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LEVELS OF FECAL CORTICOSTERONE IN SANDHILL CRANES DURING A HUMAN-LED MIGRATION

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ABSTRACT: Fourteen captive-reared greater sandhill cranes (*Grus canadensis tabida*) were conditioned to follow ultralight aircraft to promote migration between Wisconsin and Florida (USA) after release. Fecal samples were collected throughout the training period in Wisconsin and during a 1,977-km human-led migration to Florida to determine fecal corticosterone (FC) concentrations by radioimmunoassay. The mean (±SE) FC concentration during the training period was 109.5±7.5 ng/g and was representative of baseline levels recorded previously from sandhill cranes. Fecal corticosterone concentrations increased in early migration compared to concentrations 1 mo prior to departure ($P<0.01$) but were not different from baseline concentrations at the end of the 6-wk migration period. The variability of FC concentrations in individual samples was greater throughout the migration than the training period. Increases in FC during migration were modest and generally consistent with normal corticosterone elevations observed in migrating birds.

Key words: Fecal corticosterone, glucocorticoid, *Grus canadensis tabida,* health management, migration, radioimmunoassay, sandhill crane, stress.

INTRODUCTION

Corticosterone is the primary glucocorticoid produced in birds experiencing stress (Siegel, 1980; Harvey et al., 1984; Wingfield et al., 1992). Overcrowding, food deprivation, weather extremes, exposure to predators, exertion, capture, restraint, and blood sampling are known stressors for birds (Harvey and Hall, 1990; Le Maho et al., 1992; Siegel, 1995; Silverin, 1998; Cockrem and Silverin, 2002). Capture methods that result in different intervals prior to blood sampling limit the use of serum corticosterone as a marker of stress in wild birds. Measurement of fecal corticosterone (FC) has recently been validated as an alternative method to monitor stress in Florida sandhill cranes (*Grus canadensis pratensis;* Ludders et al., 1998, 2001). The advantages of this method are reduced bias in basal corticosterone levels from lack of restraint and handling for blood sampling (Schwartz et al., 1992; Goymann et al., 2002), reduced risk of morbidity from handling, and reduced labor and equipment costs for sample acquisition.

Measuring corticosterone may assist the determination of risks from a variety of stressors that could impair individual or population health and fitness (Monfort et al., 1998). Chronic activation of the stress response in birds has been associated with deleterious sequelae such as compromised cellular and humoral immunity, growth, and reproduction (Axelrod and Reisine, 1984; Harvey et al., 1984; Carsia and Harvey, 2000). These negative outcomes are important factors in the success of conservation programs of endangered species (Wasser et al., 1997).

This paper describes FC monitoring in a group of captive-bred greater sandhill cranes (*Grus canadensis tabida*) that experienced a novel, highly manipulative reintroduction technique aimed at facilitating migratory behavior (Lishman et al., 1997). The purpose of this study was to document premigratory FC concentrations in the cranes and characterize shifts

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in FC concentrations during the human-led migration.

MATERIALS AND METHODS

Fourteen fertile eggs were collected during May 2000 from nests of wild sandhill cranes in Juneau County, Wisconsin (USA; 44°N, 90°W) under a US Fish and Wildlife Service permit. The eggs were transferred to the Patuxent Wildlife Research Center (PWRC, Laurel, Maryland, USA, 39°N, 76°W) for hatching and subsequent captive-rearing. The 14 cranes (seven female and seven male) were reared by technicians wearing crane costumes and using a strict protocol to prevent early imprinting on humans (Duff et al., 2001). The cranes experienced natural light cycles and were fed formulated diets (Ziegler Brothers crane chick starter and maintenance diets, Gardners, Pennsylvania, USA, containing 90 g/907 kg monensin, a coccidiostat). The cranes were habituated to ultralight aircraft engine noise after hatching and between 3 and 9 days of age were trained to follow the moving fuselage of an ultralight aircraft driven by a crane-costumed pilot. Each crane completed a mean 7 hr 6 min±19 min (±SE) of aircraft training at PWRC. In addition, the cranes were socialized with conspecifics beginning at 1–4 days of age. Group size was gradually increased over time, with two cohorts of seven similarly aged birds of mixed sex formed by 35–45 days of age (C1 and C2).

Each bird exhibited normal clinical appearance, weight gain, and gross development throughout the captive-rearing period. No evidence of infectious disease agents of concern in cranes (avian tuberculosis, inclusion body disease of cranes, West Nile virus) was found in any bird. All birds were given ivermectin (Ivomec, Merck & Company, Rahway, New Jersey, USA) and fenbendazole (Panacur, Hoeschst Roussel, Warren, New Jersey, USA) prior to shipment to the release site.

At 40–54 days of age, the cranes were shipped in individual crates via private aircraft to the Necedah National Wildlife Refuge (NNWR, Necedah, Wisconsin, 44°N, 90°W). The cranes were evaluated by veterinarians upon arrival and found to be in good health. The two cohorts were acclimated to separate pens (1,073 and 602 m^2) consisting of mixed wetland and pond edge habitat adjacent to an ultralight compatible runway. The pens were topped with 5-cm flight netting approximately 2.5–3 m from the ground. Both pens were located on the same wetland complex and separated by 2 km. The same formulated diet fed at PWRC was provided ad libitum in sheltered containers, and fresh water was provided in shallow steel pans daily in addition to standing sources of water within the pens.

The cohorts were trained behind the ultralight aircraft at first light approximately every other day, weather permitting. The cranes were habituated to the wing of the ultralight aircraft soon after acclimation to NNWR and began to fly behind the aircraft after fledging at approximately 70 days of age. The mean duration of ultralight training during 95 days at NNWR was 22 hr 32 min±19 min. On nontraining days, each cohort would be allowed morning exercise periods in adjacent wetlands to provide natural foraging experience. Beginning in the first week of September, the two cohorts were housed in adjacent portions of a release pen to facilitate formation of a single cohort. Prior to mixing, the cranes were color marked with 7.5-cm plastic leg bands and a radio transmitter (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA) above the tarsal joint, and a prerelease veterinary evaluation was performed. All cranes were healthy based on physical, hematologic, biochemical, and disease screening examinations. Within 1 wk, all 14 birds were housed together and managed similarly to the smaller groups. One crane was euthanized shortly after due to a severe wing fracture sustained during training.

Thirteen cranes began a scheduled migration behind the aircraft in early Octo-

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ber toward St. Martins Marsh and Aquatic Preserve in Citrus County, Florida (USA; 28°N, 82°W). The 1,977-km migration occurred in 31 stages (mean 61.8±5.8 km/day; mean 56.6 min±4.2 min flight time/day). Cranes flew with the ultralight at first light and were housed in a 146 m^2 portable pen at the next location with fresh water and food offered ad libitum from two feeders until the next flight. Flights typically required active flight at 200–300 m altitude, rather than a soaring flight based on thermal activity. Interspersed among the 31 flight days were 9 days of rest due to poor weather or mechanical problems. One crane abandoned the ultralight cohort during the second stage in Wisconsin and successfully joined wild sandhill cranes in the area. Another crane was found dead in the portable pen in early November with fractured cervical vertebrae, likely from striking the pen fence during a disturbance the previous night. Veterinary evaluations of the 11 surviving cranes conducted after migration did not detect any significant abnormalities.

At NNWR, fecal samples were collected every 2 wk after allowing a 1-mo acclimation to the release pen environment, during which all birds attained fledging age. The samples were collected a minimum of 10 days following a significant stressor (veterinary examination or major social manipulation) to facilitate the determination of baseline FC levels. During migration, fecal samples were collected every other day, beginning the morning of the third day. Each sample was anonymous in origin and consisted of a single, fresh-appearing early morning fecal mass, to avoid sampling the same individual repeatedly and to coincide with expected maximal 24-hr basal corticosterone levels (Carsia and Harvey, 2000). All fecal samples were refrigerated immediately in plastic film canisters and then frozen at −20 C within 2 hr and until the time of analysis.

Radioimmunoassay detection of corticosterone (ng/g) was conducted using the methods established by Ludders et al. (2001). Briefly, 0.2 g of lyophilized feces was hydrolyzed (overnight incubation at 37 C) using 0.02 ml β-glucuronidase/aryl sulfatase (Roche Diagnostics, Mannheim, Germany), then extracted with 5.0 ml diethyl ether. After decanting, the ether was evaporated and the sample was reconstituted in 1 ml ethanol. Tritiated corticosterone was added to the samples prior to extraction to allow for correction for extraction loss. Fecal extracts (0.005 ml) were subjected to the corticosterone radioimmunoassay (ICN, Costa Mesa, California, USA). All samples were analyzed within the same assay. The intra-assay coefficient of variation was 1.6 and 4.4% at 56 and 25.8% binding (n=10 and 10).

Each weekly mean FC concentration during migration represents the mean concentration of samples collected from the preceding week. Analyses of FC concentrations were made using one- and two-way analysis of variance (ANOVA) and Student's t-tests (Statview 5, SAS Institute Inc., Cary, North Carolina, USA); significance was established at $P<0.05$.

TABLE 1. Fecal corticosterone concentrations in sandhill cranes during ultralight aircraft training and migration.

Sample set	No. of samples	Mean (ng/g)	SE	Range
Training 1	7	114.3	26.3	68.5–263.6
Training 2	8	116.3	11.1	84.3–164.7
Training 3	5	109.8	13.6	66.7–143.8
Training 4	6	85.3	7.9	61.2–113.2
Training 5	6	118.8	17.4	74.6–169.1
Migration week 1	12	191.6	23.7	94.8–324
Migration week 2	14	210.5	21.6	116.7–334
Migration week 3	12	202.0	46.4	99.7–637
Migration week 4	12	118.0	14.5	65.8–231.9
Migration week 5	16	148.3	17.5	62.6–263.3
Migration week 6	12	131.2	23.7	36.7–279.7

RESULTS

The mean FC concentration during the training period was 109.5±7.5 ng/g (n=32; median=93.1 ng/g) (Table 1). There was little variability in FC concentrations, and

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concentrations did not differ significantly across the five collection dates. Similarly, mean FC concentrations from C1, C2, and the large single group prior to migration did not differ from one another. The reference range (mean±2 SD) of FC concentration derived from the training period of this study was 25–193 ng/g.

The mean FC concentration during migration was 167.1±10.9 ng/g (n=76; median=130.8 ng/g). Weekly mean FC concentrations during migration changed as the migration progressed, with higher FC concentrations in early migration compared to the last 3 wk. The mean FC concentration of the cranes was elevated in the first week of migration compared to the month prior to departure ($P<0.01$). By the sixth week of migration, the mean FC concentration was not significantly different from premigration concentrations.

DISCUSSION

Fecal corticosterone concentrations in sandhill cranes undergoing field training at the NNWR were similar to, or less than, concentrations of clinically normal adult sandhill cranes acclimated to confinement under laboratory conditions (Ludders et al., 2001). There were few elevations and limited variability observed in the data during the training phase. A single elevated value was observed in a member of C1 from the first collection date (263.6 ng/g). The results also correlate well with observations of the cranes' behavior that suggested little overt stress within any social group and a static routine apart from gradual increases in physical performance training behind the ultralight aircraft. Banding, premigration health examinations, and integration of the smaller cohorts into one large group were not associated with lasting changes in FC concentrations.

Fecal corticosterone concentrations in the cranes increased and became more variable after migration started and were sustained for 3 wk before declining. The increases were modest and likely reflected basal corticosterone elevations common in migratory birds. Unfortunately, no established FC data are available from wild, migrating sandhill cranes for comparison.

Elevated corticosterone is believed to facilitate behavioral and physiologic changes necessary to promote successful migration, such as through effects on feeding, locomotion, and orientation (Holberton, 1999; Piersma et al., 2000; Landys-Ciannelli et al., 2002; Lohmus et al., 2003). In addition, FC concentrations may have also been affected by a combination of psychological, physical, and environmental stressors during the early phases of migration. Six of 12 dominance positions shifted in the first 3 wk of migration, likely inducing social stress in lower ranking individuals. The anonymity of the fecal samples, however, precludes direct evaluation of the relationship between social rank and the FC concentrations in the cranes. The cranes were also frequently exposed to novel environments; 15 different roosting locations were used in the first 3 wk of migration. Behavior exhibited by the cranes at takeoff and during flights was not as organized early in migration as compared to the end of migration (Duff, pers. comm.). Although the conditioning and endurance of the cranes was appropriate at departure, physical stress, possibly in combination with cold stress in early stages in Wisconsin, occurred with daily flights.

The elevations observed early in migration did not preclude the cranes' ability to respond to acute stressors. Elevated corticosterone values were observed in two of four samples collected following a long flight under warm conditions during week 3 (371 ng/g and 637 ng/g). These findings suggest the cranes were capable of mounting a stress response despite their apparent shift in basal corticosterone levels. The death of the crane during week 5 of the migration, although suspected to have involved predator disturbance, was surprisingly not associated with increases in the daily or weekly mean FC concentration of the group.

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By the end of migration, mean FC concentrations declined and became similar to those observed during the last month of training, yet individual sample variability remained elevated. We believe the variability in corticosterone concentrations can be explained by three potential factors: compromised nutritional status in some birds (four of 11 birds experienced 10% or greater weight loss over the course of the migration), continued exposure of the group to novel surroundings, and continual shifts in group social structure (seven of 11 dominance ranks changed over the last week of migration). The overall decline in FC concentrations during the last 3 wk was likely due to improvements in physical conditioning and endurance of the birds and possibly adaptation to the ultralight method through learned behavior (Harvey et al., 1984).

In summary, sandhill cranes undergoing reintroduction and field training for human-led migration exhibited normative concentrations of corticosterone in feces after environmental acclimation, regimented activity, and several days' acclimation to new social settings. The cranes exhibited modest elevations in corticosterone concentrations in feces while on human-led migration, consistent with normal elevations observed in migratory birds. The elevations did not preclude response to occasional acute stressors and did not compromise clinical health. The methodology used to obtain the samples was inexpensive, noninvasive to study subjects, and effective at establishing normal and elevated corticosterone concentrations in feces. With further validation, this technique shows promise for application in health monitoring programs for a variety of endangered bird species.

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