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SERUM IRON AND SELECTED BIOCHEMICAL VALUES IN FREE-RANGING BLACK RHINOCEROS (*DICEROS BICORNIS*) FROM SOUTH AFRICA

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Abstract: Serum samples collected from 20 black rhinoceros (*Diceros bicornis*) were analyzed for iron values from six different areas in South Africa. In addition, biochemical profiles were performed on individual samples. Comparisons of iron values from free-ranging black rhinoceros and from 28 free-ranging white rhinoceros (*Ceratotherium simum*) were conducted by location and age. Among the free-ranging black rhinoceros, samples were compared from different regions to a set of samples from black rhinoceros that had been captured and held in bomas. Serum iron levels were not significantly different ($P = 0.55$) among the three locations with more than one animal (medians 5.57, 5.70, 6.47 ppm), but the median value from the boma group was significantly lower (2.91 ppm; $P = 0.042$), contrary to previous studies. Similar to reports in captive black rhinos, serum iron levels appeared to show a trend toward increasing values between subadult and adult animals, although differences were not statistically significant among black rhinoceros. Comparison of serum iron levels between free-ranging black and white rhinoceros showed significantly higher median value in black rhinoceros (5.73 ppm) versus white rhinoceros (3.38 ppm, $P = 0.001$). Other significant differences ($P < 0.05$) in biochemical values between species included lower median aspartate aminotransferase (37 versus 76.5 U/L), higher copper (1.50 versus 1.34 ppm), higher zinc (1.36 versus 0.37 ppm), lower total protein (8.0 versus 10.35 g/dL), higher gamma glutamyltransferase (13.0 versus 12.5 U/L), and lower globulin (6.6 versus 7.6 g/dL) in black rhinoceros. Further investigations should be conducted to examine the role of age, location, and time in boma confinement on iron values in South African rhinoceros to understand iron metabolism in these species.

Key words: black rhinoceros, *Diceros bicornis*, iron, serum biochemistry, white rhinoceros.

INTRODUCTION

The role of iron in diseases of black rhinoceros (*Diceros bicornis*) has been the subject of research for several decades.[12] Although hemochromatosis is not commonly documented, hemosiderosis is a common finding in captive black rhinoceros at necropsy.[8] This condition has not routinely been observed in free-ranging rhinoceros except in cases in which animals have been captured and brought into captivity for translocation.[11] A similar syndrome has also occurred in the other browsing species of rhinoceros, the Sumatran rhinoceros (*Dicerorhinus sumatranesis*), but not the Asian one-horned (*Rhinoceros unicornis*) or white (*Ceratotherium simum*) rhinoceros.[14] This has led to speculation that dietary factors in captivity may play a role in increased iron absorption.[5] An alternative hypothesis is that there is a genetic predisposition to iron accumulation in these species.[2] In this study, serum iron, biochemical values related to liver health, and other trace mineral values (copper and zinc) were compared between free-ranging black rhinoceros from different locations in South Africa, different age categories, and free-ranging white rhinoceros to determine if there were any significant trends that could be further investigated to understand iron storage disorder of captive black rhinoceros.

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MATERIALS AND METHODS

Animals

Sixteen free-ranging black rhinoceros were immobilized in South Africa between 2005 and 2010 in the Eastern Cape (Addo National Park, Sam Knotts National Reserve, and Kleinvlak

Province), Mpumalanga (Kruger National Park), Limpopo (Marakele National Park), Northern Cape (Vaalbos National Park, Karoo National Park), and Northwestern province (Pilanesberg Game Reserve), and four rhinoceros were held in bomas in Skukuza in Kruger National Park. The four black rhinoceros held in the bomas were confined for 3, 9, and 25 days and approximately 7 months. Twenty-eight free-ranging white rhinoceros were immobilized in Kruger National Park during 2010. All animals were captured according to the Standard Operating Procedure for the Capture, Transportation and Maintenance in Holding Facilities of Wildlife for South African National Parks. This Standard Operating Procedure was approved by the South African National Parks Animal Use and Care Committee. Animals appeared healthy based on body condition and physical examination. Of the black rhinoceros, there were 2 juveniles, 6 subadults, and 12 adults. The white rhinoceros sample group consisted of 19 subadults and 9 adults. Animals were grouped into age categories based on size: juveniles (calf with dam), subadult (2.5–6 years of age), adult (older than 6 years).

Sample analyses

All samples used in this study had been collected for other purposes and stored at −4°C until analyzed retrospectively. A single sample was taken from each rhinoceros at the time of immobilization. Sufficient serum was not available from one adult black rhinoceros, and the four boma-confined rhinoceros were excluded for comparisons to free-ranging white rhinoceros. Therefore, only 15 black rhinoceros samples were analyzed for serum chemistry. Samples from all 28 white rhinoceros were analyzed. Sera were used for chemistry panel analyses with the ABAXIS VetScan2 chemistry analyzer (ABAXIS Inc., Union City, California 94587, USA) using a large animal chemistry rotor (ABAXIS). Measured analytes included albumin (alb), alkaline phosphatase (alk phos), aspartate aminotransferase (AST), calcium (Ca), gamma glutamyltransferase (GGT), total protein (TP), globulin (glob), blood urea nitrogen (BUN), creatinine phosphokinase (CPK), phosphorus (P), and magnesium (Mg). Trace mineral analyses were performed at the Agricultural Research Council Institute for Soil, Climate and Water (Pretoria, South Africa) using inductively coupled plasma-atomic emission spectroscopy methodology as previously described.[3]

Statistical analyses

STATA 11 SE (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, Texas 77840, USA) was used for statistical analysis. Descriptive tables and scatter plots were generated. Mean and standard deviations were calculated for each parameter and presented for descriptive purposes. However, due to the relative small sample size available for this study, nonparametric analyses were used to compare median iron (and other analytes) values between groups (black versus white rhinoceros) by using the Wilcoxon rank sum test. The Kruskal-Wallis test using posthoc pair comparisons was used when comparing black rhinoceros iron values among different geographic locations. The Wilcoxon rank sum test was used to compare iron median values among subadult and adult rhinoceros. A multivariable linear regression analysis using ranks was used to compare median values of iron (and other analytes) while controlling (adjusting) for the differences in age distribution among rhinoceros of different species (black versus white rhinoceros). Statistical significance was declared at $P < 0.05$.

RESULTS

Serum iron results from 20 black rhinoceros were plotted based on age category (Fig. 1). Adult rhinoceros had higher median (5.34 ppm) iron values than subadult rhinoceros (3.22 ppm), although this difference was not statistically significant at the 5% level (Wilcoxon rank sum test $P = 0.08$) (Fig. 1). The two samples from juvenile rhinoceros had relatively high iron levels compared with other rhinoceros (7.04 and 8.20 ppm). A comparison of subadult and adult white rhinoceros iron values showed that adults also had higher levels (3.78 ppm) than subadults (3.21 ppm), which was statistically significant (Wilcoxon rank sum test $P = 0.048$). Therefore, further analyses (multivariable analysis) were performed to control for age.

Individual black rhinoceros samples were also compared by location. Three individuals showed higher values than the overall median (two rhinoceros from the Northern Cape with iron values of 12.25 and 21.22 ppm and one rhinoceros from the Eastern Cape with an iron value of 47.35 ppm). Because there was a single rhinoceros in two of the other locations, these were not included in the analyses. When a subset of the rhinoceros were grouped by location and median iron values compared between three groups of

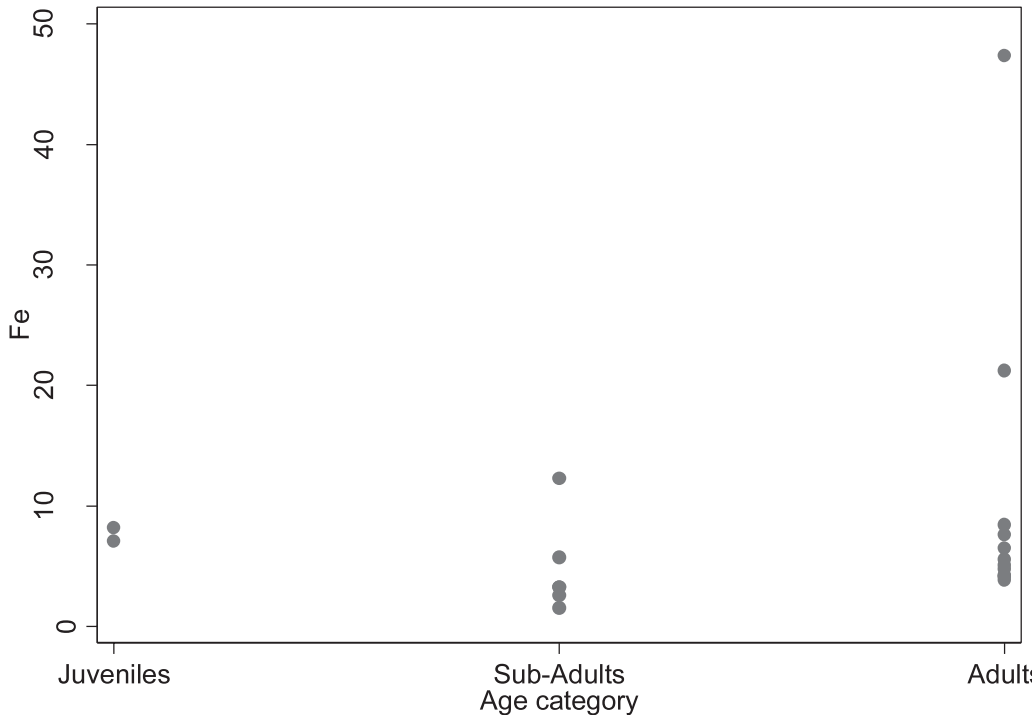

Figure 1. Serum iron levels (ppm) among black rhinoceros from three different age categories (juveniles: calf with dam; subadults 2.5–6 years of age; adults: older than 6 years).

free-ranging rhinoceros (Eastern Cape, Limpopo and Northern Cape), no significant different was found between the iron median values (Kruskal-Wallis test, $\chi^2 = 1.19$, df = 2, $P = 0.55$) (Table 1). However, the analysis showed a significant difference in median values when the group of rhinoceros that had been held in bomas after capture were included and compared with the other three groups (Kruskal-Wallis test, $\chi^2 = 8.2$, df = 3, $P = 0.042$) (Table 1). Posthoc pair comparison analyses indicated that boma-confined black rhinoceros had a significantly ($P < 0.05$) lower median value (2.91 ppm) than the Eastern Cape (5.57 ppm) and Northern Cape (6.47 ppm) black rhinoceros.

Because iron accumulation can lead to hepatic dysfunction, changes in serum liver values and trace minerals were evaluated in the free-ranging black rhinoceros and compared with white rhinoceros captured in Kruger National Park adjusting for differences in age profile (distribution) among black and white rhinoceros. Free-ranging black rhinoceros had significantly ($P = 0.002$) higher serum iron levels (median 5.73 ppm) compared with white rhinoceros (median 3.38 ppm) (Table 2). Other significant differences ($P < 0.05$) in biochemical values between species included lower median aspartate aminotransferase (37 versus 76.5 U/L), higher copper (1.50 versus 1.34 ppm), higher zinc (1.36 versus 0.37 ppm), lower total protein (8.0 versus 10.35 g/dL), higher gamma glutamyltransferase (13.0 versus 12.5 U/L), and lower globulin (6.6 versus 7.6 g/dL) in black rhinoceros.

DISCUSSION

Although this study had a relatively low sample ($n = 20$) of black rhinoceros, there were a number of interesting findings that require additional investigation. This is the first study that has evaluated age and iron levels in free-ranging black rhinoceros. Similar to previous results in captive animals, there appeared to be a trend toward higher serum iron levels in adult versus subadult animals, suggesting accumulation with age in both wild black and white rhinoceros.[5] However, the two juvenile black rhinoceros had the highest median iron value (7.62 ppm), which may be age or diet related, an artifact of the small sample number, or an effect of unmeasured factors. Most previous studies in free-ranging rhinoceros have examined a single point in time or only a single age category, making assessment of iron accumulation with age difficult.[13]

Table 1. Summary statistics for iron values depending on rhinoceros location.

	Eastern Cape	Boma	Northern Cape	Limpopo
Mean	14.09	2.86	10.09	5.76
SD	18.65	1.09	6.87	2.63
25th%	5.11	2.05	5.73	3.52
Median	**5.57**	**2.91**	**6.47**	**5.70**
75th %	8.20	3.67	12.25	8.01
n	**5**	**4**	**5**	**4**

There has been some indication that boma confinement or translocation stress leads to increased risk for hemosiderosis.[11,13] However in this study, three subadult and one adult rhinoceros that had been held in bomas between 3 and 25 days (up to 7 months in the case of one animal) had significantly lower serum iron levels than similarly aged rhinoceros that had just been caught. This result may have been confounded by diet, age, parasite burden, and environmental conditions, such as season, gender, and other underlying conditions. It does indicate that further studies should be conducted to examine the role that captive management of black rhinoceros plays in iron metabolism, especially in boma situations.

Another finding was the variation in iron levels between black rhinoceros captured in different locations within South Africa. Geographically, the terrain, habitat, and vegetation differ widely between the study sites. The black rhinoceros with the highest individual serum iron value (47.35 ppm) was a mature male captured in the Eastern Cape, where soil consists of light red clay loam.[1] The other high individual values were found in a subadult and adult rhinoceros from the Northern Cape (12.55 and 21.22 ppm, respectively), where the soil consists of 15–35% clay.[18] Overall median values for the various locations were not significantly different. However, soil content and thus plant iron content may play a role in individual free-ranging rhinoceros serum values, which varies by location.

Although serum iron is considered an insensitive measure of total body iron stores, elevations are observed in such conditions as hereditary hemochromatosis in humans and hemosiderosis in other species.[15,17,21] Other measures of iron status include total iron-binding capacity, ferritin, and transferrin saturation. However, some limitations include lack of availability of these assays, especially outside the USA, and influence of inflammatory response on production of acute phase proteins, such as ferritin.[22] A rhinoceros-specific ferritin assay has been developed.[16] Unfortunately, this was not available in South Africa at the time of the study, although future work should include evaluation of ferritin, total iron-binding capacity, and transferrin saturation in free-ranging black rhinoceros. In addition, future work with hepcidin, a key hormonal regulator of iron metabolism, may be useful in understanding differences in rhinoceros species and the comparison of captive and free-ranging populations.[22] In

Table 2. Comparison of free-ranging black and white rhinoceros serum values.[a]

Species	Fe (ppm)	Cu (ppm)	Zn (ppm)	AST (U/L)	GGT (U/L)	TP (g/dL)	Glob (g/dL)	P (mg/dL)
Black rhino								
Mean	7.19	1.57	1.31	42.67	15.07	8.11	5.39	5.01
SD	4.52	0.31	0.55	20.78	3.67	0.75	2.86	1.04
25%	4.24	1.29	0.85	36.00	13.00	7.70	5.80	4.10
Median	5.73	1.50	1.36	37.00	13.00	8.00	6.60	4.90
75%	8.20	1.80	1.78	46.00	19.00	8.50	7.00	5.80
n	15	15	15	15	15	15	15	12
White rhinos								
Mean	3.65	1.36	0.60	78.61	13.50	10.12	7.66	5.01
SD	1.49	0.40	1.00	13.45	4.06	1.06	1.01	0.52
25%	2.70	1.20	0.34	70.50	10.00	9.80	7.25	4.60
Median	3.38	1.34	0.37	76.50	12.50	10.35	7.60	5.10
75%	4.13	1.50	0.48	87.00	15.00	10.85	8.40	5.30
n	28	28	28	28	28	28	28	28

[a] Multivariable linear regression analysis using ranks to compare median values between free-ranging black and white rhinoceros (adjusting for age) ($P < 0.05$).

Fe: iron; Cu: copper; Zn: zinc; AST: aspartate aminotransferase; GGT: gamma glutamyltransferase; TP: total protein; Glob: globulin; P: phosphorus.

humans, increased hepcidin levels correlate with increased ferritin and decreased serum iron.[10]

Iron is critical for cellular function, including oxygen transport and energy metabolism. However, excessive iron can be detrimental if available to microbial organisms or as free iron that can combine with oxygen to form free radicals and cause lipid peroxidation. The form and amount of serum or tissue iron becomes important in determining whether these accumulations may cause potential cellular dysfunction.[9] Effects of elevated iron may be reflected clinically as changes in serum biochemical values indicating inflammation, necrosis, or altered metabolism of the affected tissue. When the median values of measured serum biochemical parameters were evaluated in the 15 black rhinoceros, none were outside of International Species Information System reference ranges used for captive black rhinoceros, although there were some notable differences, such as lower AST and higher globulin in the free-ranging animals.[7] This may be due to differences in laboratories used, stimulation of the immune system due to parasites, environmental antigens, or possible subclinical disease. Although GGT and AST are used to assess hepatocellular damage in horses, AST is not liver specific.[6] Serum iron and gamma globulins are often elevated in horses with severe chronic hepatic disease. However, there was no evidence that the free-ranging black rhinoceros in this study had abnormal changes indicating concurrent pathology.

One hypothesis for reported lower iron levels and lack of hemosiderosis in free-ranging rhinoceros is that they are not exposed to the same level of oxidative stress as captive black rhinoceros. A variety of circumstances in captivity may lead to inflammatory responses that release free radicals, damaging cell membranes, resulting in hemolysis and hemosiderin deposition in the reticuloendothelial cell system. This may manifest clinically as ulcerative dermatitis, hepatopathy, and anemia.[4]

Another hypothesis is that this is a species-specific difference in iron metabolism. There have been numerous studies suggesting that black rhinoceros have innate differences in red blood cell metabolism and immunologic capabilities.[5,14,20] Therefore, comparison with free-ranging white rhinoceros from one of the same capture locations was also made to examine species differences in serum iron and biochemical values. Although there were significant differences, especially lower iron in white rhinoceros as has been shown previously, these values were all considered to be within healthy ranges, similar to those reported for other wild populations. [19] This may be due to genetic, physiologic, dietary, or other adaptive mechanisms for iron metabolism observed in the nonbrowsing rhinoceros species.

In summary, it appears that free-ranging black rhinoceros may be subject to the same potential issues that occur in captive rhinoceros, such as progressive accumulation of iron with age and variation with location-based dietary intake. However, no obvious adverse effects were apparent based on a very small sample size using serum biochemical analyses. These questions should be further investigated in free-ranging animals to determine if these preliminary results are consistent in a larger rhinoceros population sample when using additional measurement tools and controlling for potential confounding factors.

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