Research Note: Quantifying corticosterone in turkey (*Meleagris gallopavo*) feathers using ELISA

Emily M. Leishman,[*] Nikole E. Freeman,[†] Amy E. M. Newman,[†] Nienke van Staaveren,[*,‡] Benjamin J. Wood,[*,§,#] Alexandra Harlander-Matauschek,[‡] and Christine F. Baes[*,ǁǁ,1]

[*]*Department of Animal Biosciences, Centre for the Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario N1G 2W1, Canada;* [†]*Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada;* [‡]*Department of Animal Biosciences, The Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario N1G 2W1, Canada;* [§]*Research & Development, Hybrid Turkeys, Kitchener, Ontario N2K 3S2, Canada;* [#]*School of Veterinary Science, University of Queensland, Gatton, Queensland 4343, Australia; and* [ǁǁ]*Vetsuisse Faculty, Institute of Genetics, University of Bern, Bern 3001, Switzerland*

ABSTRACT The measurement of corticosterone in feathers is an appealing tool for assessing glucocorticoids in wild and domestic bird species. Feather corticosterone measurements can be performed noninvasively and can provide a means for comparing glucocorticoid secretion between individual birds; thus, such measurements can be used to assess the welfare of domestic poultry. The focus of this study was to assess the validity of detecting corticosterone in turkey (*Meleagris gallopavo*) feathers using an ELISA and investigate differences between genetic lines. Primary feather 9 was obtained at a processing plant from 123 individuals from 3 different purebred turkey lines (line A [N = 46], line B [N = 24], and line C [N = 53]). Assay validation tests were performed using a commercially available ELISA kit (Cayman Chemicals, Cedarlane Labs, Canada). Indicators of accuracy, recovery, precision, and sensitivity were sufficient. Significant differences in feather corticosterone concentration between the 3 lines were observed. Line C had significantly higher feather corticosterone than line A ($P < 0.0001$) and line B ($P = 0.036$). These results indicate that the quantification of feather corticosterone using an ELISA is a valid method for assessing glucocorticoid levels in turkeys. This is the first report of differences in feather corticosterone between different purebred turkey lines. Differences observed between purebred lines provide an intriguing basis for further investigation into the genetic parameters of glucocorticoid levels in turkeys.

Key words: glucocorticoid, turkey, feather corticosterone, welfare, genetics

2020 Poultry Science 99:5261–5264
https://doi.org/10.1016/j.psj.2020.06.075

INTRODUCTION

The release of glucocorticoids can be acutely adaptive, allowing an animal to overcome disturbances and re-establish homeostasis; however, chronic elevation of glucocorticoids can have a detrimental effect on fitness (Sapolsky et al., 2000). Quantification of corticosterone, the primary avian glucocorticoid, in feathers may be less sensitive to acute disturbances and less invasive than blood sampling (Bortolotti et al., 2008). Corticosterone is integrated into the feather during its growth over a long period of time (i.e., over months) and so can provide insight into past hypothalamic–pituitary–adrenal axis activity (Bortolotti et al., 2008).

In a variety of bird species, feather corticosterone has been used to make connections between glucocorticoid levels and factors such as laying performance (Shini et al., 2009) and individual fitness (Harris et al., 2017). However, the investigation of feather corticosterone in domestic bird species, especially turkeys, is limited. In wild turkeys, feather corticosterone has been measured using the traditional radioimmunoassay method (Freeman and Newman, 2018); however, this method is associated with health hazards, license requirements, and limited stability. The use of ELISA for the measurement of feather corticosterone in turkeys could reduce these issues but has not been evaluated. Therefore, this study aimed to validate the measurement of corticosterone in turkey feathers using an ELISA. Furthermore, we investigated if differences in feather corticosterone levels can be observed between different purebred turkey

Received January 27, 2020.
Accepted June 4, 2020.

[1]Corresponding author: cbaes@uoguelph.ca

lines as part of a larger project aimed at improving turkey welfare and productivity through genomic selection.

MATERIALS AND METHODS

Subjects

Adult male turkeys (*Meleagris gallopavo*) from 3 purebred lines (line A [N = 46], line B [N = 24], and line C [N = 53]) were sampled between 18 and 21 wk of age (N_{Total} = 123). All birds were reared under standard commercial conditions and fed a standard commercial diet. Each line was selected for various breeding goals; lines A and B were focused on reproductive traits (i.e., egg production), and Line C was focused on production traits (i.e., body weight). Each genetic line was marked with a unique food dye color diluted in water before shipping to a commercial poultry processing plant. The ninth primary feather from the right wing was collected using shears postmortem and before defeathering. Feathers were rinsed with water and left to dry overnight before being stored in paper envelopes until analysis. All protocols complied with the guidelines of the Canadian Council on Animal Care and were approved by the University of Guelph Animal Care Committee (AUP 3782).

Feather Corticosterone Extraction and Assay

The feather corticosterone extraction protocol is based on methods described by Freeman and Newman (2018) and Bortolotti et al. (2008). The calamus was removed using shears at the time of sample collection. The inner vane was minced into pieces <5 mm^2 and further ground using a bead mill with ceramic beads (Bead Blaster: Benchmark Scientific, Edison, NJ). The ground feather was weighed using an analytical balance (15 ± 0.1 mg, model accu-124D Dual Range, accuracy to 0.1 mg: Fisher Scientific, Toronto, Canada) into a test tube and suspended in 5 mL of methanol (HPLC grade, Fisher Scientific). The tubes were placed in a sonicating water bath for 30 min and moved to a shaking incubator at 50°C for 12 h. Vacuum filtration with #4 Whatman filter paper was used to separate feather particles from methanol, and the empty test tube was rinsed with 1 mL of additional methanol twice. The additional methanol was then added to the vial containing the extracted methanol for a total of 7 mL of methanol. The methanol was evaporated under nitrogen gas using an evaporation plate at 40°C. Extract residues were reconstituted with 500 µL of assay buffer immediately before the assay. Samples were run across 5 ELISA plates (mouse anti-rabbit IgG, Corticosterone ELISA kit, number 501320, Cayman Chemicals, Cedarlane Labs, Canada) in duplicate.

Assay Validation

The assay was validated by testing accuracy, steroid recovery, precision, and sensitivity. Testing for parallelism between the slopes of the standard curve and serial dilutions indicates assay accuracy (Chard, 1995). If parallelism is not found between the standard curve and serial dilution, it may point to assay interference (Freeman and Newman, 2018). Serial dilutions were performed using a pool of pulverized feathers. Some feathers were covered with dye, so 2 pools of feathers were created to determine if there was an effect of dye on feather corticosterone concentrations. One pool was created using feathers with dye visible (N = 10), and the other pool did not contain any visible dye (N = 7). Each feather pool was created by mincing feathers using scissors and mixing thoroughly to control for intraindividual and interindividual variation in feather corticosterone concentrations. Corticosterone was extracted from the pool, and then extracts were diluted 2-fold, ranging from 2.5 mg to 80 mg. The serial dilution was also performed to determine the optimal mass of the feather sample required to quantify corticosterone within the quantitative range of the assay. Steroid recovery was determined by spiking a pool of pulverized feather with a known quantity of corticosterone. Precision was measured through intra-assay and interassay variability. Sensitivity was defined as the smallest amount of hormone measured within the range of the assay. All validations were run on the same ELISA plate except for spike recovery.

Statistical Methods

For all analyses, R Studio, version 3.5.3 (2019), was used. P-values < 0.05 were considered significant. Parallelism between the slopes of the standard curve and serial dilutions was determined using an ANCOVA. Parallelism was considered to be achieved if there was a nonsignificant interaction between the percent binding of the standard curve and serial dilution. To ensure that the presence of dye did not affect the binding of the assay, a post-hoc t test was performed using a Bonferroni correction to compare the binding of the dyed and nondyed feather pools. To test for differences in feather corticosterone concentration between genetic lines, a one-factor ANOVA was used with a post-hoc Tukey's Honest Significant Difference test for pairwise comparisons between lines.

RESULTS AND DISCUSSION

Assay Validation

While previous work has validated using ELISA for other poultry species (Carbajal et al., 2014) and other methods in wild turkeys have been used (Freeman and Newman, 2018), this is the first time an ELISA has been validated for domestic turkeys. The optimal feather mass for analyzing a single sample (~50% binding) was 15 mg (Figure 1), showing that a relatively small amount of feather is needed. The serial dilutions for corticosterone extracted from turkey feathers with and without dye were parallel to the standard curve (ANCOVA: F = 1.5116, P = 0.2669, Figure 1), indicating assay accuracy (Chard, 1995). Furthermore, the presence of

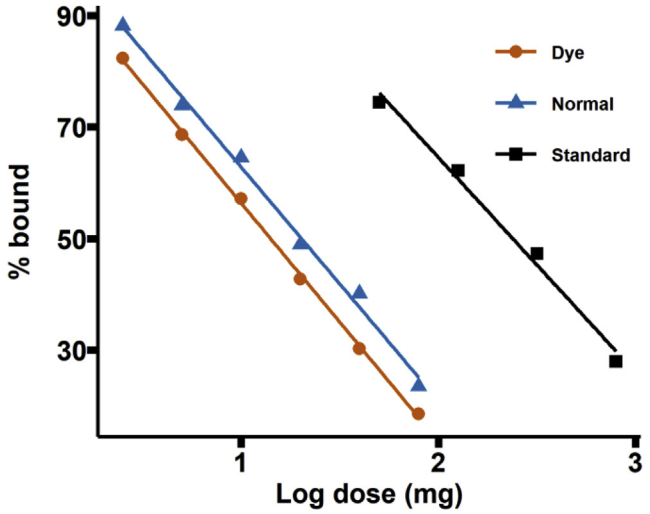

Figure 1. Serial dilutions of corticosterone in pooled turkey feather from dyed (N = 10) and undyed feathers (N = 7) demonstrated parallelism with the standard curve.

dye on the feathers did not significantly impact the binding of the assay ($P > 0.05$). Intra-assay and interassay CV of the ELISAs were 1.4 ± 2.48% and 12.1 ± 0.02%, respectively. The average recovery was 65 ± 2.5%, suggesting that that other steroids (e.g., 11-Deoxycorticosterone 15.8% cross-reactivity) or unidentified substances in the sample matrix might be interacting with the assay as shown in the ELISA kit cross-reactivity table (Corticosterone ELISA kit, number 501320, Cayman Chemicals, Cedarlane Labs, Canada). Finally, corticosterone was detected within the range of the assay to 2.5 mg. These findings show that an ELISA method is suitable for detecting corticosterone in turkey feathers.

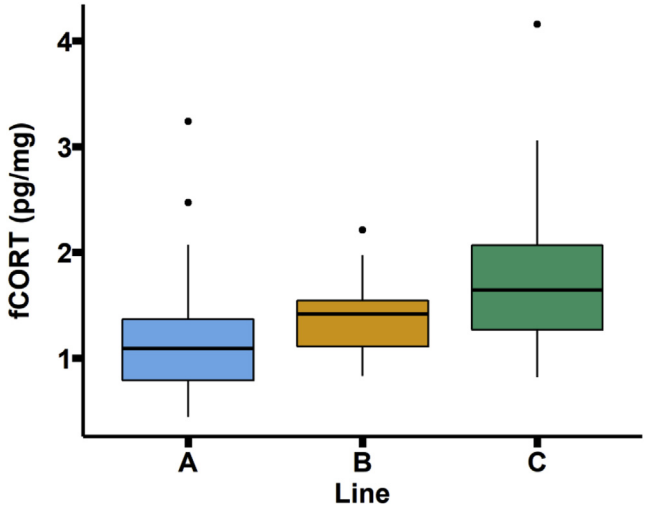

Figure 2. Corticosterone concentrations (fCORT, pg/mg) of turkey primary feathers from 3 different purebred lines: line A (N = 46), line B (N = 24), and line C (N = 53). Each boxplot reflects the 25th, 50th, and 75th percentiles. Box whiskers represent the 10th and 90th percentiles.

Line Differences

Mean feather corticosterone concentrations for line A, line B, and line C were 1.16 ± 0.08 pg/mg, 1.38 ± 0.07 pg/mg, and 1.73 ± 0.09 pg/mg, respectively. The differences in corticosterone level observed between our 3 lines (ANOVA: $F_{(2,120)} = 12.71$, $P < 0.001$, Figure 2) merits further investigation. Line C had significantly higher feather corticosterone than line A (Tukey HSD: 95% confidence interval **[CI]** [0.29, 0.83], $P < 0.0001$) and line B (Tukey HSD: 95% CI [0.02, 0.67], $P = 0.036$), whereas no difference was observed between line A and line B (Tukey HSD: 95% CI [−0.11, 0.55], $P = 0.366$). Brown and Nestor (1973) previously showed that corticosterone levels in turkey lines can be manipulated through direct selection and is associated with changes in growth and reproductive performance. The commercial purebred lines in the current study were kept under identical conditions but were selected for different breeding objectives (e.g., reproductive [A and B] and growth traits [C]), which could have led to associated changes in feather corticosterone. For example, there is a known negative relationship between corticosterone levels and reproduction in poultry. Birds with elevated corticosterone levels have been shown to have a delayed onset of laying (Salvante and Williams, 2003) and decreased egg production (Shini et al., 2009). There may also be an underlying relationship between corticosterone deposition in the feather and body size or growth rate. A positive relationship between plasma corticosterone and growth rate has been found in turkeys (Hocking et al., 1999). Owing to these relationships, purebred lines of turkeys selected for improved reproductive or growth traits might result in lower corticosterone levels compared with lines that are not as intensely selected for these traits. However, the relationship between these traits and feather corticosterone is not yet established, and further work is needed to explain the differences between the lines. The fact that differences are observed show promise to develop feather corticosterone as a potential phenotypic trait for genomic selection in turkeys.

In conclusion, this study showed that an ELISA can be used to measure feather corticosterone in turkeys. It must be acknowledged that further biological and pharmacological validations (i.e., chromatography of feather extracts, radiolabeled corticosterone during feather growth [Lattin et al., 2011]) must be completed to confirm that the ELISA using feather samples is indeed detecting levels of corticosterone from the turkey adrenal gland. Differences in feather corticosterone between turkey lines were observed in this study, opening the door for future studies on the genetic basis of feather corticosterone in domestic turkeys and the phenotypic consequences of varying levels of feather corticosterone. The potential for using genetic selection to select for a more favorable level of corticosterone in domestic turkeys has the potential to improve their health, welfare, and productivity.

ACKNOWLEDGMENTS

The authors would like to acknowledge Sarah Adams, Emma Hyland, Cristian Mastrangelo, Jacob Maxwell, and Elizah McFarland for their help with sample collection and processing. The authors extend their gratitude to the managers and personnel of the Hybrid Turkeys pedigree farm (Kitchener, Ontario) and Hayter's Farm (Dashwood, Ontario) collaborating on this study. This project was funded by the Government of Canada through Genome Canada and the Ontario Genomics Institute (OGI-133). This study was part of the project entitled "Application of genomic selection in turkeys for health, welfare, efficiency and production traits" funded by the government of Canada through the Genome Canada Genomic Application Partnership Program and administered by Ontario Genomics (recipients: B.J. Wood (Industry) and C.F. Baes (Academic)). The authors would also like to acknowledge NSERC and Hybrid Turkeys for financial support. The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Conflict of Interest Statement: The authors declare that B. J. Wood was an employee at Hybrid Turkeys at time of the study. All remaining authors declare that they have no known competing financial interests or personal relationships that influence the work reported in this article.

REFERENCES

Bortolotti, G. R., T. A. Marchant, J. Blas, and T. German. 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct. Ecol. 22:494–500.

Brown, K. I., and K. E. Nestor. 1973. Some physiological responses of turkeys selected for high and low adrenal response to cold stress. Poult. Sci. 52:1948–1954.

Carbajal, A., O. Tallo-Parra, M. Sabes-Alsina, I. Mular, and M. Lopez-Bejar. 2014. Feather corticosterone evaluated by ELISA in broilers: a potential tool to evaluate broiler welfare. Poult. Sci. 93:2884–2886.

Chard, T. 1995. An Introduction to Radioimmunoassay and Related Techniques. Elsevier, New York, NY.

Freeman, N. E., and A. E. M. Newman. 2018. Quantifying corticosterone in feathers: validations for an emerging technique. Conserv. Physiol. 6:1–9.

Harris, C. M., C. L. Madliger, and O. P. Love. 2017. An evaluation of feather corticosterone as a biomarker of fitness and an ecologically relevant stressor during breeding in the wild. Oecologia 183:987–996.

Hocking, P. M., M. H. Maxwell, and M. A. Mitghell. 1999. Welfare of food restricted male and female turkeys. Br. Poult. Sci. 40:19–29.

Lattin, C. R., J. M. Reed, D. W. Desrochers, and L. M. Romero. 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. J. Avian Biol. 42:247–252.

Salvante, K. G., and T. D. Williams. 2003. Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. Gen. Comp. Endocrinol. 130:205–214.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21:55–89.

Shini, S., A. Shini, and G. R. Huff. 2009. Effects of chronic and repeated corticosterone administration in rearing chickens on physiology, the onset of lay and egg production of hens. Physiol. Behav. 98:73–77.