

# Androgen metabolite measurement in droppings from adult male Eastern Bluebirds (*Sialia sialis*)

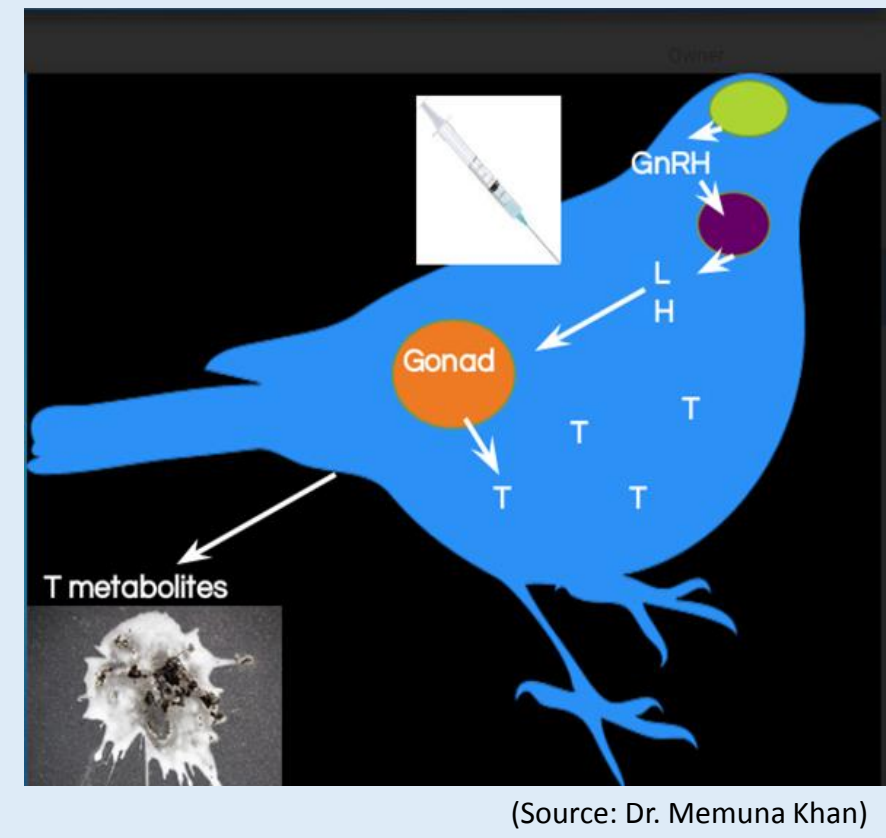


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## Hypothesis

**Hypothesis:** Non-invasive analytical techniques can reliably measure androgen metabolites in bluebird excrement

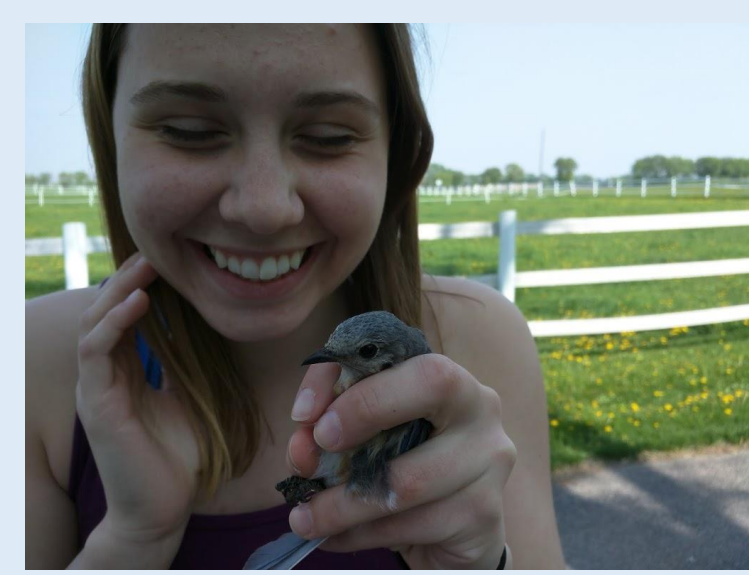


(Source: Dr. Memuna Khan)

## Introduction

**Aim:** To validate non-invasive measurement of testosterone metabolites in excrement from Eastern Bluebirds

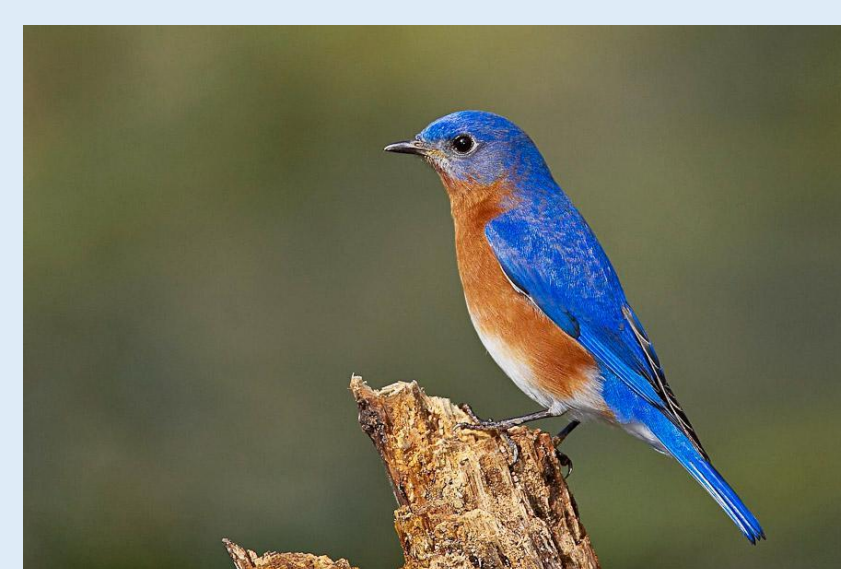
- Validation is essential because metabolic pathways may differ by species<sup>1</sup>, sex<sup>2</sup>, and age<sup>3</sup>
- Non-invasive techniques to assess the physiology of free-living birds using steroid hormone metabolite measurements in droppings are attractive because blood sampling has a myriad of pitfalls:
  - Hormones in the blood change in response to stress<sup>4</sup>
  - Large amounts of blood or numerous samples are not possible due to the small size of Eastern Bluebirds
  - It only provides information on hormone levels at one specific point in time



(Source: Dr. Memuna Khan)

- Consequently, there are benefits to measuring excrement:

- It is easily obtained
- Is less harmful to collect from the bird
- It provides a better view of the pattern of hormone levels over time



(Source: The Internet Bird Collection)

## Methods

### Sample Collection:

15 subjects were obtained through the Ripon College Bluebird trail from April 14 to May 22, 2014

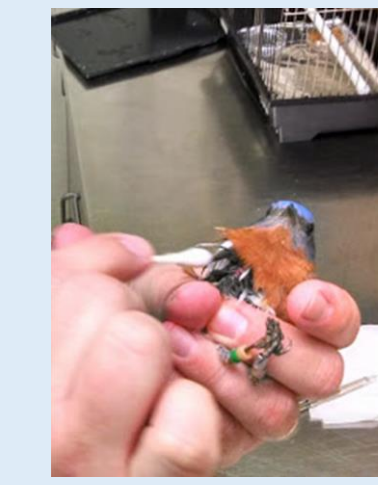
Pretreatment samples of blood and droppings were taken before the birds were injected with either GnRH to stimulate the HPG axis or saline as a control



(Source: Dr. Memuna Khan)



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Further samples were taken at 30 minutes and 60 minutes for blood and at 1, 2, 4, 6, and 8 hours for droppings

### Purification and Analysis:

Blood samples were separated into plasma while droppings samples were freeze dried; both were stored at -20°C

Droppings were homogenized and extracted in MeOH



(Source: Dr. Memuna Khan)

Droppings were then purified through Waters Oasis HLB cartridges

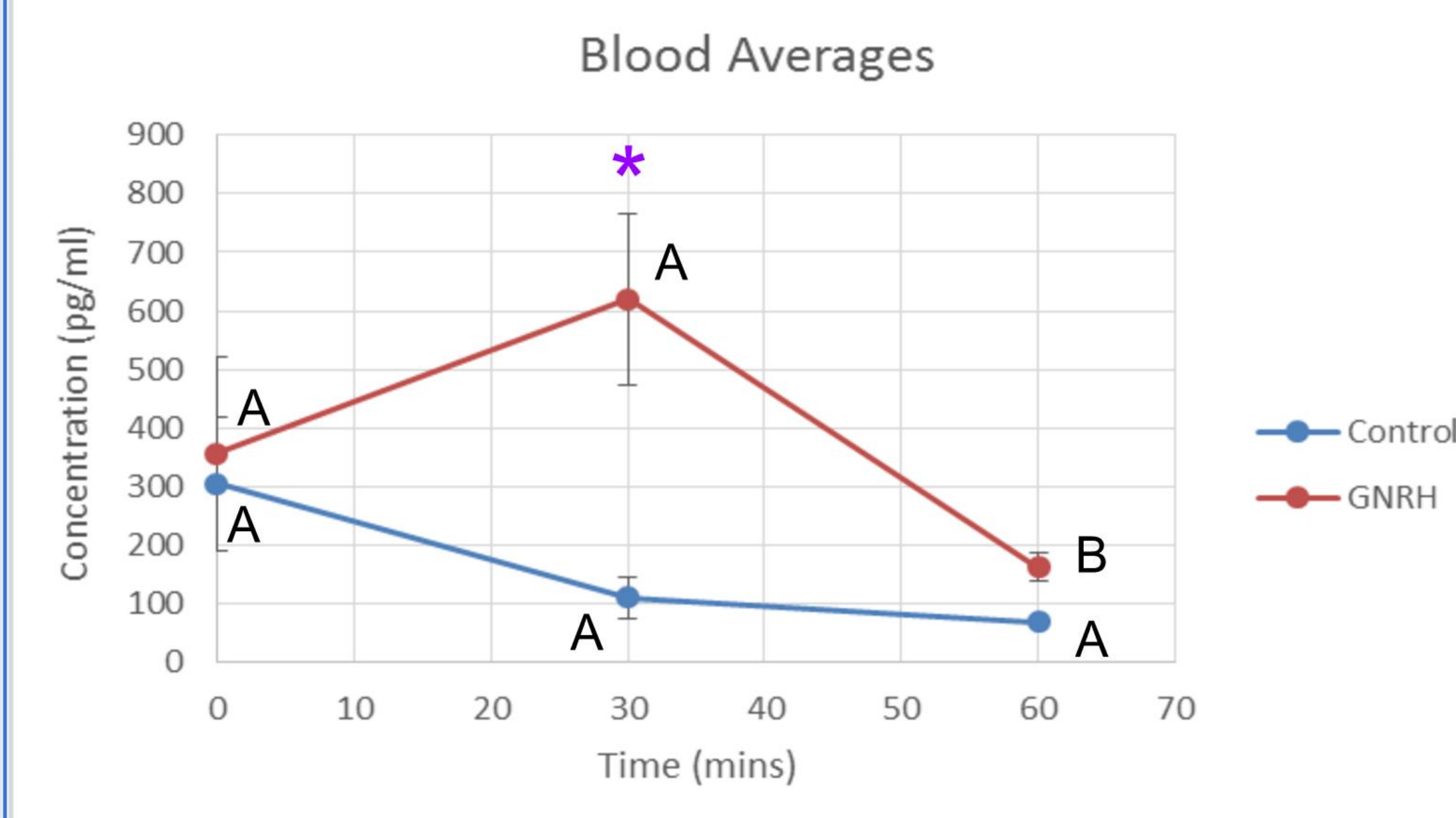


(Source: Dr. Memuna Khan)

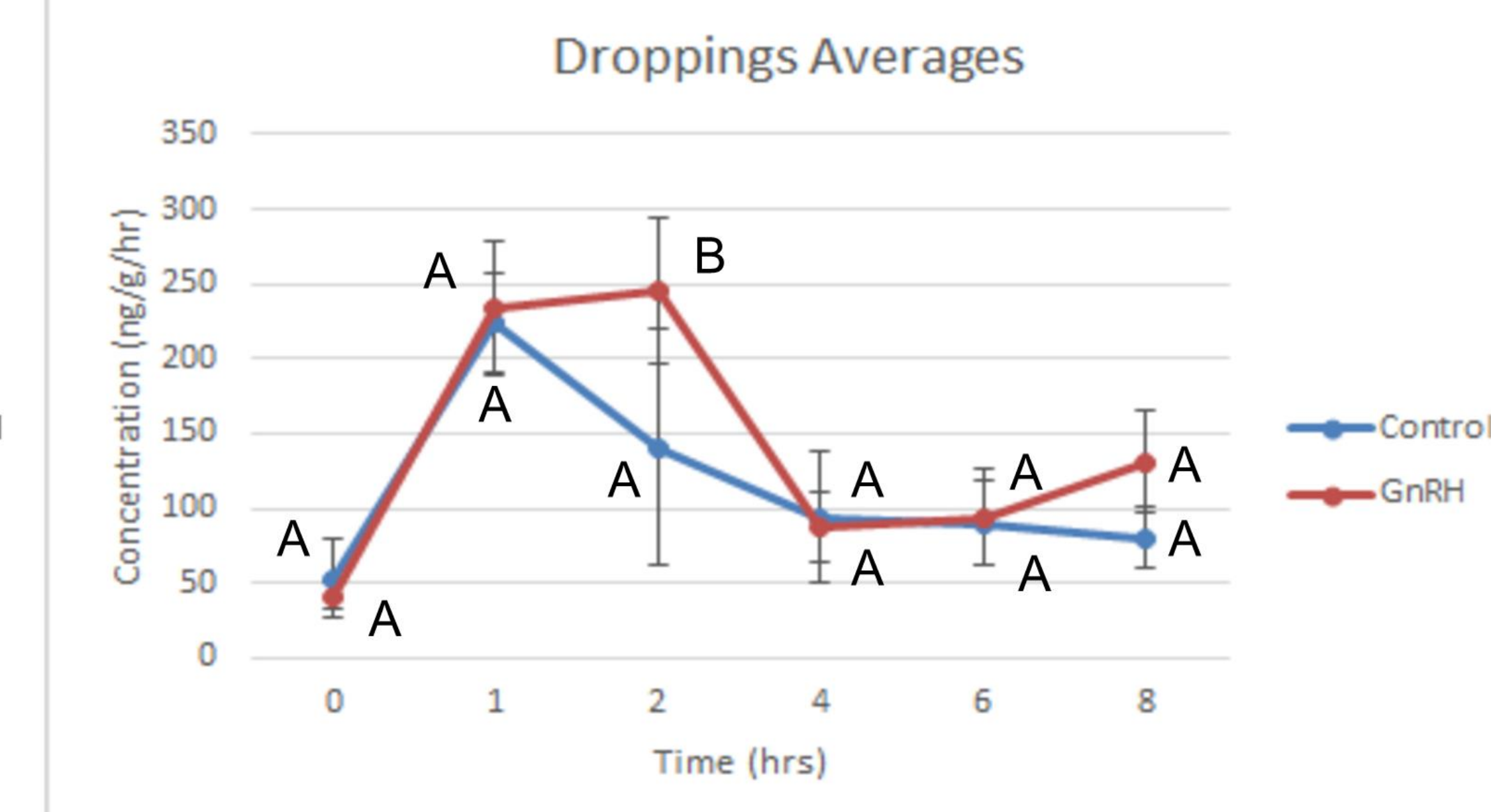
All samples were run through an Enzyme Immunoassay to get T or T-metabolite concentrations of blood and droppings, respectively

## Results

The resulting concentrations were calculated from the EIA and were further analyzed using a two-way ANOVA to check for statistical significance among multiple comparisons.



**Figure 1.** Average concentration of T in experimental males injected with GnRH (n=8) and control males injected with saline (n=7). Control males did not show a significant change (F (2, 36)=6.729, p=0.014) while GnRH males did show a significant change (F (2, 36)=3.445, p=0.043). There was a peak increase for GnRH males at 30 minutes that is significantly different from the average measurement at 60 minutes (p=0.036). There is also a significant difference between the control average and the GnRH average a 30 minutes (p=0.015).



**Figure 2.** Average concentrations of T-metabolites for experimental males injected with GnRH (n=8) and control males injected with saline (n=7). The control males did not show a significant change (F (5, 55)=1.065, p=0.307) while the GnRH males did show a change, but the overall difference was not significant (F (5, 55)=5.738, p=0). However, the experimental GnRH average at 2 hours showed a significant difference from 0 hours (p=0.012). There were no significant differences within the control averages or between the control and the GnRH averages at any of the time points.

## Conclusions

- The blood plasma results indicate that there is a significant peak in testosterone levels for the birds injected with the GnRH challenge as a result of HPG axis stimulation.
  - This confirmed that adult male Eastern Bluebirds responded to a GnRH challenge
- Droppings results indicate males injected with the GnRH challenge showed a peak in T-metabolites in accordance with the rise in blood testosterone levels.
  - Excrement data also showed a response to the GnRH challenge
- There was an unusual peak at 1 hour for droppings data that indicates there may be a problem with the methodology
  - High variation in the results could be affected by inefficient sample size
  - Concentration measurements could be altered by the efficiency of the antibody in our EIA (Salimetrics #2402, Expanded Range Salivary Testosterone EIA kit)
- Further analysis of procedural contents is needed before practical application

## References

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