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# Fecal Corticosterone Reflects Serum Corticosterone in Florida Sandhill Cranes

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ABSTRACT: Florida sandhill cranes (*Grus canadensis pratensis*) were conditioned to confinement 6 hr/day for 7 days. On day 8, each bird's jugular vein was catheterized, blood samples were drawn, and each crane was confined for 6 hr. Using a randomized, restricted cross-over design, cranes were injected intravenously with either 0.9% NaCl solution or ACTH (cosyntropin; Cortrosyn®; 0.25 mg). During the 6 hr of confinement, fecal samples (feces and urine) were collected from each of five cranes immediately after defecation. Individual fecal samples were collected approximately at hourly intervals and assayed for corticosterone. We showed previously that serum corticosterone did not vary significantly following saline injection, but peaked significantly 60 min after ACTH injection. Maximal fecal corticosterone concentrations (ng/g) were greater ($P < 0.10$; median 1087 ng/g) following ACTH stimulation compared to maximal fecal corticosterone concentrations at the end of acclimation (day 7; median 176) and following saline treatment (median 541). In cranes under controlled conditions, fecal corticosterone concentration reflects serum corticosterone levels.

*Key words:* Adrenocorticotropic hormone, fecal corticosterone, *Grus canadensis pratensis*, sandhill cranes, serum corticosterone.

Corticosterone, the single best hormonal indicator of stress in birds, increases following stressful events (Harvey, 1984; Le Maho et al., 1992). Plasma corticosterone can indicate stress in birds except that physical restrain and collection of blood are known stressors. Alternatively, if fecal corticosterone concentrations reflect serum concentrations, then it would allow for non-invasively, non-stressfully determining corticosterone levels in birds. Studies in chickens (Schwartz et al., 1992b) and one northern spotted owl (Wasser et al., 1997) suggest that this is the case.

Previously, we studied the serum corticosterone response of cranes to saline and adrenocorticosterone hormone (ACTH) treatments (Ludders et al., 1998). Saline treatment did not affect serum corticosterone. Serum corticosterone concentrations peaked 60 min after ACTH treatment and were greater ($P \leq 0.04$; 1-tailed) than concentrations immediately before treatment and following saline treatment (both $P \leq 0.02$).

We undertook the fecal phase of the blood study (Ludders et al., 1998) to determined if fecal corticosterone concentrations reflect serum corticosterone concentrations in sandhill cranes. We believed that fecal corticosterone concentrations would be higher after ACTH than after saline and higher than at the end of the acclimation period.

The Institutional Animal Care and Use Committee of the International Crane Foundation (ICF; Baraboo, Wisconsin, USA) approved this study. Ten captive-reared adult Florida sandhill cranes (*Grus canadensis pratensis*) housed at the International Crane Foundation (43°30′N, 89°42′W) were used in this study from 21 October 1996 through 21 January 1997. The cranes (5 males and 5 females) had a median age of 21 yr (range 2 to 27 yr) and an average weight of 4.7 kg (range 3.8 to 5.6 kg). All cranes were healthy at the time of study (physical, hematological, and biochemical examinations).

Feeding, watering and cleaning routines were completed between 0800 and 0900 hr. The cranes were given food (Garver Crane Grower—Maintenance; Garver Feed and Supply Co., Inc., Madison, Wisconsin, USA) and water ad libitum. Each pair had free access to indoor and outdoor runs and an exercise yard. All cranes were



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exposed to natural light cycles and ambient temperatures.

Each pair of cranes was studied for 9 consecutive days: 7 days for acclimation to confinement in a wooden enclosure and 1 day each for treatment with saline and ACTH (Ludders et al., 1998). The order in which crane pairs were studied was randomized using a randomization table and a restricted cross-over design so that cranes were equally allocated by gender to saline or ACTH as first treatment. This approach was used to avoid imbalance of treatment order.

Each crane was acclimated to confinement for 6 hr per day (09:00 to 15:00, Central Standard Time) for 7 consecutive days. Daily, each crane was captured by hand, weighed, and an intravenous line (Medex, Inc., Duluth, Georgia, USA; length 152 cm) was bandaged to the right side of the neck. The crane was placed in a wooden enclosure with a floor of wire mesh and a top that consisted of plastic mesh affixed to a centrally positioned piece of wood that extended the full length of the enclosure and that had a centrally located slot extending for most of its length. The free end of the intravenous line was passed through the slot to the outside of the enclosure. Fecal samples (feces plus urine) were collected from wax paper placed under each enclosure, or from the wire mesh floor of each enclosure at the time that a bird defecated. Individual fecal samples were frozen at −20 C within 2 hr of sample collection. At the end of each day, the bandage and IV lines were removed and the cranes were returned to their housing unit.

On days 8 and 9, the right jugular vein in each crane was catheterized (18 gauge, 5 cm; Angiocath, Becton, Dickinson, Sandy, Utah, USA), a 2-ml venous blood sample (Time 0) was collected, and an intravenous line (152-cm long) pre-filled with heparinized saline (heparin 4 u/ml; Elkins-Sinn, Inc., Cherry Hill, New Jersey, USA) was attached to the catheter. This line was used to collect additional 2-ml blood samples at 60 min after the start of confinement, and at 30, 60, 120, 180, 240, and 300 min after injection of saline and ACTH. The IV line also was used for giving ACTH and saline treatments. Blood samples were processed for serum as previously described (Ludders et al., 1998). Individual fecal samples were collected and stored as described above, but only samples that were collected approximately at hourly intervals were analyzed for fecal corticosterone.

On day 8, cranes were injected intravenously with Cortrosyn® (cosyntropin, 0.25 mg, average of 0.053 mg/kg; Organon Inc, West Orange, New Jersey, USA) or with 0.9% sodium chloride solution. Each crane received the alternative treatment on day 9.

All fecal and serum samples were shipped frozen on dry ice to the Center for the Reproduction of Endangered Species (CRES; Zoological Society of San Diego, San Diego, California, USA). A radioimmunoassay method was used for determining the serum corticosterone concentrations (Ludders et al., 1998). Fecal samples were lyophilized to eliminate variability due to water. Dried feces (0.2 g) were mixed with buffer (1 ml, pH 5) and 0.02 ml glucuronidase/arylsulfatase (Boehringer Mannheim, Germany), and incubated overnight at 37 C. To account for extraction variability, 10,000 cpm of tritiated corticosterone was added to each sample. Samples were extracted using 5 ml of diethyl ether. The ether portion was decanted, dried and re-suspended in 1 ml of anhydrous ethanol of which 0.2 ml was taken for extraction efficiency calculations and 0.05 ml was taken for corticosterone I-125 radioimmunoassay (ICN, Costa Mesa, California, USA). ICN reports the following cross-reactivities: desoxycorticosterone, 34%; testosterone, 10%; cortisol, 5%; aldosterone, 3%; progesterone, 2%; dihydrotestosterone, 1%; and cholesterol, 1%. All other steroids tested at <1% cross-reactivity. Dose response of serially diluted crane fecal extracts in the radioimmune

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analysis (RIA) yielded a parallel slope to the corticosterone standard curve. A fecal-sample extract was separated by high performance liquid chromatography (Rossi et al., 1987) indicating that the predominant compound measured in this assay system was corticosterone.

Fecal-corticosterone concentrations (ng/g) were measured in samples that were collected 1 hr after the start of confinement on days 7, 8 and 9. Wilcoxon's signed-rank test (one-tailed) (Zar, 1996) compared the maximum fecal corticosterone concentration measured for each crane on day 7 (end of acclimation period) and following saline treatment, day 7 and following ACTH, and the two treatments. Because of the small sample size in this pilot study, alpha was assumed at $P < 0.1$, and without corrections for multiple comparisons.

Five cranes were dropped from the study: Crane A1 had catheter failures after both treatments, and produced too few fecal samples for meaningful analysis as was the case for three other cranes (D1, E1, E2); crane D2 developed subcutaneous emphysema 4 days into the acclimation period.

Two cranes (B2 and C1) became anemic at the end of the study because of abrasions on their feet as a result of pacing in their enclosures. Crane B2 (a female) had hematocrits of 28% and 26% on days 8 and 9, respectively. Crane C1 (a male) had hematocrits of 30% and 28% on days 8 and 9, respectively.

Maximum fecal corticosterone concentrations (ng/g) following saline treatment did not differ significantly ($P = 0.28$) from fecal corticosterone concentrations on day 7. Following ACTH treatment, maximum fecal corticosterone concentrations were significantly greater ($P = 0.063$) than maximum fecal corticosterone concentrations on day 7, and following saline treatment (Table 1). Minimum fecal corticosterone concentrations for day 7 did not differ significantly ($P = 0.22$) from those following saline treatment, but they were significantly ($P = 0.062$) lower compared to corticosterone concentrations following ACTH treatment (Table 1).

TABLE 1. Minimum and maximum corticosterone concentration (ng/g) in fecal samples collected from each of five sandhill cranes.

| | Day 7 | | Saline | | ACTH[a] | |
|---|---|---|---|---|---|---|
| Crane | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum |
| A2 | 17 | 110 | 51 | 266 | 133 | 1,087 |
| B1 | 18 | 34 | 15 | 311 | 379 | 1,043 |
| B2 | 313 | 637 | 278 | 734 | 479 | 2,620 |
| C1 | 80 | 722 | 178 | 541 | 16 | 455 |
| C2 | 86 | 176 | 139 | 716 | 174 | 1,513 |
| Median | 80 | 176 | 139 | 541 | 174[b] | 1,087[c] |

[a] Adrenocorticotropic hormone.
[b] Differs significantly from fecal corticosterone concentrations on day 7 ($P = 0.062$; Wilcoxon's signed-rank test, one-tailed).
[c] Differs significantly from feral corticosterone concentrations on day 7 and following saline treatment ($P = 0.063$; Wilcoxon's signed-rank test, one-tailed).

Four of five cranes had "flat," low fecal corticosterone concentrations on day 7 (the last day of acclimation), but crane C1 had spikes (Fig. 4). For three cranes (Fig. 1–3), the fecal pattern after saline treatment was similar in appearance to that of day 7, and also mimicked the lack of serum response to saline. By inspection, crane C2 (Fig. 5) had fecal corticosterone concentrations higher than on day 7, but that mimicked the serum pattern. Other than crane C1 (which continued to have "bizarre" patterns), each crane had its highest fecal corticosterone concentrations after ACTH treatment. When we were able to trace both serum and fecal corticosterone concentrations after ACTH treatment, the peak in fecal corticosterone was detected at or within 2 hr after the serum peak.

A possible explanation for the bizarre fecal corticosterone results obtained from crane C1 may be due to the fact that this bird was anemic and thus more stressed compared to the other cranes. However, crane B2 was more anemic than C1, but both of her serum and fecal corticosterone concentration profiles were similar to those of the other three cranes. Crane C1

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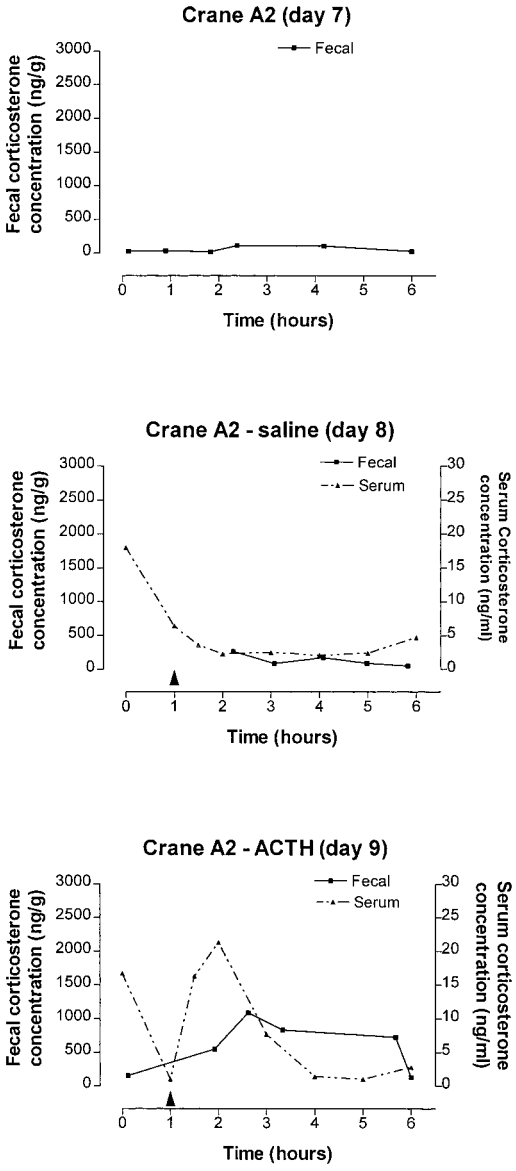


FIGURE 1. Fecal corticosterone concentrations for crane A2 on day 7 (top panel), and following treatment with saline or ACTH (middle and lower panels, respectively). Arrow at the 1 hr mark indicates when the crane received saline or ACTH treatments.

tended to pace in the enclosure more than the other four cranes and he was more aggressive.

In cranes A2, B2 and C1, the patterns of serum and fecal corticosterone following ACTH show increases in serum corticosterone followed by increases in fecal corticosterone. In two cranes (B1, C2), for which there are no serum corticosterone data following ACTH, the maximum fecal concentrations were greater than those measured on day 7 and following saline treatment (Figs. 2, 5).

In these cranes, ACTH treatment caused a fivefold increase in serum corticosterone by 60 min after injection that returned to baseline concentrations 180 min after drug injection (Ludders et al., 1998). This pattern and magnitude of response are similar to those reported in other avian species (Zenoble et al., 1985; Beuving and Vonder, 1986; Spellman et al., 1995). Following saline treatment, serum corticosterone in eight cranes did not differ from baseline over the 5 hr of study (Ludders et al., 1998). Maximum fecal corticosterone concentrations on day 7 and following saline treatment did not differ significantly from each other. However, the minimum and median values for fecal corticosterone on day 7 were lower than those following saline treatment and suggest that the cranes acclimated to confinement. The higher fecal values following saline compared to day 7 also suggest that physical restraint and catheterization were stressors. Following ACTH treatment, fecal corticosterone concentrations were significantly ($P = 0.063$) greater than those measured either on day 7 or following saline treatment.

In chickens studied over two 24 hr periods, there were no differences in fecal (pooled samples) or plasma corticosterone concentrations in birds that were bled but not heat stressed. Fecal and plasma corticosterone were higher in birds that were both bled and heat stressed (Schwartz et al., 1992b). Our results suggest that fecal corticosterone concentrations for individual fecal samples from individual birds reflect serum corticosterone concentrations. However, our data include only five cranes, one of which had a "bizarre" set of data, and the timing of defecation and the volume defecated could not be controlled.

The relationship between serum and fe-

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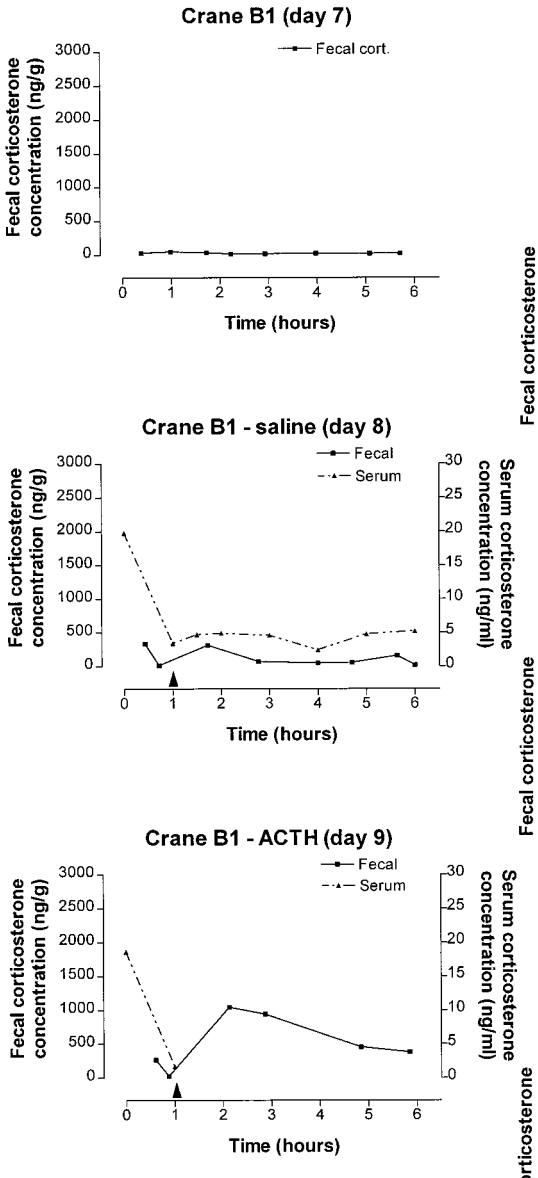


FIGURE 2. Fecal corticosterone concentrations for crane B1 on day 7 (top panel), and following treatment with saline or ACTH (middle and lower panels, respectively). See Figure 1 for details.

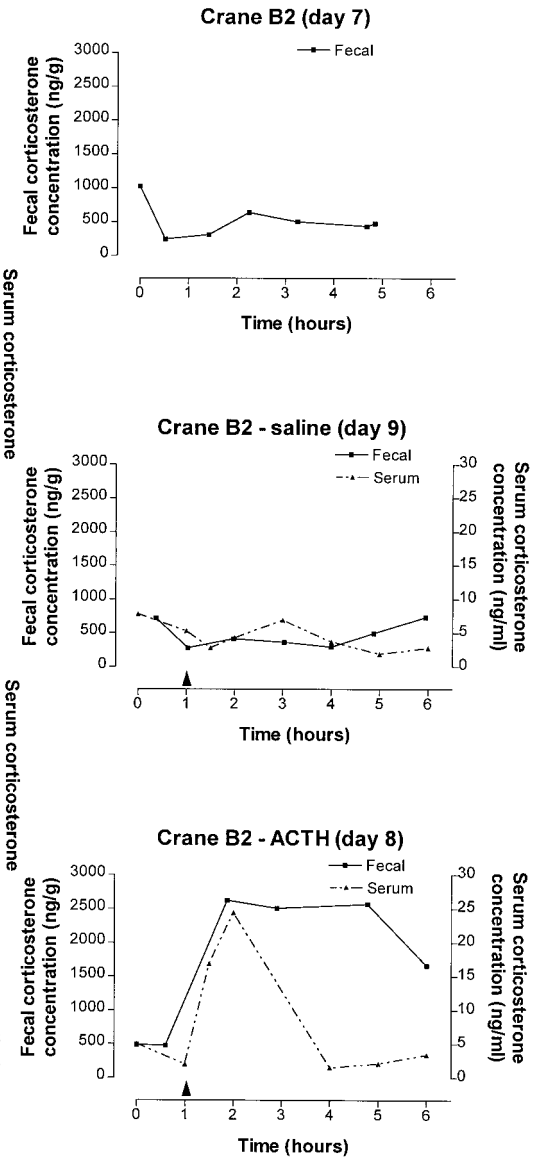


FIGURE 3. Fecal corticosterone concentrations for crane B2 on day 7 (top panel), and following treatment with saline or ACTH (middle and lower panels, respectively). See Figure 1 for details.

cal corticosterone concentrations is affected both by the transfer time of corticosterone from blood to the intestinal tract, and by the transit time of ingesta through the alimentary tract. We did not determine

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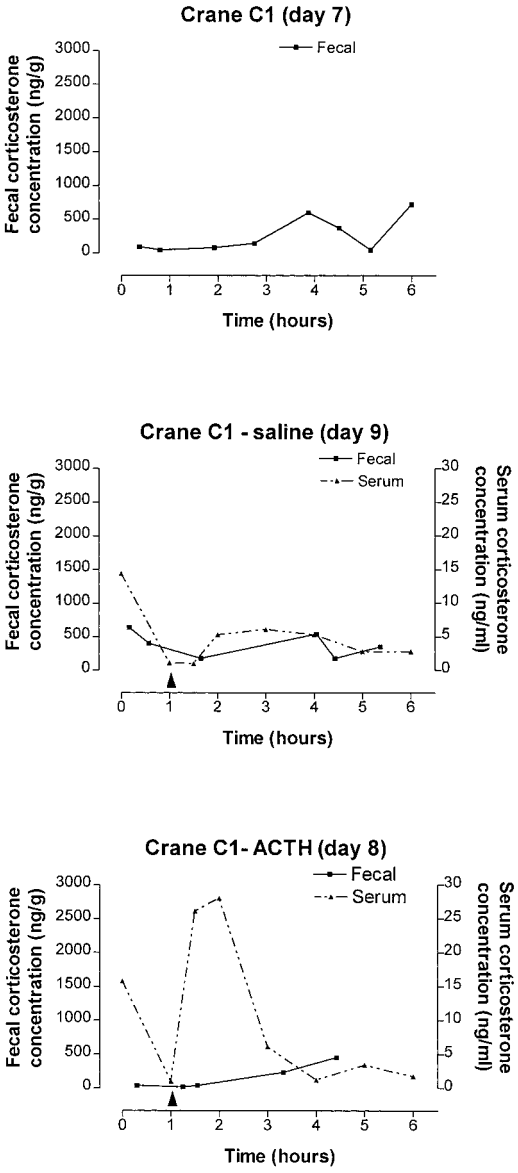


FIGURE 4. Fecal corticosterone concentrations for crane C1 on day 7 (top panel), and following treatment with saline or ACTH (middle and lower panels, respectively). See Figure 1 for details.

transfer time of corticosterone from blood to gut nor the rate of passage of ingested material through the intestinal tract of the sandhill cranes. In cattle egrets (*Ardeola ibis*), intravenously injected radiolabeled steroids were rapidly excreted in feces (feces plus urine) within 6 hr after injection (Schwartz et al., 1992a). In general, dyes

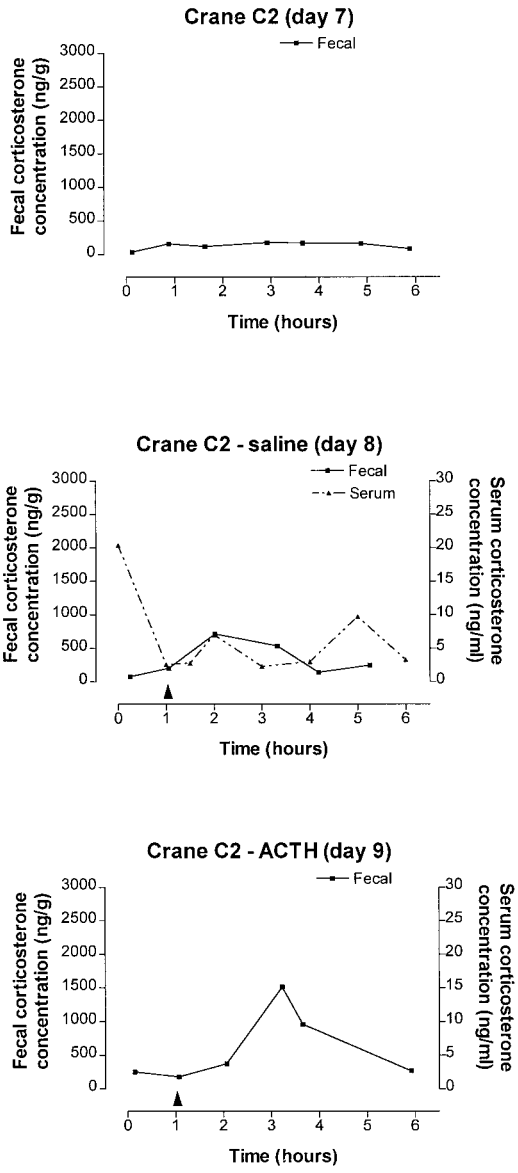


FIGURE 5. Fecal corticosterone concentrations for crane C2 on day 7 (top panel), and following treatment with saline or ACTH (middle and lower panels, respectively). See Figure 1 for details.

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or radiolabeled isotopes fed to birds begin to appear in feces within 2 to 4 hr after ingestion. In whooping cranes (*Grus americana*), an inert chromic oxide tracer included in a variety of diets indicated that food passed through their intestinal tract quickly and was almost completely eliminated within 7 hr (Nelson et al., 1997). In white leghorn hens fed a variety of diets that included chromic oxide or 144Ce, the time of first appearance of the markers in feces varied between 2.1 and 3.2 hr after ingestion (Mateos et al., 1982). Those studies suggest (as we saw in this study) that changes in serum corticosterone could be detected in feces within 5 hr.

The fecal samples from these cranes consisted of feces and urine. It is quite likely that as the proportion of feces to urine varies, so too would the total "fecal" concentration. In male cattle egrets injected with radiolabeled estrogen, progesterone and testosterone, approximately 83% of the steroids were excreted in the urinary component by 4 hr after injection (Schwartz et al., 1992a). For a female northern spotted owl (*Strix occidentals caurina*) that was injected with ACTH, fecal material was separated from the urinary component (Wasser et al., 1997). In this single owl, blood samples were not collected, but there was a rapid and significant increase in fecal corticosterone within 2 hr after ACTH injection with a peak at 12 hr that returned to baseline by 26 hr (Wasser et al., 1997).

In summary, under controlled conditions and after acclimating sandhill cranes to confinement, serum and feces collected during a 5 hr sampling period suggest a positive relationship between serum and fecal corticosterone levels. Thus fecal corticosterone might serve as a non-invasive indicator of stress in birds.

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