Black Rhinoceros

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Black rhinoceros under human care are predisposed to Iron Overload Disorder that is unlike the hereditary condition seen in humans. We aim to address the black rhino caretaker community at multiple perspectives (keeper, curator, veterinarian, nutritionist, veterinary technician, and researcher) to describe approaches to Iron Overload Disorder in black rhinos and share learnings.

This report includes sections on (1) background on how iron functions in comparative species and how Iron Overload Disorder appears to work in black rhinos, (2) practical recommendations for known diagnostics, (3) a brief review of current investigations on inflammatory and other potential biomarkers, (4) nutrition knowledge and advice as prevention, and (5) an overview of treatment options including information on chelation and details on performing large volume voluntary phlebotomy. The aim is to use evidence to support the successful management of this disorder to ensure optimal animal health, welfare, and longevity for a sustainable black rhinoceros population.

Keywords: chelation ; ferritin ; hemochromatosis ; hemosiderosis ; oxidative stress ; phlebotomy ; transferrin saturation

1. Introduction

Black rhinoceroses (BR; *Diceros bicornis*) under human care are predisposed to non-hemochromatosis Iron Overload Disorder with laboratory and histopathologic evidence of cellular injury, necrosis, and clinical signs similar to human iron overload disorders [1][2]. BR are native to eastern and central Africa and are Critically Endangered [3]. Poaching has reduced the wild population by >90% since 1970, and ~240 individuals are managed under human care with ~87 individuals in North America [4]. Over the last 30 years, BR have been documented with diseases that have either been induced by or exacerbated by IOD, prompting significant efforts in diagnostic, treatment, and prevention strategies [2][5][6].

Iron overload is an abnormal and chronic imbalance of iron metabolism with iron accumulation occurring over the course of years, saturating iron transport proteins and leading to organ damage and failure (reviewed in [7]). BR can live many years with IOD and typically do not show overt signs of illness until late in disease progression resulting in a shortened life span and reduced fecundity in this endangered species [8]. A lack of acute clinical symptoms in this species is not an honest marker of animal health nor iron balance.

IOD is a multi-factorial disease process requiring an evidence-based and integrative approach for successful prevention and treatment. The aim of this report is to highlight accumulated evidence supporting the successful management of this disorder to ensure optimal animal health, welfare, and longevity. Specifically, as representatives of the Center for the Study of Iron in Rhinos (CSI-R), the authors will provide specific practical recommendations to treat this disorder in BR. Strategies and recommendations build on the collective expertise of colleagues and empirical data from experiences in BR nutrition, clinical veterinary medicine, husbandry, and operant conditioning. This report also integrates evidence from human medicine (Figure 1), as iron overload is a common clinical problem [9], and the management of the disorder has been studied extensively; thus, we use these learnings to supplement our strategies in BR under human care.

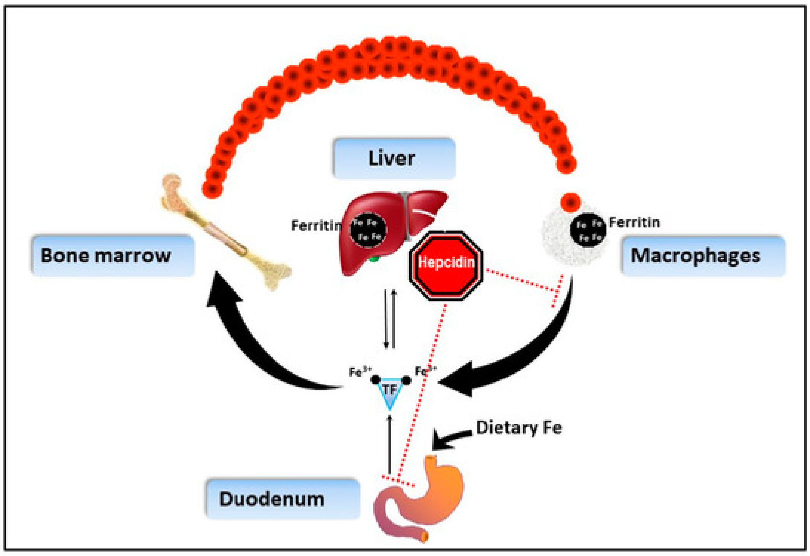

Figure 1. Regulation of iron homeostasis in humans (adapted and simplified from Knutson and Wessling-Resnick 2003) [10]. Dietary iron is absorbed in the duodenum, part of the small intestine. After regulated passage into the body, iron is transported primarily on transferrin (TF) to the bone marrow for red blood cell production. As red blood cells are broken down by phagocytic macrophages where iron is contained in ferritin (black circles), iron is primarily recycled to the bone marrow. Excess iron is stored in the liver, mainly within ferritin protein, until needed. Hepcidin, the iron regulating peptide hormone produced by the liver, blocks entry of iron from the small intestine and release of iron from macrophages by signaling the internalization of transport protein ferroportin. Once iron has entered the body, there is no route of excretion except forms of blood loss.

2. Evidence-Based Nutrition Practices

Nutrition is an integral component of preventing IOD; thus, the assessment of dietary iron concentration and other nutritional factors is critical for iron balance in BR. BR likely evolved with a low-iron diet due to low iron concentrations (<215 ppm) [11] and limited availability of iron in leaves and stems of browse plants that contain high concentrations of iron-binding phenolic compounds [12][13]. The diet commonly offered to BR often has excessive iron concentrations (>300 ppm) as well as a poor representation of the nutrient and plant defense chemical compositions (e.g., polyphenols and alkaloids) found in wild browse [14]. The source of high iron concentrations in diets is pelleted feed, as dietary constituents and soil contamination can produce feeds high in iron, even when formulated to be low in iron (e.g., <200 mg/kg) [5][15][16][17][18]. It is not practical and may not be possible to provide a nutritionally complete diet under human care for BR without a pelleted energy source [19]. Furthermore, exhibit soil and vegetation can contain variable concentrations of iron, including those in excess to BR [5][20].

2.1. Could We Make the Diet More Like the Wild?

Studies on adding iron-binding compounds (e.g., polyphenols and concentrated tannins) to BR diets thus far lack compelling evidence to warrant inclusion, though no negative effects were found [21][22]. A better understanding of the biological activity of phenolic and tannin compounds is needed before routinely incorporating them. Naturally occurring iron-binding compounds such as those found in tea, grape pomace, quebracho, curcumin, etc., vary widely in composition and can bind other minerals such as zinc, copper, and calcium, further complicating nutrient balance. While supplemental iron-binding compounds and iron chelators can reduce iron absorption, the benefits should outweigh risks and must be considered with welfare and health outputs monitored. Assessing the nutrient bioavailability in vivo is invasive and obtaining total urinary and fecal iron output is challenging; these constraints hinder the development and subsequent inclusion of dietary iron-binding compounds in BR diets.

2.2. Has Diet Ever Changed the Impact of IOD?

There is evidence that a diet formulated to be low in iron can improve biomarkers of iron in BR. In particular, a reformulated pellet made by the Disney Animal Kingdom® Nutrition team with Mazuri Exotic Animal Nutrition®, with decreased iron concentration from 772 mg/kg to 222–306 mg/kg, and a reduction of the amount of pellet offered to less than 30% of the total diet in four BR impacted biomarkers [19]. These nutritional modifications resulted in a total dietary iron concentration of 135 mg/kg. Thus, the majority of the diet was forage, with about 30% as fresh browse. The overall diet, including the reformulated pellet, was high in fiber (neutral detergent fiber (NDF) = 58.9%), and thus more consistent with a wild diet. The four BR consumed the reformulated diet, including two rhinos that were markedly iron-overloaded (ferritin >4000 ng/mL and transferrin saturation at 100%) [19]. Within a month post-diet change, serum ferritin decreased in all four animals, regardless of concomitant phlebotomy [23]. The other two younger animals showed reduced IOD biomarkers. Currently, both animals show no evidence of IOD compared to age-matched conspecifics under a more traditional diet. These results highlight the significant impact that diet modification can have on iron loading in the BR (Figure 2 and Figure 3).

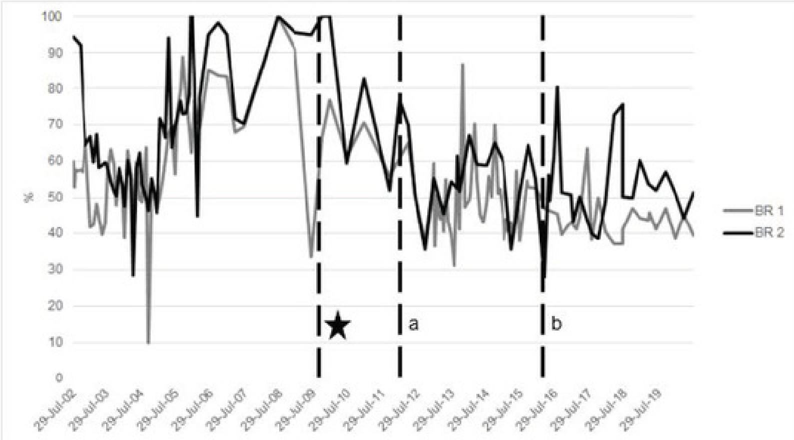

Figure 2. Transferrin Saturation (%) across time of two male black rhinoceros (BR1, BR2) at Disney's Animal Kingdom®. * Major diet change occurred on 11 October 2009 for both animals. [a] Phlebotomy began on BR1—March 4, 2012; [b] Phlebotomy began on BR2—20 May 2016.

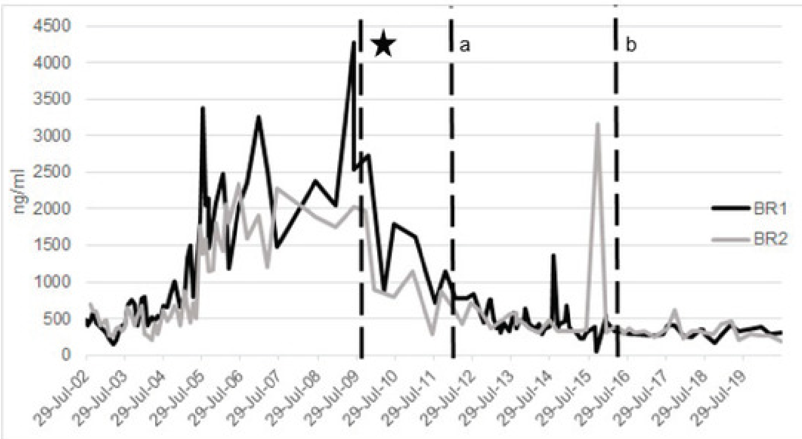

Figure 3. Serum ferritin (ng/mL) across time of two male black rhinoceros (BR1, BR2) at Disney's Animal Kingdom®. * Major diet change occurred on 11 October 2009 for both animals. [a] Phlebotomy began on BR1—4 March 2012; [b] Phlebotomy began on BR2—20 May 2016.

2.3. What Are the Best Practices for Feeding Black Rhinos?

An appropriate diet for BR maximizes animal welfare, lifespan, and reproductive output. Recommendations for feeding must consider not only iron but also the many interconnected nutrients and metabolic concerns which may exacerbate iron-related issues, such as obesity. Based on empirical evidence, the following specific feeding guidelines for BR are recommended [23]:

- **Iron should be limited.** Iron concentration in the diet is recommended to not exceed 300 mg/kg dry matter (DM) or about 6 g of iron per day for a 1300 kg BR fed 1.5% BW in DM [5]. Based on the availability of feed items such as low-iron pelleted feed and browse (Table 1), a dietary iron concentration less than 300 mg/kg (dry matter basis) is a practical recommendation for BR [5][24]. Consider testing exhibit soil and vegetation to ensure they are not significant sources of iron in the diet of BRs [5][20].
- **Monitor individual body weight and body condition.** Feed 1–3% of body weight (BW) on an as fed basis, 1–2% on a dry matter basis. Maintaining appropriate body weight is critical as iron imbalance and obesity are presumed to be related; iron balance also is implicated with metabolic syndrome and associated negative health impacts [25]; however, the exact mechanism is not yet clear. Individual assessment of BR for bodyweight regularly across time (ideally weekly) and tracking with diet consumption is recommended [23][14]. Body condition scoring systems (e.g., a 1–5 scoring system based on ~seven body areas) are subjective, with varied recommendations on what is considered ideal depending on

housing conditions along with medical and physiological considerations. Typically, a score of 3–4 out of a 5-point scale is considered ideal under human care. [26][27]. To optimize health, adjust diets as needed to ensure animals are within a target body weight range set for the individual animal.

- **Feed at least twice daily.** It is recommended to feed pellets in two feedings each day with forage to ensure maximum absorption of macronutrients [24]. Iron is not the only nutrient to consider in feeding complex diets to rhinos under human care. A single feeding would not be ideal for multiple reasons, including digestive efficiency, microbial community maintenance, satiety, and natural foraging behaviors.

- **Feed appropriate pelleted feeds**. The pellets milled for zoo animals vary widely in nutrient composition, and not all available pellets are appropriate for browsing species. Pellet formulations for BR are recommended to be high in fiber and low in starch and soluble sugars (Neutral Detergent Fiber (NDF) = 40–60%) [5]. Starch and sugar must be limited as these items can be associated with severe dental plaque, have metabolic impacts, and contribute to obesity [23][28][29]. A maximum of one-third of total calories is recommended to come from a pelleted concentrate. This limit avoids high pellet inclusion rates, which could be negative for dental health, body weight, and proper digestive health due to lack of long particle fibers [13].

- **Alfalfa hay should be limited**. This recommendation is due to its high protein, calcium, and iron, which can also create diarrhea and colic [5][23][24]. Conversely, low-quality hay (straw, wet/moldy, low nutrient content) is also not recommended due to the risk of intestinal impaction and/or colic. The iron in alfalfa is also held in a potentially highly bioavailable form (plant ferritin) [23].

- **Maximize browse and provide access to hay**. Preferably high quality roughage, ideally grass hay — not legume-based, as well as clean water and salt *ad libitum* [24]. Browse options may vary based on season and region, with options to freeze or ensile [27][30]. As browse best approximates the natural physical form of BR diets; it has the potential for iron-binding [31].

- **Total dietary vitamin E concentrations should be 150–200 IU/kg diet.** Extra supplementation may be necessary in addition to vitamin E in pelleted feed dependent on serum evaluation [32]. Vitamin E is a critical antioxidant that protects against ROS created by and including iron [23][33][34]. As BR lack some natural antioxidant production, ensuring dietary alpha-tocopherol (vitamin E) serves as a necessary preventive [23][33][34].

- **Phosphorus levels in the serum should be monitored and supplemented where appropriate.** BR have a predisposition to deficiency and continued concern for hemolytic issues; additionally, there is a link between phosphorus and iron metabolism [8][35]. Supplementation of monosodium phosphate and/or wheat bran in addition to phosphorus provided in a pelleted diet is recommended based on serum assessment. Naturally low phosphorus carriage in BR RBC (2–5% other mammals) [34] is thought to be connected to RBC fragility and potentially elevated RBC turnover [8][36][37]. In support of supplementation, higher levels of dietary phosphorus have been documented to combat anemic hemolytic crises in this species [8][35][37].

- **The calcium to phosphorus (Ca:P) ratio of the diet should be 2:1 (no less than 1:1).** A well-formulated pellet will provide appropriate calcium and phosphorus to meet the nutrient requirements of BR. An appropriate ratio eliminates the need for calcium supplementation, which can be contaminated with iron [16]. Grass hay typically is 1:1 and alfalfa 3:1, the latter of which can lead to hypercalcemia and hypophosphatemia. The amount of phosphorus added as a supplement should not unbalance the Ca:P ratio in the diet, in the amounts recommended based on body weight. The diet is balanced primarily with the pelleted portion, which is the majority of the dry matter of the diet and typically has the optimal 2:1 ratio (Table 1). Inverted serum Ca:P ratios are incredibly rare in rhinos; instead, hypercalcemia cases are far more common. As black rhinos physiologically appear to have an increased need for phosphorus, which is utilized for RBC turnover, they appear able to maintain serum Ca:P ratios of 2:1 despite a potential intake between 1:1 to 2:1.

- **Avoid non-specific mineral supplements and mineral salt blocks.** Plain salt blocks have minimal to no iron content and are appropriate [23].

- **Limit high vitamin C diet items (e.g., citrus fruits)**. Also, avoid feeding these foods at the same time as pellets due to increased iron availability in the presence of acidic foods such as vitamin C (Table 1) [38].

- **Training and enrichment diet items should be low in sugar, starch, and iron.** Target less than 10% of the total diet comprising of training and enrichment foods. Take into consideration high-sugar, high-starch, and high-iron items (such as molasses-based foods), which often are included in balanced diets for BR (Table 1) [23].

Table 1. Moisture (%), dry matter (DM;%), iron (ppm DM), and vitamin C (mg/100 g as fed; AF), calcium (%DM), and phosphorus (%DM) values in example diet items commonly used for black rhinoceros. Feed composition can be quite variable depending on harvest location, manufacturer, and season. Nutrients included in the table are not sufficient to balance an animal's diet or to evaluate inclusion as training or enrichment food items; thus, it is recommended to consult a nutritionist. For example, although bananas are lower in iron and vitamin C than cauliflower, bananas are high in starch (~23% DM) and thus should be limited in favor of metabolic health. Another cautionary example is appreciating the high moisture in produce such as leafy greens, which dilutes nutrient concentrations in the AF product. Thus, produce items generally are not used to balance nutrient concentrations such as calcium or phosphorus.

	Moisture	Dry Matter	Iron	Vitamin C	Calcium	Phosphorus
Food Item	**%**	**%**	**ppm (DM) ***	**mg/100 g (AF) ****	**(%DM)**	**(%DM)**
Pelleted feed examples:						
Mazuri ADF 25 Herbivore diet	10.5	89.5	652	nd	1.51	0.95
Mazuri ADF 16 Herbivore diet	8.2	91.8	490	nd	1.16	0.84
Mazuri Browser Rhino Cube 5Z1P	10.4	89.6	222	nd	1.22	0.68
Produce examples:						
Cucumber (raw, whole)	97.6	2.4	86	3.2	0.89	1.35
Carrot (raw, whole)	88.9	11.1	35	5.9	0.31	0.25
Celery (raw, whole)	95.1	4.9	29	3.1	0.98	0.51
Sweet potato (raw, whole)	79.8	20.2	22	2.4	0.36	0.29
Apple (raw, whole)	88.0	12.0	11	4.6	0.05	0.08
Produce with higher vitamin C or iron level examples:						
Green Leaf lettuce (fresh, raw)	94.9	5.1	278	9.2	0.74	0.66
Spinach (fresh, raw)	91.4	8.6	264	28.1	1.02	0.73
Romaine (fresh, raw)	95.6	4.4	152	11.5	0.73	0.67
Green beans (fresh, raw)	92.2	7.8	101	12.2	0.55	0.53
Cantaloupe melon (fresh, whole)	93.1	6.9	74	36.7	0.11	0.17
Cauliflower (raw, whole)	94.0	6.0	60	48.2	0.55	0.75
Tomatoes (raw, whole)	95.7	4.3	53	13.7	0.18	0.47
Honeydew melon (fresh, whole)	91.6	8.4	37	18.0	0.11	0.33
Watermelon (fresh, whole)	92.3	7.7	33	8.1	0.14	0.33
Banana (raw, whole with peel)	82.4	17.6	15	8.7	0.07	0.12
Orange (raw, whole with peel)	82.6	17.4	14	71.0	0.57	0.13
Hay/Fresh Browse examples:						
Alfalfa Hay	12.0	88.0	386	nd	1.84	0.31
Coastal Bermudagrass Hay	10.0	90.0	52	nd	0.57	0.19
Timothy Hay	11.0	89.0	48	nd	0.54	0.09
Mulberry (whole branch fresh)	67.6	32.4	84	nd	1.7	0.38
Willow (whole branch fresh)	63.2	36.8	63	nd	0.77	0.13
Spineless cactus pads (Opuntia)	91.5	8.5	22	nd	3.67	0.17
Supplement examples:						
Dicalcium Phosphate	4.6	95.4	12,200	nd	23.4	19.7

	Moisture	Dry Matter	Iron	Vitamin C	Calcium	Phosphorus
Food Item	**%**	**%**	**ppm (DM) ***	**mg/100 g (AF) ****	**(%DM)**	**(%DM)**
Trace mineral block	**0.1**	**99.9**	**1790**	**nd**	**0.3**	**0.05**
Dried Beet Pulp with molasses	**7**	**93**	**731**	**nd**	**1.15**	**0.1**
Molasses	**8.0**	**92.0**	**577**	**0.0**	**0.18**	**0.02**
Wheat bran	**8.4**	**91.6**	**186**	**nd**	**0.15**	**1.48**
Steamed Rolled Oats	**8.5**	**91.5**	**41**	**0.0**	**0.05**	**0.48**

* Dry matter, moisture, calcium, phosphorus, and iron determined on feed at Disney's Animal Kingdom®, analyzed by Dairy One Laboratories (Ithaca, NY). ** Vitamin C values sourced from the USDA database (https://www.nal.usda.gov/usda-food-composition-database). "nd" indicates the nutrient value for the food item was not determined.

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