Comparison of serum electrolytes, minerals and fat soluble vitamins between wild, captive concentrate fed, and captive forage only fed White rhinoceros (*Cerathotherium simum simum).*

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Introduction:

Recent concerns about processed food items fed to herbivores, especially those containing starch, in captive herbivores has led to the development of lower starch feed items. In addition, several species of herbivores in captivity have been managed on forage and produce items without supplement processed food items. These species, Lowland gorilla (Ball 2008, Bergl 2009) and Southern white rhinoceros (*Cerathotherium simum simum)* (Ball 2006) have shown health benefits to this style of feeding. The goal is to reduce the overall inflammatory potential that a processed feed may introduce, either by starch or a novel protein source, and to provide a higher level of physically effect fiber that is essential for optimal gastrointestinal health in these hindgut-fermenters. Additional health benefits come in the form of reduced stereotypical behavior as more time is spent foraging. Commercial produce and forages provided are not identical to ones found in an animal's home range. While similarities may exist, caution must be exercised in making too much genrality about what may be adequate for any given species. While palatability is often the first hurdle, matching the animal's nutrient requirements is a challenge.

Methods and Materials:

In this ongoing project, a herd of white rhinos managed for 10 years on grass hay and grass pasture had the serum values compared to other captive rhinos managed with a concentrate supplement. Both of these groups were then compared to published literature values for selected nutrients. Serum was collected voluntarily from captive Southern white rhino in this study. The first study group (Forage fed) consisted of Southern white rhino that are on a grass hay only diet with access to a large pasture of fresh grasses (n=11) and a group of 4 rhinos recently converted to all hay diet (n=4). The second group, is hay and concentrate fed zoo rhino (n=34 from 4 other facilities). All captive rhinos are housed in Florida and blood samples were collected voluntarily. All the samples were screened to include only healthy rhinos at the time of sampling that were over 18 months of age. This age was chosen as a time when nursing if occurring is not contributing a significant part of the intake of the young rhino. The third group consisted of Southern white rhino (n=38) from South African national parks reported in several publication (Dierenfeld 2005), (Clauss 2002), (van Heerdan 1985), (Keep 1976). The wild white rhino are involved in a managed care systems, but they are allowed to feed naturally with no supplements. Some samples from wild rhinos were stored as plasma but are included in this comparison as they are the only readily available samples analyzed. Samples were collected after the rhinos were immobilized for other management procedures and found to be healthy by the respective authors at the time of sampling.

Blood samples from both captive rhino groups and the wild rhino group were analyzed for calcium, chloride, cobalt, copper, iron, manganese, magnesium, molybdenum, phosphorus, potassium, selenium, sodium, zinc, and vitamin A and E content. T-test with p = 0.05 was used to compare the means of the forage only population to the concentrate supplemented population. A T-test was used at p= 0.05 to compare amongst the concentrate fed group and if found to be significant, the groups were pooled to generate one large concentrate fed data set. To compare the wild rhino values, a 99% confidence interval was generated for each item measured. If the means of the published values fell outside that value, it was consider differing. The mean values for the wild rhinoceros taken from the literature were taken directly from published summations in a recent Master thesis (Wunder 2011).

Results:

Results are summarized in Table 1. Molyybdenum and managanese showed no difference between the captive groups or with the wild means. Concentrate fed rhinos had higher values of cobalt. Chloride was different between the captive groups with the forage fed also higher than wild values. Sodium was equal between the two captive groups with the forage group lower than wild rhinos. Magnesium was higher in the forage fed rhinos compared to concentrate fed ones and to wild rhinos. There was no difference between potassium levels in the captive rhinos but both were lower than wild rhinos. Phosphorus was not different between the captive groups and no data was available in the reports cited in this project. Calcium was lower in the forage fed rhinos compared to the concentrate rhinos. Both captive groups had higher calcium levels than the reported wild values. Iron was equal between the captive rhinos and both higher than wild rhinos. Copper was also equal between the captive groups with the forage group having a lower level than the wild rhinos. Zinc was equal between the captive rhinos but both lower than wild rhinos. Selenium was lower in the forage fed captive group than the concentrate fed group and both groups lower than wild rhinos. Vitamin E was also lower in the forage fed group compared to the concentrate fed group. The forage fed group had lower values compared to the wild rhinos. Total protein was lower in the forage fed rhinos compared to the concentrate fed group. Both captive groups were lower than the reported values for wild rhinos. Albumin and globulins were examined between the active groups as well but not reported in Table 1. The albumin in the forage fed rhinos had a mean of 2.82 gm/dl (n=62, SD = 0.45) and the concentrate fed rhinos averaging 3.14gm/dl (n=68, SD= 0.43) with a p value of $6.01\text{^}10^{-5}$. Globulin in the forage fed rhinos had mean of 4.5gm/dl (n=44, SD =44) and the concentrate group having a mean of 5.15gm/dl (n=91, SD=0.91) with a p value of $1.06\text{^}10^{-5}$.

Discussion:

The higher cobalt levels in concentrate fed rhinos may be simply a result of addition of this element to the concentrate but both groups appear to meet requirements. The statistical differences in sodium, chloride, and magnesium are not believed to be clinically important. The higher level of potassium in the wild rhinoceros may be explained by the fact that they were chemically captured and had most likely some degree of muscle excretion. While phosphorus levels were the same between the captive groups, hypophosphotemia is a recognized concern in both black and white rhinos. The cause is not likely nutritional in origin but supplemental phosphorus is therapeutic. Copper levels were the lowest in the forage fed rhinoceros. The

reported levels of copper in wild rhinos in this report are higher than previous reports and equal with the concentrate fed rhinos. Clinical reports of copper toxicity or deficiency are not found but there is considerable question about the role of copper in relation to iron storage disease in black rhinos. While not statistically significant it is very interesting to find the mean for serum iron higher in the forage fed rhinos than the concentrate fed ones. Both groups are higher than the wild rhinos. Overall iron status requires ferritin and transferritin saturation analysis and is the topic of further investigation. Selenium and Vitamin E levels are both lower in the forage fed rhinos. No clinical events have been reported that can be attributed to lower levels of either of these two nutrients. One possibility that has been speculated upon is that with lower inflammatory stimulus, the requirement for both selenium and Vitamin E may be reduced. The total protein elevation in wild rhinos is somewhat unexpected. Some hemoconcentration during chemical immobilization may explain some of this elevation. The fact that the forage fed rhinos had the lowest serum protein is not surprising but the fractionation may help define the differences in the groups. While the statistical difference between the groups cannot be disputed, the clinical importance in this difference (2.82gm/dl vs. 3.15gm/dl) seems insignificant. The difference in the globulins is both statistically significant and may have some clinical importance. Elevations of inflammatory mediators between the groups would warrant further investigations to see if there is any credibility to one diet being less inflammatory than another. The difference in zinc between the captive groups and the wild rhinoceros may be the only nutritional problem identified clinically and supported by this study. Elevations in calcium are notable from the wild data in both captive groups. Diet may explain a substantial amount of this elevation but other causes of hypercalcemia may need to be examined as well especially renal health. In 10 years of feeding white rhinoceros a forage only diet, clinical skin lesions that resolved with forages high in zinc and a reduction in alfalfa hay used for training resulted in a resolution of the skin lesion and elevation of the serum zinc levels. This problem was noted twice only in late term pregnant females.

Conclusion:

White rhinoceros can be managed successfully in captivity with forages alone. Specific recommendations for this management program include monitoring all minerals, electrolytes, and vitamins as possible. Zinc deficiency is a known potential problem but can be easily discovered and corrected. Suggestions of difference in regards to inflammatory nidus between forage fed and concentrate fed rhinoceros deserve consideration. Follow-up has begun on a subset of this study to follow white rhinoceros as they transition from concentrate to forage only feeding. In addition to the above parameters, such inflammatory markers as ferritin will be examined.

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4. Clauss, Marcus, et. al. “Fat soluble vitamins in blood and tissues of free-ranging and captive rhinoceros.” Journal of Wildlife Diseases 38.2 (2002): 402-413.
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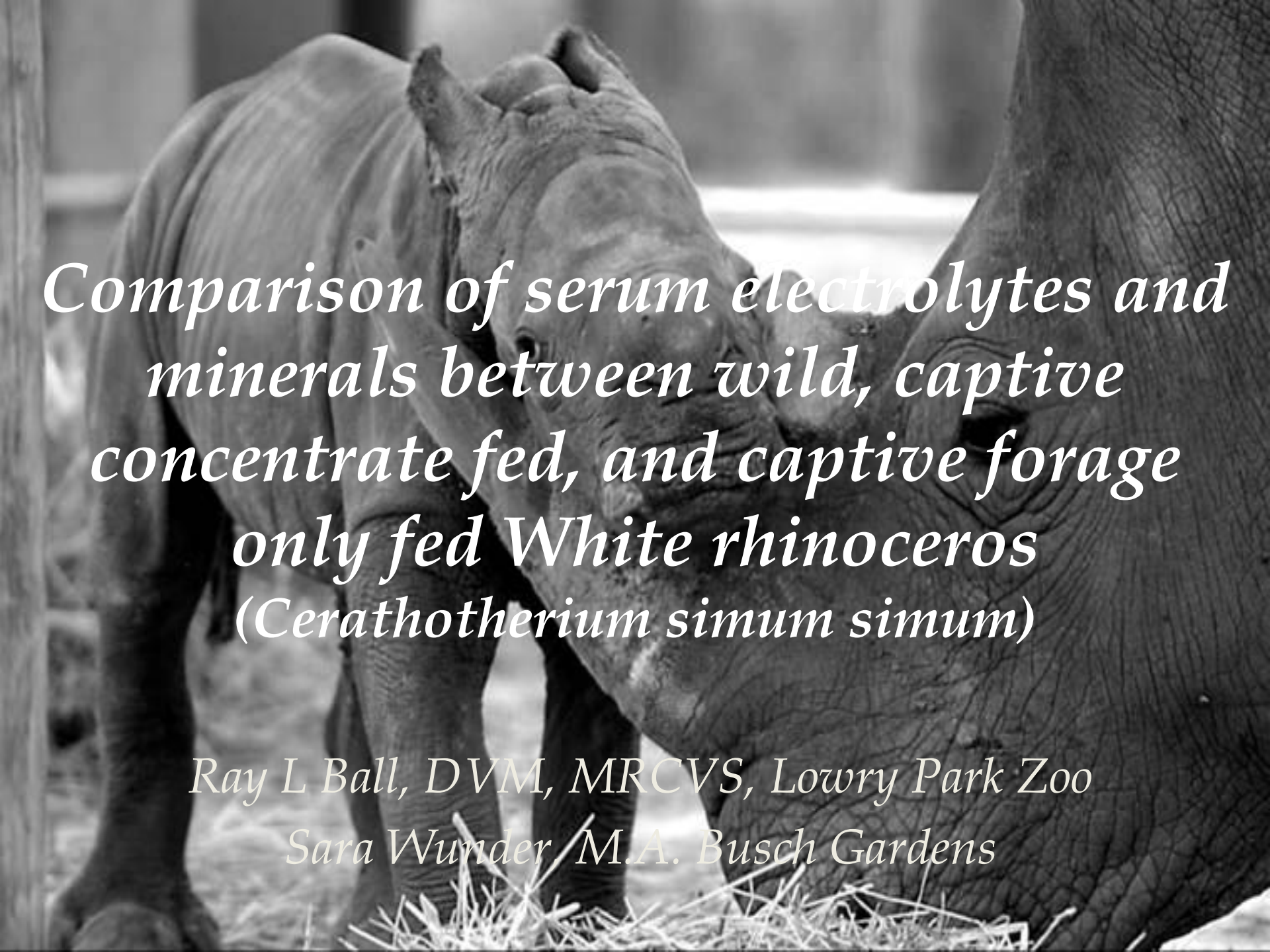
8. Van Heerden J, Keffen RH, Dauth J, Dreyer MJ. 1985. Blood chemical parameters in free-living white rhinoceros. J S Afr Vet Assoc 56:187–9.
9. Wunder, S. Nutrition of the Southern White Rhinoceros, *Cerathotherium simum simum*: Managed Care vs. Wild Population. Thesis for Master of Arts in Interdisciplinary Studies at George Mason University. 2011

Table 1. Mean, standard deviation (SD), and number of samples (n) compared with T-test results between forage fed and concentrate. 99% Confidence intervals (CI) shown with comparison to published means for wild white rhinoceros.

	Calcium mg/dl			**Chloride mEq/L**			**Magnesium mg/dl**			**Molybdenum ng/mL**		
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild
Mean	11.61	12.43	10.78	94.2	96.2	94.2	2.56	2.19	2.06	38.6	30.78	28.35
SD	0.628	1.1		3.31	4.54		0.369	0.389		24.35	8.85	
n	62	200		54	95		55	21		20	7	
Differ at p 0.05	Yes			Yes			Yes			No		
99% CI +/-	0.205438	0.2003525		1.160241	1.19980628		0.12816283	0.2186538		14.02494114	8.61611189	
	11.40456	12.2296475		93.039759	95.0001937		2.43183717	1.9713462		24.57505886	22.1638881	
	11.81544	12.6303525		95.360241	97.3998063		2.68816283	2.4086538		52.62494114	39.3961119	
Diff from wild	Higher	Higher		Same	Higher		Higher	Same		Same	Same	
	Potassium mEq/L			**Sodium mEq/L**			**Total Protein gm/dl**			**Manganese ng/mL**		
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild
Mean	4.41	4.46	5.44	132.7	132.5	136	7.57	8.5	9.27	2.34	2.51	2.47
SD	0.29	0.32		4.18	15		0.79	0.97		1.9	2.2	
n	58	176		57	96		62	95		19	7	
Differ at p 0.05	No			No			Yes			No		
99% CI +/-	0.098085	0.06213134		1.4261196	3.94341716		0.25843321	0.25634628		1.122777963	2.14185832	
	4.311915	4.39786866		131.27388	128.556583		7.31156679	8.24365372		1.217222037	0.36814168	
	4.508085	4.52213134		134.12612	136.443417		7.82843321	8.75634628		3.462777963	4.65185832	
Diff from wild	Lower	Lower		Lower	Same		Lower	Lower		Same	Same	

	Copper ug/mL			**Iron** ug/dL			**Zinc** ug/mL			**Phosphorus mg/dl**	
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate
Mean	1.427	1.7	1.62	1.43	1.24	0.79	0.93	0.9	1.67	4.41	4.34
SD	0.2	0.238		0.32	0.89		0.24	0.2		1.05	1.3
n	25	7		26	7		25	7		63	99
Differ at p 0.05	Yes			No			No			No	
99% CI +/-	0.103033	0.23171013		0.1616517	0.86647905		0.12363981	0.19471439			
	1.323967	1.46828987		1.2683483	0.37352095		0.80636019	0.70528561			
	1.530033	1.93171013		1.5916517	2.10647905		1.05363981	1.09471439			
Diff from wild	Lower	Same		Higher	Higher		Lower	Lower			
	Selenium ng/mL			**Vitamin E** ug/mL			**Cobalt** ng/mL				
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate			
Mean	59.45	118	200.8	0.41	1.93	0.77	0.348	0.635			
SD	16.35	40.2		0.255	1.19		0.25	0.256			
n	20	4		27	6		20	7			
Differ at p 0.05	Yes			Yes			Yes				
99% CI +/-	9.417158	51.774169		0.1264082	1.25137771						
	50.03284	66.225831		0.2835918	0.67862229						
	68.86716	169.774169		0.5364082	3.18137771						
Diff from wild	Lower	Lower		Lower	Same						

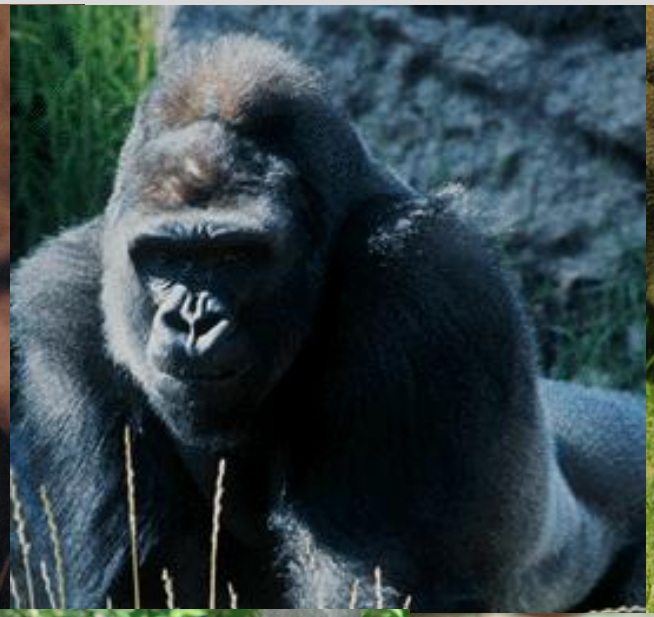
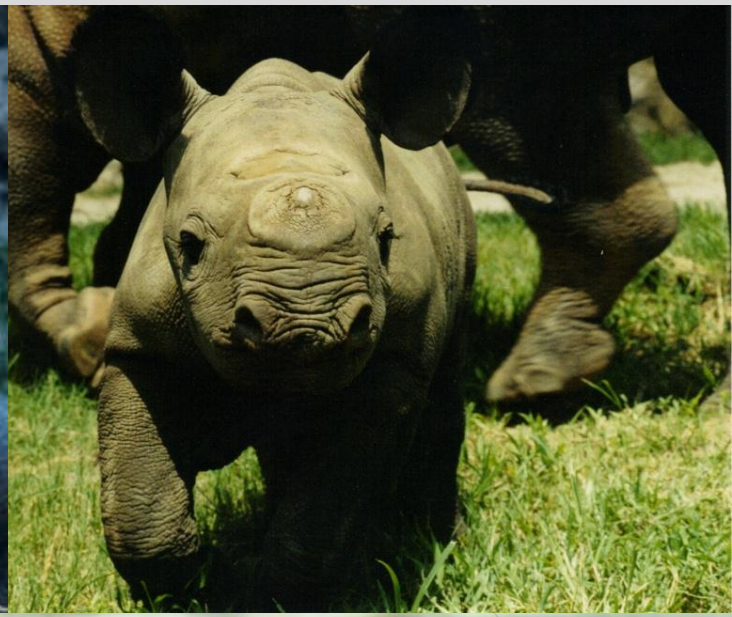
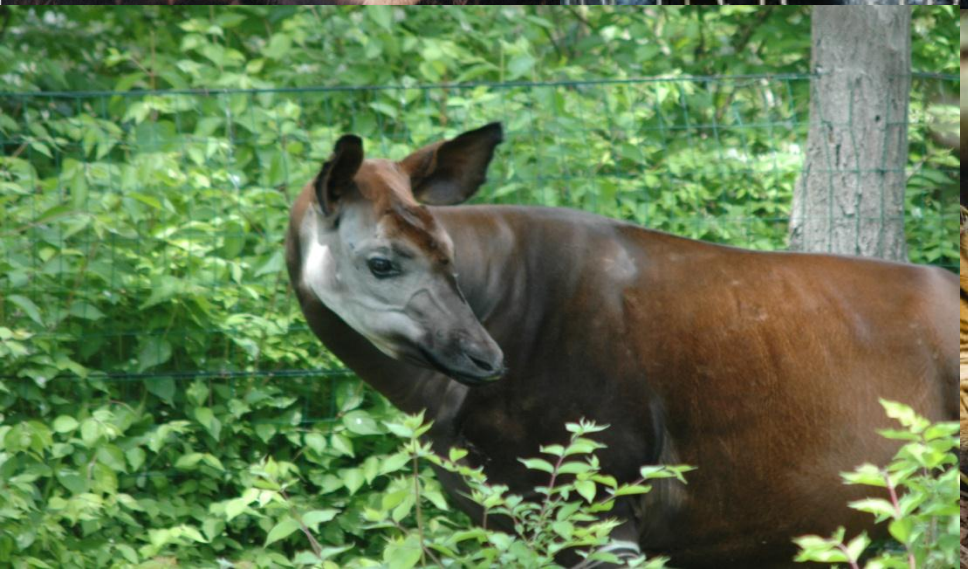


Comparison of serum electrolytes and minerals between wild, captive concentrate fed, and captive forage only fed White rhinoceros (Cerathotherium simum simum)

Ray L Ball, DVM, MRCVS, Lowry Park Zoo

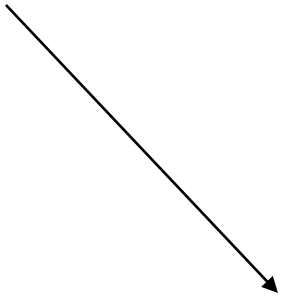
Sara Wunder, M.A. Busch Gardens

Lower inflammatory stimulus with diet management


Captive Diets

- Fatty Acids
- Starches
- Novel proteins



Inflammation

Herbivores

- More forage
- Less starch
- Less sodium??
- Higher peNDF
- Less antigenic stimulus

- Lessen concentrates
- Eliminate concentrates

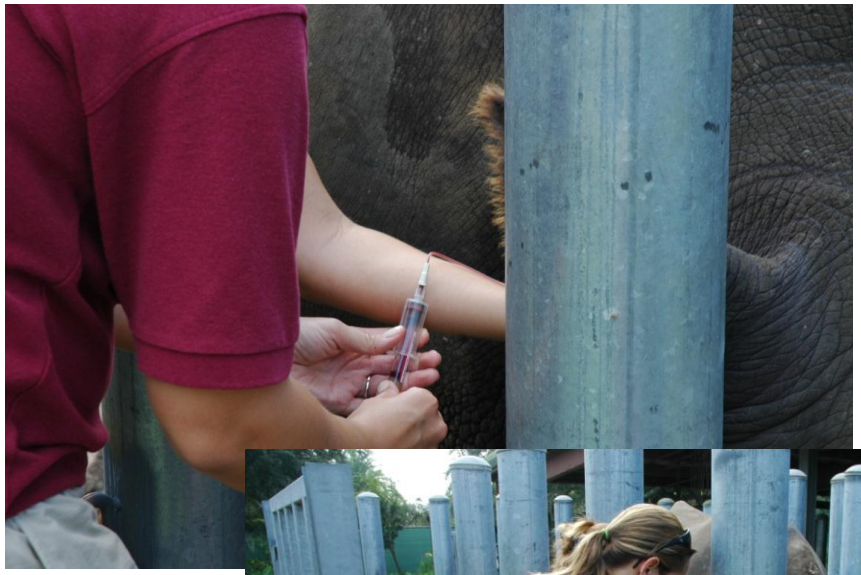
MAINTANENCE OF WILD BORN WHITE RHINOS (CERATOTHERIUM SIMUM SIMUM) ON FORAGE ONLY DIETS IN CAPTIVITY.

- May 2001 currently
- Grass and grass hay
- Successful reproduction
  - 6/7 female offspring
  - 2 pregnant F2 females
- 1.2% BW intake

Matching nutritional requirements to constraints of managed care

- Data from wildlife
- Domestic models
- Feeding trials
- Scientific studies

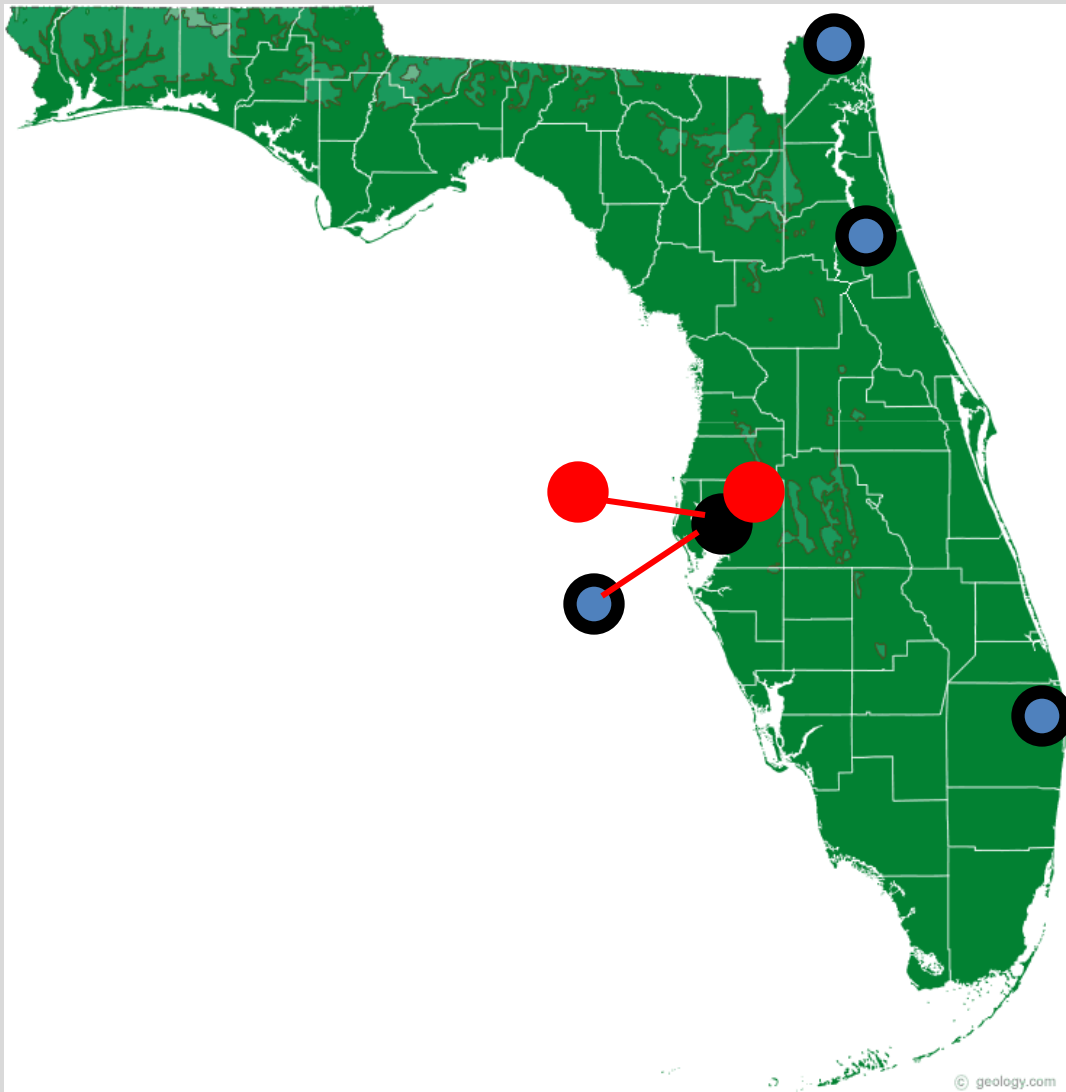
Sara Elizabeth Wunder, Nutrition of the Southern White Rhinoceros, *Cerathotherium simum simum*: Managed Care vs. Wild Population. George Mason University, Master of Arts in Interdisciplinary Studies, Interdisciplinary Studies, Zoo and Aquarium Leadership



- Expanded the sampling
- Eliminated rhinos <18mos
- Eliminated any considered un-healthy at the time of the blood draw
- Serum samples
- Voluntary bloods

Comparison among the three feeding groups: Managed care

- Forage fed
  - N=15 rhinos
  - 2 facility
  - Data from 2002-2011
- Concentrate fed
  - N=34 rhinos
  - 4 facilities
  - Data from 2007-2010



Comparison among the three feeding groups: Wild rhinos

- Literature derived values
  - Forage only
  - Protected care
  - N=38 rhinos
  - All immobilized
- South African facilities
  - Keeper (1976)
  - Heerden (1985)
  - Clauss (2002)
  - Dierenfeld (2005)

Parameters measured

- Calcium
- Chloride
- Sodium
- Phosphorus
- Potassium
- Serum Proteins
  - Albumin
  - Globulin

- ***Selenium***
- ***Zinc***
- ***Vitamin E***
- ***Cobalt***
- ***Copper***
- ***Iron***
- ***Manganese***
- ***Magnesium***
- ***Molybdenum***
- ***25-OH Vit D***

Statistics

- T-test of means between all the concentrate fed at p=0.05
  - Same so pooled these samples into one
- T-test of means between concentrate and forage fed at p= 0.05
- 99% CI of means between two captive groups and wild literature values

	Calcium mg/dl			Chloride mEq/L			Magnesium mg/dl			Molybdenum ng/mL		
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild
Mean	11.61	12.43	10.78	94.2	96.2	94.2	2.56	2.19	2.06	38.6	30.78	28.35
SD	0.628	1.1		3.31	4.54		0.369	0.389		24.35	8.85	
n	62	200		54	95		55	21		20	7	
Differ at p 0.05	Yes			Yes			Yes			No		
99% CI +/-	0.205438	0.2003525		1.160241	1.19980628		0.12816283	0.2186538		14.02494114	8.61611189	
	11.40456	12.2296475		93.039759	95.0001937		2.43183717	1.9713462		24.57505886	22.1638881	
	11.81544	12.6303525		95.360241	97.3998063		2.68816283	2.4086538		52.62494114	39.3961119	
Diff from wild	Higher	Higher		Same	Higher		Higher	Same		Same	Same	

	Potassium mEq/L			Sodium mEq/L			Total Protein gm/dl			Manganese ng/mL		
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild
Mean	4.41	4.46	5.44	132.7	132.5	136	7.57	8.5	9.27	2.34	2.51	2.47
SD	0.29	0.32		4.18	15		0.79	0.97		1.9	2.2	
n	58	176		57	96		62	95		19	7	
Differ at p 0.05	No			No			Yes			No		
99% CI +/-	0.098085	0.06213134		1.4261196	3.94341716		0.25843321	0.25634628		1.122777963	2.14185832	
	4.311915	4.39786866		131.27388	128.556583		7.31156679	8.24365372		1.217222037	0.36814168	
	4.508085	4.52213134		134.12612	136.443417		7.82843321	8.75634628		3.462777963	4.65185832	
Diff from wild	Lower	Lower		Lower	Same		Lower	Lower		Same	Same	

	Copper ug/mL			**Iron** ug/dL			**Zinc** ug/mL			Phosphorus mg/dl	
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate
Mean	1.427	1.7	1.62	1.43	1.24	0.79	0.93	0.9	1.67	4.41	4.34
SD	0.2	0.238		0.32	0.89		0.24	0.2		1.05	1.3
n	25	7		26	7		25	7		63	99
Differ at p 0.05	Yes			No			No			No	
99% CI +/-	0.103033	0.23171013		0.1616517	0.86647905		0.12363981	0.19471439			
	1.323967	1.46828987		1.2683483	0.37352095		0.80636019	0.70528561			
	1.530033	1.93171013		1.5916517	2.10647905		1.05363981	1.09471439			
Diff from wild	Lower	Same		Higher	Higher		Lower	Lower			

	Selenium ng/mL			**Vitamin E** ug/mL			**Cobalt** ng/mL	
	Forage	Concentrate	Wild	Forage	Concentrate	Wild	Forage	Concentrate
Mean	59.45	118	200.8	0.41	1.93	0.77	0.348	0.635
SD	16.35	40.2		0.255	1.19		0.25	0.256
n	20	4		27	6		20	7
Differ at p 0.05	Yes			Yes			Yes	
99% CI +/-	9.417158	51.774169		0.1264082	1.25137771			
	50.03284	66.225831		0.2835918	0.67862229			
	68.86716	169.774169		0.5364082	3.18137771			
Diff from wild	Lower	Lower		Lower	Same			

No clinical significance

Chloride

- Wild = forage
- Wild < concentrate
- Forage < concentrate

- Sodium
  - Wild = forage
  - Wild < concentrate
  - Forage = concentrate

Magnesium

- Wild < forage
- Wild = concentrate
- Forage > concentrate

Calcium mg/dl

	Forage	Concentrate	Wild
Mean	11.61	12.43	10.78
SD	0.628	1.1	
n	62	200	
Differ at p 0.05	Yes		
99% CI +/-	0.205438	0.2003525	
	11.40456	12.2296475	
	11.81544	12.6303525	
Diff from wild	higher	higher	

Calcium

- Managed groups hypercalcemic to wild
- Diet composition
- Renal health concerns
  - Look at renal specific parameters in these populations
- Similar trends in Asian elephants
  - Silva, I.D., Kuruwita, V.Y., 1993. Hematology, plasma, and serum biochemistry values in free-ranging elephants in Sri Lanka. Journal of Zoo and Wildlife Medicine 24, 434-439.

Phosphorus mg/dl

	Forage	Concentrate	Wild
Mean	4.41	4.34	N/A
SD	1.05	1.3	
n	63	99	
Differ at p 0.05	No		

- Hypophosphatemia in managed care
  - Renal insufficiency
  - Chronic glucocorticoids
  - Re-feeding syndrome

Potassium mEq/L

	Forage	Concentrate	Wild
Mean	4.41	4.46	5.44
SD	0.29	0.32	
n	58	176	
Differ at p 0.05	No		
99% CI +/-	0.0980847	0.06213134	
	4.3119153	4.39786866	
	4.5080847	4.52213134	
Diff from wild	lower	lower	

Possibly a direct consequence of the immobilization and elevated muscle enzymes activity

Cobalt ng/ml

	Forage	Concentrate	Wild
Mean	0.348	0.635	
SD	0.25	0.256	
n	20	7	
Differ at p 0.05	Yes		

- Difference in supplementation in concentrates

Copper mcg/ml

	Forage	Concentrate	Wild
Mean	1.427	1.7	1.62
SD	0.2	0.238	
n	25	7	
Differ at p 0.05	Yes		
99% CI +/-	0.103	0.23171013	
	1.324	1.46828987	
	1.53	1.93171013	
Diff from wild	lower	same	

- No clinical support for toxicity or deficiency

Iron mcg/dl

	Forage	Concentrate	Wild
Mean	1.43	1.24	0.79
SD	0.32	0.89	
N	26	7	
Differ at p 0.05	No		
99% CI +/-	0.162	0.86647905	
	1.268	0.37352095	
	1.592	2.10647905	
Diff from wild	Higher	same	

- Iron alone is not the most significant item in iron storage disease

Selenium ng/mL

	Forage	Concentrate	Wild
Mean	59.45	118	200.8
SD	16.35	40	
n	20	4	
Differ at p 0.05	Yes		
99% CI +/-	9.417158	51.774169	
	50.03284	66.225831	
	68.86716	169.774169	
Diff from wild	Lower	Lower	

Vitamin E ug/mL

	Forage	Concentrate	Wild
Mean	0.41	1.93	0.77
SD	0.255	1.19	
n	27	6	
Differ at p 0.05	Yes		
99% CI +/-	0.126408	1.25137771	
	0.283592	0.67862229	
	0.536408	3.18137771	
Diff from wild	Lower	Same	

Vit E and Selenium

- Forage fed very low level
  - No clinical issues with this level

- Less inflammatory stressors require less antioxidant??

Zinc ug/mL

	Forage	Concentrate	Wild
Mean	0.93	0.9	1.67
SD	0.24	0.2	
n	25	7	
Differ at p 0.05	No		
99% CI +/-	0.12364	0.19471439	
	0.80636	0.70528561	
	1.05364	1.09471439	
Diff from wild	Lower	Lower	

- Zinc responsive dermatopathy noted
- Late term pregnant females (0.25ug/mL)

Total protein gm/dL

	Forage	Concentrate	Wild
Mean	7.57	8.5	9.27
SD	0.79	0.97	
n	62	95	
Differ at p 0.05	Yes		
99% CI +/-	0.258433212	0.25634628	
	7.311566788	8.24365372	
	7.828433212	8.75634628	
Diff from wild	lower	lower	

- Hemoconcentration??

Total protein

Albumin

- 2.82 gm/dl (28.2 g/L) (n=62, SD = 0.45) ;
- 3.14gm/dl (31.4g/L) (n=68, SD= 0.43)
- P= $6.01\text{^}10^{-5}$

Globulin

- 4.5gm/dl (45.0 g/L) (n=44, SD =44)
- 5.15gm/dl (51.5g/L) (n=91, SD=0.91)
- P=$1.06\text{^}10^{-5}$.

- Both differ
- Albumin reflect lower protein intake
- Globulin reflects inflammation

25-OH Vit D ng/mL

	Forage	Concentrate
Mean	107.05	68.77
SD	35.22	23.84
n	12	3
Differ at p 0.05	Yes	

- Diet
- Housing/Sunshine
- Abnormal Vit D handling

Conclusion

- Supports the forage only feeding of white rhinos appears to be sound
- Suggest renal health should be investigated
- Extend forage only feeding to other rhinoceros species
- Zn supplement for pregnant females
- Suggest inflammation may need to be investigated

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