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Physiological stress in captive Greater rheas (*Rhea americana*): Highly sensitive plasma corticosterone response to an ACTH challenge

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ABSTRACT

Up to the present no studies have been conducted either on baseline concentrations of adrenal hormones or on hormonal responses to stress in Greater rhea (*Rhea americana*) and most ratite species. The aims of this work were to assess the presence of corticosterone in plasma of Greater rhea, to validate a corticosterone ^{125}I-radioimmunoassay for determining corticosterone levels in plasma samples and to study the activation of the adrenal gland after an adrenocorticotrophic hormone (ACTH) challenge. Six captive Greater rhea juveniles of 10 months of age received an intravenous ACTH injection. Blood samples were taken at 0 min (baseline pre-ACTH levels), and post-injection at 15, 30, 60 min and at 24 and 48 h. The high pressure liquid chromatography (HPLC) analysis of pooled plasma showed that corticosterone is the glucocorticoid found in the plasma of Greater rhea. Biochemical assays of standard validation (e.g., parallelism, exogenous corticosterone recovery) showed that measurements of corticosterone present in the plasma of the Greater rhea provided by commercial corticosterone ^{125}I-radioimmunoassay were accurate and precise. ACTH challenge induced a more than 40-fold increase in plasma corticosterone at 60 min post-ACTH (from 4.0 to 166.5 ng/ml, on average). The corticosterone response to ACTH in Greater rhea was higher than is usual in birds, an apparently typical characteristic of ratites.

1. Introduction

Interest in animal's welfare is increasing, not only among scientists but also lay people. Exposure of vertebrates to a wide variety of adverse stimuli (stressors) triggers the called stress responses. These are integrated neuroendocrine responses (Ellis et al., 2006) that involve activation of the hypothalamic–pituitary–adrenal (HPA) axis and secretion of cortisol or corticosterone. While activation of the HPA axis is considered to be an adaptive response to stress (Cockrem, 2007), prolonged stimulation of the HPA axis that accompanies certain stress states can exert many deleterious effects on the performance and welfare of the animals (Jones, 1996; Faure and Mills, 1998; Jones and Hocking, 1999; Sapolsky et al., 2000; Marín and Satterlee, 2003, 2004). Glucocorticoid release as a measure of stress response is increasingly used in studies in ecology and conservation biology as a biomarker to reflect the combined effects of health, physiological constraints, energy allocation, habitat quality and anthropogenic disturbances on the individual and on the population (Romero, 2004).

Greater rhea (*Rhea americana*) is a bird species endemic to South America. Wild populations are declining due to several anthropogenic factors, including habitat fragmentation and loss, poaching and excessive egg gathering (Bellis et al., 2004; Martella and Navarro, 2006; Giordano et al., 2008). As a consequence, the species has been included in the "Near threatened" IUCN category (IUCN, 2008). Although captive breeding of Greater rhea for both commercial and conservation purposes has grown considerably in the last years (Martella and Navarro, 2006), the causes of certain diseases and of the high chick mortality—and adult mortality to a lesser degree—are not fully understood.

Quantifying basic physiological parameters, such as adrenal gland activity in species of *in situ* and *ex situ* conservation concern, is important to generate a database and define tools that can be used to evaluate problems occurring in wild or captive populations. Given that glucocorticoids have not been measured in Greater rhea up to the present, this first attempt has the following objectives: (1) to confirm that corticosterone is the main glucocorticoid released by adrenal glands in Greater rhea; (2) to conduct a biochemical and physiological validation of a commercial radioim-

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munoassay to assess corticosterone changes in blood; and (3) to characterize adrenocortical response through an activation of the adrenal gland using an ACTH challenge.

2. Materials and methods

2.1. Animals

Samples were obtained from six 10-month old subadult Greater rhea individuals bred in captivity on the Experimental Farm of the Zoo of Córdoba city, Argentina (31S, 64W). Juveniles were selected for the experiment because individuals older than 12 months are difficult to handle. One month before the experiment, three randomly chosen males of body mass of 12.2 ± 1.04 kg [mean ± standard error] and three females of 11.03 ± 1.13 kg of body mass were housed in a pen (5 × 10 × 2 m) with natural soil floor, and were offered food (Vasquetto® processed feed for chicken) and water *ad libitum*. Greater rheas were exposed to natural light and temperature conditions and were provided with a 10 m^2 roofed shelter.

2.2. Experimental design

Two experienced assistants captured and immobilized each individual, while a third one administered the injections and performed the blood sampling. Blood samples were always collected within 3 min of capture and immobilization, following the protocol of Romero and Reed (2005). Through injection in the right jugular vein, animals received synthetic ACTH (Laboratorios Elea, Buenos Aires, Argentina) (5 IU/kg of body mass) diluted in 0.9% saline solution (Rettenbacher et al., 2004; Thiel et al., 2005). Blood samples were taken immediately before ACTH administration (baseline value) and 15, 30 and 60 min, and 24 and 48 h after administration. The experiment was performed during spring (October).

2.3. Blood sample collection

Blood samples (2 ml) were taken from the right jugular vein of Greater rheas using heparinized syringes (21 g needle), between 7:00 and 8:00 am to control any effect of circadian variation on corticosterone levels. Blood samples were centrifuged (2500G) and the resulting plasma was collected and stored at −20 °C until assayed.

2.4. High pressure liquid chromatography (HPLC)

The nature of glucocorticoids present in plasma of Greater rhea was analyzed using HPLC. The elution positions of two reference standards, corticosterone and cortisol, were determined by HPLC and then compared to the rhea samples. Post-ACTH plasma samples were used to create a 4 ml pool presumably of high glucocorticoid concentration; steroids from this pool were extracted with dichloromethane, following the protocol of Wong et al. (1994). For chromatographic sample runs, 100 µl of plasma extract was injected on to a reversed-phase column (LiChrospher 100/RP-18/5 µm; 4.6 × 250 mm; Merk, Argentina), with an isocratic mobile phase (1 ml/min) composed of: 35% acetonitrile, 65% water and 0.05% glacial acetic acid (Wong et al., 1994). Furthermore, thirty HPLC fractions (1 ml) were also collected to determine immunoreactive corticosteroid component in Greater rhea plasma in the validated radioimmunoassay (RIA) kit (see the following section). All the HPLC fractions were dried and reconstituted in 250 µl steroid diluent provided in the RIA kit. An addition of 5% EtOH in the resuspension volume was used to improve recovery of steroids (Newman et al., 2007). Finally, an immunoreactivity profile was obtained from analysis of collected eluates.

2.5. Radioimmunoassay

Corticosterone concentrations present in plasma of Greater rhea and in HPLC fractions were quantified using a commercially available ^{125}I corticosterone-radioimmunoassay kit (MP Biomedicals, Costa Mesa, California, USA) developed for mice and used in similar studies with other animal species (Washburn et al., 2002; Nilsson et al., 2008). RIA was used following the procedure described in Washburn et al. (2002). Manufacturer cross-reactivity with other steroids were: desoxycorticosterone (0.34%), testosterone (0.10%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%), and less than 0.01% for all other steroids tested.

Corticosterone concentration was expressed as nanograms of hormone per ml of plasma (ng/ml). To ascertain biochemical validity of this assay the following tests were performed: parallelism, accuracy and precision (Washburn et al., 2002; Monfort, 2003; Busso et al., 2005).

2.5.1. Parallelism

This test was performed to determine whether plasma dilutions of Greater rhea behave in a similar manner to the corticosterone standards. This test is an indirect way to measure assay specificity (Biddlecombe and Law, 1996). Two pooled plasma samples (baseline: $n = 6$ and baseline + post-ACTH: $n = 36$) were serially diluted (1:2–1:32, and 1:25–1:400, respectively). The slopes of the log-logit transformed curves of those samples were then compared to that of the kit standard curve.

2.5.2. Accuracy

This test was carried out to determine if plasma components affect the antibody-hormone binding of RIA. The recovery curve was generated by adding a similar volume of plasma (small and known hormone concentration according to parallelism test, dilution 1:400) to each point of the RIA standard ($n = 3$ for each point). The recovered amounts include a percentage of the added standard plus the amount contained in the original sample. Finally, a linear regression analysis was performed to determine the relationship between x (added hormone) and y (recovered hormone).

2.5.3. Precision

This characteristic was assessed by calculation of intra and inter-assay coefficients of variation (CV) in the hormonal measurements performed in "high" and "low" controls provided with the kit and two Greater rhea plasma controls. High and low Greater rhea plasma controls were obtained from all pooled samples and were calculated from the parallelism test (20% and 80% binding as the lower and upper ends of the sensitivity range).

2.6. Statistical analyses

The data were statistically analyzed with Infostat (Infostat, Grupo Infostat, FCA-UNC, Argentina). Tests for equal slopes (parallelism) between the baseline and baseline + post-ACTH pooled plasma samples from Greater rhea and kit corticosterone standards were used (Neter et al., 1990). Results of the ACTH challenge were analyzed by repeated-measures ANOVA to examine the effects of sex (male and female), post-administration time (time since ACTH challenge) and the interaction between these two factors. In this experimental design, each animal served as its own control, since their pre- versus post-ACTH corticosterone levels were compared. ANOVA assumptions of homogeneity of variances and normality were tested using Levene's and Shapiro-Wilk test, respectively. LSD Fisher test was used for post-hoc analysis. Values were ex-

pressed as the mean ± standard error (SEM), and significance level was set at 0.05.

3. Results

3.1. High performance liquid chromatography (HPLC)

Chromatographic separation of pooled post-ACTH Greater rhea plasma samples revealed that a peak co-eluted with the reference standard corticosterone. No peak was found at the elution position of the standard cortisol (Fig. 1).

The elution pattern of immunoreactive substances measured after HPLC using the corticosterone RIA show that plasma immunoreactivity co-eluted (100%) with added corticosterone reference standard (Fig. 1).

3.2. Radioimmunoassay

Log-logit transformed curves of serially diluted pools of plasma samples were found to parallel the log-logit transformed standard curves. Standard curve: $y = -0.07x + 167.18$; pool of baseline samples: $y = -0.07x + 168.13$ ($r^2 = 0.98$, $n = 13$, $p = 0.76$); standard curve: $y = -0.07x + 166.75$; pool of baseline + post-ACTH samples: $y = -0.09x + 171.61$ ($r^2 = 0.97$, $n = 12$, $p = 0.19$).

A 1:4 dilution for baseline samples and 1:50 for post-ACTH samples displaced the labeled hormone of the antibody by 50% (for this reason they were selected as optimum dilutions). Regarding accuracy test, quantitative exogenous recovery of corticosterone was: $y = 19.72 + 1.11x$ ($r^2 = 0.95$). Intra-assay CV were 9.14 ± 2.16% and 5.76 ± 1.69% for high and low concentration controls supplied in the kit ($n = 9$), respectively, and 9.9 ± 3.57% and 5.32 ± 1.91% for high and low concentration controls obtained from plasma of Greater rhea ($n = 7$), respectively. Inter-assay CV were lower than 10% and no significant differences were observed between them.

3.3. ACTH challenge

Repeated-measures ANOVA showed a significant main effect of time since ACTH administration ($F_{(5,20)} = 47.43$, $p = 0.0000$), but did not reveal neither a significant effect of sex ($F_{(1,4)} = 0.09$, $p = 0.77$); nor an interaction between time since ACTH administration and sex; therefore, male and female data were pooled for post-hoc analysis of time since ACTH administration effect. The increase of corticosterone was significant over time, with values between 3.98 ± 1.04 ng/ml ("Time 0" of injection) and 89.83 ± 12.42, 141.6 ± 21.98 and 166.54 ± 16.01 ng/ml (at 15, 30 and 60 min post administration, respectively). An over 40-fold increase in corticosterone levels was observed in post-ACTH values. No significant differences between corticosterone concentrations were found at 30 and 60 min post-ACTH administration. Furthermore, corticosterone levels at 24 and 48 h post-ACTH did not show significant differences with baseline value ($F_{(2,8)} = 0.03$, $p = 0.97$) (Fig. 2).

4. Discussion

The results of HPLC and of the different biochemical assays performed (specificity, accuracy and precision), which provide reliable measurements, confirm that corticosterone was the major glucocorticoid in Greater rhea plasma. The co-elusion of the corticosterone standard and the immunoreactive peak observed in plasma samples, together with the parallelism, accuracy and precision tests and with the increase detected in response to ACTH administration, validated the radioimmunoassay procedure as an adequate method for measuring adrenocortical activity in Greater rhea, as it is in other bird species (Washburn et al., 2002; Nilsson et al., 2008). Moreover, baseline values of plasma corticosterone concentrations (3.98 ng/ml) (range: 0.52–7.66) obtained in Greater rhea are comparable to those found in other bird species (Müllner et al., 2004; Romero et al., 2006), including other ratites, such as the ostrich (4.9 ± 2.9 ng/ml) (Mitchell et al., 1996). Peak plasma corticosterone concentrations after an ACTH challenge were found, for example, at 60 min in Florida sandhill cranes (Grus canadensis pratensis) (Ludders et al., 1998), at 60–90 min in bald eagles (*Haliaeetus leucocephalus*) (Zenoble et al., 1985) and at 90 min in harlequin ducks (*Histrionicus histrionicus*) (Nilsson et al., 2008). In Greater rhea, corticosterone levels in response to ACTH challenge were still high at 60 min.

The significant increase in corticosterone levels reached in Greater rhea is noticeable compared to the increases reported in the literature for other avian species. For example, increases of 4- and 16-fold were found in *Zonotrichia leucophrys gambelii* and *Gallus domesticus*, respectively (Astheimer et al., 1994; Dehnhard et al., 2003). Interestingly, a high magnitude (about 50-fold) corticosterone response was also observed in the Brown kiwi (*Apteryx australis*) after capture and immobilization for 30 min (Cockrem, personal communication). Corticosterone is a hydrophobic hormone, and the majority circulates in the plasma bound to a carrier protein called corticosterone binding globulin

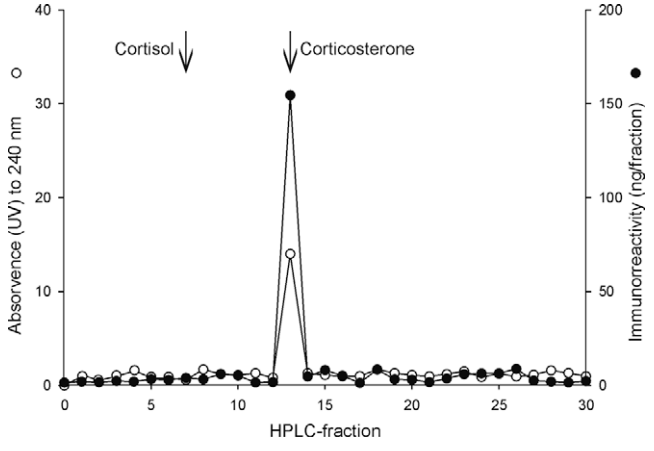

Fig. 1. HPLC separation (○) of pooled Greater rhea plasma samples. Cortisol and corticosterone standards (arrows) were added as a reference. Immunoreactivity (●) of each fraction was determined by corticosterone RIA.

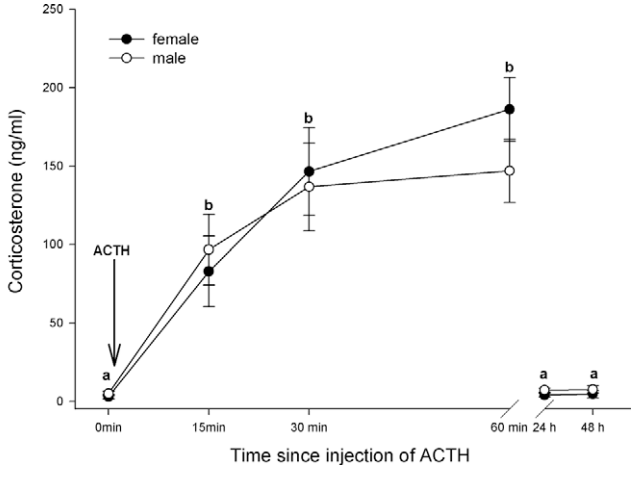

Fig. 2. Plasma corticosterone concentrations (mean ± SE) in Greater rhea ($n = 6$) after administration of ACTH. ACTH injection at 0:00, first sample collected prior to injection.

(CBG) (Rosner, 1990). Only unbound, or free, steroid is thought to interact with receptors, so that free corticosterone may be the fraction of corticosterone that is biologically available to tissues (Breuner and Orchinik, 2002). Thus, further studies should be carried out to assess the levels of corticosterone bound to CBG in Greater rheas since free corticosterone may not be higher than in other birds. However, regardless of whether the high levels of corticosterone are due to bound or free corticosterone, the results are consistent with a biochemist mechanism to maintain a high level of free corticosterone during a prolonged stress response (by maintaining the bound and free corticosterone concentration equilibria) (Berg et al., 2002). The findings are therefore also consistent with the fast running antipredatory strategy of this species, where they probably need a greater amount of available blood glucose (from the hyperglycemic action of corticosterone) to maintain the running capacity for long periods of time during their flight-type stress response.

In summary, we determined that corticosterone is the glucocorticoid present in Greater rhea plasma. The commercial corticosterone RIA employed provides highly reliable results. This assay has several advantages: it is simple; it can be performed easily and rapidly, since it does not require extracting corticosterone from the plasma sample before the assay is performed; and RIA components are available in the market. Exogenous administration of ACTH produced a rapid 40-fold increase in plasmatic corticosterone concentration, biologically supporting the use of RIA as a sensitive method to assess adrenocortical activity. The magnitude of the adrenocortical response observed suggests that the HPA axis in this species is highly sensitive, which could be a characteristic of ratites.

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References

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1994. Gender and Seasonal differences in the adrenocortical response to ACTH challenge in an arctic passerine, *Zonotrichia leucophrys gambelii*. Gen. Comp. Endocrinol. 94, 33–43.

Bellis, L.M., Martella, M.B., Navarro, J.L., Vignolo, P., 2004. Home range of Greater and Lesser Rhea in Argentina: relevance to conservation. Biodivers. Conserv. 13, 2589–2598.

Berg, J.M., Tymoczko, J.L., Stryer, L., 2002. Biochemistry, fifth ed. W.H. Freeman and Company, New York.

Biddlecombe, R.A., Law, B., 1996. Validation of an immunoassay. In: Law, Brian (Ed.), Immunoassay a Practical Guide. Taylor & Francis Ltd., London, pp. 171–203.

Breuner, C.W., Orchinik, M., 2002. Beyond carrier proteins: plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175, 99–112.

Busso, J.M., Ponzio, M., Dabbene, V., Cuneo, M., Ruiz, R., 2005. Assessment of urine and fecal testosterone metabolite excretion in *Chinchilla lanigera* males. Anim. Repr. Sci. 86 (3–4), 339–351.

Cockrem, J.F., 2007. Stress endocrinology and conservation. In: Maitra, S.K. (Ed.), Hormone Biotechnology. Daya Publishing House, Delhi, pp. 346–353.

Dehnhard, M., Schreer, A., Krone, O., Jewgenow, K., Krause, M., Grossmann, R., 2003. Measurement of plasma corticosterone and fecal glucocorticoid metabolites in the chicken (*Gallus domesticus*), the great cormorant (*Phalacrocorax carbo*), and the goshawk (*Accipiter gentilis*). Gen. Comp. Endocrinol. 131, 345–352.

Giordano, P.F., Bellis, L.M., Navarro, J.L., Martella, M.B., 2008. Abundance and spatial distribution of Greater rhea *Rhea americana* in two sites on the pampas grasslands with different land use. Bird Conserv. Internat. 18, 63–70.

Ellis, B.J., Jackson, J.J., Boyce, W.T., 2006. The stress response systems: universality and adaptive individual differences. Dev. Rev. 26, 175–212.

Faure, J.M., Mills, A.D., 1998. Improving the adaptability of animals by selection. In: Grandin, T. (Ed.), Genetics and the Behavior of Domestic Animals. Academic Press, San Diego, CA, pp. 235–264.

IUCN, 2008. IUCN Red List of Threatened Species. IUCN, Gland, Switzerland. Available from: <http://www.iucnredlist.org> (accessed 29.09.08).

Jones, R.B., 1996. Fear and adaptability in poultry: insights, implications and imperatives. World Poult. Sci. J. 52, 131–174.

Jones, R.B., Hocking, P.M., 1999. Genetic selection for poultry behaviour: big bad wolf or friend in need? Anim. Welf. 8, 343–359.

Ludders, J.W., Langenberg, J.A., Czekala, N.M., Erb, H.N., McCormick, H., 1998. Serum corticosterone response to adrenocorticotropic hormone stimulation in Florida sandhill cranes. J. Wildl. Dis. 34, 715–721.

Marín, R.H., Satterlee, D.G., 2004. Cloacal gland and testes development in male Japanese quail selected for divergent adrenocortical responsiveness. Poult. Sci. 83, 1028–1034.

Marín, R.H., Satterlee, D.G., 2003. Selection for contrasting adrenocortical responsiveness in Japanese quail influences sexual behavior in males. Appl. Anim. Behav. Sci. 83, 187–199.

Martella, M., Navarro, J., 2006. Proyecto Ñandú. Manejo de *Rhea americana* y *R. pennata* en la Argentina. In: Bolkovic, M.L., Ramadori, D.E. (Eds.), Manejo de Fauna en Argentina: Proyectos de Uso Sustentable. Dirección de Fauna Silvestre—Secretaría de Ambiente y Desarrollo Sustentable, Buenos Aires, Argentina, pp. 39–50.

Mitchell, M., Kettlewell, P., Sandercock, D., Maxwell, M., Spackman, D., 1996. Physiological stress in ostriches during road transportation. In: Deeming, D.C. (Ed.), Improving our Understanding of Ratites in a Farming Environment. Reino Unido, pp. 79–80.

Monfort, S.L., 2003. Non-invasive endocrine measures of reproduction and stress in wild population. In: Holt, W.V., Pickard, A.R., Rodger, J.C., Wild, D.E. (Eds.), Reproductive Science and Integrated Conservation. Cambridge, London, pp. 146–165.

Müllner, A., Linsenmair, K.E., Wikelski, M., 2004. Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (*Opisthocomus hoazin*). Biol. Conserv. 118 (4), 549–558.

Neter, J., Wasserman, W., Kutner, M., 1990. Applied Linear Statistical Models: Regression, Analysis of Variance and Experimental Designs. Irwin, Homewood, IL. pp. 165–167.

Newman, A., Chin, E., Schmidt, K., Bond, L., Wynne-Edwards, K., Soma, K., 2007. Analysis of steroids in songbird plasma and brain by coupling solid phase extraction to radioimmunoassay. Gen. Comp. Endocrinol. 155, 503–510.

Nilsson, P.B., Hollmén, T.E., Atkinson, S., Mashburn, K.L., Tuomi, P.A., Esler, D., Mulcahy, D.M., Rizzolo, D.J., 2008. Effects of ACTH, capture, and short term confinement on glucocorticoid concentrations in harlequin ducks (*Histrionicus histrionicus*). Comp. Biochem. Physiol. A. 149, 275–283.

Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., Palme, R., 2004. Measurement of corticosterone metabolites in chicken droppings. Brit. Poult. Sci. 45 (5), 704–711.

Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19 (5), 249–255.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A 140, 73–79.

Romero, L.M., Cyr, N.E., Romero, R.C., 2006. Corticosterone responses change seasonally in free-living house sparrows (*Passer domesticus*). Gen. Comp. Endocrinol. 149 (1), 58–65.

Rosner, W., 1990. The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. Endocr. Rev. 11, 80–91.

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

Thiel, D., Jenni-Eiermann, S., Palme, R., 2005. Measuring corticosterone metabolites in droppings of capercaillies (*Tetrao urogallus*). Ann. NY Acad. Sci. 1046, 1–13.

Washburn, B., Millspaugh, J., Faaborg, J., Schulz, J., 2002. Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. Condor 104, 558–563.

Wong, N., Chien, B., D'mello, A., 1994. Analysis of corticosterone in rat plasma by high-performance liquid chromatography. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 661, 211–218.

Zenoble, R.D., Kemppainen, R.J., Young, D.W., Carpenter, J.W., 1985. Effect of ACTH on plasma-corticosterone and cortisol in eagles and condors. J. Am. Vet. Med. Assoc. 187, 1119–1120.