COMPARISON OF CIRCULATING IRON, TOTAL IRON BINDING CAPACITY, AND PERCENT TRANSFERRIN SATURATION IN WILD AND CAPTIVE KORI BUSTARDS (*ARDEOTIS KORI*)

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Abstract: The kori bustard (*Ardeotis kori*) is one of the largest extant flighted birds and is displayed in zoos primarily in North America and Europe. In captivity, kori bustard diets are primarily based on animal proteins, whereas in the wild these birds eat a wide variety of plants, insects, and small vertebrate prey. The purpose of this study was to compare circulating iron, total iron binding capacity, and percent transferrin saturation levels in apparently healthy wild and captive kori bustards. Adult captive kori bustards had slightly higher percent transferrin saturation levels than juvenile captive birds, although this finding was not statistically significant. This information can be referenced as a guide for the assessment of nutrition and health in captive birds.

Key words: *Ardeotis kori*, hemosiderosis, iron, kori bustard, percent transferrin saturation, total iron binding capacity.

BRIEF COMMUNICATION

Kori bustards (*Ardeotis kori*) are one of the heaviest extant flighted birds.[2] They inhabit the dry savannas and grasslands of southern and eastern Africa, including areas in Ethiopia, Kenya, Tanzania, Mozambique, Zimbabwe, Botswana, Namibia, Angola, and South Africa.[6] Kept in captivity since the 1930s, kori bustards are fed primarily nutritionally complete feeds, whole prey (vertebrate and invertebrate), and produce.[3,4,6] Wild diets have been observed to be more omnivorous, including lizards, insects, leaves, seeds, acacia gum, and flowers.[10,11] In captivity, kori bustards have been diagnosed with hemosiderosis, an excessive accumulation of stainable iron without morphologic or biochemical evidence of toxicity (T. Walsh, pers. comm.). Body iron stores are controlled predominantly via enteric iron absorption, and iron overload may be primary (hereditary) or secondary (excessive intake, toxicities, metabolic diseases, and malnu-

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trition).[7,8] Dietary sources of iron from myoglobin and hemoglobin are more highly bioavailable than non–mammal-derived iron sources.[8] Therefore, differences between primarily carnivorous captive kori bustard diets and more omnivorous wild diets may influence iron homeostasis in captive kori bustards.

The purpose of this study was to compare circulating iron, total iron binding capacity (TIBC), and percent transferrin saturation in apparently healthy wild kori bustards and captive kori bustards. These data serve as a baseline for comparison of iron status of healthy captive kori bustards.

Wild kori bustards in this study were captured in Laikipia District of central Kenya, on Mpala Research Centre and Wildlife Foundation (0.2922°N, 36.8980°E) and surrounding properties, in May 2006 and November 2009–January 2010. The landscape at Mpala is characteristic of semiarid African savannas, predominated by grassy savanna bushland with patches of woodland and open grassland, with over 800 plant species documented in this area (www.mpala.org). Using approved Institutional Animal Care and Use Committee protocols, all animals were humanely captured by gently herding them with a slow-moving motor vehicle or persons on foot into large mist nets placed strategically in the native habitats. Upon capture, each bird was immediately weighed, a physical examination was performed, morphometric measurements were obtained, and birds were sexed via physical

characteristics. All birds were released after no more than 27 min.

Captive birds were housed at the Smithsonian National Zoological Park, Washington, DC, USA, in large outdoor enclosures that mimicked their natural environment, with access to free-ranging insects, plant matter, and small vertebrates. Captive birds were fed a commercial meat-based diet (Premium Beef Feline, Central Nebraska Packing, North Platte, Nebraska 69103, USA), frozen–thawed whole small rodent prey, a combination of complete pelleted diets (exotic gamebird maintenance pellets, Ratite diet, Mazuri Exotic Animal Nutrition, St. Louis, Missouri 63166, USA; crane diet, Zeigler Bros., Inc. Gardners, Pennsylvania 17324, USA), fresh produce, and invertebrates (crickets or mealworms). Animals were evaluated during routine exams and determined to be healthy based on examination by a veterinarian and, when indicated, additional diagnostic testing such as radiographs, hematology, and plasma biochemical analysis.

Blood samples were collected from the right jugular or the medial metatarsal veins via standard collection techniques.[1] Blood samples in Kenya and the United States were collected into lithium heparin or serum clot tubes and were stored at room temperature or in a cooler under field conditions until processing, which occurred at least 20 min following collection. Samples were centrifuged at 2,200–2,500 rpm for 10–15 min to separate serum or plasma from cellular components. In the field, separated serum or plasma samples were aliquoted and stored in liquid nitrogen until return to the United States. All samples were stored frozen at −80°C prior to analysis at the Animal Health Diagnostic Center at Cornell University, Ithaca, New York, USA. Iron and TIBC measurements were performed with a Roche/Hitachi Modular analyzer via a colorimetric assay (Roche Diagnostics, Indianapolis, Indiana 46250, USA). Diagnostic methodologies are validated for serum and plasma samples (Roche Iron and TIBC method sheet version V9.0, Roche Diagnostics). Percent transferrin saturation was calculated as the ratio of serum iron concentration to TIBC multiplied by 100.

Data normality was evaluated using skewness and kurtosis. All data distributions had slight to moderate skewness and mild kurtosis. Variances were compared between the samples using an F-test with ratios close to 1 in all situations. The two-tailed Student's t-test was used to compare outcome measures of circulating iron, TIBC, and percent saturation of transferrin between captive and wild as well as juvenile and adult birds. The level of significance to reject the null hypothesis that no difference existed between the two samples was set at $\alpha \leq 0.05$. All data analyses were performed in Microsoft Excel 2013, part of Microsoft Office Professional Plus 2013 (Microsoft, Redmond, Washington 98052, USA).

A total of 10 wild kori bustards were captured and sampled for this study. Nine birds (five male and four female) had samples adequate for analysis. A total of 11 captive kori bustards were sampled for this study and all birds (6 male and 5 female) had samples adequate for analysis. In wild and captive kori bustards, similar distribution of males and females were noted between wild and captive groups and within each wild or captive group. In captive kori bustards, the median age was 6 yr 2 mo; no wild juvenile birds were captured in this study (Table 1).

Iron, TIBC, and percent transferrin saturation levels where highly variable between individual birds (Table 1). Mean TIBC levels were 154.1 ± 42.2 μg/dl for wild birds and 136.2 ± 42.3 μg/dl for captive birds. Mean percent transferrin saturation levels were 77.8 ± 27.8% for wild birds and 79.5 ± 24.6% for captive birds. None of the measures statistically differed between wild and captive birds, between all adult birds and juvenile captive birds, or between adult wild and adult captive birds (all $P > 0.1$; Table 2). Captive adult kori bustards showed a trend of higher percent transferrin saturation than captive juvenile birds ($P = 0.08$), but neither mean iron nor mean TIBC differed between juvenile and adult captive birds (Table 2). The sample size did not give necessary power to definitely rule out differences in these three outcomes between populations (Table 2).

In avian species, total body iron stores are controlled via absorption of iron in the intestine.[10] Hemosiderosis has been documented at the time of necropsy in captive kori bustards but has not been documented in wild kori bustards (T. Walsh, pers. comm.). Kori bustards and other Otidiformes have not typically been considered to be highly sensitive to iron storage–related diseases.[7] Unfortunately, diagnosis of these conditions is often not apparent until the time of necropsy. Noninvasive antemortem assessment of iron levels includes iron, TIBC, percent transferrin saturation, and serum ferritin levels.[7] Although serum ferritin is considered the best noninvasive test for iron body stores in mammals, this test requires a species-specific immunologic assay that has not been optimized for any avian species. At

Table 1. Wild and captive kori bustard (*Ardeotis kori*) demographics and results of iron concentration, total iron binding capacity (TIBC), and percent transferrin saturation.

Bird	Husbandry status[a]	Age[b]	Sex[c]	Iron (µg/dl)	TIBC (µg/dl)	% Transferrin saturation
1	C	J	M	67	148	45
2	C	J	F	171	181	94
3	C	J	M	78	147	53
4	C	J	M	61	168	36
5	C	J	F	129	129	100
6	C	A	M	72	72	100
7	C	A	F	143	143	100
8	C	A	F	70	70	100
9	C	A	F	82	105	78
10	C	A	M	200	207	97
11	C	A	M	92	128	72
12	W	A	F	127	127	100
13	W	A	M	89	123	72
14	W	A	M	114	135	72
15	W	A	F	113	113	100
16	W	A	M	158	172	92
17	W	A	M	63	126	50
18	W	A	M	131	149	88
19	W	A	F	41	235	17
20	W	A	F	200	207	97

[a] C indicates captive housed birds; W, wild-caught and released birds.
[b] J indicates juvenile birds (age <2 yr); A, adult birds (age >2 yr).
[c] M indicates birds sexed as male based on physical features; F, birds sexed as female based on physical features.

present, the only antemortem iron test available to confirm iron storage diseases in avian species is a liver biopsy, which was considered too invasive to pursue in the population of kori bustards utilized in this study. Further, the logistics of collecting a liver biopsy can be limiting in some avian species based on their small size or anes-thetic risk if medically compromised; thus, a less invasive diagnostic tool, such as the blood tests utilized in this study, provides a limited but clinically useful indication of the iron storage status in an individual bird.

In this study, there was no significant relation-ship between iron values and whether kori bustards were from the wild or housed in captivity. In addition to dietary intake, many factors are known to affect iron levels in avian species, including reproductive status, starvation (due to molting, migration, or pathologic causes), and hereditary factors.[9] In this study, adult birds were observed to have a slightly higher mean percent transferrin saturation level than their juvenile counterparts, which has been documented in other species.[5,7] If percent transferrin saturation in captive kori bustards truly does increase with age, studying iron analyses over time by repeated sampling from hatchling to adult on consistent diets would help to shed light on a possible diet predisposition to iron accumulation in adult birds over time.

Although this study is limited by small sample size and an uneven distribution of age between captive and wild birds, it does provide a baseline for interpretation of iron analytes in kori bustards. Ideally, liver iron levels and histopathologic evaluation would be paired with iron levels to provide a more complete picture of iron homeo-stasis in kori bustards, but this was impractical to perform on the wild study population. Repetitive liver biopsies are rarely used in a clinical setting; the noninvasive parameters presented here may provide the clinician with a relevant baseline of information with which to approach a suspect case of iron overload in this species.

At this time, a link between wild versus captive diets and serum iron evaluation in kori bustards could not be established. Although this is not

Table 2. Comparisons of mean serum iron concentration, total iron binding capacity (TIBC), and percent transferrin saturation between demographic groups wild and captive kori bustards (*Ardeotis kori*) using Student's *t*-tests.

Demographic groups	Iron Mean (µg/dl)	Iron SD (µg/dl)	Iron P-value	TIBC Mean (µg/dl)	TIBC SD (µg/dl)	TIBC P-value	% Transferrin saturation Mean (%)	% Transferrin saturation SD (%)	% Transferrin saturation P-value
Wild (n = 9)	115.1	47.8	0.67	154.1	42.2	0.36	77.8	27.8	0.88
Captive (n = 11)	105.9	47.4		136.2	42.3		79.5	24.6	
Captive juvenile (n = 5)	101.2	47.4	0.78	154.6	20.2	0.2	65.6	29.4	0.08
Captive adult (n = 6)	109.8	51.7		120.8	51.4		91.2	12.7	
Adult (all) (n = 14)	113	47.6	0.64	140.8	47.4	0.54	83.1	23.4	0.19
Captive juvenile (n = 5)	101.2	47.4		154.6	20.2		65.6	29.4	
Captive adult	109.8	51.6	0.84	120.8	51.4	0.19	91.2	12.7	0.29
Wild	115.1	47.9		154.1	42.2		77.8	27.8	

statistically significant, kori bustards in captivity do demonstrate a trend of increasing percent transferrin saturation as compared to juveniles. Kori bustards may be at risk for developing hemosiderosis, but a link between iron levels and these disease processes was outside the scope of this work. Additional investigation is needed to further evaluate the role of iron in kori bustard health.

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