Summary Of Mineral And Iron Binding Polyphenolic Plant Compound Levels In Diets Offered Captive Black Rhinoceros *(Diceros bicornis)* In 3 Zoos And 1 Ranch In Texas

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Hemosiderosis is commonly noted in captive black rhinoceros but not free-ranging animals. Animals held in a ranch setting, offered a diet containing at least 46% browse on a dry matter basis, do not experience the same health problems as animals in zoos. It is known that plants contain polyphenolic compounds that can bind iron and make it unavailable for absorption. It is proposed that the significant contribution of browse and consequently, polyphenolic compounds that can bind iron, facilitates better health and lower serum ferritin values in ranch animals. At the time of data collection zoo diets ranged in total iron binding polyphenolic levels from 0.11 mgE gallic acid/g to 0.65 mgE gallic acid/g. The ranch diet was 0.20 mgE gallic acid/g. These levels do not reflect intake over the year. Iron levels in diets for zoos and the ranch were 183 ppm to 264 ppm, and 375 ppm, respectively. A calf had the lowest serum ferritin level at 84 ng/ml. Zoo ferritin values ranged from 716 ng/ml to 9,560 ng/ml while ranch animals ranged from 477 ng/ml to 725 ng/ml.

Key words: browse; gallic acid; hemosiderosis; polyphenolic compounds

INTRODUCTION

Hemosiderosis is known to occur in captive black rhinoceroses (*Diceros bicornis*) but not free-ranging or recently captured animals [Kock et al., 1992; Paglia and Dennis, 1999]. Although hemosiderosis can occur secondary to other disease processes, it has been documented that most cases in black rhinoceros do not appear to be the result of hemolysis. Whether dietary levels of iron or other dietary factors that affect iron absorption, including iron-binding polyphenolics, play a role in this disease has not been investigated.

While some data are available on the nutrient content of plants consumed by free-ranging animals each ingredient's contribution to the overall diet has not been determined [Dierenfeld et al., 1995; Goddard, 1968; Goddard, 1970; Loutit et al., 1987]. Consequently, the level of iron and iron binding polyphenolics in the diet of free-ranging animals is not known. Qualitative analysis indicates that black rhinoceros prefer trees, shrubs, and herbs to grasses. The polyphenolic compounds in the aforementioned plants are virtually nonexistent in most captive diets consisting of manufactured feeds and dried hays. Similar to lemurs, it is suggested that the lack of these compounds is a key factor in the development of hemosiderosis in captive black rhinoceros [Spelman et al., 1989].

The purpose of this study was to quantify mineral and iron binding polyphenolic compound levels in diets offered black rhinoceros consisting predominately of manufactured feed, dried hay and little browse, (6% or less/low browse), as well as levels in diets offered animals held on a ranch containing 46% fresh browse (high browse). Serum parameters assessing iron stores were measured to determine if a link existed between dietary iron, iron binding polyphenolic compounds, and iron stores.

MATERIALS AND METHODS

Animals included in the study were eight black rhinoceros fed a low browse diet from the Dallas Zoo, Fort Worth Zoo, and Fossil Rim Wildlife Center and three black rhinoceros fed a high browse diet from El Coyote Ranch. Each zoo had one animal that had a poor health history and/or occurrences of idiopathic hemorrhagic vasculopathy syndrome (IHVS/ a newly described syndrome in black rhinoceros that is suspected to be the result of an immune-mediated response to an infectious agent possibly causing non-hemolytic anemia) [Murray et al., 2000]. All ranch animals were considered to have a good health history.

Each institution completed a survey noting the diet offered. Supplements and produce items were considered a small portion of the diet and consequently not analyzed for nutrient content. Composition of the diets by food group is presented in Table 1. The remaining diet ingredients were collected and analyzed for calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, copper and manganese by inductively coupled plasma spectrometry at the Fort Worth Zoo Nutrition Laboratory as well as total iron binding phenolics (TIBP) and iron binding tannins (IBT) at Miami University, Oxford OH and Smithsonian's National Zoological Park, Washington DC [AOAC, 1996; Hagerman et al., 1997].

Blood samples were taken from eight adult animals and one calf from the zoos and three adults from the ranch. Serum samples were frozen and shipped to Kansas State University for analysis. Analyses included iron by a coulometrical assay and ferritin by an enzyme linked immunoabsorbent assay (Table 2) [Smith et al., 1981; Smith et al., 1995].

RESULTS

Diet analysis

Mineral levels in the diets offered met or exceeded known nutrient requirements for horse and ponies (Table 3) [NRC, 1989]. Requirement ranges noted for these equids encompass maintenance, growth, pregnancy and lactation. Iron most notably exceeded the domestic requirement ranging from 183 ppm to 375 ppm compared to a requirement of 40 to 50 ppm. The Dallas Zoo diet contained the smallest amount of iron, 183 ppm, while the El Coyote Ranch diet contained the greatest, 375 ppm. Analysis of the individual ingredients within each diet, while there was a significant amount of variation, was in increasing order of iron content: browse, hay, nutritionally complete feeds (Tables 4-6). The diet offered animals at Fossil Rim contained the highest levels of TIBP (0.65 mgE gallic acid/g) and IBT (0.35 mgE gallic acid/g). Iron binding tannins were not at a detectable level in any of the other diets. Total iron binding polyphenolics in the remaining diets ranged from 0.11 mgE gallic acid/g in the Fort Worth Zoo diet to 0.20 mgE gallic acid/g in the El Coyote Ranch diet. Analyses of individual ingredients within each diet, while there was a significant amount of variation, were in increasing order if TIBP content: nutritionally complete feeds, hay, and browse (Tables 4-6).

Serum parameters

Small sample sizes precluded grouping animals together for comparison. Animals varied in sex, age, time in captivity as well as health histories. However, each zoo had one animal that had experienced at least one episode of IHVS. These animals, for their zoo, had the highest or similar to the highest serum ferritin values: 9560 ng/ml., 3625 ng/ml, and 1217 ng/ml for Fossil Rim, Fort Worth Zoo and Dallas Zoo, respectively. The same trend was not seen for similarities in serum iron and transferrin saturation. A 4-month-old calf at Fossil Rim had the lowest serum ferritin level, 84 ng/ml. Animals from El Coyote Ranch followed with serum ferritin levels ranging from 477 ng/ml to 751 ng/ml. The ranch animals had the lowest serum iron ranging form 78 to 122 ug/dl.

DISCUSSION

Diet analysis

Nutrient levels required by horses and ponies may be considered an appropriate target for rhinoceros considering similarities in gastrointestinal tract characteristics and lack of information on requirements specific to rhinoceros. Most of the mineral levels in the diets in this study exceeded these requirements. Most notably, iron was approximately eight times the equid requirement. None of the mineral levels in the diet can be considered harmful for equids. The maximum tolerable level of iron for equids is twenty-five times the requirement. The diet containing the most iron is approximately nine times the low end of the requirement range (El Coyote Ranch). The browse offered in the diet appeared to make a large contribution to the overall high iron value for El Coyote Ranch. Mineral analysis of *Acacia farnesiana* from the ranch conducted by other researchers was not similar to values determined in this study [Graffam et al., 1997]. Iron values differed by four fold. It is not clear why values differed though location of the sampling site and environmental conditions at time of sampling between studies may not have been the same and could have affected the mineral levels. All other browse analyzed in this study had an iron content ranging from 25 to 232 ppm. These values were considerably lower than that of *Acacia farnesianan*. Hay iron content appeared similar to browse but both appeared much lower in iron than the nutritionally complete feeds. Large standard deviations indicate a significant amount of variation across ingredients and within food ingredient categories.

In general, iron absorption is regulated by the body's needs. When stores are adequate, less iron is absorbed than when stores are low. However, endogenous controls can be overridden in idiopathic hemochromatosis, which is the result of a genetic defect, and also in continuous over-consumption of iron [Linder, 1991]. Consequently, feeding iron levels closer to the requirement may be warranted. Unfortunately, ingredients commonly used in formulating nutritionally complete feeds for herbivores naturally contain levels of iron in excess of equid requirements. Many hays also contain high levels of iron. Consequently, it is difficult to formulate a more moderate iron level in diets for these animals.

Because many factors affect iron absorption besides chronic over-ingestion of iron, it is important to consider non-iron factors as agents in reducing or increasing iron absorption. Factors that enhance iron uptake include: ascorbic acid, fructose, citric acid, lysine, histidine, cysteine, methionine, EDTA, NTA, natural chelation as in heme groups, conditions resulting in increased erythropoiesis, low iron stores, and idiopathic hemochromatosis [Linder, 1991]. Factors known to inhibit iron uptakes include: oxalic acid, polyphenolic plant compounds including tannins, phytate, carbonate, phosphate, excess of other metals such as cobalt, copper, zinc, cadmium, manganese, lead, high iron stores, and infection/inflammation [Linder, 1991]. It has been postulated that a lack of inhibitory factors in diets offered animals in captivity may result in enhanced iron absorption in these animals compared to wild animals [Dierenfeld et al., 1995 ; Spelman et al., 1989]. Of these inhibitory factors, total iron binding polyphenolics and iron binding tannins, were assessed in this study. While browse contained detectable levels of these compounds, nutritionally complete feeds and hays contained little to undetectable levels of these compounds. Hay and plant sources used as ingredients in nutritionally complete feeds have been genetically altered over time to contain less of these compounds due to their negative effects on palatability and consequently feed performance [Hagerman and Butler, 1991]. Because nutritionally complete feeds and hays are the base of most diets for captive black rhinoceroses, it is apparent that most animals in captivity probably are not consuming significant levels of these iron-binding compounds. Browse offered to the zoo animals in this study contributed between 0.3% and 6.4% of the diet on a dry matter basis. The high end of the range is the Fossil Rim diet. This number can be considered misleading in that it represents the diet offered at the time of sampling, when browse was readily available. Consequently, it is not reflective of the diet offered year round, especially in the winter when browse is considerably less available. The diet offered at El Coyote Ranch contains significantly more browse, 46.3% on a dry matter basis, than the zoo diets. However, the overall TIBP and IBT contents were still higher in the Fossil Rim diet. This is due to the significantly higher content of these compounds in *Rhus ssp.* (15.0 mgE gallic acid/g and 11 mgE gallic acid/g for TIBP and IBT, respectively) compared to *Acacia farnesiana* (0.32 mgE gallic acid/g and nondetectable for TIBP and IBT, respectively).

Serum parameters

Serum ferritin has been used to assess iron stores in several species including horses and more recently, in rhinoceros [NRC, 1989; Paglia and Dennis, 1999; Smith et al., 1995]. Ferritin levels in animals in this study were within the range reported for long-term captive black rhinoceros, 2,200± 2,240 ng/ml, except for one animal at Fossil Rim with a much higher value and a history of health problems [Paglia and Dennis, 1999]. Though most animals fell within this range, this range is considerably higher than that for free-ranging black rhinoceros, 133±62 ng/ml, and domestic horses, 43 to 262 ng/ml [Paglia and Dennis, 1999]. In addition, hemosiderosis is known to occur in captive black rhinoceros but does not appear to occur in free-ranging animals [Kock et al., 1992]. The lowest ferritin, 84 ng/ml, was in a 3 month old calf at Fossil Rim. Serum ferritin levels have been shown to increase with time in captivity [Smith et al., 1995]. Ferritin levels over time for all animals in this study are not available. However, two wild born animals, both 27 years of age, and in captivity since 1984 have considerably different values. One of the animals has permanently resided on a ranch consuming a high browse diet. The other has been held at both a ranch and a zoo, receiving a very low browse diet at the zoo. The animal receiving the lower browse diet over time had one episode of IHVS as well as 5 fold higher serum ferritin values. It is suspected that although at the time of this study the ranch diet did not contain the highest levels of TIBP, it may provide the highest level of these compounds over the course of the year. Different conditions exist between the ranch and zoo setting as well as diet. Even though the sample size in this study is small, diet cannot be ruled out as a factor affecting ferritin values until more research is conducted.

Serum iron values were similar to those for free-ranging animals, 101±19 ug/dl, and the domestic horse, 50±198 ug/dl [Paglia and Dennis, 1999]. Since serum iron values are affected by several factors such as diurnal variation in humans as well as corticoseroids in dogs they should not be considered the best measures of iron stores [Alpers, 1988; Spelman et al., 1989]. Transferrin saturation was similar for all animals compared to long term captive animals 65±22 but again higher than values for free-ranging animals, 28±6 [Paglia and Dennis, 1999].

Several links have been proposed between excess iron ingestion and disease in black rhinoceros [Paglia and Dennis, 1999]. Hemosiderosis has been identified histologically in black rhinoceros that have died with a history of hemolytic anemia and IHVS [Paglia and Dennis, 1999; Murray et al., 2000]. Recent findings suggest hemolytic anemia alone could not be the cause of hemosiderosis [Paglia and Dennis, 1999]. If too much iron is absorbed and available it may result in iron available for infectious agents (affecting immunity) and iron available for production of and disposal of oxygen radicals and peroxide (affecting cell integrity) [Linder, 1991]. Animals in this study with poor heath histories, including IHVS, were those in the zoo setting with low browse diets and presumably low levels of TIBP over the year. The inclusion of TIBP in diets and their effects on iron absorption warrants further investigation.

CONCLUSIONS

1. Browse analyzed in this study was lower in iron and higher in iron binding polyphenolics than manufactured feeds and hays.
2. A large amount of variation exists in iron and iron binding polyphenolic levels in feeds.
3. Other polyphenolic compounds, in addition to tannins, exist that bind iron.
4. Lowest serum ferritin values were found in animals consuming the greatest amounts of browse but not with the greatest levels of iron binding polyphenolics at the time of the study.
5. Long term, controlled studies are needed to quantify the effects of iron binding polyphenolics on iron absorption in black rhinoceros.

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TABLE 1. Composition of diets offered to black rhinoceros at 4 institutions by food group[1]

	Percent of the diet on a dry matter basis			
Ingredient	Dallas	El Coyote	Fort Worth	Fossil Rim
Nutritionally complete feeds[2]	14.31	11.04	33.03	46.48
Alfalfa hay	68.75	30.91	40.48	30.47
Grass hay[3]	13.05	10.43	24.68	16.45
Browse	1.66	46.26	0.33	6.35
Produce[4]	1.49	1.36	1.27	0.15
Supplements[5]	0.74	0.00	0.20	0.11

[1]Nutritionally complete feeds, hays and browse species listed in Tables 4-6.
[2]Nutritionally complete feeds included: Mazuri ADF 16, Mazuri Browser, Purina Athlete, HMS Elephant Supplement.
[3]Grass hays included: coastal bermuda and red top cane.
[4]Produce included: apples, carrots, sweet potatoes, bananas.
[5]Supplements included: biotin, TPGS, Emcelle E, bonemeal.

TABLE 2. Health status and serum parameters of black rhinoceros at four institutions

Institution/ Animal name		Age yr	Health status at sampling	Unhealthy Episodes In History	Ferritin ng/ml	Iron ug/dL	Transferri n saturation
Dallas							
Nyakaskana	M	16	healthy	no	1351	120	49
Moyo	M	3	heathy	no	716	126	48
Independence	M	5	healthy	yes	1217	104	50
Mean					1095	117	49
Standard deviation					335	11	1
El Coyote							
Macho	M	27	healthy	no	751	78	40
Margarita	F	10	healthy	no	725	105	60
Salsa	F	3	healthy	no	477	122	60
Mean					651	102	53
Standard deviation					151	22	12
Fort Worth							
Chula	F	27	healthy	yes	3625	195	82
Mtoto	F	10	healthy	no	2408	197	84
Mean					3017	196	83
Standard deviation					861	1	1
Fossil Rim							
Gota Gota	M	10	healthy	no	926	112	55
Coco	F	9	healthy	no	1498	98	49
Sinampande	F	12	healthy	yes	9560	148	76
Mean					3995	119	60
Standard deviation					4828	26	14
Mupani (calf)	F	0.25	healthy	no	84	140	52

TABLE 3. Iron binding polyphenolic and mineral levels (dry matter basis, DMB) in diets offered to black rhinoceros at four institutions

	Level in the diet (DMB)[1]						
Nutrient	Dallas	El Coyote	Fort Worth	Fossil Rim	Horse Recs.[2]	Mean	SD
Calcium, %	1.07	0.83	1.17	0.92	0.24-0.6	1.00	0.15
Phosphorus, %	0.32	0.399	0.38	0.45	0.17-0.34	0.39	0.05
Magnesium, %	0.23	0.24	0.27	0.24	0.09-0.13	0.25	0.02
Potassium, %	2.92	1.76	1.84	1.64	0.3-0.4	2.04	0.59
Sodium, %	0.29	0.12	0.12	0.34	0.1-0.3	0.22	0.11
Iron, ppm	183.2	374.9	264.1	250.2	40-50	268.1	79.5
Zinc, ppm	89.96	77.67	142.7	76.37	40	96.7	31.3
Copper, ppm	14.19	14.67	19.73	21.31	10	17.48	3.58
Total iron binding polyphenolics, mgE gallic acid/g	0.15	0.195	0.11	0.65	-	0.28	0.25
Iron binding tannins, mgE gallic acid/g	ND	ND	ND	0.35	-	-	-
Manganese, ppm	52.12	77.67	66.98	82.23	40	69.75	13.38

[1]Analysis does not include produce and supplements.
[2]NRC, 1989.

TABLE 4. Total iron binding polyphenolic (TIBP), iron binding tannins and minerals levels in commercial food items offered black rhinoceros at four institutions

	Concentrations on a dry matter basis										
	TIBP	Iron Binding Tannins	Ca	P	Mg	K	Na	Fe	Zn	Cu	Mn
Food item	mgE gallic acid/g	mgE gallic acid/g	%	%	%	%	%	ppm	ppm	ppm	Ppm
Mazuri ADF 16/Dallas	0.08	ND[1]	1.04	0.91	0.39	2.07	0.50	505.00	418.00	32.00	118.00
Mazuri ADF 16/Fossil Rim	0.05	ND	0.78	0.75	0.27	1.65	0.73	391.00	133.00	34.00	119.00
Mazuri Browser	0.08	ND	0.95	0.61	0.36	1.09	0.74	361.00	95.00	27.00	87.00
Purina Athlete	0.09	ND	0.92	0.80	0.11	0.73	0.53	722.00	245.00	65.00	203.00
ZNN Low Fiber	0.07	ND	0.93	0.70	0.25	1.67	0.31	409.00	372.00	42.00	151.00
HMS Elephant Supplement	0.08	ND	1.09	0.79	0.28	1.54	0.08	525.00	241.00	29.00	276.00
Mean	0.08		0.95	0.76	0.28	1.46	0.48	485.50	250.67	38.17	159.00
Standard deviation	0.01		0.11	0.10	0.10	0.48	0.25	132.78	127.22	14.13	69.50

[1]Not determined.

TABLE 5. Total iron binding polyphenolic (TIBP), iron binding tannins and minerals levels in harvested forages food items offered black rhinoceros at four institutions

	Concentrations on a dry matter basis										
	TIBP	Iron Binding Tannins	Ca	P	Mg	K	Na	Fe	Zn	Cu	Mn
Food item	mgE gallic acid/g	mgE gallic acid/g	%	%	%	%	%	ppm	ppm	ppm	ppm
Alfafla Hay/Dallas	0.16	ND	1.18	0.23	0.22	3.43	0.31	126.00	33.00	12.00	32.00
Alfafla Hay/ El Coyote	0.05	ND	1.14	0.24	0.26	2.33	0.02	121.00	22.00	12.00	36.00
Alfalfa Hay/Fort Worth	0.10	ND	1.75	0.26	0.35	2.34	0.02	270.00	27.00	8.53	24.00
Alfalfa Hay/Fossil Rim	0.12	ND	1.22	0.24	0.26	2.08	0.02	156.00	22.00	12.00	45.00
Coastal Hay/Dallas	0.13	ND	0.38	0.15	0.13	1.41	0.02	148.00	28.00	8.75	89.00
Coastal Hay/ Fort Worth	0.16	ND	0.55	0.16	0.18	1.26	0.02	62.00	27.00	8.00	26.00
Coastal Hay/ Fossil Rim	0.13	ND	0.53	0.18	0.16	1.48	0.01	101.00	32.00	10.00	30.00
Red Top Cane/El Coyote	0.17	ND	0.29	0.26	0.31	2.39	0.02	181.00	27.00	9.81	40.00
Mean	0.12		0.96	0.21	0.22	2.05	0.06	140.57	27.29	10.18	40.29
Standard deviation	0.04		0.49	0.04	0.07	0.76	0.11	65.04	4.31	1.80	22.57

TABLE 6. Total iron binding polyphenolic (TIBP), iron binding tannins and minerals levels in browse plants offered black rhinoceros at four institutions

	Concentrations on dry matter basis										
	TIBP	Iron Binding Tannins	Ca	P	Mg	K	Na	Fe	Zn	Cu	Mn
Food Item	mgE gallic acid/g	mgE gallic acid/g	%	%	%	%	%	ppm	ppm	ppm	ppm
Mulberry *Morus ssp*	0.44	ND[1]	2.85	0.14	0.11	0.83	0.02	52.00	19.00	3.00	36.00
Hackberry *Celtis accidentalis*	0.37	ND	3.24	0.06	0.14	0.51	0.22	65.00	249.00	5.00	17.00
Bamboo (Dallas) *Phyllostachys ssp.*	0.40	ND	0.53	0.11	0.13	1.25	0.01	139.00	42.00	7.76	21.00
Huisache *Acacia farnesiana*	0.32	ND	0.69	0.45	0.21	1.32	0.21	553.00	82.00	14.00	62.00
Cottonwood *Populus deltoids*	1.30	ND	2.40	0.15	0.14	1.06	0.01	76.00	52.00	9.60	30.00
Cedar Elm *Ulmus crassifolia*	0.82	ND	0.87	0.06	0.08	0.45	0.00	95.00	12.00	6.60	22.00
American Elm *Ulmus americana*	0.58	ND	2.78	0.13	0.15	0.96	0.00	232.00	16.00	7.98	74.00

TABLE 6 (cont'd). Total iron binding polyphenolic (TIBP), iron binding tannins and minerals levels in browse plants offered black rhinoceros at four institutions.

	Concentrations on dry matter basis										
	TIBP	Iron Binding Tannins	Ca	P	Mg	K	Na	Fe	Zn	Cu	Mn
Food Item	mgE gallic acid/g	mgE gallic acid/g	%	%	%	%	%	ppm	ppm	ppm	ppm
Elliagnus *Eleagnus angustifolia*	0.30	ND	1.26	0.15	0.14	0.99	0.01	187.00	17.00	-	135.00
Box Elder *Acer negundo*	0.68	ND	2.13	0.15	0.31	0.64	0.02	122.00	25.00	-	34.00
Privet *Ligustrum vulgare*	1.81	ND	1.00	0.12	0.11	0.76	0.00	114.00	17.00	6.22	48.00
Bamboo (Ft. Worth) *Phyllostachys ssp.*	0.10	ND	0.43	0.17	0.11	0.96	0.01	122.00	17.00	5.06	21.00
Willow *Salix ssp.*	2.52	0.66	1.24	0.08	0.11	0.65	0.01	25.00	70.00	5.27	217.00
Sumac *Rhus ssp.*	15.00	11.00	1.31	0.07	0.17	0.77	0.03	37.00	12.00	3.40	8.81
Mean	1.56		1.40	0.15	0.16	1.02	0.05	137.85	43.04	6.83	51.79
Standard deviation	3.65		0.96	0.10	0.07	0.53	0.07	124.66	59.07	3.31	53.44

[1]Not determined.