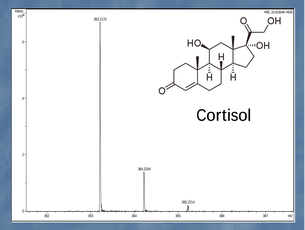


Figure 1: Cortisol Mass Spectra

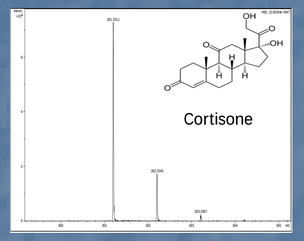


Figure 2: Cortisone Mass Spectra

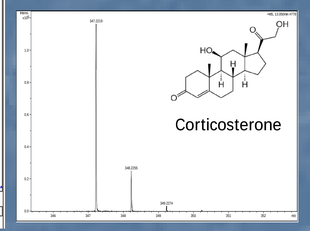


Figure 3: Corticosterone Mass Spectra

Stress Hormone Detection

Sample	Cortisol	Cortisone	Corticosterone
Spiked Sample	12.1%	10.9%	11.8%
Spiked Solvent	109.3%	99.8%	109.5%

Table 1: Percent Recovery of Cortisol, Cortisone, and Corticosterone

Method Refinement

Low percent recovery of stress hormones in fecal samples was likely caused by low extraction efficiency and ion suppression caused by complex chemical constituents of feces.

Our next step is to use Solid Phase Extraction to improve the recovery of hormones from feces by creating cleaner extracts.

Discussion

Quantifying stress levels in wildlife is currently one of the most accurate and efficient indicators of an animal's well being and habitat quality. Monitoring these levels is crucial to maintain the delicate balance between man and nature.

In the search to quench the world's need for power, alternative sources are being found that encroach upon our wilderness areas. Wildlife in these sensitive and changing habitats needs protection. Maintaining an animal's well being while harnessing nature's sources of power is a fine line and needs to be walked cautiously.

Keeping watch over the well being of the wildlife in these areas is a responsibility that falls to scientists and engineers alike. The development of our new method will aid conservation biologists and developers by providing a tool to quickly quantify stress in wildlife associated with a changing habitat and mitigation efforts.

Literature Cited

1) Cho, H.-J. et al. 2009. Quantitative metabolic profiling of 21 endogenous corticosteroids in urine by liquid chromatography-triple quadrupole-mass spectrometry. Analytica Chimica Acta. 632: 101-108.

2) Sheriff, M. J. et al. 2010. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? General and Comparative Endocrinology. 166: 614-619.

Acknowledgements

This research was funded by the National Science Foundation Grant number 0856815 through the Idaho Science Talent Expansion Program (STEP) and NSF CHE-0923535 awarded to K. Cornell.
I would also like to extend my sincere thanks to Jude Garzolini, and Dr. Joe Guarino for their support on this project.