Journal of Zoo and Wildlife Medicine 43(3): S83–S91, 2012

MANAGEMENT STRATEGIES OF IRON ACCUMULATION IN A CAPTIVE POPULATION OF BLACK RHINOCEROSES (*DICEROS BICORNIS MINOR*)

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Abstract: During routine health screens for black rhinoceroses (*Diceros bicornis minor*) in a captive setting, serum iron and ferritin were analyzed as well as total iron binding capacity and total iron saturation. Trends for ferritin and percent iron saturation showed steady increases since 2003 in four of four animals (three males; one female) with two animals (one male; one female) consistently showing higher elevations over conspecifics. The historical diet had been comprised of a commercial or in-house complete pelleted feed; several species of fresh browse, Bermuda grass, alfalfa and timothy hays, as well as enrichment and training items (apples, carrots, sweet potatoes, and a small amount of leafy greens and vegetables). In 2009, one of the three male rhinoceroses showed a threefold increase in ferritin and concurrently exhibited clinical signs of lethargy, decreased appetite, and disinterest in training. The lone female showed a twofold increase; she also became reproductively acyclic in the prior year. The male was immobilized for examination and phlebotomy. During the same time period, a new version of the complete pelleted feed, with a reduced amount of iron, was introduced. Subsequent to the diet change, the male's ferritin levels have consistently declined, and the female started cycling again. Even with these corrective steps to reduce iron levels, levels of iron saturation remained high, and ferritin levels were still above 1,500 ng/ml. Therapeutic phlebotomy was instituted via a rigorous training program that allowed phlebotomies over a 30-min time frame. This was possible because of a long-term training program for the animals, consistent training personnel, routine collection of samples on a monthly basis, and general comfort level of the animals in the restraint chute. The results of this integrated approach showed some significant improvements and an overall positive impact on the animals.

Key words: Black rhinoceros, iron storage disorder, hemosiderosis, *Diceros bicornis minor*, nutrition, therapeutic phlebotomy.

INTRODUCTION

Iron accumulation (overload) in captive black rhinoceroses (*Diceros bicornis minor*) has been a consistent finding in both serologic and pathologic evaluation.[7,11,17,21] The clinical relevance of these findings has been a topic of much discussion as death has largely been due to other causes (Miller, Black Rhinoceros Species Survival Plan, pers. comm.). Additionally, for rhinoceros, hemochromatosis has not typically been the final diagnosis but rather hemosiderosis. Regardless, it is widely accepted in the veterinary community that it is still a significant disease issue of captive rhinoceroses. Accumulation of excess iron has been linked to many of the classic rhinoceros diseases, including hemolytic anemia, mucocutaneous ulcer disorder, and leukoencephalomalacia;[17,18] as well as a few lesser-known cases such as cardiotoxicity to doxorubricin.[19] It is also known that captive black rhinoceroses, including semicaptive black rhinoceroses in Africa, have excessive amounts of iron and iron metabolites, more than that of their free-ranging counterparts.[13] Etiology of iron overload in captive black rhinoceroses continues to remain a point of debate.

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CASE REPORTS

In a collection of four captive-born black rhinoceroses (three males, one female), voluntary biannual blood collection was performed for routine health assessment. Starting in 2002, iron panels consisting of ferritin, serum iron, total iron binding capacity (TIBC), haptoglobin, ceruloplasmin, and transferrin saturation (serum iron divided by TIBC) were submitted biannually on all animals (Kansas State University Diagnostic Laboratory, Manhattan, Kansas 66506, USA). All the animals, classified as male 1 (11 yr old), male 2 (10 yr old), male 3 (15 yr old), and female (17 yr old), were observed to have elevated serum

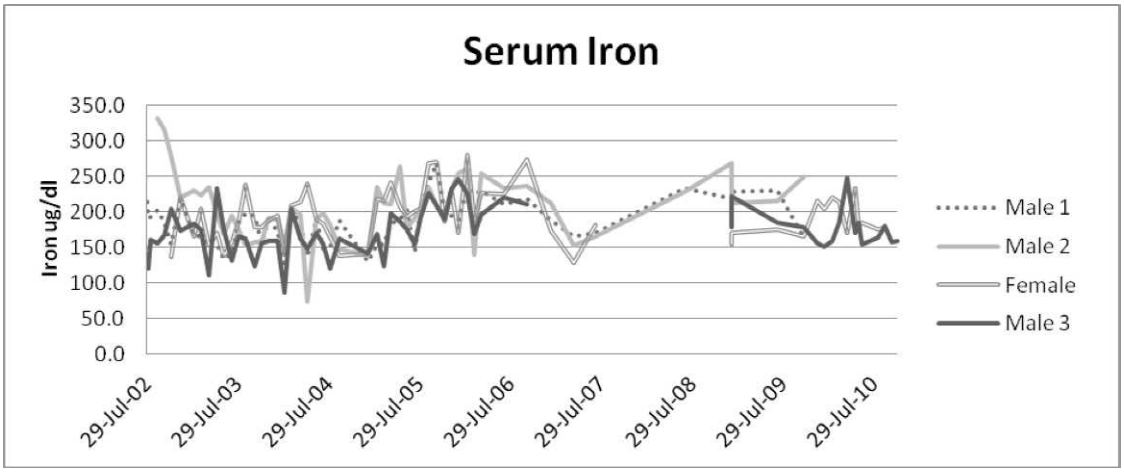

Figure 1. Total serum iron in 3:1 captive black rhinoceroses (*D. bicornis*) in a mixed-species savannah exhibit.

iron (Fe) levels (normal = 101 ± 19 μg/dL; Fig. 1), total iron binding capacity (TIBC), ferritin (normal = 133 ± 62 ng/ml; Fig. 2), and iron saturation (normal = 28 ± 6%; Fig. 3). The normal values for iron metabolites used for reference were obtained from limited data in wild animals as there are no published reference range values.[13,16] These results were consistent with excessive iron storage and prompted evaluation of the animals' clinical histories, diets, environments, and husbandry. The first goal to address these high iron levels was to decrease any potential dietary sources of iron, including environmental ones. Initially, spinach (high in iron) was eliminated in 2003 and citrus pulp (high in vitamin C, which is known to increase iron absorption) in 2006 as enrichment items from the diets, as well as eliminating the red clay wallows in the exhibit, as they were found to contain high levels of iron (13,400 ppm Fe; Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma (ICP) Radial Spectrometer, Dairy One Laboratories, Ithaca, New York 14850, USA). Two concerns about iron accumulation are: 1) the lack of natural tannins, phytate, and 2) polyphenols in the diet that would bind iron and high iron levels that are common in commercial pellets.[8,17] Investigations were initiated into developing a new pelleted diet with lower iron values, a challenging prospect based on the high iron levels (>500–1,400 ppm dry matter Fe) in many commercially processed ingredients like milled grains.[14] The lack of knowledge regarding

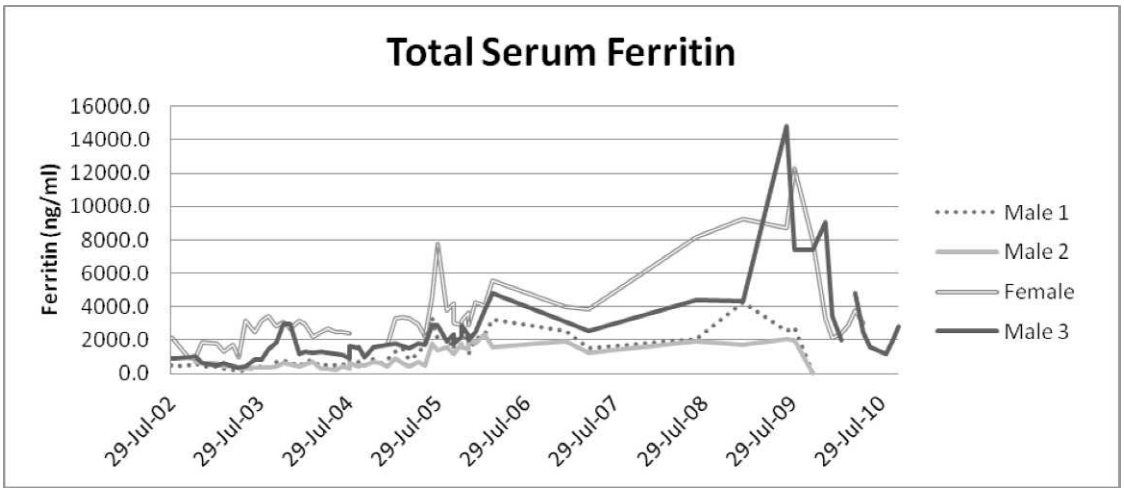

Figure 2. Total serum ferritin in 3:1 captive black rhinoceroses (*D. bicornis*) in a mixed-species savannah exhibit.

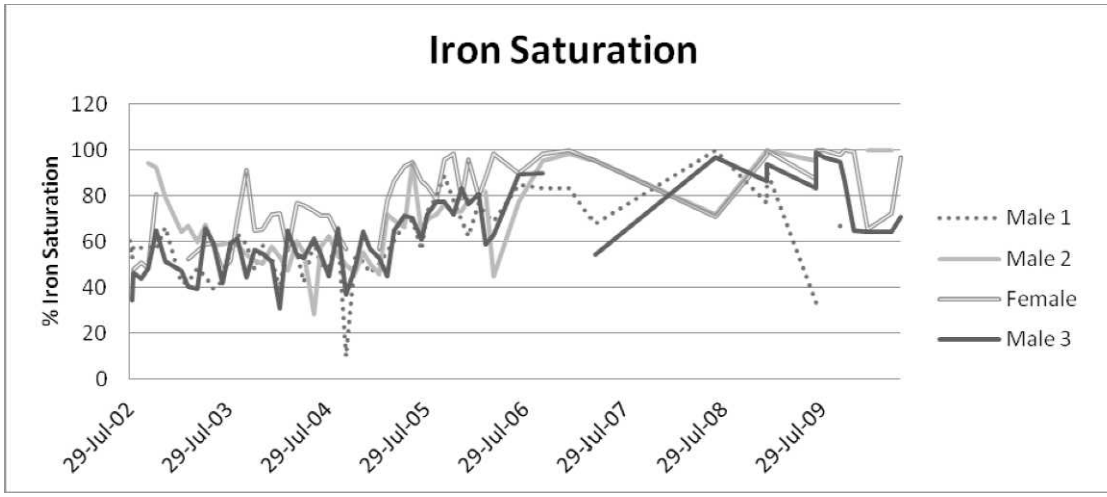

Figure 3. Total iron saturation in 3:1 captive black rhinoceroses (*D. bicornis*) in a mixed-species savannah exhibit.

the proper implementation of secondary compounds such as tannins in captive diets, continues to warrant future study, but was not an immediate course of action as some forms may be toxic or may reduce palatability.[12] Blood values continued to be monitored regularly on the animals during these changes. Overall, the values stabilized but remained high; the animals were clinically normal through 2002–2008.

Clinical cases

In 2009, one female and one male (male 3) in the group exhibited some medical issues; the other two animals continued to be normal. The female had been evaluated for its reproductive cycle for a separate study, and it was noted that the female had low fecal progesterone and thus was acyclic. This animal had normal cycles that were documented earlier in its life and had given birth to a normal calf several years prior. Ferritin and percent iron saturation were higher for this animal than its male conspecifics, but it had not exhibited any clinical signs other than being acyclic. Hematology and serum chemistries (other than iron metabolites) were considered within normal limits when compared to wild black rhinoceroses.[13,16]

In contrast, male 3 began showing signs of lethargy and abnormal training behaviors (disinterest in training sessions). Blood work at the onset of this period indicated a steep increase in ferritin in a short period of time, while all other values (other than the iron panel) stayed within normal limits. An initial attempt at standing sedation in November 2009 was unsuccessful; therefore, the rhinoceros was immobilized for a full exam and therapeutic phlebotomy. Ten liters of blood were collected from multiple venipuncture sites, including the medial radial vein, the medial saphenous, and interdigital veins over 20 min. Physical exam findings were unremarkable other than a persistent benign papilloma on the right hind limb. Within several days after the immobilization, the animal was reported to be back to exhibiting normal behavior. The subsequent blood sample collected 14 days later indicated a drop in the ferritin (Fig. 2). While it is tempting to attribute this to the phlebotomy, other factors, such as diet, were changing at this time. It also should be noted that while ferritin is a good indicator of iron stores in animals,[9,10,20,21] it is also a protein of inflammation, which confounds interpretation of these changes.

The other two males remained clinically normal in this time frame but had elevations in ferritin, serum iron, and TIBC over the wild animal data. It should be noted that the female and male 3 were also the oldest animals in the group. Liver enzymes in all the animals remained within acceptable ranges when compared to equid reference ranges. In these animals' records, iron panels were available from 2002 to the present and were collected minimally at a biannual interval. There were no iron panels for earlier time points. Even at the earliest available data points, iron and iron metabolite values of the rhinoceroses in this case series already exceeded those of other captive and free-range animals.[12]

Table 1. Measured consumption and iron content on a dry matter basis of diet items consumed by four black rhinoceroses at Disney's Animal Kingdom in July 2011 (current as of submission)

Maintenance items (fed daily)	Consumed (g/animal/day)	Fe (ppm) dry matter
Timothy hay	9,670	76
Disney's Animal Kingdom Wild Herbivore Pellet 5Z0X	6,810	306
Bermuda hay	4,280	180
Browse	~11,000	See values below
Training	~3,800	51 ± 15[a]
Enrichment	~1,800	134 ± 35[a]
Varieties of browse offered		
Willow (spring/summer months)	8,500	135
Banana (spring/summer months)	2,270	110
Elaeocarpus (winter only)	2,270	102
Ear leaf acacia (winter only)	7,500	72
Pine cones (1× a week in season)	640	251

[a] Average "Training" iron value calculated based on analysis of carrots, apples, sweet potato, watermelon, oatmeal, and low-starch primate biscuit; Average "Enrichment" iron value calculated based on analysis of cucumber, carrot, romaine lettuce, green leaf lettuce, zucchini, endive, kale, and bean paste; Both are shown as averages plus or minus standard errors.

In the historical collection, one male, not included in this case series, was euthanized due to severe liver and renal disease with hepatic hemosiderosis and glomerulonephritis. An antemortem liver biopsy confirmed moderate hemosiderosis, and sample sizes were not large enough to evaluate iron content. With this history and continued elevations of iron, liver biopsies were attempted in the animals with the highest elevations of ferritin (the female and male 3). The veterinary team was successful with one of these attempts, citing the biopsies were technically challenging due to animal size, the depth of the liver, difficulty to ultrasound, and skin thickness of the animals. Successful liver biopsy confirmed diffuse moderate hemosiderosis in the female. Muscle was obtained instead of liver in the unsuccessful sample (male 3).

Due to increasing concerns in the human medical field of immune compromise secondary to iron storage and associated secondary diseases[5] as well as relationships with captive rhinoceros disease and high levels of iron accumulation,[7,8,16,18,19] it was decided to find methods to reduce total dietary intake to prevent further accumulation and to attempt to reduce total body stores of iron.

Nutritional management

The diet had consistently comprised of a pelleted feed, timothy hay, Bermuda grass hay, fresh browse, as well as some enrichment foods that included apples, carrots, sweet potatoes, and a small amount of varied leafy greens and vegetables. The majority of the current diet consists of items low in iron with additional timothy hay and browse (Table 1). All feed items were analyzed for iron and a full mineral panel, including copper, via Thermo Jarrell Ash IRIS Advantage HX ICP Radial Spectrometer (Dairy One Laboratories). All feed items were also analyzed for a full proximate analysis as well as starch, gross energy, sugars, acid detergent, and neutral detergent fibers and lignin using the Association of Official Agricultural Chemists methodology (Dairy One Laboratories). Historically, the black rhinoceroses had been fed three different commercial pellets: Mazuri® ADF-16 (analyzed as 489 ppm Fe) when they first arrived at the park in 1997 (Mazuri PMI Nutrition International, St. Louis, Missouri 63166, USA), then transitioned to White Oak Rhinoceros Browser pellet (analyzed as 348 ppm Fe; HMS Diets, Bluffton, Indiana 46714, USA) in September of 2002, and then a formulation made in-house at Disney's Animal Kingdom (DAK; P.O. Box 10,000, Lake Buena Vista, Florida 32830, USA) by June 2003, originally called DAK High Fiber with a change in name only in 2006 to DAK Wild Herbivore (original formula analyzed as 772 ppm Fe; Mazuri PMI Nutrition International, produced from 2003). The main reason for the change from the ADF-16 diet to the browser pellet, as well as the change to the in-house wild herbivore pellet, occurred because of an ongoing interest in lowering the total starch content of the diet while providing adequate levels of digestible and indigestible fiber, especially in naturally browsing species like the black rhinoceros. High starch levels given to exotic species in the care of humans provide an unnatural dietary form of

energy that is highly absorbable and fermentable and has been associated with obesity and other health problems in a wide range of species.[6]

In October of 2009, the in-house formulated Mazuri DAK Wild Herbivore pellet was introduced to the rhinoceroses, as described in Sullivan et al.[23] This pellet was designed at DAK to have a reduced amount of iron based on a change in the basic ingredients, (analyzed as 306 ppm Fe). Iron panels were assessed at points prior to and after the introduction of this low-iron pellet. While all four black rhinoceroses showed ferritin values elevated above normal, as previously discussed, the female and male 3 had been showing marked rises in ferritin values in the year prior to the introduction of the new pellet in October 2009. Subsequent to the October 2009 diet change, the male's ferritin levels consistently declined, and the female started cycling again. Even with these positive steps, levels of iron saturation have remained high in the group. Ferritin levels remain above 2,500 ng/ml for the female and around 2,000 ng/ml for male 3, while the other two males have been under 1,000 ng/ml.

Medical management

Chelative pharmacotherapeutics were considered but were not started because of unknown reactions to the drugs, negative effects of chelators in otherwise healthy animals, and the need for daily or twice daily i.v. or i.m. administration of drug. Oral medications are available outside of the U.S. but are difficult to import and volumes for this species would be large, costly, and difficult to justify importation to out-of-country agencies. Even in treatment for humans, although parenteral drugs are available, the preferred treatment continues to be phlebotomy, only occasionally combining a parenteral chelating agent.[1,2]

Therapeutic phlebotomy in the rhinoceros is not a widely attempted treatment as it can involve serial immobilization, specialized facilities, or intensive training management. Recommendations have been outlined for the black rhinoceros,[16] and techniques have been successfully used in other large vertebrates, namely the dolphin.[10] Because the process of voluntary phlebotomy could be very involved, the initial focus was on two of the four animals to determine the feasibility of the treatment. The criteria selected for choosing which animal would undergo phlebotomy included choosing the animals that had the highest ferritin and transferrin saturations, animals that had an indication of some clinical signs associated with the condition, and animals that were ideal choices from a training perspective (good participants in operant conditioning sessions). With this in mind, the female and male 3 were chosen.

Management of venesection in humans involves an induction phase followed by maintenance therapy. The induction phase targets 7 ml/kg body weight not exceeding 550 ml, presumably based on a 70-kg human. Clinical pathologic models for success are transferrin saturations at $<75\%$ and ferritin levels of <300 ng/ml (μg/L).[3] Maintenance therapy involves regular phlebotomy, every 1–4 mo with further management based on the ferritin levels. Interestingly, guidelines for transferrin saturation are very flexible. The percentage values normally fluctuate, and as long as values are $<75\%$, they are considered below the threshold, where no toxic iron species are present systemically, and are therefore acceptable by human standards.

In establishing phlebotomy guidelines for the rhinoceroses in this collection, it was recognized that flexibility was necessary as it was unknown how much blood could be collected, how long the animals would tolerate sessions, and how the equipment would effectively facilitate phlebotomy. For a baseline, it was decided that no more than 8 L per session would be collected initially (but with an initial goal of 2 L). Each area was surgically prepped and a 17-gauge needle was used with a vacutainer collection system. The collection system setups varied based on availability of materials using either the Hospira Latex-Free Blood Collection Set, 40 inch with swaged on 17-gauge needles (patient end) and 15 gauge (bottle end; Abbott Laboratories, Abbott Park, Illinois 60064, USA) or a Mila Blood Collection Set with swivel luer lock (Mila International, Inc, Erlanger, Kentucky 41018, USA) using either an 18 or 16 gauge (patient end) with 6-foot tubing into a chamber (bottle end), which emptied into either a 500-ml, 1-L, or 2-L Braun-Baxter empty evacuated container (Braun Medical Inc, Bethlehem, Pennsylvania 18018, USA). After using this setup for a few sessions, it was improved by using a dual male adapter (Jorgensen Laboratories, Loveland, Colorado 80538, USA) that had swaged on the setup that eliminated the chamber on the Mila set. Further, technicians began using ethyl chloride topical anesthetic skin refrigerant (Gebauer's ethyl chloride medium stream spray, Gebauer Company Cleveland, Ohio 44128, USA), either applied to a gauze sponge and then immediately pressed to the skin, or sprayed directly on the skin just before needle

insertion. This improved compliance by reducing the initial discomfort during needle placement.

Training for therapeutic phlebotomy

In order to successfully pursue medical management via phlebotomy, animal compliance for voluntary blood donation was necessary. The rhinoceroses were involved in an intensive training program since their arrival at DAK, which facilitated therapeutic phlebotomy as a management option. Three components of this program benefited training for large-volume phlebotomy. The animals were already trained on blood collection from both front legs; routine samples were collected monthly. They were also trained to be comfortable in a restraint chute. Finally, both the case animals had unrelated medical procedures in the past that required participation in sessions lasting approximately 30 min.

Training specifically for voluntary phlebotomy began for both animals in September of 2009. The ear and forefoot interdigital veins are most conveniently used with rhinoceroses; however, these vessels may be prone to thrombosis under the trauma of frequent venipuncture; additionally, they are too small for large volume blood collection.[16] So the first training tasks were to change the location of needle insertion to allow access to the larger veins in the front legs, to collect from both legs simultaneously, to increase the needle size used for blood collection, and finally to desensitize animals to equipment that would enable collection of larger volumes. Additionally, the duration of sessions was increased from 30 to 45 min.

In November 2009, a standing sedation was attempted on male 3 in the restraint chute. Prior to this, voluntary phlebotomy had not been tried in either animal because training was not completed. Therefore, the purpose of the sedation was to initiate treatment, to evaluate the equipment, and to figure out a process for getting the desired volume of blood. It was deemed unnecessary to also sedate the female; the male was chosen because of the previously mentioned ferritin spike and associated abnormal behavior. Sedation was inadequate, and the rhinoceros became agitated and uncooperative and attempts to proceed with treatment were aborted; the animal was immobilized 2 wk later (described earlier). After the standing sedation attempt, the animal would not enter the restraint chute for approximately 4 mo. However, by April 2010, both animals were operant conditioned for voluntary treatment.

The target blood volume was 8–10 L per session. For the first 5 mo, volumes of 1–4 L at each session (1 to 2 sessions per month) were obtained from each animal. Despite good cooperation from the animals, higher volumes were not achieved.

DISCUSSION

The gold standard for diagnosing iron overload in most species has been liver biopsy. In human medicine there is a sequential strategy for diagnosis. The first step is to examine comparisons to baseline serum values, which show ferritin at <300 μg/L in males and <200 μg/L in females with transferrin saturations of $<45\%$.[4] The second is to document visceral iron excess via magnetic resonance imaging (MRI) with specially adapted software that allows the determination of hepatic iron concentrations; where this is not available, liver biopsy determines iron excess. The third step is to rule out acquired overload from enteral (consumption) or parenteral sources (transfusions), and finally, the fourth is to evaluate the genetic origin of the disease (the most common cause in humans).[4] Like other large mammals, it is difficult to perform liver biopsies on the rhinoceros due to their size and anatomy. Additionally, neither MRI nor genetic testing is possible at this time in rhinoceros.

Using an applied model in cetaceans, it seems plausible that biochemical parameters alone may give indication that a rhinoceros is afflicted with iron overload. In the bottlenose dolphin, *Tursiops truncatus*, cases of suspected iron overload were detected by blood work (transferrin saturation $>80\%$, increasingly high serum iron with age above population normal reference ranges, and high aminotransferases) and were responsive to phlebotomy treatment, including a return to normal serum iron and liver enzymes.[10] Historic antemortem liver biopsy data were limited in the dolphin, but those biopsy reports that were available confirmed excessive iron deposition with pathologic changes in the liver prior to phlebotomy. Currently, dolphin cases of suspected iron overload, based upon blood work alone, are being successfully treated with phlebotomy (Venn-Watson, National Marine Mammal Foundation, San Diego, California, USA, pers. comm.). While it does not appear that liver enzyme elevation was associated with hemosiderosis in the female rhinoceros (the only case confirmed by biopsy), as it was in the dolphins, it does appear that elevations of serum iron, ferritin, and transferrin saturation may correlate with hemosidero-

Table 2. Nutrient content of a black rhinoceros total diet (including browse) before and after a change solely in pellet ingredient content (Disney's Animal Kingdom formulation of Wild Herbivore) in 2009 on a dry matter basis

Analysis	Total diet before Disney's Animal Kingdom Wild Herbivore ingredient change (Sep 2009)	Total diet after Disney's Animal Kingdom Wild Herbivore ingredient change (Oct 2009)
Dry matter (%)	77.8	77.4
Crude protein (%)	10.6	10.8
Crude fat (%)	3.1	3.2
Calcium/phosphorus ratio	2.0	2.1
Neutral-detergent fiber (%)	59.3	58.9
Iron (ppm)	239.4	135.2
Vitamin E (mg/kg)	376.4	376.6

sis in the rhinoceros. Even without biopsy confirmation of hemosiderosis, it seems reasonable to otherwise follow the same paradigm in rhinoceroses as with dolphins.

Applying measurable markers of success of this cooperative treatment process was initially daunting as it was unclear how achievable it would be; human standards do not fully apply because modalities for diagnosis are unavailable for such large animals. Initially, it was hoped to see a general trend of decline in ferritin and thus lower saturation rates. In general, serum iron will remain elevated until the storage pool nears depletion.[16] Animals with chronic iron storage disorder can potentially have a large amount of accumulated iron, thus requiring lengthy treatment regimens and high blood volume phlebotomies. Ideally, repetitive phlebotomies would return the storage pool to a normal level as well as reduce the total serum ferritin. Red cell mean corpuscular volume (MCV) has been suggested as one possible marker for success; the MCV would theoretically decline, reflecting decreased availability of stored iron. It is noted that rhinoceros reticulocytes mature before leaving the marrow; therefore, erythropoietic responses are better assessed by MCV values than reticulocyte counts.[16] In MCV analyses of the four rhinoceroses, there had been no change in MCV values, likely reflecting the storage pool of iron was still high. This was further supported by continued high saturations.

The evidence of positive impact from nutritional changes and phlebotomy treatments lies in both the general trend of ferritin decreases and clinical responses to these changes. After the dietary pelleted feed change in October 2009, which was documented as the only change for the female, it was noted that she began to have normal reproductive cycles after a long period of anestrus. Although this may be coincidental, it appears to be tied into the diet change and lower ferritin levels. It also should be noted all four black rhinoceroses showed decreases in their serum ferritin levels post pellet change, although only two had undergone phlebotomy. The two younger males not undergoing phlebotomy, showed a more than 50% decrease in serum ferritin 7 mo after the pellet change (average = 2,285 ng/ml ferritin in July 2009; n = 2; average = 823 ng/ml ferritin in July 2010; n = 2). Similar effects were noted in Egyptian fruit bats, lemurs, and starlings that showed lower ferritin levels with diet modification.[9,15,20,22] There was no correlation with haptoglobin or ceruloplasmin and ferritin or transferrin saturations. Another dietary element investigated was copper, which can be linked to iron. Deficient levels of copper in the diet may upregulate gastrointestinal mineral transporters, which would allow for increased iron absorption.[8,9] However, copper levels in the diets for these animals were within normal ranges, and this was reflected in the serum. Serum copper levels of the black rhinoceroses have remained within normal ranges for these animals over the last 10 yr (1.38 ± 0.29 μg/ml) when compared to horses and free-ranging black rhinoceroses (averages 0.85–2.0 μg/ml and 1.34–1.69 μg/ml, respectively).[8]

The difference in the nutrient content of the total diet (including browse) before and after the change in pellet is shown in Table 2. The newest pellet has been formulated with the lowest iron possible, while still meeting the nutritional needs of the animal and maximizing natural dietary physical form (e.g., offering a large percentage of the diet as browse items). Feeding browse can be challenging for many institutions due to seasonal availability, cost, and labor; however, browse items likely contain higher levels of iron-binding secondary compounds (tannins, polyphenols) than any other diet item fed in a nonwild

environment. There is much debate on the effects of physical form of the diet, as well as tannin and polyphenolic availability in black rhinoceroses diets, with further research required.[12] Overall the results indicate lowering the available iron in the diet of captive black rhinoceroses may help reduce total serum ferritin values.

Overall, training the animals to be calm in chutes and allow phlebotomy for long periods of time was very successful. Issues encountered with phlebotomy included finding adequate time with appropriate staff for training exercises, reliable rhinoceros cooperation for each session, and equipment challenges. Regardless, both rhinoceroses participated in phlebotomy sessions regularly. This was possible because of one key factor: consistently using the same two trainers and one veterinary technician for each session.

Challenges encountered with training included animal refusal to enter the chute, maintenance of animal focus and interest for prolonged sessions, and the animal's physical comfort after standing in one place for more than 30 min. To circumvent boredom, the animals were kept mentally stimulated by changing the trainer at the head of the animals, alternating which trainer asked for a behavior, and using reinforcement items that were increasingly more appealing, with the best items presented last. Animals were also regularly placed in the chute at times other than those for medical treatments. To alleviate staff concerns of physical discomfort, mats and headrest platforms were added to the chute. All these combined changes resulted in the increase of the sessions from 20 to 90 min.

Even though the animals did well with length of time in the chute and manipulation of blood vessels for treatments, low-volume blood collections continued to be a problem. Reasons for this were attributed to 1) the limitations of the equipment (collection bottles losing their vacuum midprocedure), 2) tubing from the animal to the bottle collapsing, and 3) needle size (17 gauge was the widest bore tolerated by the animals). Poor flow rate was additionally attributed to animal movement, which would slow the rate or stop it completely. Speculation about other issues with large-volume collection included abutment of the bevel of the needle in the vessel or clot formation. In general, 2–4 L were collected from each animal at each procedure.

In order to improve blood flow during phlebotomy, other options are being explored that include using larger bore needles or catheters, obtaining larger bore or stiffer tubing, safely obtaining blood from the hind legs as well as the front legs, and modification of a cardiac bypass machine or a large animal i.v. fluid administration unit to facilitate removal of blood.

CONCLUSIONS

Phlebotomy, in combination with nutritional changes, has been historically successful in a number of species in reducing the impact of disease associated with iron storage and seems to have had the same effect in this population of rhinoceros as well. Preventing further accumulation of iron by diet modification in combination with phlebotomy to reduce extant body stores has made a positive impact on this group. The larger the patient, the more technically difficult medical and nutritional management can be, especially in terms of reducing iron levels in the diet of a large herbivore, while meeting energy needs and stimulating gastrointestinal health. This report outlines the challenges and successes of medical and nutritional management strategies in managing iron storage in a small captive population of black rhinoceroses.

Acknowledgments: The authors would like to extend immense gratitude to Julia Sweet, C.V.T., Laura Wheeler, C.V.T., and Shana Lavin, Ph.D., for their contributions to the treatments and editorial guidance on this paper. Thank you also to Michelle Miller, D.V.M., M.S., Ph.D., who provided veterinary care and expertise to the animals in their earlier history. Additional acknowledgments go to Lisa Harrenstein, D.V.M., for sharing her experiences and Catherine Wheaton, Ph.D., for endocrinology input as well as the following teams for their day-to-day care of the animals: the Ituri Forest team: Jeremy Neufeld, Brandon McDermed, Brian Williams, Niki Harris, and Brandy Souders; as well as the Animal Nutrition Center team at Walt Disney World's Animal Kingdom.

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Received for publication 5 April 2012