ANALYSES OF DIET AND SERUM MINERAL CONCENTRATIONS IN AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*) HOUSED AT THE NC ZOO

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Abstract

Elephants in human care (*Loxodonta africana*) are often over conditioned. Consequently, many zoos aim for reduced caloric intake and increased activity levels. Diets containing higher dietary browse and forage percentages with lower inclusion of pelleted components may stimulate increased foraging behavior. However, feeding increased browse with unknown nutrient profiles while decreasing pelleted nutritionally complete feed could prove problematic with respect to nutritional requirements despite stimulating wanted behaviors. The NC Zoo decided to feed a new grain-free supplement (Hay Enhancer™, Mazuri®) while increasing their daily browse offerings and eliminating the prior nutritionally complete feed. There were two goals for the current research: 1) To determine the percentage and complete mineral profile of all browse species consumed by the six NC Zoo elephants from February 2016 to January 2017 and 2) To determine if the new total diet meets recommended elephant mineral requirements by assessing both diet and circulating serum mineral concentrations. Individual elephant weights and blood samples were collected monthly. Every six weeks, a four-day complete diet weigh in and out was completed for all offered browse species, produce, and hay to analyze for nutrients. Estimated daily food intakes ranged from 61-72 kg, similar to wild African elephant consumption data. Results show 39 browse species were fed between February 2016 and January 2017 and browse consumption ranged from 5-17 kg per elephant daily, which though a minor component of the overall diet represents an increase in the percentage of dietary browse offered since 2015. Browse was analyzed for minerals (Ca, Cl, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Se, and Zn). The browse species varied dramatically in mineral content due to season and species. When complete diets were analyzed for each elephant, the intake of several minerals appear marginal or of concern within the analyzed diets. Na was deficient in both summer and winter diets (0.04 %) compared to the current recommended value (0.10 %). Cu (10.5- 10.8 ppm) and Zn (43 - 47 ppm) were marginally sufficient compared to recommended intake (10 ppm, 40 ppm) for African elephants. Fe (322 - 985 ppm) and Mn intake (50 - 68 ppm) both highly exceeded recommended intake values (50 ppm, 40 ppm). Even with the removal of the Mazuri supplement for data comparisons, Fe and Mn remain high. These results indicate that specific browse species should be avoided for long term use within NC elephant diets due to mineral profiles inconsistent with species nutritional needs. Serum was analyzed for the minerals: Ca, Cl, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Na/K ratio, P, Se, and Zn. Serum mineral ranges for Ca, Cl, K, Na, Na/K ratio, and Zn were within reference ranges for African elephants. Magnesium (2.2 – 3.0 mg/dL) tended towards the upper limit (0.8 - 3.0 mg/dL). Phosphorus (3.8 - 5.9 mg/dL) was above the reference range (1.07 -2.3 mg/dL) and the range for iron (59 - 188 ug/mL) often surpassed the reference range (8.4 - 151.9 ug/mL). Copper (0.77 - 1.23 ug/mL) often fell below the reference range (0.86 - 1.34 ug/mL). The data generated will enable

the NC Zoo and other zoos within the region to better incorporate appropriate browse species into animal diets across seasons, and ensure that nutrient intakes are appropriate based on current knowledge.

Introduction

According to the International Union for Conservation of Nature and National Resources, since 2008 African elephants (*Loxodonta africana*) have been considered vulnerable (Blanc, 2008). Currently, there are approximately 352,271 to 470,000 African elephants left in the wild; with large herds existing in Kenya, Botswana, and South Africa (Chase et al., 2016; Elephant Database, 2013). Because of this status it has become increasingly important to better understand the needs of elephants in human care.

In zoos, feeding is more concentrated due to management practices and limitations (Greco et. al., 2016). Over-conditioning in elephants is inversely related to elephant foot health and to the reproductive health of female elephants (Morfeld et. al., 2014). It has been widely accepted that elephants in human care are often over conditioned due to overconsumption of highly digestible feeds and lower activity levels (Dierenfeld, 2006). A more recent study found that elephant diets containing forages with highly soluble carbohydrates and low fiber lead to elephants consuming more feed to achieve proper gut fill and becoming over conditioned (Williams et. al., 2015).

Traditionally, elephants are fed high amounts of hay with a pelleted dietary component to provide essential micronutrients. While the hay represents the majority of calories, a reduction in overall forage intake is not advisable because of the need for high fiber to maintain hindgut health and to allow the elephants to participate in natural feeding behaviors. In the wild African elephants have been observed spending a majority of the day (48-63% of daylight hours) foraging and feeding and 55% of feeding activities involved browse manipulation and consumption while the other 45% consisted of grazing activities (Dierenfeld, 2006). Therefore, attempts to modify captive diets have often encompassed replacing a portion of hay with higher levels of browse, and reducing the amount of concentrate/pellets offered.

A new low inclusion, grain-free Hay Enhancer™ dietary supplement was developed. This diet item contains relatively high fiber levels (30% max crude fiber, ~ 34% NDF, and 23% ADF) compared to "traditional" supplements designed for elephants (10% max crude fiber, ~ 22% NDF, 11% ADF), resulting in lower predicted energy content, and is fortified with micronutrients to be fed at low inclusion rates (~ 5% of the total diet). We assessed dietary and serum mineral values of elephants at North Carolina Zoo (NC Zoo) during and after a change in diet that consisted of increasing the quantity and variety of browse in combination with use of the new grain-free Hay Enhancer™. While some of the browse species fed have known nutrient quantities in zoo diet analysis programs, minerals of these plant species in North Carolina have not been analyzed previously. In addition, seasonal variations in browse mineral profiles and availabilities have not previously been reported. We proposed that this shift in diet would improve elephant welfare with benefits to elephant nutrition and conditioning. Mineral profiles of native browse would benefit diet preparation in elephants and other browse consuming species at NC Zoo.

Materials & Methods

The current study was conducted with six African elephants (*Loxodonta africana,* 2.4) at the NC Zoo from February 2016 to January 2017. The elephants ranged in age from 14 to 42 years. Animal weights ranged from 2,793 to 6,130 kg and were monitored with monthly weighing. The elephants were housed on two 3.5 acre exhibits from approximately 9 AM until 5 PM unless temperatures dropped below 40 °F. Elephants were housed in the elephant barn with attached paddocks when temperatures were below 40 °F. Timothy hay was spread throughout the exhibits for consumption during the day. Each exhibit holds an average of three elephants. Daily diets were recorded by keeper staff. The elephants were fed the grain-free pellet each morning (Mazuri® Grain Free Hay Enhancer™, #516U), a portion of browse each night, and approximately 6 kg each of varied items used in training including produce (carrots and sweet potato), timothy cubes, and very small quantities of treat items, such as peanuts, each day. Bran and psyllium were provided for one week each month. During the time the animals were on exhibit they were able to graze ad libitum on natural exhibit vegetation, mostly grass, for which intake could not be accurately assessed and an assumption of a consumption level of 1% BW was used for calculations (Table 1).

Blood and fecal samples were collected monthly using vacutainers. Blood was drawn from the ear of each animal by NC Zoo vet and keeper staff. Blood was taken to the NC Zoo veterinary clinic where serum and plasma was aliquoted. An initial hematology analysis (Equine Profile Plus) was performed by veterinary staff. The remaining aliquots were stored in a -80℃ freezer until frozen transport to NC State for continued storage at -80℃. Serum mineral analyses for Ca, Cl, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Na/K ratio, P, Se, and Zn were performed by Michigan State University's Diagnostic Center for Population & Animal Health. These analyses were used to examine nutritional status of animals before, during and after diet supplementation changes occurred.

Weights of browse, hay, and enrichment items and samples of all food items (including pasture forages) were taken to compile a database of overall animal nutrient consumption. Because of the social nature of this elephant grouping, individual browse and hay consumption was not determined but amount offered was quantified. Average weights for timothy hay bales and buckets used to distribute enrichment items were taken to determine inclusion rates. Browse was weighed before and after each consumption period to determine how much the animals consumed or discarded. Each consumption period was defined as the 4 days of quantifying elephant intake and collecting samples of browse offerings every 6 weeks over the course of the trial year. These browse materials were cut by elephant management staff or by NC Zoo horticulture staff and placed in the barn stalls for the elephants overnight or placed on exhibit if elephants had overnight access. All browse came from the zoo's wooded acres or gardens that are managed by NC Zoo horticulture staff.

Non-produce diet items samples were bagged and labeled with species and date of collection and immediately placed in a freezer. Samples were transported to NC State University for storage in a -20℃ freezer and processing. Moisture content of samples was calculated after drying at 60℃ for a minimum of 48 hours in a drying oven. A Wiley mill with a 2mm screen was used to grind feed samples. Ground samples were weighed and portioned for analysis. Non-produce diet items samples were analyzed by Dairy One Forage Lab for Ca, Cl, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Se, and Zn. Mineral profiles for produce items were taken from the USDA database.

Data from diet item analyses were used to create full diet analysis for each elephant to determine mineral intake each month. These analyses were also run with removal of the grain-free pellet and removal of grain-free pellet and doubled browse for summer (May 2016) and winter (December 2016) for comparison (Tables 3 and 4).

Results

Elephants were offered 39 different browse species between February 2016 and January 2017 with amounts "consumed" (total offered minus leftovers) ranging from 30-99 kg and therefore 5-17 kg per elephant. Feed intake ranged from 1.5-3.2% BW on a dry matter basis; coinciding with wild data (Dierenfeld, 2006). Hay ranged from 58.1% to 83.6% of the diet, browse ranged from 1.5% to 6.4%, pellets from 3.3% to 4.2%, and other feedstuffs from 3% to 4.8%. Most frequent browse species were: sweet gum (*Liquidambar styraciflua*), tulip poplar (*Liriodendron tulipifera*), and arundo (*Arundo donax*). Mineral concentrations in browse had wide ranges across species and seasons. For example, the percentage of K ranged from 0.2% to 5.5% and Fe ranged from 25 to 2830 ppm (Table 2). The percentage of Ca on a dry matter basis decreased from 1.3% to 0.9% between spring and summer tulip poplar but then increased to 1.1% in autumn. Mn differed greatly between winter sweet gum (239 ppm) and winter arundo (35 ppm). Pasture Fe values were consistently high (1380-2820 ppm).

Dietary inclusion rates for Na (0.04%) and Se (0.1 ppm) are currently deficient based on NAG elephant requirements (0.1%, 0.2 ppm). These deficiencies increase when Hay Enhancer™ is removed completely from the diet, even if the amount of browse consumed is doubled (Tables 3 & 4). Cu and Zn are marginally adequate on the current diet but become deficient with the removal of Hay Enhancer™.

Serum Ca, Cl, K, Na, Na/K, and Zn ranges for all subjects remained within reference ranges for African elephants (Table 5). Cu (0.8-1.2 ug/mL) was often below the reference range (0.9-1.3 ug/mL). Mg (2.2-3 mg/dL) trended towards the upper limit of the published reference range (0.8-3 mg/dL). Fe (59-188 ug/dL) often surpassed the reference limit (8.4-152 ug/dL) and P (3.7-5.9 mg/dL) was consistently high (1.1-2.3 mg/dL). Co ranged from 0.2-2.6 ng/mL, Mn from <0.5-2.7 ng/mL, and Mo from 2.8-33.5 ng/mL but no reference ranges in African elephants are currently available for these minerals. Se ranged from 60-156 ng/mL, though no reference values are available for African elephants, the reference range for Asian elephants (227-604 ng/mL) but within the reference range for horses (50-266 ng/mL).

Discussion & Conclusion

Data indicate that the NC elephants consume 1.6% of their body weight on a DMB. Estimated intakes of the elephants in this trial ranged from 70-111 kg, which is similar to wild elephant consumption based on a rate of 1.2-1.8% BW in dry matter daily (Dierenfeld, 2006).

While serum mineral concentrations are generally within reference ranges, dietary intake levels of minerals are abnormal in several instances. Na intake is deficient in the current diet and becomes severely deficient without Hay Enhancer™. Se is also deficient and Cu and Zn are marginally adequate in the current diet. The inclusion of a trace mineral salt supplement is recommended. Comparatively, Fe, K, and Mn intake levels all exceed current NAG dietary requirements. The high Fe values may be due to the grass samples having some soil contamination during collection although attempts were made to limit this concern. It was noted by researchers and elephant keeper staff that elephants were consuming soil while on exhibit.

Nearly all browse species were exceptionally high (>100 ppm) in Fe or Mn during at least one season. Arundo contained greater than 100 ppm Fe across all seasons and intake should be monitored due to high Fe intake from exhibit grazing.

A portion of NC Zoo staff was interested in creating a more natural diet, one that did not include pelleted feed, for the African elephants. Hypothetical diets excluding a pelleted portion of the diet were assessed to determine if browse could fulfill mineral requirements without an added vitamin and mineral supplement. Excluding pellet with no change to the amount of browse consumed lead to decreases in dietary minerals. Doubled browse could not fulfill the dietary mineral requirements of the elephants. The removal of pellet from the diet would require strict management of the species and amounts of browse provided as well as additional dietary mineral supplements to meet current estimated requirements.

Data have provided the NC Zoo with average intake levels for their elephants and the true amount of browse (and specific species) fed daily. This allows the zoo to make more accurate assessments of their browse contribution to overall diet and management programs. Thus, the browse data presented in this research project is novel and presents a more complete picture of NC Zoo elephant diet. Nutritional analyses were compiled to create a complete browse database for all seasons enabling the NC Zoo (and other zoos within the region) to better incorporate browse into animal diets, and ensure nutrient intakes are appropriate based on current knowledge.

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Table 1. Daily diet and average weights of feedstuffs for NC Zoo African elephants (*Loxodonta africana*)

Animal	**Mazuri Balancer (kg)**	**Timothy Hay (bales)**	**Timothy Hay (kg)**	**Browse (kg)**	**Enrichment/Produce (kg)**
Ele 1	4	2.93	154.82	10.18	6
Ele 2	4	2.79	147.42	10.18	6
Ele 3	3	2.51	132.63	10.18	6
Ele 4	3	2.51	132.63	10.18	6
Ele 5	3	2.5	132.10	10.18	6
Ele 6	3	2.5	132.10	10.18	6

Table 2. Mineral concentration ranges for 39 browse species fed to the 6 African elephants (*Loxodonta africana*) at The NC Zoo.

Mineral	Concentration Range	Dietary Requirement[1]
% Ca	0.12-2.34	0.3-0.7
% P	0.03-0.48	0.2-0.4
% Mg	0.05-0.29	0.1
% K	0.2-5.5	0.4-0.5
% Na	0-0.04	0.1
% S	0.05-0.55	0.15
ppm Fe	25-2830	50
ppm Cu	3-13	10
ppm Zn	8-170	40
ppm Mn	14-764	40
ppm Mo	0-2.9	unknown

[1]Ullrey et al, Nutrition Advisory Group (NAG) Fact Sheet, 1997

Table 3. Dietary breakdowns of 3 NC Zoo African elephant (*Loxodonta Africana*) diets and effects of nutrient ranges on total dietary intake in summer compared to reference values.

Overall Diet Proportions	**Diet 1**[1]	**Diet 2**[2]	**Diet 3**[3]		
Hay, %	73.1-77.1	76.1-80.3	58.2-74		
Browse, %	5-6.5	5.2-6.7	9.9-12.5		
Pasture, %	7.8-13.6	8.1-14.3	7.6-13.6		
Pellet, %	3.9-4.1	0	0		
Enrichment/Other, %	3.7-4.7	3.8-4.9	3.6-4.6		
Daily intake, % BW	1.5-2.7	1.5-2.6	1.6-2.8		
Nutrients (DMB)				**Ele. Rec. (NAG)**	**Horse Rec. (NRC)**
Ca, %	0.53-0.55	0.5-0.52	0.5-0.54	0.3	0.30
P, %	0.28-0.29	0.26-0.27	0.25-0.26	0.2	0.20
Na, %	0.04	0.01	0.01	0.1	0.15
Cl, %	0.64-0.67	0.63-0.66	0.6-0.63	NA	
K, %	1.93-1.99	1.95-2	1.85-1.93	0.4	0.37
Mg, %	0.18	0.17	0.16-0.17	0.1	0.11
Fe, ppm	322-455	316-455	304-438	50	60.00
Cu, ppm	10.5-10.8	6.4-6.6	6.2-6.4	10	14.00
Mn, ppm	59.5-67.5	56.6-6.9	63.8-80.1	40	59.00
Zn, ppm	43-44	23.6-24.5	23.8-25.1	40	59.00
Se, ppm	0.11	0.04-0.05	0.04-0.05	0.2	0.15
S, %	0.2-0.21	0.2	0.19-0.2	NA	
Mo, ppm	0.99-1.09	0.86-0.97	0.83-0.93	NA	

[1] Current diet including pasture

[2] Diet excluding Hay Enhancer™

[3] Diet excluding Hay Enhancer™ with doubled browse

[4] Ullrey et al, Nutrition Advisory Group Fact Sheet, 1997
[5] Horse NRC, 2007
[NA] = Data is not currently available

Table 4. Dietary breakdowns of 3 NC Zoo African elephant (*Loxodonta africana*) diets and effects of nutrient ranges on total dietary intake in winter compared to reference values.

Overall Diet Proportions	**Diet 1[1]**	**Diet 2[2]**	**Diet 3[3]**		
Hay, %	58.1-67.5	60.1-69.9	58.2-66.9		
Browse, %	3.2-4.4	3.4-4.6	6.5-8.8		
Pasture, %	20.4-32.3	21.1-33.4	20.2-32.3		
Pellet, %	3.2-3.6	0	0		
Enrichment/Other, %	3-4.2	3.2-4.3	3-4.1		
Daily intake, % BW	1.8-3	1.8-2.8	1.8-2.9		
Nutrients (DMB)				**Ele. Rec. (NAG)[4]**	**Horse Rec. (NRC)[5]**
Ca, %	0.4-0.41	0.35-0.37	0.37-0.38	0.3	0.3
P, %	0.19-0.2	0.17-0.18	0.17-0.18	0.2	0.2
Na, %	0.04	0.01	0.01	0.1	0.15
Cl, %	0.23-0.24	0.2-0.22	0.2-0.22	NA	
K, %	1.43-1.47	1.42-1.46	1.39-1.42	0.4	0.4
Mg, %	0.16	0.15	0.15	0.1	0.1
Fe, ppm	665-985	668-999	642-969	50	60
Cu, ppm	10.5-10.7	6.8-7.0	6.8-7.0	10	14
Mn, ppm	50-60	47-57	54-66	40	59
Zn, ppm	46-47	25-26	26-27	40	59
Se, ppm	0.13-0.14	0.06-0.07	0.06-0.07	0.2	0.15
S, %	0.17-0.19	0.16-0.18	0.16-0.18	NA	
Mo, ppm	24-37	24-38	23-37	NA	

[1] Current diet including pasture

[2] Diet excluding Hay Enhancer™

[3] Diet excluding Hay Enhancer™ with doubled browse
[4] Ullrey et al, NAG Fact Sheet 4, 1997
[5] Horse NRC, 2007
[NA] = Data is not currently available

Table 5. NC Zoo serum mineral analyse ranges for the six African elephants (*Loxodonta africana*) from Feb 2016 to Jan 2017 compared to published reference ranges.

Name	Ca mg/dL	Cl mmol/L	Mg mg/dL	K mmol/L	Na mmol/L	P mg/dL	Co ng/mL	Cu ug/mL	Fe ug/dL	Mn ng/mL	Mo ng/mL	Se ng/mL	Zn ug/mL
Ele 1	10.0-10.8	82-86	2.5-3.0	4.2-5.1	122-126	3.8-5.2	0.43-0.67	1.05-1.23	59-187	0.6-2.7	3.5-10.5	92-147	0.87-1.24
Ele 2	10.0-10.6	86-91	2.3-2.6	4.2-4.7	124-131	4.7-5.9	0.21-0.41	0.9-1.07	78-118	<0.5-1.6	4.1-14.2	60-116	0.76-1.00
Ele 3	10.0-10.7	83-88	2.3-2.9	3.9-5.1	124-129	3.7-5.2	0.25-2.61	0.77-0.93	71-110	<0.5-1.7	2.8-16.3	90-156	0.81-1.15
Ele 4	9.8-10.7	85-92	2.4-2.8	4.0-5.0	124-135	3.9-5.6	0.24-1.15	1.07-1.17	62-88	<0.5-2.1	4.6-27.1	61-121	.90-1.16
Ele 5	10.1-10.6	85-89	2.2-2.4	4.1-4.8	123-130	4.2-5.5	0.27-2.25	0.9-1.01	71-129	<0.5-1.8	4.3-33.5	72-137	0.75-1.06
Ele 6	10.0-10.6	87-90	2.3-2.7	4.1-5.0	124-128	4.0-5.7	0.32-0.69	0.98-1.11	121-188	<0.5-2.3	3.2-27.4	69-125	0.70-1.03
African Range[1]	9.7-12.3	81-97	0.8-3.0	4.0-5.8	120-139	1.1-2.3	NA	0.86-1.34	8.-152	NA	NA	NA	0.60-1.47
Horse, NRC[2]												50-266	

[1] Wiedner et al., 2011
[2] Horse NRC, 2007
[NA] = Data is not currently available