Journal of Zoo and Wildlife Medicine 43(3): S108–S113, 2012

IOD IN RHINOS—NUTRITION GROUP REPORT: REPORT FROM THE NUTRITION WORKING GROUP OF THE INTERNATIONAL WORKSHOP ON IRON OVERLOAD DISORDER IN BROWSING RHINOCEROS (FEBRUARY 2011)

Marcus Clauss, Ph.D., D.V.M., Ellen Dierenfeld, Ph.D., M.S., Jesse Goff, D.V.M., Ph.D., Kirk Klasing, Ph.D., Liz Koutsos, Ph.D., M.S., Shana Lavin, Ph.D., M.S., Shannon Livingston, M.S., Brian Nielson, Ph.D., Michael Schlegel, Ph.D., P.A.S., Kathleen Sullivan, M.S., Eduardo Valdes, Ph.D., and Ann Ward, M.S.

INTRODUCTION

Iron overload disorder (IOD) has been identified in browser rhinoceros species (eg. black rhino, *Diceros bicornis*; Sumatran rhino, *Dicerorhinus sumatrensis*), although the mechanism by which IOD occurs in these species is unclear. It is known that browser rhino species are susceptible to this disorder; however, grazing rhino species (eg. White rhino, *Ceratothenum simun*; Indian rhino, *Rhinocerus unicornis*) are not as susceptible. Although the horse has been reported to be susceptible to IOD, the incidence appears to be much less frequent, making the horse a questionable model for iron metabolism and IOD in browser rhinos.

This report provides feeding recommendations for browser rhinos maintained under the care of humans as well as directions for future research efforts.

CURRENT DIETS

It is recognized that diets fed to captive browser rhinos often contain levels of iron (Fe) that are in excess of the estimated requirements for several reasons. First, hays and grasses contain variable Fe content (legume and grass forage ranged from 10 to 2,599 mg Fe/kg forage[1]) as a result of growing conditions (e.g., soil pH), use of nitrogen or phosphorus fertilizer (generally resulting in a linear increase in plant Fe concentration),[7] or soil contamination of these forages.[8] The relative bioavailability (RBV) of Fe from soil is presumed to be low, but in vitro work[5] indicates that it can become soluble in the rumen and may be available for absorption in the small intestine. Furthermore, exposure to acidic environments, such as that found during the ensiling process, increases the solubility of Fe from soil sources.[5]

Second, ingredients that may be fed to browser rhinoceros species as a component of pelleted feeds may also contribute significant Fe levels to the total diet. For example, dicalcium phosphate can be a significant source of Fe, and RBV of Fe from limestone and dicalcium phosphate is approximately 50% that of ferrous sulfate.[6] Other ingredients may contribute significant Fe as well: corn grain can contribute 10–464 ppm Fe, beet pulp 85–600 ppm Fe, and soybean meal 110–240 ppm Fe.[1] The RBV of Fe from feedstuffs is generally about 30–70% that of ferrous sulfate or ferric chloride[6,7] and is dependent on factors such as the level of inhibitory compounds, such as phytates and polyphenolics, in the feedstuff.[9] Legumes containing ferritin, such as soybeans, provide relatively available Fe (similar to ferrous sulfate) in rodent and human models.[11] However, the actual absorption efficiency for Fe is dependent on the Fe status of the animal,[7] and the

From the Clinic of Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterhurerstr. 260, 8057 Zurich, Switzerland (Clauss); Novus International, Inc., 20 Research Park Dr., St. Charles, Missouri 63304, USA (Dierenfeld); The College of Veterinary Medicine, 2048 Vet Med. Iowa State University, Ames, Iowa 50011, USA (Goff); The Department of Animal Science, University of California, Davis, California 95616, USA (Klasing); Micronutrients/Mazuri Exotic Animal Nutrition, Danforth Dr., Gray Summit, Missouri 63039, USA (Koutsos); Disney's Animal Kingdom, 180 N. Savannah Circle, Lake Buena Vista, Florida 32830, USA (Livingston, Sullivan, Valdes); Disney's Animal Kingdom/University of Florida, Department of Animal Science, 180 N. Savannah Circle, Lake Buena Vista, Florida 32830, USA (Lavin); The Department of Animal Science, 74 S. Shaw Ln., Michigan State University, East Lansing, Michigan 48824, USA (Nielson); San Diego Zoo and Wild Animal Park, 2920 Zoo Drive, San Diego, California 92101, USA (Schlegel); and the Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, Texas 76110, USA (Ward). Correspondence should be directed to Dr. Koutsos (Liz.Koutsos@Mazuri.com).

Table 1. Sample diets for browser rhinos.[a]

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredient					
Produce (%)	5.00	5.00	5.00	5.92	16.60
Herbivore pellet (%)[b]	20.00	20.00	20.00	20.14	10.00
Alfalfa hay (%)	35.00	30.00	30.00	0.00	0.00
Coastal hay (%)	40.00	25.00	10.00	0.00	0.00
Bermuda hay (%)	0.00	0.00	0.00	12.68	36.70
Timothy hay (%)	0.00	0.00	0.00	28.64	0.00
Sudangrass hay (%)	0.00	0.00	0.00	0.00	36.70
Browse (%)[c]	0.00	0.00	0.00	32.61	0.00
Triple Crown Safe Starch (%)[d]	0.00	20.00	35.00	0.00	0.00
Total (%)	100.00	100.00	100.00	100.00	100.00
Nutrient (dry matter basis)					
Crude protein (%)	17.88	17.06	16.66	12.18	12.03
Acid Detergent Fiber, ADF (%)	28.85	28.63	28.32	33.97	32.54
Neutral Detergent Fiber, NDF (%)	49.25	46.49	43.03	55.75	58.72
Starch (%)	nr	nr	nr	2.87	nr
Ethanol-soluble carbohydrates (%)	nr	nr	nr	2.41	nr
Water-soluble carbohydrates (%)	nr	nr	nr	3.06	nr
Crude fat (%)	2.61	3.34	3.92	2.58	2.14
Iron (ppm)	300	302	310	207	237
Calcium (%)	1.07	1.11	1.18	0.75	0.58
Phosphorus (%)	0.32	0.34	0.36	0.32	0.29
Magnesium (%)	0.29	0.34	0.38	0.23	0.29
Potassium (%)	1.89	1.99	2.14	1.49	2.00
Sodium (%)	0.26	0.33	0.40	0.22	0.21
Iron (ppm)	300	302	310	135	237
Zinc (ppm)	53	72	87	89	69
Copper (ppm)	15	19	23	15	17
Manganese (ppm)	64	74	80	81	67
Selenium (ppm)	0.21	0.21	0.21	0.17	0.27
Vitamin A (IU/kg)	4,232	6,034	7,436	4,320	8,983
Vitamin D3 (IU/kg)	269	703	1040	280	481
Vitamin E (IU/kg)	90	139	177	240	113
Thiamin (ppm)	2.27	2.31	2.35	nr	9.50
Riboflavin (ppm)	3.41	3.48	3.54	nr	4.00
Pantothenic acid (ppm)	4.60	4.69	4.76	nr	10.00
Niacin (ppm)	9.25	9.44	9.59	nr	23.00
Choline (ppm)	273	279	283	nr	456
Biotin (ppm)	0.04	0.11	0.16	nr	0.04

[a] nr = Not reported.
[b] Herbivore pellet varies in iron (Fe) content: Diets 1–3 contain 345 ppm Fe dry matter basis (DMB); diet 4 = 306 ppm Fe DMB; diet 5 = 242 ppm DMB.
[c] See Table 2.
[d] Wayzata, Minnesota.

absolute values obtained in rodent, chick, or human trials may not be directly applicable to browser rhinoceros species. Nonetheless, relative values may be very useful for comparisons across species.

Finally, it has been shown that wild-type diet components of browser rhinos are generally lower in Fe than are diets offered in captivity, and they often contain compounds such as phenolics that may reduce the relative bioavailability of this nutrient. For example, browse consumed by Black rhinoceros in Zimbabwe contained 29–215 ppm Fe (dry matter basis [DMB]),[3] although browse fed to Sumatran rhinos contained 45–1,400 ppm Fe (DMB), with a mean of 314 ppm Fe.[4]

Based upon this knowledge, the Nutrition Working Group identified specific actions that may mitigate the onset or perpetuation of iron overload disorder (IOD) and that were based on the primary goal of reducing the dietary Fe concentration.

Table 2. Potential varieties of browse consumed by browser rhinos.

Scientific name	Common name	Iron (Fe; ppm dry matter basis [DMB])	Comments	References
Acacia spp.	Acacia	100		Graffam et al., 1997
Acacia adsurgens		49		Disney's Animal Kingdom (DAK), unpubl. data
Acacia auriculiformis	Ear-leafed acacia	41–96		DAK, unpubl. data
Acacia catechu	Mimosa catechu			
Acacia dealbata	Silver wattle	81		DAK, unpubl. data
Acacia farnesiana	Needlebush, huisache	126–553		Graffam et al., 1997; Ward et al., 2001, DAK, unpubl. data
Acacia holosericea	Soapbrush wattle	44–137		DAK, unpubl. data
Acacia longifolia	Golden wattle	24–52		DAK, unpubl. data
Acacia podalyriifolia	Pearl acacia	59		DAK, unpubl. data
Acacia roemeriana	Catclaw acacia			Graffam et al., 1997
Acacia saligna	Blue-leaf wattle	27–76		DAK, unpubl. data
Acer negundo	Box elder	57–383		Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998, Ward et al., 2001
Acer saccharinum	Silver maple	50–711	Fe depends on part	Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998
Acer saccharum	Sugar maple	70–358	Fe depends on part	Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998
Arundo spp.	Cane	43–92		DAK, unpubl. data
Baccharis halimifolia	Groundsel tree			
Bauhinia purpurea	Butterfly tree			
Bucida buceras	Black olive			
Celtis spp.	Hackberry	65–275	Fe depends on part	Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998, Ward et al., 2001
Eleocarpus spp.	Quandong	56–102	Winter only	DAK, unpubl. data
Elaeagnus spp.	Silverberry	71		DAK, unpubl. data
Eleagnus angustifolia	Elliagnus	187		Ward et al., 2001
Elaeagnus pungens	Thorny olive	26–34		DAK, unpubl. data
Ficus spp.	Fig	64		DAK, unpubl. data
Ficus benjamina	Ficus	37–87		DAK, unpubl. data
Helianthus annuus	Sunflower	44–46		DAK, unpubl. data
Hevea brasiliensis	Rubber tree	35–41		DAK, unpubl. data
Hibiscus acetosella	Hibiscus	12–53		DAK, unpubl. data
Hibiscus rosasinensis	Hibiscus			
Hibiscus sabdariffa	Hibiscus	12–41		DAK, unpubl. data
Ipomea batatas	Sweet potato	17–51		DAK, unpubl. data
Laurus nobilis	Bay laurel, sweet bay			
Ligustrum japonicum, L. *vulgare*	Japanese privet	114		Ward et al., 2001
Liquidambar styraciflua	Sweetgum	55–269	Fe depends on part	Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998; DAK, unpubl. data
Liriodendron tulipifera	Tulip tree			
Lonicera spp.	Honeysuckle			
Manihot esculenta	Cassava	194		DAK, unpubl. data
Malus spp.	Crabapple			
Morus spp.	Mulberry	52–256	Fe depends on part	Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998, Ward et al., 2001
Morus alca	White mulberry	123		DAK, unpubl. data
Musa acuminata	Banana	110	Spring and summer	DAK, unpubl. data

Table 2. Continued.

Scientific name	Common name	Iron (Fe; ppm dry matter basis [DMB])	Comments	References
Musa paradisiaca				
Myrica cerifera	Wax myrtle			
Nyssa sylvatica	Black tupelo, black gum			
Panicum hemitomon	Maidencane			
Persea borbonia	Red Bay	60		DAK, unpubl. data
Phoenix reclinata	Clumping palm	96–101		DAK, unpubl. data
Phyllostachys spp.	Bamboo	45–139	Fe depends on part	Dierenfeld, 1997; Ward et al., 2001
Pinus spp.	Pines			
Pinus spp.	Pine cones	251	1 time a week in season	DAK, unpubl. data
Plantanus occidentalis	American sycamore			
Populus deltoides	Cottonwood	76		Ward et al., 2001
Prosopis juliflora	Honey mesquite			
Quercus spp.	Oaks	37–79		DAK, unpubl. data
Rhus spp.	Sumac	37		Ward et al., 2001
Robinia spp.	Locust, pseudoacacia			
Saccarum officinalum	Sugarcane			
Salix spp.	Willow	23–480	Spring and summer	Nijboer and Dierenfeld, 1996; Fitzpatrick et al., 1998; Ward et al., 2001; Schlegel, unpubl. data; DAK, unpubl. data
Sassafras albidum	Sassafras			
Scheffelera spp.	Schefferlera			
Schinus terebinthifolius	Brazilian pepper	48		DAK, unpubl. data
Sorbus spp.	Mountain ash			
Ulmus americana	American elm	232		Ward et al., 2001
Zea mays	Corn	23–136		DAK, unpubl. data
General note			Leaves generally, not always, higher than twigs in Fe content	

[a] A user-friendly diet calculator was developed to help assist with diet formulation. This calculator is expected to be available on the International Rhinoceros Foundation website www.rhinos-irf.org.

SPECIFIC RECOMMENDATIONS

Diet recommendations

This working group recommends that browser rhinos be provided a dietary Fe concentration of 50–100 mg Fe/kg diet (DMB), with a maximum Fe concentration of 300 mg Fe/kg DMB, or 6 g Fe/day. These recommendations are based upon the current guidelines for feeding equids[10] and previous recommendations for rhinos,[2] as well as practical limitations on available dietary components. In order to achieve the aforementioned recommendations, institutions are encouraged to limit inclusion of any dietary component that is analyzed at >300 mg Fe/kg DMB. Reference diets are provided in Table 1. It is important to note that growing conditions and harvesting methods affect plant Fe content tremendously, so values in the table are not to be used in place of Fe analysis of browse intended for rhinos at a particular institution.

This working group recommends that browser rhinos be offered as much browse as possible. Table 2 provides an abbreviated list of browse species that have been successfully provided to rhinos. Additional lists of browse species that have been successfully provided to rhinos have been published previously[2]; other lists are also available (e.g., http://www.cnr.uidaho.edu/range/toxicplants_horses/). In addition to browse, ad libitum hay should be offered, preferably with no more than 50% alfalfa in the hay offering as a result of the potential for high Fe concentrations in alfalfa. Hays that contain higher tannins or phenolics are desirable (e.g., sanfoin, *Onobrychis viciifolia* spp.; Lespedeza, *Lespedeza cuneata*).

This working group recommends that pellets or supplements be provided only to complement the nutrition found in browse and hays. These pellets-supplements should contain no supplemental Fe (e.g., ferrous sulfate) or vitamin C. If the pellet-supplement utilizes a calcium or phosphorus source it is recommended that reduced Fe sources, such as precipitated dicalcium phosphate or monosodium phosphate, be used. Given the oxidative potential of high body Fe stores, it is recommended that pellets-supplements provide enhanced antioxidant nutrition in the pellet-supplement, although delineation of this supplement will require further effort. If salt blocks are desired, it is recommended that white, iodized salt blocks are the only source of salt, as trace mineral blocks or red salt blocks contain significant Fe concentrations.

Analytic recommendations

In order to fully evaluate the Fe status of browser rhinos, all aspects of their diet, as well as serum-tissue samples, must be analyzed for Fe parameters. To minimize variability within and between these analyses and their interpretations, it may be best to send samples to the same laboratories.

It is recommended that all institutions conduct at least one analysis of water sources offered to their browser rhinos, and it is critical that those analyses include water that has been held in the containers to which the animals are exposed. If water Fe is greater than 0.3 ppm (Environmental Protection Agency standard), provide filtration or an alternate water source.

It is recommended that routine analysis of hay and pellet-supplements be conducted in order to provide data on Fe concentration of diets being offered to browser rhinos. Pelleted feeds and hay are often the diet items highest in Fe content, and it is important to consider their levels in diet formulation, as these items often make up the majority of the diet for browser rhinos in the care of humans. The recommended frequency of sampling would be annually at a minimum; this frequency is especially necessary when there are any changes to the products-diet. This can mean a change in hay vendor or a change in the manufacturer of ingredients in the pellets.

It is recommended that liver Fe be analyzed on all necropsies, in conjunction with the institutional or Species Survival Plan (SSP) pathologist. It is further recommended that serum Fe, ferritin, transferrin saturation, and total Fe binding capacity be analyzed in all browser rhinos in a collection. Serum trace minerals may also provide useful information, but priority should be given to analysis of the serum Fe parameters mentioned above. While sampling browser rhinos can be challenging as a result of training and husbandry constraints as well as medical concerns, regular evaluation of the rhinos in the care of humans may be key to understanding and identifying the cumulative effects of this disorder. Samples should ideally be taken before any major diet changes, during any immobilization, and annually at a minimum. There should be prioritization of the evaluation of young growing animals, which may not yet have developed IOD and may offer the best potential in terms of prevention or treatment. Body condition scoring (BCS) should provide insight for dietary and management evaluations, and a standardized BCS system is an identified priority of this group.

This working group suggests the following U.S. laboratories for various diet and tissue analysis. However, it is important to note that at this time all serum tissue samples that are shipped internationally require permits. This may cause challenges to rhino holding institutions outside of the U.S.

1) Diet, water, hay, browse Fe via ICP wet chemistry
    a) Dairy One Laboratories, Ithaca, New York (http://www.dairyone.com/forage/default.asp)
    b) Full mineral panel analysis is $28/sample
    c) Single mineral analysis (just Fe) is $5/sample
    d) Water mineral analysis is available for $23, or water Fe for $5 per sample
2) Liver Fe
    a) Michigan State University Diagnostic Center for Population and Animal Health for $37, requiring 50 mg of tissue
    b) http://animalhealth.msu.edu/
3) Serum ferritin, TIBC, total Fe
    a) Kansas State University Veterinary Diagnostic Laboratory has a panel of serum Fe, TIBC, and ferritin for rhinos for $46 ($34 if >10 samples)

4) 0.5-ml Frozen serum, sent cold-packed, is required
5) http://www.vet.k-state.edu/depts/dmp/service/index.htm
   a) Other laboratories are currently validating methodology for an alternate ferritin test that may be available in the future (Dr. Pam Dennis, Cleveland MetroPark Zoo)
6) Analysis of serum trace minerals
   a) Fe, cobalt, copper, manganese, molybdenum, selenium, and zinc for $37 at Michigan State University Diagnostic Center for Population and Animal Health, requiring 0.5 ml of serum
   b) http://animalhealth.msu.edu/
7) Blood smear of whole blood
   a) Routine examination of red blood cell (RBC) number, size, and maturity (to determine if hemolytic anemia is part of this complex)
   b) Examine animals routinely for Heinz body formation in RBC, which may, with more data, prove an early indicator of Fe overload

AREAS FOR FURTHER RESEARCH

1. Prepare-refine standardized BCS system for browser rhinos.
2. Determine correlation between Fe status and age, sex, diet, reproductive history, geographic location, environment and management, health status, and ISD history from browser rhinos in captivity to better define the problem as well as the risk factors associated with ISD.
3. Gather additional reference values from free-ranging browser rhino populations (diet and animal Fe status).
4. Identify resources-individuals-locations with which Fe status data and banked tissue and serum samples may be maintained for future research endeavors.
5. Identify, characterize, and make recommendations on use of and inclusion level of compounds that sequester dietary Fe (e.g., tannins and phenolics).
6. Determine the bioavailability of various sources of Fe in diet, especially Fe from soil and from Fe contamination from manufacturing.
7. Determine the effects of seasonality on mineral intake—wild and captive.
8. Examine the role of insulin resistance in the Fe status of browser rhinos.
9. Examine the nutritional content of cut, fresh, and ensiled browse.
10. Compare the cutting heights of different hays and mineral content based on evidence that hay cut with disk bine has higher Fe content than does that cut with sickle bar and that raising the sickle bar height from the ground will reduce soil and Fe contamination of hay.
11. Examine the use of $FeSO_4$ as a standard for diet or hay analysis to allow for comparisons-standardization across labs that may be using different methodology (e.g., atomic absorption spectrophotometry versus ICP).

LITERATURE CITED

1. Adams, R. S. 1975. Variability in mineral and trace-element content of dairy-cattle feeds. J. Dairy Sci. 58: 1538–1548.

2. Association of Zoos and Aquariums (AZA). 1996. AZA Rhinoceros Husbandry Resource Manual. Fort Worth, Texas.

3. Dierenfeld, E. S., R. duToit, and W. E. Braselton. 1995. Nutrient composition of selected browses consumed by Black rhinoceros (*Diceros bicornis*) in Zimbabwe. J. Zoo Wildl. Med. 26: 220–230.

4. Dierenfeld, E. S., A. Kilbourn, W. Karesh, E. Bosi, M. Andau, and S. Alsisto. 2006. Intake, utilization, and composition of browses consumed by the Sumatran rhinoceros (*Dicerorhinus sumatrensis harissoni*) in captivity in Sabah, Malaysia. Zoo Biol. 25: 417–431.

5. Hansen, S. L., and J. W. Spears. 2009. Bioaccessibility of iron from soil is increased by silage fermentation. J. Dairy Sci. 92: 2896–2905.

6. Henry, P. R., and E. R. Miller. 1995. Iron bioavailability. *In:* Ammerman, C. B., D. H. Baker, and A. J. Lewis (eds.). Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. Academic Press, San Diego, California. Pp. 169–199.

7. Horvath, D. J. 1972. Availability of manganese and iron to plants and animals. Geol. Soc. Am. Bull. 83: 451–462.

8. Mayland, H. F., and F. A. Sneva. 1983. Effect of soil contamination on the mineral-composition of forage fertilized with nitrogen. J. Range Mgmt. 36: 286–288.

9. National Research Council. 2005. Mineral Tolerances of Animals. National Academies Press, Washington, D.C.

10. NRC. 2007. Nutrient Requirements of Horses. National Academies Press, Washington, D.C.

11. Theil, E. C. 2004. Iron, ferritin, and nutrition. Annu. Rev. Nutr. 24: 327–343.