Measuring Corticosterone Metabolites in Droppings of Capercaillies *(Tetrao urogallus)*

DOMINIK THIEL,[a] SUSANNE JENNI-EIERMANN,[a] AND RUPERT PALME[b]

[a]*Swiss Ornithological Institute, 6204 Sempach, Switzerland*

[b]*Institute of Biochemistry, Department of Natural Sciences, University of Veterinary Medicine, Vienna, Austria*

ABSTRACT: **The capercaillie *(Tetrao urogallus),* the largest grouse species in the world, is decreasing in numbers in major parts of its distribution range. Disturbances by human outdoor activities are discussed as a possible reason for this population decline. An indicator for disturbances is the increase of the glucocorticoid corticosterone, a stress hormone, which helps to cope with life-threatening situations. However, repeated disturbances might result in a long-term increase of the basal corticosterone concentration, which can result in detrimental effects like reduced fitness and survival of an animal. To measure corticosterone metabolites (CMs) noninvasively in the droppings of free-living capercaillies, first an enzyme immunoassay (EIA) in captive birds had to be selected and validated. Therefore, the excretion pattern of intravenously injected radiolabeled corticosterone was determined and ^{3}H metabolites were characterized. High-performance liquid chromatography (HPLC) separations of the samples containing peak concentrations revealed that corticosterone was extensively metabolized. The HPLC fractions were tested in several EIAs for glucocorticoid metabolites. The physiological relevance of this method was proved after pharmacological stimulation of the adrenocortical activity. Only the recently established cortisone assay, measuring CMs with a 3,11-dione structure, detected an expressed increase of concentrations following ACTH stimulation. To set up a sampling protocol suited for the field, we examined the influence of various storage conditions and time of day on concentrations of CMs.**

KEYWORDS: **capercaillie; noninvasive measurement; disturbance; conservation biology; ecology; field endocrinology**

INTRODUCTION

The capercaillie *(Tetrao urogallus),* the largest Galliform bird species in the Palearctic, is decreasing in numbers in most parts of its distribution range.[1] This decrease is particularly strong in central Europe, where many populations are already extinct or threatened with extinction.[2] Consequently, the capercaillie is classified as an endangered species on the Red List of Switzerland,[3] and as critically endangered in

Address for correspondence: Dominik Thiel, Swiss Ornithological Institute, 6204 Sempach, Switzerland. Voice: 0041 41 462 97 00; fax: 0041 41 462 97 10.
dominik.thiel@vogelwarte.ch

**Ann. N.Y. Acad. Sci. 1046: 96–108 (2005). © 2005 New York Academy of Sciences.
doi: 10.1196/annals.1343.009**

Germany[4] and Austria.[5] In Switzerland, numbers of capercaillies were reduced by more than half during the last 30 years, and the decline is still going on[6] The decrease is caused mainly by habitat loss and habitat degradation.[2,6,7] The decline of the capercaillie in areas with increasing human outdoor activities has led to the assumption that capercaillies are negatively affected by human disturbance.[2,8] First, human presence may lead to the avoidance of the disturbed area and to a change into a habitat of lower quality, a reaction that may negatively affect survival,[9] as was suggested for the capercaillies in Germany.[10] Second, human-induced disturbance can have substantial energetic consequences, particularly during winter, and may disturb the normal activity pattern and cause an energy deficit, as was shown for snow geese (*Chen caerulescens*[11]). If prolonged or repeated, human disturbance causes repeated physiological stress reactions, which may result in long-term negative effects, such as reduced reproduction or reduced immuncompetence.[12,13] However, in the Black Forest (Germany), some capercaillies live near paths, ski trails, or ski runs.[14] Whether these birds are stressed or adapted to human encounters is not known.

One means to determine whether an individual shows physiological stress reactions, even in the absence of an obvious behavioral response, is to analyze the levels of stress hormones released. The organism reacts to stress with the activation of the hypothalamo–pituitary–adrenal (HPA) axis, resulting in a release of glucocorticoids (in birds: corticosterone) into the blood, which triggers adjustments in physiology and behavior to help the organism survive.[13,15] Plasma glucocorticoid concentrations are therefore widely used to diagnose a physiological stress response.[16–19]

In free-living capercaillies, it is impossible to sample blood without causing severe stress by capture and handling. Therefore, the noninvasive method to quantify hormone production by the measurement of the hormone metabolites excreted in droppings collected in the field allows tracking the metabolic response to disturbances.[20] Because this method is feedback free, repeated measurements in the same individual are possible.[21]

Corticosterone metabolites (CMs) in droppings have been quantified in a few avian species.[22–28] Glucocorticoids are extensively metabolized before excretion, and native corticosterone was not found in the droppings.[28] The metabolism of corticosterone varies between species (and even gender), and therefore the best-suited immunoassay has to be chosen and the method validated for each species anew.[29,30] When using droppings from a free-living species, additional studies are necessary to assess whether the particular sampling conditions in the field, often suboptimal, affect the concentration of CMs in the droppings. Because of their low population densities and cryptic behavior, capercaillies cannot be followed and observed easily.[8] Droppings cannot be collected shortly after defecation, and therefore the exact time of defecation in droppings found in the field is unknown. Therefore, it needs to be evaluated whether and under which ambient conditions CMs degrade after voidance[31,32] and whether the excretion of CMs varies during the day.[33]

The aim of this study was thus to select and validate an enzyme immunoassay (EIA) for the quantification of CMs in capercaillie droppings collected in the field and to test the influence of various storage conditions. This was done by the following steps: In a radiometabolism study, the excreted CMs were characterized with reversed-phase high-performance liquid chromatography (RP-HPLC). Then, several antibodies were tested to select the best-suited EIA for the quantification of the CMs. Finally, the method was physiologically validated by inducing a corticosterone re-

lease through an adrenocorticotropic hormone (ACTH) injection. Experimentally, we tested the influence of time of day, temperature, and duration of storage on the concentration of CMs in droppings. This procedure is in accordance with the guidelines recommended previously.[34]

MATERIAL AND METHODS

Animals

All experiments with capercaillies were conducted with captive birds in the Max Planck Institute for Ornithology, Radolfzell, Germany. The birds were housed in outdoor aviaries, exposed to natural light and temperature conditions, but protected from precipitation. Each aviary had one male, whereas the much smaller females could freely move between aviaries through small openings. During the cold season, the period when we did our experiments, the birds were supplied with water, conifer needles, and maize ad libitum. The age of the birds was at least 1 year. Body mass ranged between 3 and 4 kg in males and 1.5 and 2 kg in females.

For the experiments, birds were transferred singly to aviaries with dimensions of $3 \times 3 \times 2.5$ m. The floor was covered with a plastic sheet so that droppings could be easily collected and the floor cleaned. Capercaillies feeding on needles and maize void brown, nearly nitrogen-free, dry, and compact droppings.[35,36] One female reacted very nervously when workers entered the aviary for sampling droppings during the radiometabolism study, causing her to void more liquid droppings. The droppings of the ceca, which are of a pasty consistency, a different shape, and a penetrating odor, were not sampled. They are voided only once per day, are hard to find in the field, and decay within a few days under frozen conditions.

Radiometabolism Study

The experiment was carried out during April 13–16, 2003. The day before the experiment, two males and two females were placed in separate aviaries to ensure that the droppings were not mixed up. The next day at 8 A.M., each bird was injected with 1.85 MBq (50 μCi) of radiolabeled corticosterone ([1,2,6,7,- ^{3}H(N)]-corticosterone; specific activity 76.5 Ci/mmol, Perkin-Elmer Life Sciences, Boston, MA) dissolved in 0.5 mL of 0.9% NaCl solution containing 10% ethanol into the *vena ulnaris*.

One hour before injection, droppings of each bird were collected to determine background levels of radioactivity. During the first day after injection, droppings were collected every hour until 11 P.M. and frozen immediately at –23°C. Thereafter they were collected every second hour until 7 A.M. During the second day after injection, droppings were collected in 3-h intervals until 5 P.M. and during the third and fourth day at 8 A.M., 1 P.M., and 6 P.M.

Radioactivity in the droppings was measured as described previously.[28] In brief, 0.5 g of the homogenized sample was extracted with 5 mL of 60% methanol by shaking for 30 min. After centrifugation, aliquots (0.5 mL in duplicates) of the supernatant were mixed with 6 mL of a scintillation fluid (Quicksafe A, No. 100800; Zinsser Analytic, Maidenhead, UK) and measured in a liquid scintillation counter (Packard

Tri-carb 2100TR; Meriden, CT). Radioactivity was expressed as kilobecquerels (kBq) per kilogram of sample.

To characterize the excreted metabolites, we performed RP-HPLC separations with the samples containing the highest concentrations of radioactive metabolites. Cleanup and separations were performed as described previously.[28,33,37]

Analysis of Metabolites

The immunoreactivity of the 90 fractions eluted from the RP-HPLC (diluted 1:5 with assay buffer) was measured in different EIAs. Three assays were tested: an 11-oxoetiocholanolone,[38] a cortisone,[28] and a 20-dihydrocorticosterone EIA.[39] EIAs were performed as described previously[40] on microtiter plates coated with anti-rabbit immunoglobulin G (IgG) using a double-antibody technique and biotinylated steroids as labels.

Administration of ACTH

The physiological relevance of the method was evaluated by stimulating adrenocortical activity with ACTH. Three males and two females were injected with 1 mL (0.25 mg) of ACTH (Synacthen; Novartis Pharma Schweiz SA, Bern, Switzerland) in the *vena ulnaris.*

Experiments were conducted April 13–16, 2003 (one male, one female), and February 8–12, 2004 (two males, one female). To obtain basal values, we collected droppings 1 h before injection. After injection, droppings were collected for 4 days, once per hour for the first 48 h, and thereafter sampling frequency was gradually reduced to three times per day on the last day. In 2004, droppings were also collected over the 28 h before injection to assess the diurnal rhythm of CMs in droppings.

All samples were immediately stored at –23°C until analysis. The samples were extracted as previously described, except the 2004 samples, which were lyophilized before extraction. Accounting for the water loss of lyophilization, we used a reduced weight of sample for the extraction. Results were expressed in nanomoles per kilogram of fresh weight. The aliquots of the supernatant (diluted 1:10 with assay buffer) were measured with the cortisone EIA and the 11-oxoetiocholanolone EIA[28,38] to determine the levels of CMs in the droppings.

Effect of Storage Duration, Temperature, and Gender on CMs

Droppings were collected in three different aviaries within 24 h after cleaning the floor, each holding one male but several females, the latter freely moving between aviaries. Therefore, only male droppings were specific to the individual. One hundred grams of the droppings of each gender and aviary was pooled, homogenized, and divided into subsamples of 0.5 g each. Fifteen samples of each gender and aviary were frozen immediately. Five samples of each gender and aviary were stored at 8°C for 1, 7, and 21 days, respectively, and another five samples of each gender and aviary at 21°C for 1, 7, and 21 days, respectively. CM levels were analyzed following the procedure previously described.

We tested for effects of these factors on $\log_{10}$-transformed values of CMs in a split-plot ANOVA[41] with individuals nested within aviaries and single portions of droppings nested within individuals. Because gender varies among individuals, it is

tested against the residual variation among individuals within aviaries. All other factors, including interactions, vary among portions of droppings and are therefore tested at the lowest level of the experiment, that is, using the residual mean square, which quantifies the variation among portions of droppings nested within individuals. To test the significance of particular group differences, we compared them with the least significant difference at an α level of 0.05. Analysis was conducted using the GenStat package.[42]

RESULTS

Radiometabolism Study

Excretion of ^{3}H-corticosterone started immediately because above-background radioactivity was measured in the first droppings collected 1 h after injection. All four birds had one main peak, followed by several smaller peaks. The radioactivity returned to background levels about 33 h after injection. Three individuals showed a broad peak between 1 and 4 h after injection (FIG. 1). Female B differed from the other three birds by showing one sharp peak of radioactivity 1 h and a second lower and broader peak 3–4 h after injection. This female was quite nervous and was the only bird with more liquid droppings (see MATERIALS AND METHODS).

The RP-HPLC separations of the droppings with the highest radioactivity revealed the presence of three to four major radioactive peaks (mainly between fractions 30 and 50) and many smaller peaks, indicating that several CMs were excreted

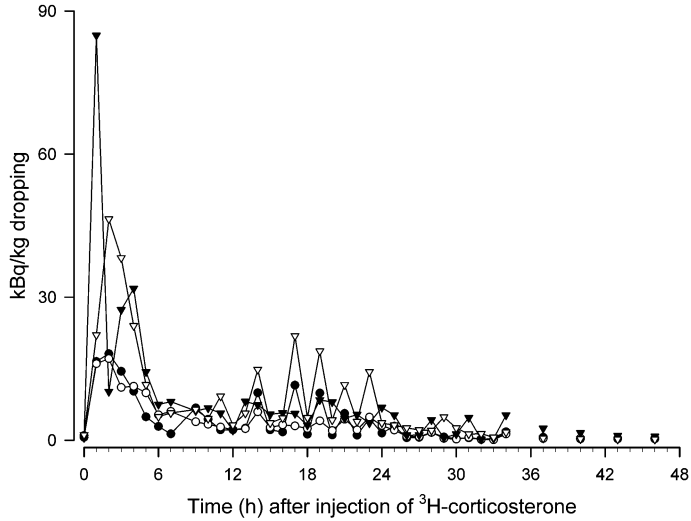

FIGURE 1. Time course of excretion of ^{3}H-corticosterone in droppings of 2 male and 2 female Capercaillies. The *circles* represent the two males, the *open triangles* female A and the *filled triangles* the results of female B.

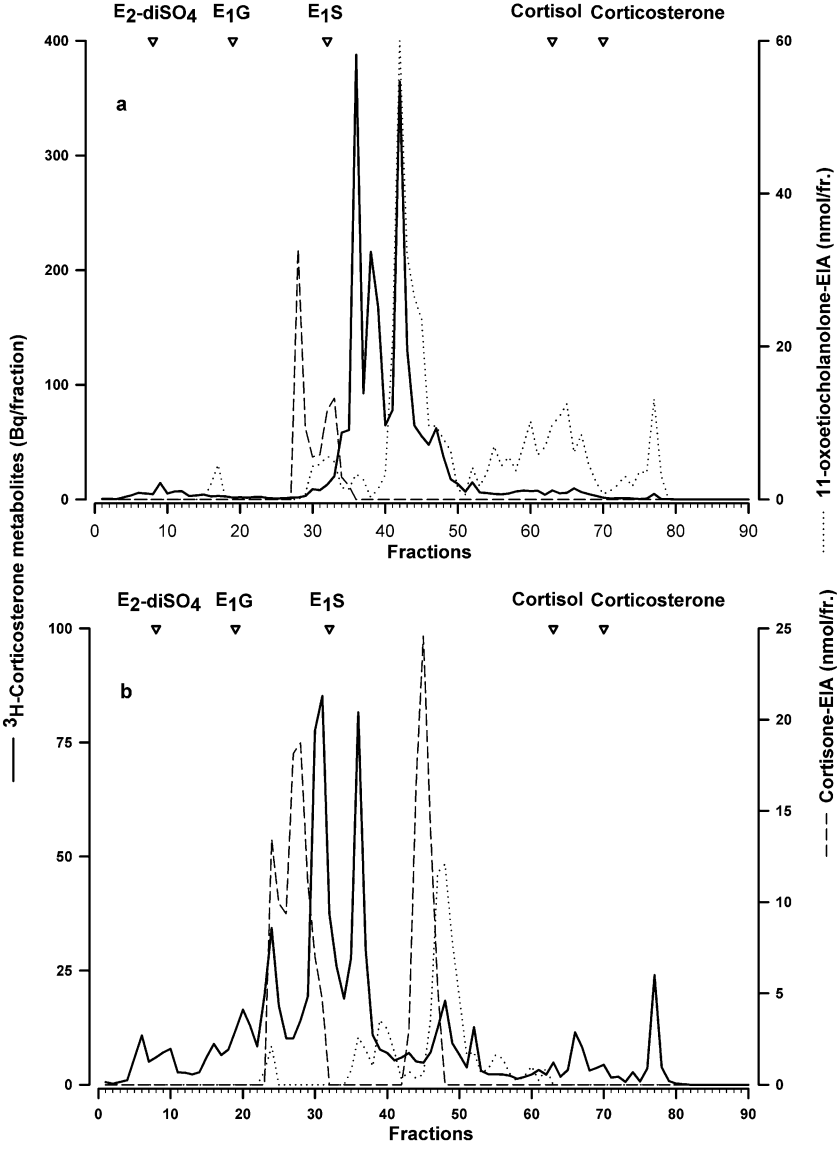

FIGURE 2. RP-HPLC separation of ^{3}H-CM in droppings of (**a**) a male and (**b**) a female Capercaillie. Radioactivity of each fraction was determined by liquid scintillation counting. The immunoreactivity of ^{3}H-CM with two different EIAs is given in nmol per fraction. *Open triangles* mark the approximate elution position of respective standards: E_2-diSO_4 = estradiol-17β-disulphate; E_1G = estroneglucuronide; E_1S = estronesulphate, cortisol, and corticosterone.

(FIG. 2). Only negligible amounts of radioactivity (if any) eluted at the position of corticosterone. In the HPLC fractions, the highest amounts of immunoreactivity were detected with the cortisone EIA, measuring metabolites with a common 3,11-dione structure, and the 11-oxoetiocholanolone assay, measuring CM with a 5-3-11-one structure (FIG. 2a and 2b).

Physiological Validation

To assess the biological relevance of the method, we injected ACTH in five birds to stimulate adrenocortical activity. The cortisone assay measured the highest concentrations in the samples. After injection, the concentration of CMs in the droppings increased sharply, peaked after 1–3 h, and returned to basal values between 4 and 13 h after injection. The increase in CMs above basal levels varied individually between a factor of about 5 up to a factor of about 60 (FIG. 3). In contrast, the 11-oxoetiocholanolone EIA detected lower concentrations and no clear response to the stimulation of the HPA axis (data not shown).

Concentrations of CMs excreted during the 28 h before injection of ACTH were regarded as basal values. Although some variation of the concentrations occurred, the pattern did not resemble a distinct diurnal rhythm (mean ± SD of the three individuals: 212 ± 70; 228 ± 178; 391 ± 192 nmol/kg of droppings).

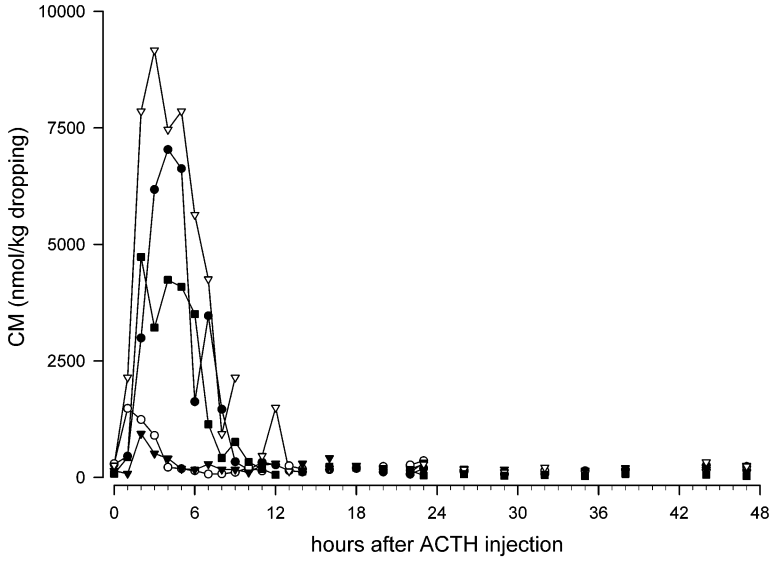

FIGURE 3. Concentrations of CM (3,11-dioxo-CM, nmol/kg droppings) after administration of ACTH in droppings of 3 male and 2 female Capercaillies.

TABLE 1. Effect of gender, ambient temperature, and storage time on the concentration of CM in droppings

Source of variation	d.f.	m.s.	F	P
Aviary stratum	2	0.084	0.28	
Individual per aviary stratum				
Gender	1	1.726	5.85	.137
Residual	2	0.295	12.86	
Units per individual per aviary				
Temperature	2	0.92605	40.37	<.001
Time	3	0.39155	17.07	<.001
Gender*temperature	2	0.22437	9.78	<.001
Gender*time	3	0.02807	1.22	.302
Temperature*time	1	0.32875	14.33	<.001
Gender*temperature*time	1	0.29662	12.93	<.001
Residual	251	0.02294		
Total	268			

NOTE: Nested ANOVA with ln[CM] as dependent variable and aviary, gender, temperatre, storage time, and interactions as independent variables. For details of the model, see the text. Units of the analysis consist of a single portion of the homogenized droppings.

ABBREVIATIONS: d.f. = degrees of freedom; m.s. = mean squares; F-value and significance P are given.

Effects of Storage

The average concentration of CMs in the samples frozen within 1 day after voidance was 537 ± 79 nmol/kg for males and 339 ± 50 nmol/kg of droppings for females. There were statistically significant effects of temperature and storage time on the concentration of CMs as well as of the gender–temperature, temperature–time, and gender–temperature–time interactions (TABLE 1). The main effects of gender and the gender–time interaction were not statistically significant.

Both temperature and storage time changed the concentration of CMs in the droppings compared with that in droppings frozen within 24 h after voidance (FIG. 4). In samples incubated at 8°C, CMs were not significantly different from those in frozen samples, but in samples exposed to a temperature of 21°C for 21 days, concentrations were significantly higher in both genders.

DISCUSSION

Radiometabolism Study

The radiometabolism study aimed to track the temporal excretion pattern of CMs and to select the droppings with the highest radioactivity to characterize the excreted CMs. The excretion of ^{3}H-corticosterone started immediately after injection. In three birds, high concentrations of radioactivity were measured during 1–4 h, ap-

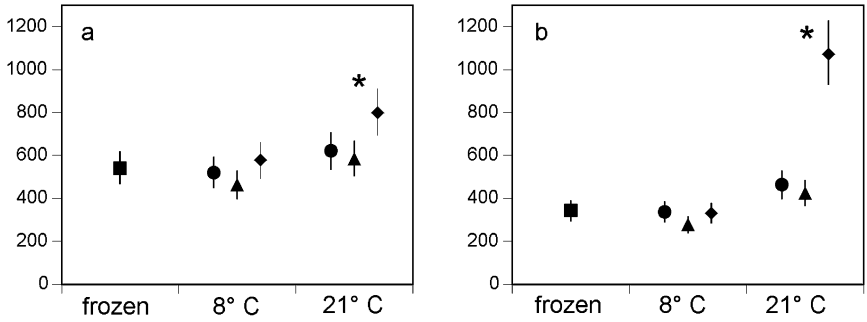

FIGURE 4. Concentrations (mean ± SE; $n = 15$) of 3,11-dioxo-CM (nmol/kg droppings) after storage at 8°C and 21°C for 1 (*circle*), 7 (*triangle*), and 21 *(rhombus*) days, respectively, for (**a**) males and (**b**) females. Control samples ($n = 45$) were frozen at −23°C within 24 hours after collection. Conditions marked with an asterisk differ significantly ($P < .05$) from control samples.

pearing as one broad peak. Only in one female could two peaks of ^{3}H-CM be observed, 1 h and 3–4 h after injection. This biphasic excretion pattern of female B agrees with that described for domestic chicken.[28] In the chicken, the first peak could be assigned to the metabolites excreted via the urine and the second peak to the excretion of the metabolites via the feces.[28] Because birds excrete urine and feces together via the cloaca, the two components cannot readily be separated, as is possible in mammals.[34,37,43]

The unimodal temporal excretion pattern found in the three normally excreting individuals might be explained by the particular diet and specific characteristics of digestion of capercaillies. During winter, capercaillies feed mainly on conifer needles (>90%). This fiber-rich diet quickly passes through the intestine, except the nutrient-rich liquid component, which enters the ceca. This diet is poor in water and protein;[8,36] thus, only small amounts of urine are excreted. Furthermore, urine is transported from the cloaca back to the ceca by reverse peristalsis for reabsorption of water.[44] Hence, urine is mixed with feces by back-and-forth movement in the rectum. Consequently, the CMs secreted by the kidney and the bile are mixed and excreted together, which results in a broad peak appearing relatively quickly. The fact that the single hen with the biphasic pattern excreted liquid (and therefore less mixed) droppings rather than the normal dry ones supports this idea.

Glucocorticoids are heavily metabolized before excretion.[34] In the RP-HPLC analysis of the droppings with the highest concentrations of radiolabeled CMs, corticosterone itself could be found only in very small amounts, if at all, in the birds. This finding is in accordance with other studies, which also found no corticosterone in droppings (black grouse *Tetrao tetrix,*[39] great tit *Parus major,*[27] European stonechats *Saxicola torquata,*[25] and domestic chicken[28]). The RP-HPLC analysis indicated that metabolites more polar than corticosterone were excreted. Because the three to four most prominent fractions were polar, conjugated or polar unconjugated metabolites were most abundant. These results agree with those in other bird species,

in which radiometabolism studies were performed (chicken, geese, and black grouse), all showing mainly polar metabolites.[22,28,39]

Physiological Validation

Stimulation of the HPA axis by injection of ACTH promotes synthesis and secretion of glucocorticoids,[45] which finally results in an increase of the CM levels in the droppings.[28] Following ACTH injection, the capercaillies' CM concentrations increased after the first hour and peaked after about 3–4 h before they slowly returned to pretreatment levels. This finding agrees with the temporal pattern of excretion in the other grouse species, the black grouse.[39] In chickens[28] and spotted owls (*Strix occidentalis caurina*[26]), peak concentrations of CMs appeared 2 h after stimulation. The delayed excretion of peak concentrations in tetranoids is probably due to urine's being transported back from the cloaca to the ceca and therefore mixed with the feces, as discussed before.

The cortisone EIA, which detects metabolites with a 3,11-dioxo structure, and the 11-oxoetiocholanolone assay, measuring CM with a 5β-3α-11-one structure, showed a high immunoreactivity in the HPLC fractions. However, only the cortisone assay showed an expressed increase of concentrations following administration of ACTH. This result demonstrates the importance of a physiological validation. Because this group-specific antibody also proved best suited for chicken and black grouse,[28,39] it seems to be the most adequate for the determination of the CMs excreted by Galliforms.

Effects of Storage

The concentration of CMs in the feces can vary with storage conditions and time.[31,32] Because the exact time since voidance of capercaillie droppings found in the field is unknown, we measured CM concentrations in droppings that were exposed for different time spans and at different temperatures. This approach may help establish a sampling protocol suited for field conditions.

The results of the storage experiment showed that the concentration of CMs varied with gender, storage time, and temperature—but only at room temperature. In both genders the concentrations increased significantly after 21 days of storage at room temperature. In comparison with the frozen samples, no significant change could be observed in droppings stored at 8°C up to 21 days. Therefore, in studies conducted in the mountains during the winter with ambient temperatures near or below the freezing point, changes in the concentration of CMs after voidance will most probably be insignificant.

An increase in glucocorticoid metabolite concentrations was also observed in mammals, when feces were incubated at room temperature. These studies measured increased concentrations after a storage time of 4 h in cattle and 24 h in horses. Most probably, naturally occurring bacteria metabolize steroids with their enzymes.[31,32]

Gender differences in pattern and amounts of excreted fecal CMs were described previously for chickens.[28] Because of the various difficulties of raising and keeping capercaillies, only a few animals could be used in our study. Therefore, statistically significant gender differences could not be proved. However, gender should be taken

into account when interpreting the results of studies of fecal samples. For the purposes of our sampling protocol in the field, this gender difference poses no problem, because the droppings of male capercaillies from roosting trees can be distinguished from those of females by their larger size.

Diurnal Pattern

The samples collected during 28 h revealed no diurnal rhythm. This finding agrees with those in the black grouse[39] but is in contrast to several studies measuring plasma corticosterone levels in other birds,[46,47] in which corticosterone concentration rises in the early morning before activity starts. Grouse species during winter typically have a particular activity pattern. They feed only twice per day, in the morning and afternoon, while they roost in snow burrows, below, or on trees during the rest of the day. This bimodal activity and feeding pattern is maintained in captivity. Hence, it is not surprising that their daily corticosterone pattern differs from that of other bird species kept in captivity.

As for the sampling protocol in the field, it seems that there is no need to observe time of day. However, because there are differences in corticosterone metabolite concentrations between droppings,[39] for example, possibly related to a pulsed excretion of corticosterone by the bile (Klasing, personal communication), several droppings from below a roost tree or a burrow should be collected and homogenized. With this method, a mean concentration of CMs excreted over a longer time span will be obtained.

CONCLUSION

This study demonstrates that the concentration of CMs can be reliably measured in droppings collected from capercaillies under field conditions during winter. CMs excreted in droppings are best determined with the cortisone EIA (measuring 3,11-dioxo CMs), as shown by the radiometabolism study and the ACTH validation experiment. Genders probably differ in the concentration of CMs in droppings, but in most cases droppings can be separated according to gender in the field. Droppings should be collected within about 20 days and should not be exposed to temperatures exceeding 8°C. The maximum age of the droppings can usually be determined when observing the last snowfall, and ambient temperatures exceeding 8°C are rare during winter in the habitat of the capercaillie. Several droppings should be analyzed together to avoid variations between droppings and to obtain a mean value over a longer period. Hence, the method to estimate whether capercaillies are physiologically stressed on the basis of contents of droppings seems to be promising.

ACKNOWLEDGMENTS

We thank Peter Berthold, Max Planck Institute for Ornithology, Radolfzell, for permission to do the experiments with his capercaillies; Alexandra Kuchar and Ruedi Meier for laboratory assistance; Marc Kéry for statistical advice; and Niklaus Zbinden and Lukas Jenni for valuable comments.

Birds were held and all experiments were carried out under license from the Regierungspräsidium Freiburg, G-03/21.

REFERENCES

1. HAGEMEIJER, W.J. & M.J. BLAIR. 1997. The EBCC Atlas of European Breeding Birds: Their Distribution and Abundance. Poyser. London.
2. STORCH, I. (compiler) 2000. Grouse status survey and conservation action plan 2000–2004. WPA/BirdLife/SSC Grouse Specialist Group. IUCN, Gland. Switzerland and Cambridge, UK and the World Pheasant Association, Reading, UK.
3. KELLER, V., N. ZBINDEN, H. SCHMID & B. VOLET. 2001. Rote Liste der gefährdeten Brutvogelarten der Schweiz. BUWAL-Reihe Vollzug Umwelt. Bundesamt für Umwelt, Wald & Landschaft. Bern & Schweizerische Vogelwarte, Sempach.
4. BAUER, H.-G. *et al.* 2002. Rote Liste der Brutvögel Deutschlands. Vogelschutz **39:** 13–60.
5. GEPP, J. 1994. Rote Listen gefährdeter Tiere österreichs. Grüne Reihe des Bundesministeriums für Umwelt, Jugend und Familie. Band 2. Styra, Graz.
6. MOLLET, P. *et al.* 2003. Verbreitung und Bestand des Auerhuhns *Tetrao urogallus* in der Schweiz 2001 und ihre Veränderungen im 19 und 20. Jahrhundert. Orn. Beob. **100:** 67–86.
7. ZEITLER, A. 1995. Skilauf und Rauhfusshühner. Orn. Beob. **92:** 227–230.
8. KLAUS, S., *et al.* 1989. Die Auerhühner. Die Neue Brehm-Bücherei. A. Ziemsen Verlag. Wittenberg Lutherstadt, Germany.
9. FRID, A. & L. DILL. 2002. Human caused disturbance stimuli as a form of predation risk. Conserv. Ecol. **6:** 11. [online].
10. MÜLLER, F. 1974. Territorialverhalten und Siedlungsstruktur einer mitteleuropäischen Population des Auerhuhns (*Tetrao urogallus major*). Ph.D. thesis, Marburg/Lahn, Germany.
11. BÉLANGER, L. & J. BÉDARD. 1990. Energetic cost of man-induced disturbance to staging Snow Geese. J. Wildl. Manage. **54:** 36–41.
12. GAVRIN, V.F. 1973. Die Wirkung des Angstfaktors auf die Produktivität von Federwildpopulationen. *In* Proc. XI. Int. Congr. Game Biol.: 401–403. Stockholm.
13. WINGFIELD, J.C. & L.M. ROMERO. 1999. Adrenocortical responses to stress and their modulation in free-living vertebrates. *In* Handbook of Physiology—Coping with the Environment. B. McEwen, Ed.: 211–239. Oxford University Press. New York.
14. VOLK, H. 1983. Winterport und Biotopschutz. Hat das Auerhuhn in Skilanglaufgebieten eine Chance? Nat. Landschaft **58:** 454–459.
15. SAPOLSKY, R.M. 1987. Stress, social status, and reproductive physiology in free-living baboons. *In* Psychobiology of Reproductive Behavior: An Evolutionary Perspective. D. Crews, Ed.: 291–392. Prentice Hall. Englewood Cliffs, NJ.
16. MUNCK A., P.M. GUYRE & N.J. HOLBROOK. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. **5:** 25–44.
17. VON HOLST, D. 1998.The concept of stress and its relevance for animal behavior. Adv. Study Behav. **27:** 1–131.
18. SAPOLSKY, R.M. 1992. Stress, the Aging Brain, and the Mechanisms of Neuron Death. MIT Press. Cambridge, MA.
19. HOFER, H. & M.L. EAST. 1998. Biological conservation and stress. Adv. Study Behav. **27:** 405–525.
20. CREEL, S. *et al*. 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conserv. Biol. **16:** 809–814.
21. PALME, R. *et al.* 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion and noninvasive measurement in fecal samples. Ann. N.Y. Acad. Sci. **1040:** 162–171.
22. KOTRSCHAL, K., K. HIRSCHENHAUSER & E. MÖSTL. 1998. The relationship between social stress and dominance is seasonal in greylag geese. Anim. Behav. **55:** 171–176.
23. NAKAGAWA, S., E. MÖSTL & J.E. WAAS. 2003. Validation of an enzyme immunoassay to measure faecal glucocorticoid metabolites from Adélie penguins (*Pygoscelis adeliae*): a non-invasive tool for estimating stress? Polar Biol. **62:** 491–493.

24. QUILLFELDT, P. & E. MÖSTL. 2003. Resource allocation in Wilson's storm-petrels *Oceanites oceanicus* determined by measurement of glucocorticoid excretion. Acta Ethol. **5:** 115–122.
25. GOYMANN, W., E. MÖSTL & E. GWINNER. 2002. Corticosterone metabolites can be measured noninvasively in excreta of European stonechats (*Saxicola torquata rubicola*). Auk **119:** 1167–1173.
26. WASSER, S.K. *et al*. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. **120:** 260–275.
27. CARERE, C. *et al*. 2003. Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and response to social stress. Horm. Behav. **43:** 540–548.
28. RETTENBACHER, S. *et al.* 2004. Measurement of corticosterone metabolites in chicken droppings. Br. Poult. Sci. **45:** 704–711.
29. MÖSTL, E. & R. PALME. 2002. Hormones as indicators of stress. Dom. Anim. Endocrinol. **23:** 67–74.
30. TOUMA, C. & R. PALME. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N.Y. Acad. Sci. **1046:** 54–74.
31. MÖSTL, E. *et al.* 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. Zentralbl. Veterinarmed. A **46:** 621–631.
32. HUNT, K.E. & S.K. WASSER. 2003. Effect of long-term preservation methods on fecal glucocorticoid concentrations of grizzly bear and African elephant. Physiol. Biochem. Zool. **76:** 918–928.
33. TOUMA, C., N. SACHSER, E. MÖSTL & R. PALME. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen. Comp. Endocrinol. **130:** 267–278.
34. PALME, R. 2005. Measuring fecal steroids: guidelines for practical application. Ann. N.Y. Acad. Sci. **1046:** 75–80.
35. GREMMELS, H.-D. 1986. Das Verdauungssystem der Rauhfusshühner—Eine Übersicht zur Physiologie und Mikroanatomie dieses Organsystems. Z. Jagdwiss. **32:** 96–104.
36. LIESER, M. 1996. Zur Nahrungswahl des Auerhuhns *Tetrao urogallus* im Schwarzwald. Orn. Beob. **93:** 47–58.
37. SCHATZ, S. & R. PALME. 2001. Measurement of faecal cortisol metabolites in cats and dogs: a non-invasive method for evaluating adrenocortical function. Vet. Res. Commun. **25:** 271–287.
38. MÖSTL, E. *et al.* 2002. Measurement of cortisol metabolites in faeces of ruminants. Vet. Res. Commun. **26:** 127–139.
39. BALTI, M., S. JENNI-EIERMANN, R. ARLETTAZ & R. PALME. 2005. A noninvasive technique to evaluate human-generated stress in the black grouse. **1046:** 81–95.
40. PALME, R. & E. MÖSTL. 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int. J. Mammal. Biol. **62**(Suppl II): 192–197.
41. SNEDECOR, W.G. & W.G. COCHRAN. 1989. Statistical Methods. 8th ed. Iowa State University Press, Ames.
42. PAYNE, R.W. *et al.* 1993. Genstat 5, Release 3. Reference Manual. Clarendon Press. Oxford.
43. PALME, R., P. FISCHER, H. SCHILDORFER & M.N. ISMAIL. 1996. Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim. Reprod. Sci. **43:** 43–63.
44. MOSS, R. & I. HANSSEN. 1980. Grouse nutrition. Nutr. Abstr. Rev. B **50:** 555–567.
45. WINGFIELD, J.C. & M. RAMENOFSKY. 1999. Hormones and the behavioral ecology of stress. *In* Stress Physiology in Animals. P.H.M. Balm, Ed.: 1–51. Academic Press. Sheffield, UK.
46. SMOAK, K.D. & G.P. BIRRENKOTT. 1986. Daily variation of corticosterone and thyroid hormones in the broiler cockerels. Poult. Sci. **65:** 197–198.
47. BREUNER, C., J.C. WINGFIELD & L.M. ROMERO. 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. J. Exp. Zool. **284:** 334–342.