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BROWSE DIVERSITY AND IRON LOADING IN CAPTIVE SUMATRAN RHINOCEROSES (*DICERORHINUS SUMATRENSIS*): A COMPARISON OF SANCTUARY AND ZOOLOGICAL POPULATIONS

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Abstract: Iron storage disease (ISD) is now recognized as a serious clinical disorder acquired by two species of browsing rhinoceroses, the African black (*Diceros bicornis*) and the Asian Sumatran (*Dicerorhinus sumatrensis*) rhinoceroses, when displaced from their natural habitats. The most complete knowledge of ISD comes from studies of the black rhinoceros, but the Asian species is also at risk. Sumatran rhinoceroses housed in traditional zoological settings outside of range countries have suffered significant morbidity and mortality potentially related to ISD induced by diet and/or other confinement conditions. With so few animals in captivity, very little information exists on iron loading in the Sumatran rhinoceros. To better characterize the problem, we retrospectively compared captive management conditions of Sumatran rhinoceroses housed under traditional zoological care with those in two native sanctuary environments. In general, zoo rhinoceroses are offered a paucity of plants and browse species compared with their sanctuary and wild counterparts managed in native rainforest habitats. Iron analyte levels and limited histopathologic observations in these populations suggest variable tendencies to overload iron, dependent upon differences in managed diet and individual food preferences. More detailed investigation of these markedly dissimilar ex situ populations is warranted to better understand the role of nutrition and other conditions affecting iron loading in browser rhinoceroses.

Key words: Sumatran rhinoceros, iron storage disease, hemosiderosis, hemochromatosis, iron, ferritin, nutrition, browser.

INTRODUCTION

In 1993, the dedication honoree of this JZWM Special Issue, Dr. Joseph E. Smith, first called attention to the possibility of an iron storage disorder (ISD) affecting African black rhinoceroses (*Diceros bicornis*). At the First International Workshop on Diseases of Black Rhinos and subsequently, he and his colleagues presented unequivocal evidence of significant disparities in iron loading between captive African black and white (*Ceratotherium simum*) rhinoceroses and suggested that they might be due to dietary differences between browsers and grazers.[36,37] At that time, attention was largely focused on other disorders of high morbidity and mortality affecting black rhinoceroses, but additional evidence supported and eventually confirmed the rapid development of clinically significant ISD in this species under captive conditions.[5,14,19,22,24-28]

Similar studies indicated that Sumatran rhinoceroses (*Dicerorhinus sumatrensis*) were also susceptible to iron overloading, a problem that was not apparent in African white or Asian (Indian) greater one-horned rhinoceroses (*Rhinoceros unicornis*).[21,22,24-27] Because so few *D. sumatrensis* remain either in captivity or the wild, we retrospectively reviewed available data on three distinct, but heterogeneous, populations in hope of detecting any differences in iron stores that might be related to dietary or environmental conditions.

The Sumatran rhinoceros is the smallest living member of the family Rhinocerotidae and is considered a nonselective browser foraging on a great variety of plant species.[39] Sumatran rhinoceroses held in traditional zoological settings outside of range countries are offered a paucity of botanical species compared to their sanctuary and wild counterparts managed in native rainforest habitats.

Compared to rhinoceroses in North American zoological parks, range-country rhinoceroses (those housed in the Sumatran Rhinoceros Sanctuary in Indonesia and the Sungai Dusun Reserve in Peninsular Malaysia) have access to a far

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greater diversity of browse. At the Sumatran Rhinoceros Sanctuary in Way Kambas National Park more than 100 browse varieties have been recorded in the diet of captive rhinoceroses, and 8 to 10 varieties are routinely fed on a daily basis (Table 1). Species composition is based largely on time of year combined with both rhinoceros and keeper preferences. Sanctuary rhinoceroses in Indonesia also browse freely in 10-hectare enclosures and select additional browse items on their own. The ability to browse freely was limited in Malaysian rhinoceroses and nonexistent in zoological settings outside of range countries.

North American zoological facilities have access to only a handful of browse options (primarily *Ficus* spp., *Acacia* spp., mulberry, bamboo, and banana), and these few suitable plant species must often be imported from other geographic regions. Additionally, zoological diets for Sumatran rhinoceroses regularly include grass and alfalfa hays and pellets that are significantly higher in iron than a strict browse diet.[1,4] Early studies suggested that the nutritional composition of browse grown in North America differed from those in Indonesia with the latter having higher fiber, particularly hemicellulose (5% vs. 23%), and variable iron content.[3,4] More recent comparisons between North American and locally available (Indonesian and Malaysian) browses indicate that iron content of these temperate and tropical browses is not that different, and other factors may affect total rhinoceros iron stores.[6]

MATERIALS AND METHODS

To facilitate comparisons of iron stores among Sumatran rhinoceroses housed under differing environmental and feeding management conditions, levels of captive management in the ex situ population from zoo to sanctuary were organized into three groups. Group 1 represents animals imported into North America and their offspring ($n = 7$ rhinoceroses; two male and five female), Group 2 represents animals housed in a conservation center in Peninsular Malaysia ($n = 6$ rhinoceroses; one male and five female), and Group 3 represents sanctuary animals living in native rainforest habitat in Way Kambas National Park ($n = 3$ rhinoceroses; one male and two female). Animals in Groups 1, 2, and 3 varied in age from <1 to 30, 15 to 25 and 20 to 25 years, respectively. Feeding management comparisons among the three groups were evaluated in five areas: 1) browse diversity as measured by number of plant species locally available, 2) browse diversity as measured by number of plant species

TABLE 1. Comparison of browse diversity and feeding management between three distinct ex situ populations of Sumatran rhinoceros (*D. sumatrensis*).

Rhinoceros facility location and group number	Browse Diversity A (# of species available locally)	Browse Diversity B (# of species fed per day)	Time spent free-range browsing in tropical forest (# of hours browsing per day)	Transit time of harvested browse (# hours from cutting to feeding)	Nonbrowse items in diet (% of diet from hay or pellets as fed)
North American zoo rhinoceroses, Group 1 ($n = 7$)	8	2–3	0	>72	20–38[a]
Peninsular Malaysia Center rhinoceroses, Group 2 ($n = 6$)	12	4–5	2 (variable)	<24	7–10[b]
Way Kambas Indonesia Sanctuary rhinoceroses, Group 3 ($n = 3$)	>100	8–10	6	<12	0[c]

Feeding management references: [a] Dierenfeld et. al., 2000; [b] Zahari et. al., 2005; [c] Radcliffe et. al., 2004.

fed daily, 3) access to free-ranging browse in a forest, 4) browse handling time from cutting to feeding, and 5) feeding of non-browse items such as pelleted feed or hay (Table 1).

All blood samples were collected from non-sedated animals during routine venipuncture procedures using a 23-gauge butterfly catheter inserted into the auricular or caudal tail vein.[29] Blood samples were collected into 6-ml syringes and immediately transferred into serum clot tubes; sera were separated from red cell fractions within 30 minutes and stored under refrigeration or frozen (–4°C) until analyzed.

Iron analytes were assayed in serum samples collected between 1997 and 2010 and shipped by air under refrigeration to the UCLA Hematology Research Laboratory (Los Angeles, California, USA) and Kansas State University Veterinary Diagnostic Laboratory (Manhattan, Kansas, USA). Only one or two specimens were available from animals in Groups 2 and 3. In Group 1, single samples were obtained from two rhinoceroses, but multiple samples (3, 4, 7, 8, and 17) were taken from the remainder. Serum iron, unsaturated and total iron-binding capacities, transferrin saturation and ceruloplasmin were determined by quantitative colorimetry. Ferritin concentrations were measured by enzyme-linked immunoabsorbant assay using reagent standards derived from black rhinoceros ferritin.[37] Haptoglobin was assayed by a modification of the method of Makimura and Suzuki.[17] Available necropsy specimens and pathology reports from 17 Sumatran rhinoceroses were reviewed for histopathologic evidence of ISD using hematoxylin and eosin and Prussian-blue stains specific for ferric iron. These included nine animals from North American and European zoos, seven from Malaysia and one from Indonesia.

RESULTS

Feeding management comparisons

Group 1 (n = 7 rhinoceroses): Sumatran rhinoceroses housed in North American zoological institutions received the lowest browse diversity of the three groups with just eight plant species available on a regular basis. Of these, one species (*Ficus rubiginosa*) made up 80% of the diet as fed on a wet matter basis (Table 1).[34] Rhinoceroses held in North American facilities were typically fed just two or three different browse species on any given day. Zoological rhinoceroses had no access to free-range (nonharvested) browse and most, if not all, browse was shipped to the institution by air (or more rarely by ground transport). Zoological rhinoceroses were fed both a pelleted diet (Mazuri® Browser Wild Herbivore or ADF-16; Purina Mills, Gray Summit, Missouri 63039, USA) and grass-legume hay.[3,4]

Group 2 (n = 6 rhinoceroses): Sumatran rhinoceroses housed in the Sungai Dusun Conservation Center in Peninsular Malaysia received a moderate level of browse diversity with an estimated 12 plant species available on a regular basis, and feeding of 4 to 5 different species per day (Table 1).[30] These rhinoceroses had limited access to non-harvested browse with free-range foraging opportunities provided 1–2 times per week. Browse was cut locally and transported to the Center over short distances by truck. Rhinoceroses were fed 30 to 40 kg of browse forage per day plus 3 kg of a pelleted herbivore diet (Cargill Equine Diet; Cargill Indonesia, Jakarta 10220, Indonesia), but no grass or legume hay was offered.[41]

Group 3 (n = 3 rhinoceroses): Sumatran rhinoceroses housed in a 100-hectare sanctuary in native rainforest habitat within Way Kambas National Park showed the highest browse diversity of the three groups with more than 100 plant species available locally and 8 to 10 varieties offered on a daily basis (Table 1). Additionally, sanctuary rhinoceroses had free-range access to non-harvested rainforest browse on a daily basis, and these animals made extensive use of the opportunity to select browse species on their own. Keeper staff monitored and recorded the browse choices of each rhinoceros and observed that food preferences changed dependent upon time of year and emergence of ripe fruiting trees. Sanctuary rhinoceroses received no supplemental hay or pelleted rations.[29]

All three groups of rhinoceroses had free choice access to water and an iron-free salt-mineral block. Additionally, all animals were offered a few kilograms of fresh fruits and vegetables daily for training and management purposes.

Iron analyte comparisons

As illustrated in Table 2, serum iron concentrations, total iron-binding capacities and transferrin saturations were comparable in Groups 2 (Sungai Dusun) and 3 (Way Kambas), but each of these values was 30–50% higher in Group 1 (North American zoos). Even greater differences were apparent in serum ferritin concentrations with the North American captive population (Group 1) exhibiting two- to four-fold increases compared to Groups 2 and 3.

TABLE 2. Comparative serum analytes representing the mean of mean values in three distinct ex situ populations of Sumatran rhinoceros (*D. sumatrensis*).

Rhinoceros facility location and group number	Mean iron (μg/dl)	Mean TIBC (μg/dl)	Mean transferrin saturation (%)	Mean ferritin (ng/ml)	Mean haptoglobin (mg/dl)	Mean ceruloplasmin (mg/dl)
North American zoo						
Rhinoceroses, Group 1 ($n = 7$)						
Mean	247	271	91	2835	124	121
SD	105	99	10	2951	86	71
n	7	7	7	7	3	3
Peninsular Malaysia Center						
Rhinoceroses, Group 2 ($n = 6$)						
Mean	129	217	60	1783	95	99
SD	43	28	18	1261	39	18
n	6	6	6	6	6	6
Way Kambas Indonesia Sanctuary						
Rhinoceroses, Group 3 ($n = 3$)						
Mean	116	201	59	680	92	155
SD	39	61	14	168	12	23
n	3	3	3	3	3	3

Small sample sizes in this retrospective evaluation precluded reliable statistical analyses. Nonetheless, these observations indicate that Sumatran rhinoceroses under disparate feeding management conditions are susceptible to development of pathological body iron burdens that correlate with time in captivity, as previously demonstrated in African black rhinoceroses.[5,14,19,22-24,26,27,37]

Rhinoceroses at the Center in Peninsular Malaysia died of a suspected outbreak of trypanosomiasis in 2003, and data from that population were collected in the years prior to this mortality event.[18,40] One animal died at the Sanctuary in Indonesia from complications of posterior paresis. Among other Sumatran rhinoceroses whose necropsy materials were available for review, the most common causes of death were sepsis and/or gastrointestinal complications, such as torsions.

Hemosiderosis of varying severity in multiple organs was often noted in necropsy reports but was either considered an incidental finding common to rhinoceroses in general or attributed to previous or recent hemolytic events. Our review found histopathologic evidence of moderate to severe ISD in seven of the eight deceased animals that had been quartered for three years or more in North American and European zoos (Paglia and Radcliffe, unpubl. data), including one that succumbed to complications directly attributable to ISD (Roth, pers. comm.). By contrast, only three of eight animals deceased in Malaysia or Indonesia exhibited hepatic iron loads that could be considered moderate, with four others showing only mild to equivocal changes.

DISCUSSION

In the absence of physiological mechanisms for excretion, iron homeostasis in vertebrates is primarily maintained by modulation of dietary uptake.[7,9,10,16] This provides the rationale for Dr. Smith's original suggestion (cited in the Introduction) that disparities in iron loading between black and white rhinoceroses might be due to dietary differences between browsers and grazers, since many browse components (such as tannins, fiber, phytates, phenolics, etc.) are well known to reduce bioavailability of iron by binding it into insoluble chelates.[36,37] Some of the complex interactions of iron and dietary factors were thoroughly reviewed by Spelman et al. and postulated to be responsible for ISD acquired by lemurs in captivity.[38]

The probability that similar mechanisms might logically contribute to development of ISD in Sumatran rhinoceroses prompted us to review data available from three groups of *D. sumatrensis*, one confined to North American zoos and the other two sheltered in Southeast Asian reserves. These retrospective observations must be interpreted with caution, however, because valid statistical analyses were precluded by the scarcity of subjects, the heterogeneity of the populations and their confinement conditions, and the inability to study them prospectively with appropriate controls. Nonetheless, these findings justify concerns that dietary characteristics can influence development of ISD in this and other susceptible species, strongly supporting the need for further research in this area.

Sumatran rhinoceroses residing in native habitats have far greater nutritional variety and food choices than those confined in zoos, and iron analyte levels appear to reflect these differences in diet, location and food preferences. All iron analytes were higher in rhinoceroses housed in North American zoos and lower in animals quartered in native rainforest settings (sanctuary rhinoceroses with high browse diversity). Among rhinoceroses managed within range countries, body iron stores reflected by serum ferritin levels were lower in Way Kambas rhinoceroses than those at Sungai Dusun, consistent with greater food variety and browsing freedom in the former environment. Serum ferritin concentrations, which provide the most reliable indirect measure of total body iron stores,[2,11,12,15,35] showed far greater differences with the North American captive population (Group 1) exhibiting two- to four-fold increases compared to Groups 2 and 3. This finding is consistent with previous observations that Sumatran (like African black) rhinoceroses develop progressive overburdens of iron under captive conditions.[21-28] Ceruloplasmin levels were comparable to those found in other rhinoceros species in captivity and in the wild (Paglia, unpublished data), eliminating ceruloplasmin deficiency as a contributor to ISD. Since both are acute-phase reactant proteins, normal or low serum concentrations of ceruloplasmin support the interpretation of elevated ferritin values as reliable reflections of total-body iron loads rather than nonspecific reactions to inflammatory conditions.

The subjective histopathologic observations of this current study support the quantitative iron analyte data indicating that Sumatran rhinoceroses are highly susceptible to development of ISD when quartered in North American or European zoos compared to those in peninsular Malaysia or Indonesia. These findings suggest that disparate iron stores among various populations of Sumatran rhinoceroses may be linked to dietary differences and feeding management. The nutritional composition of browses grown in North America differs somewhat from local browses grown in Southeast Asia with the latter having higher fiber particularly hemicellulose (5% NA browse; 23% Indonesia browse), and variable iron content.[3,4] More recent comparisons of North American, Indonesian and Malaysian browses indicate that the iron content of these subtropical and tropical browses is similar. Since iron content of browses does not differ markedly among captive facilities, non-browse items may be significant contributing factors promoting iron storage in zoo rhinoceroses that receive supplemental hay forage and pelleted feeds. Equine pelleted feeds can be quite high in iron (500-1400 mg/kg DM)[20] while Mazuri® browser herbivore pellets are lower at 300–350 mg/kg DM iron.

The ability of foodstuffs to bind or chelate iron may be more important than the total iron content ingested.[9,13,33,38] Although various browses are similar in iron content, other factors including anti-nutritional chemicals (i.e., natural iron chelators) and body condition and fitness of the rhinoceroses do differ and could contribute to the observed disparities in iron stores among these populations.[6] Given the importance of anti-nutritional factors to browser rhinoceroses, the large diversity of browse species available to those managed within in situ forest sanctuaries would likely enhance natural iron chelation by offering them greater opportunity to forage on plant varieties that favor iron binding. In this regard, it is notable that two animals with the lowest iron analyte markers of ISD in Group 2, (257 and 438 ng/ml ferritin, 60% and 36% transferrin saturation, 9.3 and 16.5 yr after capture, respectively), were recorded to be the group's most avid foragers of native browse; whereas the most reluctant natural browser had the group's highest values (4,250 ng/ml ferritin and 89% transferrin saturation, 6.5 yr in captivity).

Environmental factors including measures of fitness and disease may also impact the timeline and severity of iron storage. Sanctuary, but not zoo rhinoceroses, carry notable hemoparasite burdens that are endemic, and these infections increase red blood cell loss and turnover and thereby impact iron balance. Sanctuary rhinoceroses are able to move about their rainforest enclosures (10–20 hectares) at will and these animals are consequently more heavily muscled and in better body condition than zoo rhinoceroses. One male Sumatran rhinoceros was moved back to Indonesia after 5+ yr in a North American zoological environment. Comparison of iron stores before and after transport would be useful to better understand how such rhinoceroses adapt to significant changes in diet and environment. More detailed investigation of these markedly dissimilar ex situ populations is warranted to better understand the role of nutrition in iron loading in browser rhinoceroses. Since these populations are so few in number, serial monitoring of ferritin and/or transferrin saturation beginning at birth would allow each animal to serve as its own control to evaluate rates of iron accumulation more accurately.

Today, the Sumatran rhinoceros is one of the world's most endangered large mammals. Efforts to conserve this unique and ancient species have focused primarily on physical protection of the animals and their habitats. Historically, attempts to propagate the species ex situ have been discouraging with the loss of 39 of 41 rhinoceroses originally transferred from the wild into captive breeding programs. Many of these animals died of diseases or events that can be directly attributable to inadequacies in captive husbandry and feeding management.[8] Recently, however, major advances in understanding reproductive physiology of the species have successfully resulted in several live births, justifiably acclaimed as the first in over a century.[31,32] Two of these calves in which serial specimens have been available have shown 10-fold or greater elevations in serum ferritin within three years, indicative of developing ISD, so the overall value of captive propagation as a conservation strategy for this species remains arguable.

Retrospective observations presented here suggest that higher browse diversity and/or native environmental conditions (e.g., temperature and humidity), along with seasonal variations in browses, are associated with lower metrics of iron loading in Sumatran rhinoceroses. These environmental factors can be met far better in range countries. We suggest that if captive propagation for this species continues to be viewed as a viable conservation strategy, efforts should be focused on preserving them in their native countries where suitable habitat and feeding management can be offered, possibly avoiding the rapid development of ISD pathology characteristic of conventional zoo confinements.

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