

TCVM for Avian, Laboratory and Exotic Species

Effects of *An-shen* Aquapuncture on Heart Rate and Salivary Corticosterone Concentrations in Domestic Chickens (*Gallus gallus domesticus*) and Japanese Quail (*Coturnix japonica*)

Christian M. Capobianco, Corinne P. Kozlowski, Elizabeth G. Duke, Tara M. Harrison*

ABSTRACT

Stress contributes to significant morbidity and mortality in birds impacting growth, reproductive activity, healing, and immune function. Aquapuncture has been used anecdotally for the treatment of stress-associated clinical signs (*Shen* Disturbance) in birds. The goal of this study was to evaluate the effects of cyanocobalamin (vitamin B₁₂) aquapuncture at *An-shen* on salivary corticosterone concentrations, stress-induced behaviors, and heart rate over a 60-minute period in domestic chickens (*Gallus gallus domesticus*) and Japanese quail (*Coturnix japonica*). A group of 21 chickens and 10 quail were randomly assigned to either a Treatment Group, receiving bilateral *An-shen* aquapuncture with a 1:1 vitamin B₁₂ and 0.9% sterile saline solution, or a Control Group, receiving no treatment. Physical examinations were performed on each animal, which served as the initial stressor followed by aquapuncture in the Treatment Group. Saliva samples were obtained at 0-, 15-, 30-, 45-, and 60-minutes post-examination. Animals were restrained in hand for the duration of the 60-minute experiment to provide a consistent stressor. Samples were frozen and salivary corticosterone concentrations were quantified using commercially available enzyme immunoassay (EIA). Subjective sedation was observed in the majority of treated chickens (8/11; 72.7%) and quail (4/5; 80%). In all treated birds, there was a significantly larger decrease in heart rate when compared to control animals. While quail had limited saliva yield, precluding meaningful analysis, salivary corticosterone proved to be an effective minimally invasive biomarker in chickens, with median salivary concentrations consistently lower than those of controls at each time point. Collectively, these findings indicate that bilateral *An-shen* aquapuncture resulted in sedation, decreased heart rate, and lower salivary corticosterone concentrations consistent with decreased stress, supporting its potential as a practical and clinically relevant intervention for stress reduction in the studied species.

Keywords: acupuncture, *An-shen*, corticosterone, domestic chickens, Japanese quail, stress

Avian veterinary visits are important for maintaining optimal health and treating disease; however, these visits can be stressful events for a prey species in an unfamiliar environment. Stress contributes to significant morbidity and mortality amongst birds under managed care.¹ In client-owned and wildlife birds where prolonged hospitalization is necessary, chronic stress can impact growth, reproductive activity, healing, and immune function.² Pacheco's disease (psittacine herpesvirus), proventricular dilatation disease (avian bornavirus), aspergillosis, and feather destructive behavior among others have described stress as an important contributing factor for disease.³⁻⁶ For those reasons, an ability to ameliorate the stress response with a combination of behavioral modification, environmental modification, pharmacologic intervention, and alternative therapies is integral.

Administration of sedative medications either prior to or during the veterinary visit is common in avian medicine with typical pharmaceuticals including gabapentin,

midazolam, diazepam, and butorphanol among others. Though these medications are commonly utilized and side effects are uncommon, they are still possible and can be detrimental to a patient.⁷ Additionally, responses to sedative doses appear to be highly variable by individual and may be ineffective in reducing stress, necessitating

Author Professional Degrees and Certifications: DVM, CVA (Capobianco); PhD (Kozlowski); DVM (Duke); DVM, MPVM, Dipl ACZM, Dipl ACVPM, Dipl ECZM (ZHM), CVA (Harrison); **From:** Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA (Capobianco); Department of Reproductive and Behavioral Science, Saint Louis Zoo, Saint Louis, Missouri, USA (Kozlowski); Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA (Duke, Harrison); *Address correspondence to Dr. Harrison (tmharri7@ncsu.edu).

dosage adjustment or medication change. Alternatively, there are non-pharmacological methods for reducing stress in birds including environmental modifications, behavioral modification, and alternative therapies such as acupuncture.⁸⁻¹⁰ Though these methods do not have the potential side effects that medication administration does, they also have varied effectiveness based on the individual. Acupuncture has been used to reduce stress in both humans and animals. The duration of effect may be prolonged using aquapuncture, an acupuncture technique involving the injection of liquids (e.g. saline, cyanocobalamin), at an acupuncture point.^{11,12} Common acupoints used for sedation in veterinary patients include GV-20 and *An-shen*.¹³

Previous studies conducted in dogs have concluded that aquapuncture at *An-shen* was beneficial in decreasing stress parameters such as heart rate, panting, and other stress behaviors; however, there was no significant change in salivary cortisol between the study groups.^{14,15} *An-shen* aquapuncture has been used anecdotally in birds to provide calming effects for several hours or days.^{8,9} Though there has been reported success with *An-shen* aquapuncture in birds, to the authors' knowledge, there have been no controlled studies evaluating the corticosterone response to stressful stimuli in birds treated with *An-shen* aquapuncture when compared to untreated individuals. Previous studies have demonstrated strong correlations between salivary and plasma corticosterone levels in large-billed crows (*Corvus macrorhynchos*) and common gulls (*Larus canus*), making salivary corticosterone a useful biomarker in birds.^{16,17} This prospective study investigated the effects of bilateral *An-shen* vitamin B₁₂ aquapuncture on behavior, physiologic parameters, and salivary corticosterone concentrations in domestic chickens (*Gallus gallus domesticus*) and Japanese quail (*Coturnix japonica*) under managed care. It was hypothesized that treated birds would show fewer signs of stress and have lower salivary corticosterone concentrations when compared to those that were not treated.

MATERIALS AND METHODS

Animals

The study population comprised a convenience sample of seventeen adult domestic chickens ($n_{\text{male}}=0$, $n_{\text{female}}=17$) maintained as a research colony at North Carolina State University in addition to ten privately-owned adult quail ($n_{\text{male}}=5$, $n_{\text{female}}=5$) and four adult domestic chickens ($n_{\text{male}}=0$, $n_{\text{female}}=4$). Owners of private animals signed informed consent for their animals to be included in the study. The median weights of the chickens and quail were 2.3 kg and 0.28 kg, respectively. This study was approved by the North Carolina State University College of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC #24-170).

Experimental Design

Study participants were randomly assigned to either a Treatment Group ($n_{\text{chickens}} = 11$, $n_{\text{quail}} = 5$), which received bilateral subcutaneous *An-shen* aquapuncture with a 1:1

vitamin B₁₂^a and 0.9% sterile saline solution^b, or a Control Group ($n_{\text{chickens}} = 10$, $n_{\text{quail}} = 5$), not receiving any treatment. The animals were manually restrained for brief physical examinations of less than 5 minutes duration and were excluded from the study if there was any evidence of significant clinical disease, including but not limited to heart murmur, arrhythmia, respiratory distress, and presence of fractures or soft tissue injuries. The manual restraint for physical examination served as the initial stressor ($T = 0$) and initial heart rates were obtained during this examination. Immediately following the examination, the quail in the Treatment Group received a dose of 0.05 mL of the diluted vitamin B₁₂ solution per point and chickens in the Treatment Group received a dose of 0.1 mL per point (Figure 1). The solution was administered with a 25-gauge, 5/8-inch needle and a 1.0 mL syringe. The avian *An-shen* acupoint is located immediately caudal to the ear opening at the level of the ear opening's midpoint. All aquapuncture treatments were performed by the first author, who is certified in veterinary acupuncture.

Animals were kept in hand for the duration of the study period to prevent additional stress associated with repeated capture. Saliva samples were obtained using infant saliva swabs^c by placing the swab into the oral cavity for 60 seconds at 0-, 15-, 30-, 45-, and 60-minutes post-examination to achieve a target of 75-100 uL of saliva per sample (Figure 2). Saliva samples were immediately placed into the corresponding labeled tubes and were stored at -20°C. Following the 60-minute experiment and collection of the final saliva sample, each individual had a final heart rate obtained prior to returning them to their habitat. All individuals were assessed for mentation, level of activity, feeding behavior, and respiratory rate 10 minutes after being returned to their habitat and the morning following the experiment.



Figure 1: Diluted vitamin B₁₂ being administered in the left *An-shen* acupuncture point in a domestic chicken (*Gallus gallus domesticus*).



Figure 2: An infant saliva swab^c placed into the oral cavity of a domestic chicken (*Gallus gallus domesticus*) for saliva sample collection.

Corticosterone ELISA

Saliva samples were assayed for corticosterone using a commercial enzyme immunoassay^d. The range of the standard curve for the corticosterone assay was 39.062 pg/mL to 10,000 pg/mL. Prior to assay, saliva samples were centrifuged for 20 minutes at 2500 RPM. Samples were then diluted 1:2 with an assay buffer, and enzyme immunoassays were performed in duplicate according to the manufacturers' protocols.

Statistical Analyses

Statistics were performed using IBM SPSS^e and R Statistics^{f,18,19}. A *p*-value of less than or equal to 0.05 was considered significant for all statistical tests performed. Results were analyzed using the Wilcoxon rank sum for one-tailed hypotheses.

RESULTS

Behavior and Heart Rate

All birds tolerated the procedures well, with no adverse effects noted at the conclusion of sample collection period and at 24 hours post-treatment. Subjective sedation, characterized by closed eyes and drooping head, were observed in the majority of treated chickens (8/11; 72.7%) and quail (4/5; 80%); while this behavior was not observed in any of the control individuals (Figure 3). In combining the heart rates of the chickens and quail, those in the Treatment Groups had a median decrease in heart rate of 40 beats per minute (14.8%) while those in the Control Groups had a median decrease in heart rate of 4 beats per minute (5.2%). There was a statistically significant difference in the decrease in heart rate between the treatment and control groups ($p < 0.001$) (Figure 4).



Figure 3: Domestic chicken (*Gallus gallus domesticus*) showing signs of adequate sedation after bilateral *An-shen* aquapuncture administration.

Salivary Corticosterone

Maximum salivary corticosterone concentrations in chickens and quail were 1.44 ng/mL and 1.23 ng/mL, respectively, while the minimum detectable concentration for both chickens and quail was 0.025 ng/mL. Of the total saliva samples obtained, 93 of 105 chicken samples (89%) and 22 of 50 quail samples (44%) were of sufficient volume for corticosterone analysis.

Chickens in the Treatment Group had a significantly lower overall salivary corticosterone concentration

compared to the Control Group ($p=0.031$). The median salivary corticosterone concentration peaked in control chickens at 30 minutes post-treatment, while it was highest in treated chickens at 15 minutes post-treatment. At 30 minutes post-treatment, corticosterone concentrations remained significantly lower in the Treatment Group ($p=0.030$) and neared significance at 45 minutes post-treatment ($p=0.173$). Across all time points, median salivary corticosterone concentrations were consistently lower in treatment chickens when compared to controls (Figure 5).

In contrast, the limited number of adequate quail samples precluded meaningful analysis of salivary corticosterone concentrations over time. Due to insufficient sample availability, corticosterone concentrations in quail were not considered a reliable endpoint and were not further analyzed.

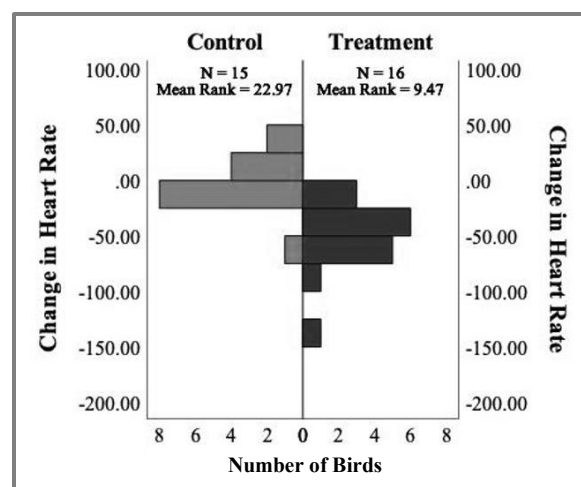


Figure 4: Change in heart rate over 60 minutes in treated and control domestic chickens (*Gallus gallus domesticus*) and Japanese quail (*Coturnix japonica*).

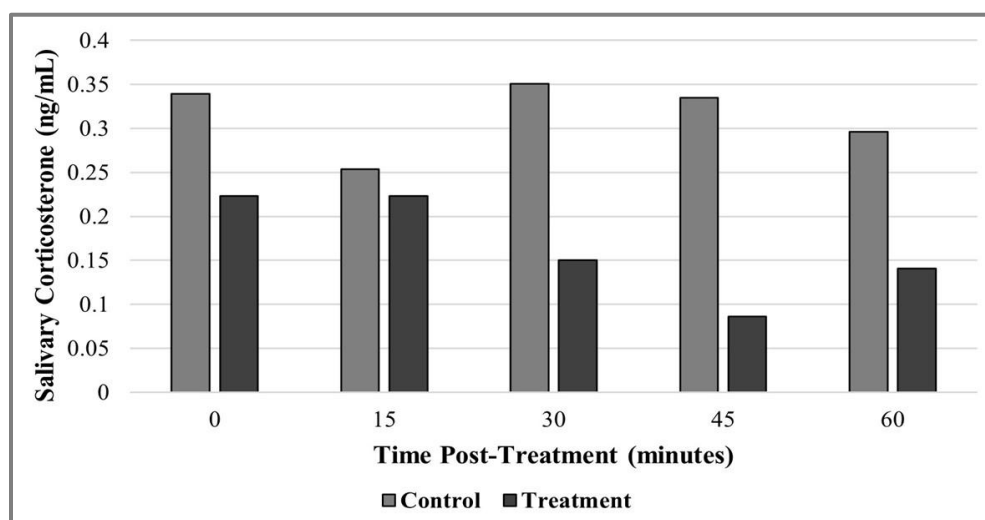


Figure 5: Median salivary corticosterone concentrations of domestic chickens (*Gallus gallus domesticus*) after either bilateral *An-shen* aquapuncture ("Treatment") or no aquapuncture ("Control") over time.

DISCUSSION

The importance of minimizing stress in avian patients during veterinary visits is being increasingly recognized by veterinary professionals, with pharmacologic intervention often being necessary to prevent the physiologic responses to stressful situations.^{7,20-22} Following a perceived stressor in birds, there is activation of the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axes.¹ Sympathetic catecholamine release in birds results in tachycardia, tachypnea, hypertension, and hyperthermia.²³⁻²⁵ In both the Treatment Group and Control Group, initial heart rates during physical examination were similarly elevated. After the initial physical examination and aquapuncture treatment, heart rates decreased in both groups as expected; however, the decrease was greatest in treated animals compared to control animals. This significant decrease in heart rate, in addition to the subjective sedation observed in treated animals, is consistent with a response to the bilateral *An-shen* aquapuncture.

Stress induced hypothalamic-pituitary-adrenal activation causes secretion of corticosteroid hormones including corticosterone.²⁶ This hormone is responsible for minimizing non-essential metabolic and physiologic activities as an animal faces persistent stressors. Avian corticosterone can be measured from plasma, feces, feathers, eggs, urine, and saliva with advantages and disadvantages to using each.²⁷⁻³³ Although plasma corticosterone is often considered the gold standard, its measurement is invasive, requiring blood collection, and circulating hormone concentrations can be rapidly altered by stress within two to three minutes.³⁴ In the present study, this method was not employed as repeated blood sampling would have been an additional stressor at each collection time point, and the volumes needed for analysis would not have been safe for collection from each individual. Though a sparse sampling study design was considered, this study design would have drastically

decreased the number of samples obtained at each time point. Additionally, the stress response is highly individual specific and would require the use of the same individual sampled repeatedly throughout the experiment to draw significant conclusions.

Fecal, feather, urine and egg corticosterone is less invasive and can be helpful for tracking long term stress (hours to days), but is not useful for assessing the acute stress response, which was necessary for the present study. Salivary corticosterone was utilized in this study as it is minimally invasive and requires approximately 30 minutes from the onset of a stressful stimulus to display increases in corticosterone concentrations.^{33,35} In the present study, median salivary corticosterone peaked between 15 and 30 minutes following a stressful stimulus in chickens, which is consistent with previous reports. Using saliva to measure corticosterone allowed for measurement of both baseline and stress-induced corticosterone concentrations easily throughout the study period. One significant limitation of salivary corticosterone measurement, however, is the need to collect adequate volumes of saliva for analysis. In the present study, although the swabs were left in each animal's oral cavity for the recommended amount of time, the vast majority of the quail samples did not have a sufficient volume for measurement. As there was a decreased number of adequate saliva samples achieved from quail when compared to the chickens in this study, it is inappropriate to draw significant conclusions from the quail corticosterone data.

Limitations of this study included an inconsistent sample size for salivary corticosterone concentrations at each time point associated with many quail and some chickens not producing adequate saliva volumes for measurement as previously discussed. Additionally, though attempts were made to minimize confounding stressors, it was impossible to completely remove all stressors. For that reason, it was important to provide a uniform amount of mild stress to each individual, which was the manual restraint. Even with those guidelines in place, every animal's interpretation of their environment and subsequent level of concern is different, making it impossible to prevent small spikes in stress throughout the sampling period, which could have skewed results. This study also did not have a placebo treatment in control individuals, such as aquapuncture performed at a different location that was not an acupoint. As a full acupoint map is not available in avian species and there was concern for regional stimulation of an acupoint that may influence the results, this sham procedure was not performed. Finally, it is important to remember that the study animals came from different sources and their level of stress associated with handling was likely very different; however, this was controlled for by having a large sample size within each group.

This is the first study to evaluate the effects of aquapuncture on the stress response in birds. The hypothesis was satisfied as the birds that were treated with bilateral *An-shen* aquapuncture showed subjective sedation as well as a significant decrease in heart rate and salivary

corticosterone concentrations when compared to the Control Group. Though there were changes in these variables, the present study did not investigate the effects of the treatment after 60 minutes. Future studies should seek to evaluate the duration of the calming effects seen with aquapuncture in birds. For birds that need to be hospitalized for prolonged periods of time, knowing the duration of effect would be necessary to recommend a frequency for aquapuncture treatment. This study design could be modified for use in other commonly treated zoological companion mammals where aquapuncture may be useful. Overall, the results of the present study are promising, and aquapuncture should be considered by practitioners when attempting to decrease stress and provide sedation in avian species within a clinical setting.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Olivia Petritz, Dr. Maria Serrano, Helen Clawitter, McKenzie Tsaousis, Lauren Dulin, Mary Emfinger, Stephen Xiao, Nicole D'Avignon and Jessica Kern for their assistance in carrying out this study.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

Declaration of Interest and Funding

The research reported in this publication was supported by the World Association of Traditional Chinese Veterinary Medicine Research Grant Program. The authors declare there were no conflicts of interest.

FOOTNOTES

- ^a Vitamin B₁₂, 3000 mcg/mL injection, Vedco Inc., Saint Joseph, MO, USA
- ^b 0.9% Sodium Chloride Inj, Hospira Inc., Lake Forest, IL, USA
- ^c SalivaBio infant swab, Salimetrics LLC, State College, PA, USA
- ^d DetectX Corticosterone K014 enzyme immunoassay, Arbor Assays Inc., Ann Arbor, MI, USA
- ^e IBM SPSS Statistics, version 32, International Business Machines Corporation, Armonk, NY, USA
- ^f R Core Team, version 4.3.2, The R Foundation for Statistical Computing, Vienna, Austria

REFERENCES

1. Blas J. Stress in birds. Sturkie's Avian Physiology 6th Ed. Scanes C (ed). Cambridge, MA: Academic Press 2015:769–810. <https://doi.org/10.1016/B978-0-12-407160-5.00033-6>
2. Janssen K, Marsland C, Barreto M et al. Identifying the stressors impacting rescued avian wildlife. *Animals* (Basel) 2020; 10(9):1500. <https://doi.org/10.3390/ani10091500>
3. Cacciuttolo E, Rossi G, Nardoni S et al. Anatomopathological aspects of avian aspergillosis. *Vet Res Commun* 2009; 33(6):521–527. <https://doi.org/10.1007/s11259-008-9199-7>
4. Panigrahy B, Grumbles L. Pacheco's disease in psittacine birds. *Avian Dis* 1984; 28(3):808–812. <https://doi.org/10.2307/1590254>
5. Rubbenstroth D. Avian bornavirus research—A comprehensive review. *Viruses* 2022; 14(7):1513. <https://doi.org/10.3390/v14071513>
6. Rubinstein J, Lightfoot T. Feather loss and feather destructive behavior in pet birds. *Vet Clin North Am Exot Anim Pract* 2014; 17(1):77–101. <https://doi.org/10.1016/j.cvex.2013.10.002>
7. van Zeeland Y. Medication for behavior modification in birds. *Vet Clin North Am Exot Anim Pract* 2018; 21(1):115–149. <https://doi.org/10.1016/j.cvex.2017.08.005>
8. Harrison T, Churgin S. Acupuncture and traditional Chinese veterinary medicine in zoological and exotic animal medicine: A review and introduction of methods. *Vet Sci* 2022; 9(2):74. <https://doi.org/10.3390/vetsci9020074>

9. Koh R, Harrison T. Acupuncture in zoological companion animals. *Vet Clin North Am Exot Anim Pract* 2023; 26(1):257-280. <https://doi.org/10.1016/j.cvex.2022.07.008>
10. Mattison S. Training birds and small mammals for medical behaviors. *Vet Clin North Am Exot Anim Pract* 2012; 15(3):487-499. <https://doi.org/10.1016/j.cvex.2012.06.012>
11. Chen C, Lin C, Chern R et al. Neuronal activity stimulated by liquid substrates injection at zusanli (ST36) acupoint: The possible mechanism of aquapuncture. *Evid Based Complement Alternat Med* 2014; 627342. <https://doi.org/10.1155/2014/627342>
12. Luna S, Angeli A, Ferreira C et al. Comparison of pharmacopuncture, aquapuncture and acepromazine for sedation of horses. *Evid Based Complement Alternat Med* 2008; 5(3):267-272. <https://doi.org/10.1093/ecam/nel096>
13. Xie H, Preast V. Xie's Veterinary Acupuncture. Ames, IA: Blackwell Publishing 2007:290-292.
14. Kintz Konegser A. Comparison between aqua-acupuncture and trazodone on mitigation of fear, anxiety and stress in healthy dogs during routine veterinary examination. *Am J Trad Chin Vet Med* 2022; 17(1):27-35. <https://doi.org/10.59565/001c.83389>
15. Mier H. Effectiveness of aqua-acupuncture for reducing stress of canine patients in veterinary clinics. *Am J Trad Chin Vet Med* 2021; 16(1):41-50. <https://doi.org/10.59565/001c.82230>
16. Aota I, Yatsuda C, Izawa E. Salivary corticosterone measurement in large-billed crows by enzyme-linked immunosorbent assay. *J Vet Med Sci* 2023; 85(1):71-75. <https://doi.org/10.1292/jvms.22-0383>
17. Carbillet J, Saks L, Sepp T. Salivary corticosterone reflects plasmatic levels in a wild seabird. *Gen Comp Endocrinol* 2024; 345:114390. <https://doi.org/10.1016/j.ygcen.2023.114390>
18. Hofner B, Boccuto L, Göker M. Controlling false discoveries in high-dimensional situations: Boosting with stability selection. *BMC Bioinformatics* 2015; 16:144. <https://doi.org/10.1186/s12859-015-0575-3>
19. The R Core Team. R: A language and environment for statistical computing: The R manuals [computer software]. The R Project for Statistical Computing. 2023; <https://cran.rstudio.com/manuals.html>
20. Day T, Roge C. Evaluation of sedation in quail induced by use of midazolam and reversed by use of flumazenil. *J Am Vet Med Assoc* 1996; 209(5):969-971.
21. Vesal N, Eskandari M. Sedative effects of midazolam and xylazine with or without ketamine and detomidine alone following intranasal administration in Ring-necked Parakeets. *J Am Vet Med Assoc* 2006; 228(3):383-388. <https://doi.org/10.2460/javma.228.3.383>
22. Vesal N, Zare P. Clinical evaluation of intranasal benzodiazepines, alpha-agonists and their antagonists in canaries. *Vet Anaesth Analg* 2006; 33(3):143-148. <https://doi.org/10.1111/j.1467-2995.2005.00244.x>
23. Cabanac A, Guillemette M. Temperature and heart rate as stress indicators of handled common eider. *Physiol Behav* 2001; 74(4-5):475-479. [https://doi.org/10.1016/s0031-9384\(01\)00586-8](https://doi.org/10.1016/s0031-9384(01)00586-8)
24. Cabanac M, Aizawa S. Fever and tachycardia in a bird (*Gallus domesticus*) after simple handling. *Physiol Behav* 2000; 69(4-5):541-545. [https://doi.org/10.1016/s0031-9384\(00\)00227-4](https://doi.org/10.1016/s0031-9384(00)00227-4)
25. Greenacre C, Lusby A. Physiologic responses of Amazon parrots (*Amazona species*) to manual restraint. *J Avian Med Surg* 2004; 18(1):19-22. <https://doi.org/10.1647/2003-011>
26. Doss G, Mans C. Avian sedation. *J Avian Med Surg* 2021; 35(3):253-268. <https://doi.org/10.1647/20-00045>
27. Dantzer B, Fletcher Q, Boonstra R et al. Measures of physiological stress: A transparent or opaque window into the status, management and conservation of species? *Conserv Physiol* 2014; 2(1):cou023. <https://doi.org/10.1093/conphys/cou023>
28. Kouwenberg A, McKay D, Fitzsimmons M et al. Measuring corticosterone in feathers using an acetonitrile/hexane extraction and enzyme immunoassay: Feather corticosterone levels of food-supplemented Atlantic Puffin chicks. *J Field Ornithol* 2015; 86(1):73-83. <https://doi.org/10.1111/jof.12090>
29. Leishman E, Freeman N, Newman A et al. Research note: Quantifying corticosterone in turkey (*Meleagris gallopavo*) feathers using ELISA. *Poult Sci* 2020; 99(11):5261-5264. <https://doi.org/10.1016/j.psj.2020.06.075>
30. Mormède P, Andanson S, Aupérin B et al. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiol Behav* 2007; 92(3):317-339. <https://doi.org/10.1016/j.physbeh.2006.12.003>
31. Ode M, Asaba A, Miyazawa E et al. Sex-reversed correlation between stress levels and dominance rank in a captive non-breeder flock of crows. *Horm Behav* 2015; 73:131-134. <https://doi.org/10.1016/j.yhbeh.2015.07.012>
32. Royo F, Mayo S, Carlsson H et al. Egg corticosterone: A noninvasive measure of stress in egg-laying birds. *J Avian Med Surg* 2008; 22(4):310-314. <https://doi.org/10.1647/2008-001.1>
33. Sheriff M, Dantzer B, Delehanty B et al. Measuring stress in wildlife: Techniques for quantifying glucocorticoids. *Oecologia* 2011; 166(4):869-887. <https://doi.org/10.1007/s00442-011-1943-y>
34. Romero L, Reed J. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comp Biochem Physiol A Mol Integr Physiol* 2005; 140(1):73-79. <https://doi.org/10.1016/j.cbpb.2004.11.004>
35. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology* 1994; 19(4):313-333. [https://doi.org/10.1016/0306-4530\(94\)90013-2](https://doi.org/10.1016/0306-4530(94)90013-2)