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Authors: Spelman, Lucy H., Fleming, W. James, Davis, Gary S., and Stoskopf, Michael K.

Source: Journal of Wildlife Diseases, 31(2) : 136-141

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-31.2.136>

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## EFFECT OF EXOGENOUS ADRENOCORTICOTROPIC HORMONE ADMINISTRATION ON PLASMA CORTICOSTERONE CONCENTRATIONS IN AMERICAN BLACK DUCKS (*ANAS RUBRIPES*)

Lucy H. Spelman,<sup>1</sup> W. James Fleming,<sup>2</sup> Gary S. Davis,<sup>3</sup> and Michael K. Stoskopf<sup>1</sup>

<sup>1</sup> Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, 4700 Hillsborough St., Raleigh, North Carolina 27606, USA

<sup>2</sup> National Biological Service, North Carolina Cooperative Fish and Wildlife Research Unit, North Carolina State University, Raleigh, North Carolina 27695, USA

<sup>3</sup> Extension Poultry Science, College of Agriculture and Life Sciences, North Carolina State University, Scott Hall Box 7608, Raleigh, North Carolina 27695, USA

**ABSTRACT:** A protocol for the adrenocorticotrophic (ACTH) stimulation test in American black ducks (*Anas rubripes*) was established with synthetic ACTH, cosyntropin (Cortrosyn®); ACTH stimulation testing was conducted on 31 adult ducks (14 males, 17 females) in September 1993. Plasma corticosterone concentrations were measured on heparinized blood samples collected 30 min, and 1, 2, and 4 hr post-injection. In comparison with saline controls, cosyntropin (0.25 mg/duck) produced a two- to three-fold increase in corticosterone 30 min after administration. Maximal concentrations ranged from 132 to 312 ng/ml and occurred between 1 and 2 hr post-injection. Corticosterone concentrations declined to basal, pre-injection values after 4 hr. Endogenous ACTH release in response to handling stress was evident in control ducks after saline injection but did not interfere with interpretation of the stimulation test. Recommendations for the ACTH stimulation test in black ducks include a 30 min acclimatization period for recently captured or relocated ducks and determination of plasma corticosterone concentration 1 to 2 hr following intramuscular injection with 0.25 mg cosyntropin.

**Key words:** Adrenocorticotrophic hormone, ACTH, American black duck, *Anas rubripes*, corticosterone, ACTH stimulation test, radioimmunoassay, stress.

### INTRODUCTION

Decreasing populations of American black ducks (*Anas rubripes*) prompted investigation into the effects of environmental stress such as noise and water pollution on the long-term survival of this species. Circulating plasma corticosterone concentrations and the response to adrenocorticotrophic hormone (ACTH) stimulation were measured to assess adrenal function in domestic chickens (*Gallus domesticus*) (Rees et al., 1985b; Beuving and Vonder, 1986), domestic turkeys (*Meleagris gallopavo*) (Davis and Siopes, 1987), and domestic ducks (*Anas platyrhynchos*) (Harvey et al., 1980; Rees et al., 1985a) under a variety of stressful conditions such as food deprivation, restraint, blood sampling, temperature extremes, salinity, crowding, and exercise. The ACTH stimulation test has been described in healthy psittacines (Lothrop et al., 1985; Zenoble et al., 1985a), pigeons (*Columbia livia domestica*) (Lumeij et al., 1987), and raptors (Zenoble et

al., 1985b) but has not been documented in wild waterfowl species.

Our objectives were to establish a reproducible method for the ACTH stimulation test in healthy, captive black ducks and to generate baseline data for future evaluation of chronic stress in this species.

### MATERIALS AND METHODS

Formulation, dose, and route of ACTH administration, timing and magnitude of peak corticosterone concentrations, and method of corticosterone assay vary in reports of ACTH-stimulation testing in birds. Therefore, a pilot study was performed in black ducks to determine a dose of ACTH that provided consistent stimulation of the adrenal gland 1 hr after intramuscular injection. Plasma corticosterone concentrations were measured with a commercially available radioimmunoassay kit (Coat-A-Count <sup>125</sup>I corticosterone RIA kit, Diagnostic Products Corp., Los Angeles California, USA). Synthetic cosyntropin (Cortrosyn®, Organon Inc., West Orange New Jersey, USA) at 0.125 and 0.25 mg/duck and natural porcine corticotropin A (ACTH 1-39, Sigma Chemical Company, St. Louis, Missouri, USA) at 2, 5 and 10 IU/kg were compared

with saline-injected controls. Each dose was evaluated in at least six ducks. All ducks responded to synthetic cosyntropin with an increase in corticosterone concentration. The higher dose (0.25 mg) consistently produced a two- to three-fold increase in corticosterone; the magnitude of the response did not vary with the weight of the duck. No response was observed in ducks receiving 2 or 5 IU/kg of porcine ACTH. Some ducks responded to the highest dose of porcine ACTH (10 IU/kg) but the results were variable. Synthetic cosyntropin (0.25 mg) was chosen for further investigation of ACTH stimulation testing in black ducks.

Testing was conducted in September 1993 on 31 (14 males, 17 females) adult American black ducks. The ducks were 1- to 2-yr old, captive-reared (genetic wildstrain), and ranged in weight from 1.1 to 1.5 kg. They were housed outdoors in a 10 × 60 m netted enclosure along the edge of a pond with access to natural vegetation. The diet was supplemented with commercial pellets (Mazuri® Waterfowl Maintenance, Purina Mills, Inc., St. Louis, Missouri, USA).

The study was conducted between 0700 and 1200 hr. On the morning of testing, the ducks were corralled into a narrow end of the pen, captured, and immediately placed in 2 × 2 m pens in groups of three to five for the duration of the test. Ducks were assigned to one of four post-injection sampling groups: 30 min ( $n = 12$ ), 1 hr ( $n = 26$ ), 2 hr ( $n = 12$ ), and 4 hr ( $n = 12$ ). Half of the ducks in each group received 0.25 mg of cosyntropin reconstituted in 1.0 ml of 0.9% NaCl, and the other half received 1.0 ml of 0.9% NaCl as controls. Injections were given intramuscularly in the right pectoral muscle.

Pre- and post-injection blood samples were collected from each duck for plasma corticosterone determination. Blood was sampled only once from each duck after ACTH or saline administration (either at 30 min, 1, 2, or 4 hr) to minimize the effects of handling and venipuncture stress. Those whose blood was sampled at longer intervals were tested first to complete data collection within the morning hours.

Blood samples (1.0 ml) were drawn from the jugular vein into heparinized syringes (Sodium heparin, 1,000 IU/ml, Elkins-Sinn, Inc., Cherry Hill, New Jersey). Heparinized blood was centrifuged within 4 hr of collection, and the plasma stored in 0.25 ml aliquots in plastic containers at  $-70^{\circ}\text{C}$ . The corticosterone assay was performed within 2 wk of sample collection.

Plasma corticosterone concentration was measured with the Coat-A-Count® radioimmunoassay kit, an assay specific for corticosterone. The highest level of cross reactivity occurred with 11-deoxycorticosterone (1.6%) and the lowest level with dehydroepiandrosterone

(0.12%), as determined by Diagnostic Products Corporation. Test samples were evaluated in three assays. Plasma samples from American black duck, bob-white quail (*Colinus virginianus*), domestic laying turkeys, and chickens were used to determine the intraassay (4.3%) and interassay (6%) coefficient of variation. Serial dilutions of corticosterone (1 ng to 1 pg) were added to the assay buffer and filtered plasma to determine the minimum detectable dose of corticosterone (0.01 ng/ml). To test parallelism and recovery, 50  $\mu\text{l}$  of pooled black duck plasma was added to the kit standards and compared with the standard curve (logit-log plot of percent bound versus concentration). The slopes of the standard and the standard plus black duck plasma curves were identical ( $-0.91$ ). The observed and expected values of corticosterone (ng/ml) were used to calculate the percent recovery (observed/expected × 100), which ranged from 85 to 102%.

Pre- and post-injection corticosterone concentrations in the saline and ACTH groups were compared with the Wilcoxon-signed rank test (Noether, 1991). Differences in pre- and post-injection corticosterone concentrations between treatment groups were considered significantly different when  $P < 0.05$ . The data were not normally distributed and, therefore, median, 25th, and 75th percentiles were used as summary statistics.

## RESULTS

American black ducks had little or no adrenocortical responses to saline injections (Fig. 1). Corticosterone concentrations in control ducks increased slightly 30 min post-saline injection, remained similar to basal values after 1 and 2 hr, and decreased by 4 hr (Table 1). Basal corticosterone concentrations were higher in control ducks sampled at four hours (median 135 ng/ml) than in the other control and experimental groups (median 49.7 to 87.6 ng/ml) (Table 1).

Black ducks responded to intramuscular injection of exogenous synthetic ACTH (0.25 mg cosyntropin/duck) with a two- to three-fold increase in plasma corticosterone concentration 30 min post-administration (Table 1). Concentrations of corticosterone were maximal between 1 and 2 hr post-injection of ACTH (Fig. 1). After 4 hr, corticosterone declined to basal, pre-injection concentrations (Table 1). There

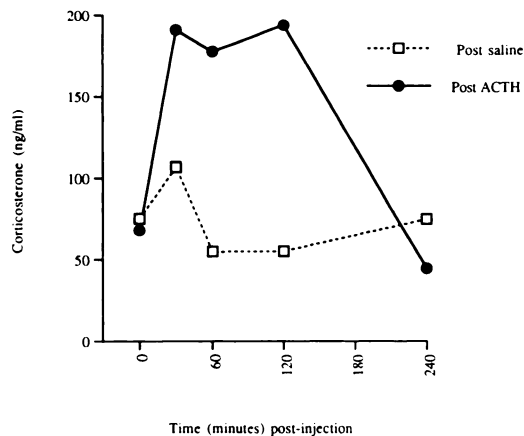


FIGURE 1. Median concentration of plasma corticosterone (ng/ml) in American black ducks at 0 min ( $n = 31$  per group), and following administration of 0.9% saline or 0.25 mg cosyntropin (synthetic ACTH) intramuscularly at 30 min ( $n = 6$  per group), 60 min ( $n = 13$  per group), 120 min ( $n = 6$  per group), and 240 min (saline,  $n = 6$  and ACTH,  $n = 5$ ).

were no clinically apparent effects of cosyntropin administration in black ducks.

#### DISCUSSION

The ACTH stimulation test has potential application for black ducks subject to chronic stress in captive and free-ranging conditions. Exposure of wild waterfowl populations to food shortages, environmental contaminants, or weather extremes may alter adrenal function. Chronic stressors may enhance (adrenal hypertrophy) or reduce (adrenal atrophy) adrenocortical reactivity in birds (Ritchie et al., 1994). Similar changes in adrenal function have been documented in various species of mammal exposed to chronic stress (Sakellaris and Vernikos-Danellis, 1975; Friend et al., 1977; Ortize et al., 1985; Von Borell and Ladewig, 1989).

Diurnal variation, seasonality, reproductive activity, genetics, and endogenous ACTH release also can affect circulating glucocorticoid concentrations in birds (Edens and Siegel, 1975; Harvey and Hall, 1990; Wingfield et al., 1992). Therefore, evaluation of adrenocortical function requires application of an acute stressor or direct stimulation of the adrenal gland with

exogenous ACTH. An exaggerated response may indicate overproduction of corticosteroids (hyperadrenocorticism), whereas little or no response may indicate insufficient production (hypoadrenocorticism). Both excessive and inadequate concentrations of corticosterone have been associated with deleterious effects in birds, including impaired cellular and humoral immunity, growth, and reproduction (Harvey et al., 1984).

Synthetic ACTH administration (cosyntropin, 0.25 mg/duck) provided reliable stimulation of the adrenal gland in adult black ducks. For ducks weighing 1.1 to 1.5 kg, the dose range of cosyntropin was 0.11 to 0.23 mg/kg, or 11 to 23 IU/kg. Although more costly than porcine origin ACTH, cosyntropin requires minimal preparation (available in 0.25 mg vials) unlike natural preparations, and has decreased antigenicity. Comparable doses of cosyntropin have been used for the ACTH stimulation test in mammals (0.125 mg/cat to 0.25 mg/dog) (Plumb, 1991) and several avian species including psittacines and raptors (0.125 to 0.16 mg/bird, Lothrop et al., 1985; Zenoble et al., 1985a, b), pigeons (*Columba livia*) (0.05 to 0.125 mg/bird, Lumeij et al., 1987) and domestic ducks (0.08 to 0.25 mg/kg, Harvey et al., 1980).

Concentrations of plasma corticosterone (132 to 312 ng/ml) measured in captive black ducks 1 hr after ACTH administration were higher than those reported for various nondomestic (22 to 150 ng/ml) (Lumeij et al., 1987; Zenoble et al., 1985a, b) and domestic avian species (15 to 40 ng/ml) (Harvey and Phillips, 1980; Harvey et al., 1980; Freeman et al., 1980; Rees et al., 1985a, b; Koelkebeck et al., 1986; Beuving and Vonder, 1986; Davis and Siopes, 1987; Lumeij et al., 1987). Basal corticosterone concentrations in black ducks prior to ACTH administration also were higher than in other avian species, including one study on captive black ducks in which ACTH stimulation was not performed (Rattner et al., 1983). Differences

TABLE 1. Comparison of median plasma corticosterone levels (ng/ml) in American black ducks (*Anas rubripes*) before and after intramuscular injection with 0.9% saline (controls) or 0.25 mg cosyntropin (ACTH stimulation).

Sampling time	Pre-injection			Post-injection			P value <sup>a</sup>
	Median	Percentiles		Median	Percentiles		
		25th	75th		25th	75th	
ACTH stimulation							
30 min (n = 6)	78.0	65.6	126.4	190.8	140.6	245.0	0.03
1 hr (n = 13)	66.4	39.4	99.0	177.3	156.0	235.0	<0.01
2 hr (n = 6)	69.8	54.9	96.4	193.8	116.5	250.8	0.03
4 hr (n = 6)	49.7	31.7	124.1	44.7 <sup>b</sup>	17.8	189.0	0.89
Controls							
30 min (n = 6)	87.6	66.9	110.5	106.6	85.5	149.4	0.24
1 hr (n = 13)	52.7	44.7	72.9	54.9	39.6	77.9	0.92
2 hr (n = 6)	63.3	44.8	101.4	55.2	44.3	72.9	0.69
4 hr (n = 6)	135.2	103.9	181.3	75.1	39.5	106.9	0.03

<sup>a</sup> Probability based on Wilcoxon-signed rank test.

<sup>b</sup> Unable to sample one duck in this group.

in testing methodology account for much of the variation in absolute corticosterone concentrations among studies in birds. Therefore, the diagnosis of adrenal disorders in birds is based upon a relative increase in corticosterone after ACTH administration.

Species differences in adrenal responsiveness may also affect corticosterone concentrations after ACTH stimulation (Wingfield et al., 1992). High values for corticosterone in captive black ducks may be secondary to an increased capacity for corticosterone production or decreased sensitivity of the hypothalamic-pituitary-adrenal axis to negative feedback inhibition by elevations in circulating corticosterone. Corticosterone concentrations post-ACTH administration may be even higher in wild black ducks and in other free-ranging waterfowl.

Saline-injected control ducks had a mild increase in plasma corticosterone 30 min post-administration and a subsequent return to basal concentrations. The transient elevation in corticosterone in controls was not significant and was attributed to physiologic endogenous ACTH release in response to restraint and manipulation. Increases in corticosterone from handling

stress have been observed in many avian species (Harvey et al., 1980; Beuving and Vonder, 1986; Zenoble et al., 1985b; Wingfield et al., 1992). The rise in corticosterone concentrations from exogenous ACTH administration in black ducks far exceeded the increase in controls and persisted for at least 2 hr. Endogenous ACTH release in ducks receiving cosyntropin did not interfere with interpretation of the stimulation test. Similar findings were documented in domestic ducks in which serial blood sampling and treadmill exercise increased plasma corticosterone but also maintained the response to ACTH stimulation (Rees et al., 1985a).

Endogenous ACTH release was most evident in one of the control groups in which post-injection samples were collected at 4 hr. This group of ducks had <30 min to adjust to the temporary pens prior to initiation of testing. Corticosterone concentrations prior to saline administration were higher than those at 4 hr post-injection, and higher than pre-injection concentrations in the remainder of the ducks in control and experimental groups. Based on these data, we believe that black ducks became acclimated to the testing conditions after 30 min and that activity in ad-

jacent pens did not adversely affect response to saline or ACTH injection. In ducks receiving exogenous ACTH, any increase in corticosterone from handling stress was obscured by the much greater increase produced by the stimulation test.

We recommend the following protocol for the ACTH stimulation test in American black ducks. Acclimate ducks that have been recently captured or relocated for a minimum of 30 min prior to the test. Collect heparanized blood from the jugular vein for plasma corticosterone determination before and 1 to 2 hr after intramuscular injection of 0.25 mg corticotropin (Cortrosyn®). A relative rise in corticosterone concentrations two- to three-fold above basal values is evidence for adequate adrenocortical activity in captive-reared black ducks.

#### ACKNOWLEDGMENTS

For their advice and review of the manuscript, the authors thank Jay F. Levine, Michael R. Loomis, and Craig A. Harms.

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*Received for publication 17 February 1994.*