Research Note

Corticosterone stress response of Greater Rhea (*Rhea americana*) during short-term road transport

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ABSTRACT The effect of transport stress on blood corticosterone levels in captive Greater Rheas was investigated. Twelve adult individuals (7 males; 5 females) were loaded in pairs inside wooden crates and transported along a paved road for 30 min. Blood samples were taken before the individuals were introduced into the crate (baseline value) and immediately after they were unloaded (30 min after capture). To assess whether corticosterone levels were affected by the blood sampling procedure per se, another 6 (nontransport) control birds (3 males; 3 females) were also captured and sampled at the same times as their transported counterparts. Plasma corticosterone concentrations were measured using a commercially available corticosterone ^{125}I radio-immunoassay kit. Baseline corticosterone levels were similar in the control and transported birds (9.0 ± 1.6 and 10.4 ± 0.8 ng/mL, respectively). Transportation induced a highly significant ($P < 0.001$), more than 40-fold increase in the corticosterone levels (433.6 ± 35.4 ng/mL) that was about 5 times higher ($P < 0.001$) than in their nontransported counterparts (88.4 ± 14.8 ng/mL). The present findings suggest that Greater Rhea is a species highly sensitive to stressful manipulations. Both blood sampling and transportation induced highly significant adrenocortical responses. Considering that transportation is one of the unavoidable common practices in the management of Greater Rheas and, as shown in the present study, that it induces a significant 40-fold corticosterone stress response, efforts should focus on helping to generate management transport standards for optimization of the welfare of this ratite.

Key words: Greater Rhea, transportation, stress response, plasma corticosterone

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INTRODUCTION

Handling and transportation are considered major stressors for farm animals (as reviewed by Grandin, 1997; Knowles and Warriss, 2000) and might have deleterious effects on health, welfare, performance, and, ultimately, product quality (Pérez et al., 2002; Karaman, 2009; Yue et al., 2010). Transport stress can cause huge economic losses to the animal industry because of stress-induced injuries, animal mortality, and poorer quality animal products (Warriss et al., 2005; Gosálvez et al., 2006; Voslářová et al., 2007). Thus, there is a growing interest among scientists and producers to evaluate and understand the effects of transportation. This understanding will contribute to design transport procedures that enhance animal welfare.

Greater Rhea (*Rhea americana*) is bred in captivity for conservation purposes—as genetic reservoirs and source of individuals for reintroduction into the wild—and for commercialization of meat, skin, feathers, fat, and eggs (Navarro and Martella, 2008, 2011). Greater Rhea transportation is a frequent practice for management or commercial purposes; however, there are no studies evaluating the effects of transport in this species. Although captive breeding of ratites has been practiced for many years, scientific studies comparing the effects of stress or other effects of handling and transport procedures on welfare are very scarce and have been focused on ostriches (*Struthio camelus*; Mitchell et al., 1996; Wotton and Hewitt, 1999; Minka and Ayo, 2007). Recently, we have reported a particularly high adrenocortical stress response in captive Greater

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Rheas after an adrenocorticotropic hormone (**ACTH**) challenge (Lèche et al., 2009, 2011). This highly sensitive hypothalamic-pituitary-adrenal axis suggests that transport would be a potent stressor for this species. The aim of the present study was to investigate the corticosterone stress response of adult Greater Rheas during short-term road transportation.

MATERIALS AND METHODS

Birds and Experimental Design

Eighteen adult Greater Rheas (10 males; 8 females) bred in captivity on an experimental farm in the Zoo of Córdoba City, Argentina (31°S, 64°W), were used in the present study. On the day of transportation, 2 experienced assistants captured and immobilized each individual, whereas a third assistant collected a blood sample. Each blood sampling procedure was completed within 3 min of capture and immobilization, following the protocol of Romero and Reed (2005). After blood collection, 3 males and 3 females were released and remained undisturbed (nontransport control group). This group served to determine whether corticosterone levels were affected by the blood sampling procedure per se. Another 7 males and 5 females (transport group) were loaded into wooden crates 1.5 m high × 1.5 m long × 0.90 m wide, which had several holes (2 cm diameter) drilled at the top of each side panel and were provided with a layer of 3 to 4 cm of hay. The birds were loaded in same-sex pairs by selecting animals of the same size. Crates were loaded individually by 2 to 3 workers and then placed in the back of a pickup truck. The vehicle traveled 30 min along a paved road, at an average speed of 60 km/h. Upon arrival, crates were immediately unloaded and before being released, a second blood sample was collected following the same procedure described above. At the same time, a second blood sample was also collected from the nontransport control group. All Greater Rheas were loaded and transported on the same day between 0700 and 1000 h. Four animals (2 crates) were simultaneously transported in each of the 3 trips that were included in the experiment. The loading process, which consisted in capture, blood collection, placement of birds into the wooden crates, and the carrying and loading of the 2 crates onto the truck, was completed within 5 min, by 2 teams of 4 assistants (each one handling 2 animals and one crate).

Sample Collection and Corticosterone Determination

Blood samples (2 mL) were taken via jugular venipuncture. After centrifugation (2,500 × g for 15 min at ambient temperature), plasma samples were frozen at −20°C until assayed. Corticosterone concentrations in plasma of Greater Rheas were measured using the commercially available ^{125}I corticosterone-radioimmunoassay kit (MP Biomedicals, Costa Mesa, CA), which was previously validated for plasma corticosterone determinations in this species (Lèche et al., 2009).

Statistical Analyses

Blood samples were analyzed using a 2-way repeated measures ANOVA to determine possible differences in plasma corticosterone concentrations between sexes (male vs. female), stress treatment (transport vs. nontransport control), and blood sampling time [prestress exposure (baseline) vs. 30 min after stress exposure]. Analysis of variance assumptions of homogeneity of variances and normality were evaluated. The LSD Fisher test was used for post hoc analysis. Data were expressed as mean ± SE. Values of $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Corticosterone levels in Greater Rheas were significantly affected both by stress treatment ($F_{(1, 14)} = 94.6$, $P < 0.001$) and by time of blood sampling ($F_{(1, 14)} = 198.3$, $P < 0.001$). No significant differences were found between male and female corticosterone stress responses ($F_{(1, 14)} = 0.29$, $P = 0.59$). The baseline corticosterone levels were similar in the control and transported birds (9.0 ± 1.6 and 10.4 ± 0.8 ng/mL, respectively). Transportation induced a highly significant ($P < 0.001$), more than 40-fold increase in the corticosterone levels (433.6 ± 35.4 ng/mL), whereas blood sampled from nontransported rheas showed an approximately 10-fold significant ($P < 0.02$) increase (88.4 ± 14.8 ng/mL). Thus, the increase observed in the transported rheas was about 5 times higher ($P < 0.001$) than in their nontransported counterparts (Figure 1). These results suggest that greater rheas are highly sensitive to stressful manipulations. A simple blood sampling procedure that in several bird species induces moderate corticosterone changes (Eskeland and Blom, 1979; Noirault et al., 1999) and in other species is not even able to affect corticosterone levels (Wingfield et al., 1982; Sockman and Schwabl, 2001), in rheas induced a significant 10-fold corticosterone increase.

To our best knowledge, the only published scientific study evaluating transport stress in ratites is that of Mitchell et al. (1996), who transported 10-mo-old ostriches and found only a 2-fold increase ($P < 0.05$) in plasma corticosterone levels after transportation. That much lower stress response than the present one may be due to the time of posttransport blood collection (4.5 h) set in that study. It is possible that Mitchell et al. (1996) may have missed the peak of corticosterone concentration, and the blood levels they found represented a point toward the recovery of pretreatment corticosterone plasma levels. The high adrenocortical stress response level observed in Greater Rheas after transportation in the present study is comparable with the reported corticosterone response recorded after an ACTH challenge (Lèche et al., 2009, 2011). Indeed,

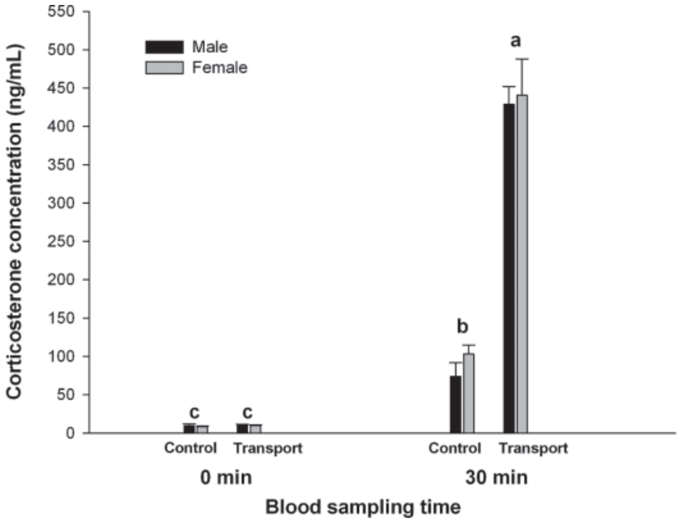

Figure 1. Baseline and 30-min poststress plasma corticosterone levels (mean ± SE) in adult Greater Rheas. Different letters (a–c) indicate significant ($P < 0.001$; Fisher least significant difference test) differences between groups. Bars represent treatment means, and the lines represent SE.

Lèche et al. (2011) reported a sharp increase in plasma corticosterone levels from 7.8 ± 2.6 ng/mL to 215.8 ± 12.2 ng/mL 2 h post-ACTH injection, followed by levels of 245.0 ± 26.5 and 166.0 ± 18.9 ng/mL after 4 and 6 h post-ACTH administration, respectively. Corticosterone levels finally dropped to pretreatment levels 8 h after injection (10.7 ± 2.5 ng/mL). However, it should be noted that the mentioned study using ACTH in rheas did not include a dose response curve; therefore, no conclusions should be drawn about the size of corticosterone responses to transport in comparison with corticosterone responses to ACTH. The present study suggests that transportation is a potent stressor for this species because corticosterone levels increased more than 40-fold after only 30 min of exposure (an increase about 5 times higher than the induced by a capture and blood sampling manipulation). A similar increase (about 50-fold) was reported by Cockrem et al. (2004), also in another ratite species (Brown kiwi, *Apteryx australis*) after capture and immobilization during 30 min. The significant increase in corticosterone levels reached in these ratites is noticeable compared with the corticosterone increases reported in the literature for other avian groups. 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). The consistency of the mentioned ratite results suggests that this type of adrenocortical response might be characteristic of this group of birds, possibly associated with the high energetic costs involved in their antipredator strategy that includes fast and prolonged running. Further studies that evaluate the impact of transportation on behavioral and physiological parameters of Greater Rhea, and the time required for return to basal activity and physiological levels are currently underway.

The present findings suggest that Greater Rhea is a highly sensitive species to stressful manipulations. Both blood samplings and transportation induced highly significant adrenocortical responses. Considering that transportation is one of the unavoidable common practices in the management of Greater Rheas and, as shown in the present study, that it induces a significant 40-fold corticosterone stress response, efforts should focus on helping to generate management transport standards for optimization of the welfare of this ratite.

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