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RESEARCH ARTICLE

Analyses of African elephant (*Loxodonta africana*) diet with various browse and pellet inclusion levels

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Abstract

To more closely simulate the diet of free-ranging elephants, the diet of six (2.4) African elephants (*Loxodonta africana*) was altered to include more browse and less pelleted complete feed (5% total diet). Dietary proximate compounds, minerals, vitamins A (and carotenoids), D and E, and fatty acids were analyzed on pelleted diet items and forages including hay, grass, and browse. A total of 42 browse species were offered over 1 year with an average total diet inclusion of 5.2% (dry matter basis) per day. Dietary Na and Se were low while Fe and Mn were high compared to published intake levels for elephants. Analyzed nutrients within browse varied widely among seasons and species. Ingredient analyses were used to create predicted elephant nutrient intake for (a) the current diet, (b) a diet excluding pellets, and (c) a diet excluding pellets and providing browse at doubled levels. Formulated diets excluding pellets had lower mineral levels than the current diet and doubled browse did not alter mineral inclusions of concern. This study provides seasonal data on the nutrient levels of Southeastern browse species important for various pachyderm and herbivorous species. Predicted nutrient intake with new diet scenarios does not support the exclusion of pellets in the diets of African elephants without greater browse quantity availability, strict diet management, or additional supplements.

KEYWORDS
forages, nutrients, pachyderm, zoo

1 | INTRODUCTION

Free-ranging African elephants (*Loxodonta africana*) spend 48–63% of their day foraging and feeding, consisting of 45% grazing and 55% browse manipulation (Dierenfeld, 2006). Comparatively, feeding within human care facilities is more structured in terms of time, amounts of feed, and variability of feed items with 47.5% of North American zoos having notably predictable feeding schedules (Greco et al., 2016). Predictable feeding schedules, increased feed diversity, overconsumption of highly digestible feeds, and lowered activity are correlated with over-conditioning (Dierenfeld, 2006; Morfeld, Meehan, Hogan, & Brown, 2016). Williams, Tollefson, and Valdes (2015) found that elephants consuming diets containing forages with highly soluble carbohydrates and low fiber required more feed for proper gut fill and thus were over-conditioned. Over-conditioning is a concern because it may negatively affect elephant foot and reproductive health (Clauss, Wang, Ghebremeskel, Lendl, & Streich, 2003; Morfeld, Lehnhardt, Alligood, Bolling, & Brown, 2014).

It is difficult to formulate a diet for a species in human care when the nutrient content of offered feed items is unknown. Traditionally, elephants are fed like horses, with high amounts of hay and a nutritionally complete pellet fed at approximately 10% on a dry matter basis (DMB) of the total diet (Ullrey, Crissey, & Hintz, 1997). While hay represents most calories, a reduction in overall forage intake is not advisable because high fiber is needed to maintain hindgut health and natural feeding behaviors. Prior attempts to modify elephant diets include replacing a portion of hay with higher levels of browse and/or reducing the amount of nutritionally complete pellets (Stoinski, Daniel, & Maple, 2000; Hatt & Clauss, 2006). The inclusion of browse in elephant diets is expected to increase foraging, by increasing feed manipulation time, as seen in free-ranging African elephants (Dierenfeld, 2006; Stoinski et al., 2000).

We assessed diet nutrient values of six African elephants at the North Carolina Zoo (NC Zoo) during a diet change that consisted of increasing the quantity and variety of browse in combination with the use of a grain-free pellet (Mazuri® Hay Enhancer™). The study objectives were: (a) Determine the number of browse species fed to the six African elephants yearly and the total amount of browse offered compared to uneaten browse to determine what was potentially consumed for representative periods. (b) (i) Determine the nutrient profiles of various NC Zoo non-produce diet items (NPDI) (processed feeds, hay, pasture, and browse species) fed over four seasons thus establishing parameters for previously unstudied nutrients and feed items; (ii) Determine if season and feed type affect nutrient concentrations in NPDI. (c) Calculate nutrient intake using analyzed NPDI and diet intake estimates for comparison to current recommended intake levels. (d) Evaluate other dietary programs, including reduction/elimination of pellets, in addition to increased browse offering.

These investigations are important because it is possible that increasing dietary browse and reducing pellets could improve elephant welfare by offering more fiber and foraging behavior opportunities (Dierenfeld, 2006; Stoinski et al., 2000). Having known nutrient profiles of native browse species across all four seasons can benefit diet preparation in elephants and other browsing species at NC Zoo and within the Southeastern United States.

2 | MATERIALS AND METHODS

2.1 | Animals

Six African elephants (2.4) at the NC Zoo in Asheboro, NC were studied from February 2016 to April 2017. This herd was 14–42 years old with five mature animals and one growing female. Males weighed 5,388 to 6,227 kg and females 2,578 to 4,528 kg. Elephants had access to a barn (5,783 sq. ft) with four attached outdoor paddocks (2,550 sq. ft each; two single male paddocks and two paired female paddocks) at night or were housed in the barn during the day if temperatures were below 4.4°C. If temperatures were higher than 4.4°C, animals were provided access to two 3.5-acre habitats from approximately 9:00 to 17:00. If temperatures remained above 4.4°C throughout the night, elephants stayed on habitat. Each habitat supports three elephants. Elephant welfare was monitored by keeper staff throughout the study by visual exams and monthly serum and plasma collections. This study was approved by the NC Zoo Animal Research Committee.

2.2 | Diet

Daily diets were recorded by NC Zoo keeper staff for the study duration. This included number of timothy hay (*Phleum pratense*) bales, enrichment food items, and browse species. Enrichment food items included a variety of produce, primarily sweet potatoes and carrots, processed timothy cubes, shelled peanuts, popcorn, and alfalfa hay (*Medicago sativa*). Average weights of daily diet components are in Table 1. The Mazuri® Hay Enhancer™, weighed daily, was offered to animals individually during shifting or training before the remainder was fed on the barn stall floor. Timothy hay was fed in the barn and on exhibit. Various species of browse were cut from pesticide and herbicide-free NC Zoo grounds by keeper and/or horticulture staff daily then divided by number of branches and size of branches among elephants for overnight feeding in the barn or on exhibit. Enrichment food items were scattered on exhibit for foraging purposes or used as additional training incentives. The exhibit pasture was composed of fescue grass (*Festuca arundinacea*), annual ryegrass (*Lolium multiflorum*), and Bermuda grass (*Cynodon dactylon*) with some wild white clover (*Trifolium repens*) and limited weed species. Although pasture samples were analyzed for nutrient content, elephants graze ad libitum while on exhibit and exact pasture intake per animal could not be determined. Free-ranging African elephants have been reported to consume approximately 4–6% of their body weight (as fed) daily with a range of 15–90% coming from grasses due to environmental and seasonal effects

TABLE 1 Daily diet and average weights (kg) of feedstuffs (as fed basis) for six NC Zoo African elephants (*Loxodonta africana*) from February 2016 to April 2017[a]

Animal	Mazuri® Hay Enhancer™	Timothy hay	Browse	Enrichment/produce	Pasture (1% BW)[b,c]	Total diet
Elephant 1	4	155	11	6	59	234
Elephant 2	4	147	11	6	55	222
Elephant 3	3	133	11	6	43	196
Elephant 4	3	133	11	6	43	196
Elephant 5	3	132	11	6	39	191
Elephant 6	3	132	11	6	27	179

[a]Elephants 1 and 2 are males and Elephants 3–6 are females.
[b]1% BW determined from halving NRC pasture intake of horses to accommodate browse consumption.
[c]Pasture intake on a DMB rather than as fed.

(Colbert, 1993; Laws, 1970). Grass diets higher than 50% grass (as fed) are not thought to be optimal for health and reproduction (Laws, 1970). Our current research diets were estimated to be a lower percentage of African elephant BW (2.5–4.4% as fed [1.5–3.1% DM]) to accommodate for the decreased levels of grazing within human managed elephants. We estimated a grass consumption level for the current work of 1% BW as fed (0.5% DMB) by halving the minimum horse DM recommendations of (1.0–2.0% DMB) and utilizing the available African elephant data (Colbert, 1993; Laws, 1970; National Research Council, 2007). The only alteration made to the diet for this study was a transition from Mazuri® Wild Herbivore™ and Mazuri® Wild Herbivore Plus™, fed at approximately 10–15% of the diet to Mazuri® Hay Enhancer™, fed at approximately 5%.

2.3 | Sample collection and analyses

Elephant body weights were recorded monthly by keeper staff using a barn floor scale. One female elephant was not weighed throughout the entire study due to a pre-existing behavior, thus total diet calculations could not be accurately determined for this animal, but her weight is predicted to be like her female companion.

Each sampling/consumption period was defined as a 4-day period of quantifying elephant intake and collecting samples of browse offerings, pasture, and both hay species every 6 weeks from February 2016 to April 2017. Browse samples included all parts of the plant (stems, leaves, bark, and thick diameter (≤ 2 in) branches) because elephants were seen eating all parts. Browse was weighed before and after being offered during each consumption period to determine how much was consumed or discarded. Weights of browse, hay, and other food items and samples of all NPDI were taken to compile a database of predicted overall diet consumption. Samples of timothy cubes and Mazuri® Hay Enhancer™ were collected once for analysis. Samples of produce and human grade enrichment items were not collected because nutrient values are available through the USDA. Because of the social nature and housing of the elephants, individual browse and hay offering was not determined but total amount offered was quantified.

NPDI samples were bagged and labeled with species and date of collection and immediately placed in a freezer (−18°C). Samples were transported to NC State University for storage at −20°C. Moisture content was calculated after drying at 60°C for a minimum of 48 hr in a Shel Labs FX28-2 drying oven (Sheldon Manufacturing, Cornelius, Oregon). A Model 4 Wiley mill (Thomas Scientific, Swedesboro, New Jersey) with a 2 mm screen was used to grind dried samples. Ground samples were weighed and portioned for lab analyses. Proximate compounds, minerals, and fatty acids were analyzed for all seasons. NPDI samples from winter and summer were sent for α-tocopherol, carotenoids and ergocalciferol (D_2) and cholecalciferol (D_3) analysis, since summer and winter have the most notable differences in plant age and sun exposure, these two seasons were hypothesized to show the greatest differences in vitamins. The most appropriate method for drying feedstuffs for carotenoid and tocopherol analyses has been debated although literature suggests the method used in the current study is acceptable (Chuyen, Roach, Golding, Parks, & Nguyen, 2016; Saini, Shetty, Prakash, & Giridhar, 2014). One summer sample of alfalfa hay did not contain enough weight to run both vitamin D and carotenoid and α-tocopherol analyses thus there is one less vitamin D sample than carotenoid and α-tocopherol samples. Due to issues with the initial spring 2016 collection period, insufficient weight was collected, and fatty acid analysis was not run due to prioritization. Seasons were defined as: (a) Spring: March–May, (b) Summer: June–August, (c) Autumn: September–November, and (d) Winter: December–February.

A minimum of 50 g of ground sample was sent to Dairy One Forage Lab (Ithaca, New York) for a wet chemistry package containing: dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, crude fat (CF), ash, starch, nonfibrous carbohydrates (NFC), digestible energy (DE), Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, S, and Se. A minimum of 20 g was sent to Arizona State University (Tempe, Arizona) for analysis of α-tocopherol and carotenoids including lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and α-carotene and to Lipid Technologies (Austin, Minnesota) for a full fatty acid profile. A minimum of 50 g was sent to Heartland Assays (Ames, Iowa) for vitamin D_2 and D_3 analysis.

2.4 | Diet calculations

Using average weights of NPDI and the associated analyses, nutrient profiles for predicted diets for summer (July 2016) and winter (January 2017) were calculated in Microsoft Excel (Microsoft Office 365, Redmond, Washington) to create: (a) a diet to determine current estimated nutrient intake; (b) a diet with no pellets; and (c) a diet with no pellets and the weight of browse doubled. Results from these diet assessments were evaluated against current recommended dietary intake of nutrient ranges (National Research Council, 2007; Ullrey et al., 1997) to assess if any of these diets indicated nutrient levels of concern.

2.5 | Statistical analysis

Statistical analysis was performed in SAS 9.4 (Cary, NC) to evaluate (a) if there were significant difference between nutrient content of different feed items among species and (b) if there were seasonal differences in nutrient content of browse, pasture, and hay, with special consideration for browse species. GLIMMIX mixed models for browse, pasture, and hay values (proximates, minerals, vitamins/carotenoids, and fatty acids) includes season, and species as fixed-effect terms. Null hypothesis that a fixed-effect is zero was tested against the residual variance after accounting for the rest of fixed-effects in the model. Pairwise t-test between relevant predicted means (least squares [LS] means) was used for significance ($p < .05$). Manufactured or nutrient fortified feed items (timothy cubes and Mazuri® Hay Enhancer™) were not included in statistical models.

3 | RESULTS

3.1 | Total diet overview results

All animals maintained good health throughout the study. Total feed intake coincided with data from free-ranging elephants (Colbert, 1993; Dierenfeld, 2006, Laws, 1970). During the study, 42 species of browse were fed (Table 2) with four additional generic groups including "bamboo," "maple," "oak," and "pine," Thirty-two species were collected and analyzed with at least one season represented. The five most frequently fed browse species were sweet gum (*Liquidambar styraciflua*), wax myrtle (*Morella cerifera*), bamboo (*Phyllostachys aurea* & *Phyllostachys nigra*), tulip poplar (*Liriodendron tulipifera*), and thorny elaeagnus (*Elaeagnus pungens*). Nutrient analyses of these five most frequently fed species are included in corresponding nutrient tables (Tables 5,6,8, and 9) as species specific seasonal change examples. LS Means for each season include all sampled species within seasonal collection periods. Browse "consumption" (total offered minus leftovers) on an as fed basis ranged from 30 to 99 kg per day (5–17 kg per elephant per day). Total elephant feed intake averages and ranges (DMB) and dietary inclusion rates on a DMB and an as fed basis for summer (July 2016) and winter (January 2017) are in Table 3.

3.2 | Proximate analyses compounds and minerals

Total means and ranges for dietary proximate and mineral analyses are presented across season (Table 4), and LS means for between seasons (Tables 5 and 6). Dry matter was higher in winter than spring ($p = .005$) and summer ($p = 0.009$). All proximate nutrients were found to be different when compared between feed items, but none differed between the five most frequently fed browse species. Magnesium was higher in summer than in spring ($p = .022$). All minerals, except for Se ($p = .1$), were different when compared by NPDI type. Of the minerals discussed above, the only mineral that displayed NPDI type differences between the five most frequently fed browse species was Ca. Calcium in sweet gum ($p = .02$) and tulip poplar ($p = .005$) was higher than Ca in mixed species bamboo.

A 2015 mineral content (mg/L) report for all the water accessible to the elephants (NC Laboratory of Public Health (Raleigh, NC)) included values for Ca (13), Cl (13), Cu, (<.05), Fe (<.1), Mn (<.03), K (2.1), Se (<.005), Na (23), Zn (<.05), and sulfate (27).

TABLE 2 Complete list of browse species recorded as fed to six NC Zoo African elephants (*Loxodonta africana)* from March 2016 to April 2017 with number of times recorded in daily diets

Arundo (*Arundo donax*) *n* = 59[a]	Mulberry (*Morus alba*) *n* = 13[a]
Bamboo (*Phyllostachys sp.*) *n* = 95[a]	Oak (*Quercus* sp.) *n* = 1[a]
Beech (*Fagus grandifolia*) *n* = 11[a]	Pecan tree (*Carya illinoinensis*) *n* = 4
Black Bamboo (*Phyllostachys nigra*) *n* = 1[a]	Pignut hickory (*Carya glabra*) *n* = 23[a]
Black gum (*Nyssa sylvatica*) *n* = 2	Pine (*Pinus* sp.) *n* = 48[a]
Black willow (*Salix nigra*) *n* = 1	Red bud (*Cercis canadensis*) *n* = 11
Boxelder (*Acer negundo*) *n* = 1	Red cedar (*Juniperus virginiana*) *n* = 10
Chestnut oak (*Quercus montana*) *n* = 21[a]	Red tip (*P. glabra* × *P. serratifolia*) *n* = 5
Common Persimon (*Diospyros virginiana*) *n* = 3[a]	River birch (*Betula nigra*) *n* = 2
Contorted Mulberry (*Morus bombycis "Unryu"*) *n* = 27[a]	Russian olive (*Elaeagnus angustifolia*) *n* = 42[a]
Corn (*Dracaena fragrans*) *n* = 3	Sesbania (*Sesbania grandiflora*) *n* = 2
Dogwood (*Cornus florida*) *n* = 30[a]	Sugar maple (*Acer saccharum*) *n* = 29[a]
Eastern Hemlock (*Tsuga canadensis*) *n* = 1[a]	Sumac (*Rhus* sp.) *n* = 1
Ficus (*Ficus benjamina*) *n* = 19[a]	Sunflower (*Tithonia diversifolia*) *n* = 1
Golden bamboo (*Phyllostachys aurea*) *n* = 0[a,b]	Sweet gum (*Liquidambar styraciflua*) *n* = 117[a]
Grapevine (*Vitis* sp.) *n* = 23[a]	Sycamore (*Platanus occidentalis*) *n* = 13[a]
Green banana (*Musa basjoo*) *n* = 19[a]	Thorny elaeagnus (*Elaeagnus pungens*) *n* = 82[a]
Honey Locust (*Gleditsia triacanthos*) *n* = 1	Tulip poplar (*Liriodendron tulipifera*) *n* = 84[a]
Honeysuckle (*Lonicera japonica*) *n* = 5[a]	Viburnum (*Viburnum rafinesquianum*) *n* = 1[a]
Loblolly pine (*Pinus taeda*) *n* = 2[a]	Virginia pine (*Pinus virginiana*) *n* = 11[a]
Magnolia (*Magnolia* sp.) *n* = 15	Wax myrtle (*Morella cerifera*) *n* = 97[a]
Maple (*Acer* sp.) *n* = 3[a]	Willow oak (*Quercus phellos*) *n* = 54[a]
Mimosa (*Albizia julibrissin*) *n* = 39[a]	Winged elm (*Ulmus alata*) *n* = 19[a]

[a]Species that were analyzed in at least one or more seasons.
[b]Golden Bamboo was often listed as green bamboo on daily diet sheets or only as bamboo. The exact species was verified with the NC Zoo horticulture department.

TABLE 3 Total average daily dietary intake and summer (July 2016) and winter (January 2017) daily dietary intake ranges on a dry matter basis (DMB) and as fed basis for five of the six african elephants (*Loxodonta africana*) housed at the NC Zoo[a]

Feed type	Total average daily intake (range)	Season	Seasonal ranges DMB (as fed)
Hay, %	74.5 (58.1–83.1)	Summer	73.5–80.2 (48.6–61.8)
		Winter	70.3–78.3 (48.3–62.2)
Browse, %	5.18 (2.0–13.1)	Summer	4.8–5.3 (6.8–8.6)
		Winter	5.0–5.6 (5.6–7.2)
Pasture, %	12.6 (6.0–32.3)	Summer	6.9–13.7 (22.7–38.5)
		Winter	8.9–17.3 (23.6–40.1)
Pellet, %	3.8 (3.0–4.4)	Summer	3.5–4.1 (2.7–2.8)
		Winter	3.3–3.9 (2.7)
Enrichment, %	3.9 (3.0–4.8)	Summer	3.8–4.1 (3.4–4.3)
		Winter	3.5–3.9 (3.4–4.4)

[a]One elephant could not be weighed due to a pre-existing behavioral aversion to the barn scale.

3.3 | Vitamins and fatty acids

Dietary vitamins and fatty acid analyses total means and ranges are presented across season (Table 7), and LS means for between seasons (Tables 8 and 9). Neither D_2 nor D_3 were different between summer and winter nor were there differences among feed items. Summer LS means for α-tocopherol were higher than winter ($p = .002$). Additionally, NPDI type was different over time ($p = .01$) but did not differ between the five most commonly fed browse species. None of the carotenoids displayed seasonal differences or differences between NPDI types. Palmitic acid was higher in summer ($p = .005$) and autumn ($p = .015$) compared to winter. There were no differences across season for the n6:n3 ratio, but autumn was lower in PUFAs compared to spring ($p = .002$) and winter ($p = .02$) and lower in n3 when compared to spring ($p = .03$). There were no species differences among the five most commonly fed browse species for any fatty acids.

3.4 | Total diet

Total diet formulations were run for summer and winter months to determine dietary nutrient highs or lows and to compare nutrients between the two seasons assumed to have the highest and lowest nutrient concentrations respectively (Tables 10,11). Crude protein, ash, CF, and lignin percentages were similar between seasons. Inclusion rates of K, Fe, and Mn in summer were higher than their corresponding recommended intake levels (Ullrey et al., 1997). Copper, S, P, and Mg were marginally adequate. Dietary inclusion rates for Na and Se are below elephant recommendations (Ullrey et al., 1997). Winter inclusions of K, Fe, and Mn were also high compared to recommended intakes. In winter, S and Cu were marginally adequate along with Ca, P, and Mg. Like summer, Na and Se were very low in winter. Vitamin D_3 was below the recommended intake level in the current diet and reached negligible levels when pellets were removed (Ullrey et al., 1997).

TABLE 4 Proximate nutrient concentrations ($n = 64$) and Mineral Concentrations ($n = 64$) in non-produce feed (browse, hay items, and pasture) fed from February 2016 to April 2017 to six African Elephants (*Loxodonta africana*) at the NC Zoo

Nutrient	Mean ± *SD*	Range
Dry matter (DM)	50.4 ± 18.0	16.0–91.6
Crude protein (CP)	10.2 ± 5.2	3.8–23.5
Acid detergent fiber (ADF)	44.0 ± 9.9	24.6–65.1
Neutral detergent fiber (NDF)	60.2 ± 9.6	41.2–79.8
Crude fat (CF)	2.9 ± 1.6	0.9–9.3
Ash	7.0 ± 4.0	1.7–17.6
Lignin	12.5 ± 6.2	2.9–30.6
Starch	2.1 ± 1.2	0.3–5.1
Nonfibrous carbohydrates (NFC)	19.6 ± 8.5	2.0–37.3
Digestible energy (DE)	2,221.0 ± 375.0	1,540–2,930
Calcium	0.8 ± 0.5	0.1–1.9
Phosphorus	0.2 ± 0.1	0.0–0.4
Magnesium	0.1 ± 0.1	0.0–0.3
Potassium	1.2 ± 1.0	0.2–5.5
Sodium	0.00 ± 0.01	0.00–0.10
Iron	311 ± 596	29–2,483
Zinc	30 ± 25	8–168
Copper	6.5 ± 2.2	3.0–12.5
Manganese	227.4 ± 221.4	14.0–744.0
Molybdenum	0.6 ± 0.6	0.1–2.6
Sulfur	0.2 ± 0.1	0.1–0.5
Selenium	0.1 ± 0.0	0.0–0.2

When the diet was evaluated without pellets, there was no change in the marginality of Mg, but Cu and Zn became low in both summer and winter (Ullrey et al., 1997). The levels of Na and Se became even lower without pellets. Even when the amount of browse fed was doubled, these marginal and low ranges did not change. The formulated winter diet without pellets was similar to the summer diet. Phosphorus, Mg, and S were marginal. Copper and Zn became low and Na and Se levels became even lower. When browse was increased, there was little change in marginalities and low levels of minerals except for Zn which exceeded the recommended level in winter (Ullrey et al., 1997).

4 | DISCUSSION

All adult African elephants measured at the NC Zoo ($n = 5$) are maintaining a consumption level of 1.5–2.2% BW DMB. The consumption range overlaps with free ranging elephants (1.2–1.8% BW DMB) (Colbert, 1993, Dierenfeld, 2006; Laws, 1970). The youngest elephant in the NC Zoo herd had an average consumption

TABLE 5 Seasonal proximate analyses of five most common browse species fed to six NC Zoo African elephants (*Loxodonta africana*) from spring 2016 to spring 2017 with seasonal least square (LS) means and standard errors[a,b,c]

Units	%									kcal/kg
Feed item	**DM[d]**	**CP**	**ADF**	**NDF**	**Cfat**	**Ash**	**Lignin**	**Starch**	**NFC**	**DE[e]**
Mixed sp. bamboo	54.3	7.8	41.6	69.0	2.4	7.4	8.6	3.0	13.1	2,185
Sweet gum	45.5	5.1	45.9	55.9	1.8	5.2	14.9	1.2	32.0	2,020
Thorny elaeagnus	55.9	11.7	58.2	70.9	1.7	3.3	21.6	2.7	12.4	1,695
Tulip poplar	42.5	5.3	43.0	58.2	5.8	5.1	10.0	2.9	25.7	2,475
Wax myrtle	54.2	8.5	42.6	56.8	2.9	3.1	14.9	2.4	28.8	2,245
Spring LS mean[f]	**45.0 ± 1.6[*]**	**10.4 ± 0.6**	**43.6 ± 1.6**	**58.9 ± 1.6**	**3.0 ± 0.2**	**7.0 ± 0.3**	**11.8 ± 0.8**	**2.0 ± 0.2**	**20.5 ± 1.3**	**2,271 ± 264**
Spring total range	**19.0–91.0**	**3.8–23.2**	**29.9–60.4**	**45.3–77.9**	**0.9–6.3**	**2.5–17.6**	**3.3–21.6**	**0.3–4.6**	**7.8–35.4**	**1,540–2,930**
Mixed sp. bamboo	55.4	6.0	55.8	79.8	1.7	5.5	16.3	1.9	6.6	1,660
Sweet gum	35.1	6.6	39.7	49.1	2.3	4.8	11.2	2.2	37.3	2,325
Tulip poplar	37.1	6.1	39.8	52.2	5.4	4.7	13.6	4.8	31.8	2,365
Summer LS mean	**44.3 ± 2.0[*]**	**10.2 ± 0.8**	**45.3 ± 1.9**	**59.5 ± 1.9**	**3.6 ± 0.3**	**6.9 ± 0.4**	**13.9 ± 1.0**	**2.2 ± 0.3**	**19.6 ± 1.6**	**2,207 ± 275**
Summer total range	**23.9–89.2**	**5.4–18.3**	**31.9–58.6**	**46.1–79.8**	**1.7–9.3**	**1.7–14.3**	**3.5–30.6**	**0.3–4.8**	**6.6–37.3**	**1,600–2,680**
Sweet gum	44.1	6.4	36.8	51.1	1.9	5.8	10.5	3.0	35.0	2,240
Thorny elaeagnus	45.0	14.6	50.4	66.6	3.0	3.9	19.2	0.8	11.9	1,958
Tulip poplar	42.5	7.5	40.6	61.4	4.4	5.2	10.8	3.7	21.7	2,340
Autumn LS mean	**50.3 ± 1.9[*,**]**	**10.0 ± 0.7**	**40.3 ± 1.8**	**60.1 ± 1.8**	**3.0 ± 0.2**	**7.2 ± 0.3**	**11.9 ± 0.9**	**2.5 ± 0.3**	**20.2 ± 1.5**	**2,230 ± 283**
Autumn total range	**16.0–89.6**	**6.1–21.4**	**24.6–52.8**	**42.0–73.0**	**1.8–7.5**	**2.9–17.4**	**2.9–19.2**	**0.5–5.1**	**2.0–35.0**	**1.705–2,795**
Mixed sp. bamboo	58.5	8.5	51.5	74.5	1.9	7.7	12.4	1.9	7.9	1,915
Sweet gum	50.1	4.8	56.3	67.4	1.5	4.5	15.6	2.3	21.9	1,863
Thorny elaeagnus	48.0	15.8	56.0	66.7	2.3	4.3	22.0	1.6	11.2	1,805
Wax myrtle	54.0	7.9	54.7	65.0	2.7	3.4	27.3	1.6	21.0	1,590
Winter LS mean	**55.1 ± 2.4[**]**	**10.5 ± 0.9**	**48.8 ± 2.4**	**59.4 ± 2.3**	**2.8 ± 0.3**	**7.3 ± 0.4**	**15.1 ± 1.2**	**2.3 ± 0.3**	**18.7 ± 2.0**	**2,038 ± 305**
Winter total range	**39.6–91.6**	**4.8–22.3**	**31.7–65.1**	**41.2–75.0**	**1.1–6.0**	**3.1–17.3**	**3.2–27.3**	**0.8–2.9**	**7.9–31.9**	**1,590–2,780**

Abbreviations: ADF, acid detergent fiber; Cfat, Crude fat; CP, crude protein; DE, digestible energy; DM, dry matter; NDF, neutral detergent fiber; NFC, nonfibrous carbohydrates.

[a]Seasons are defined as: Spring (March–May), Summer (June–August), Autumn (September–November), Winter (December–February).

[b]Differing superscripts (*,**) in seasonal least squares mean columns are significantly different at ($p = .05$).

[c]Seasonal least square means and ranges include all sampled browse species, hay, and pasture.

[d]DM on an as fed basis.

[e]DE calculated based on ruminants.

[f]Spring samples include spring 2016 and spring 2017.

level of 3.2% BW DMB; this higher level was to ensure proper caloric intake for growth.

A low inclusion, grain-free Mazuri® Hay Enhancer™ dietary supplement was developed to contain higher fiber levels (30% max crude fiber, ~ 36% NDF, and 25% ADF) compared to traditional elephant supplements such as Mazuri® Elephant Supplement™ (12% max crude fiber, ~ 22% NDF, 11% ADF), resulting in lower predicted energy. Compared to traditional elephant diets, such as Mazuri® Wild Herbivore Plus™ (22% max crude fiber, ~ 38% NDF, 23% ADF), Mazuri® Hay Enhancer™ contains lower NDF and ADF since it is included in diets with higher forage intake levels. It also is fortified with micronutrients, so it can be fed at low dietary inclusion rates (~ 5% DMB of the total diet). During this study, Mazuri® Hay Enhancer™ was fed at an average of 3.8% of the total NC Zoo elephant diet.

A total of 42 individual browse species were fed during this study. Despite browse only contributing an average of 5.2% DMB of the daily diet, diversity of browse being fed to these elephants daily and seasonally helped to provide a more varied diet. Observation by researchers and keeper staff noted browse preferences specific to individual animals and a decrease in interest of specific species if fed for extended time periods. The number of browse species fed per day depended on accessibility and the amount of keeper time available for cutting browse. While all elephants would regularly strip and consume bark, one female elephant was more likely to consume thicker branches and small tree trunk pieces. Manipulation of large branches after stripping was seen, but very large branch trunk portions (>5 in diameter) were often ignored. These larger branches and trunk portions may offer behavioral enrichment, but do not offer nutritional benefits. If weight loss is desired for animals, including

TABLE 6 Seasonal mineral analyses of five most common browse species fed to six NC Zoo African elephants (*Loxodonta africana*) from Spring 2016 to Spring 2017 with seasonal least square (LS) means and standard deviations[a,b,c]

Units	%						mg/kg					
Feed item	**Ca**	**P**	**Mg**	**K**	**Na**	**S**	**Fe**	**Zn**	**Cu**	**Mn**	**Mo**	**Se**
Mixed sp. bamboo	0.3	0.1	0.1	0.8	0.0	0.2	294	25	6	98	0.8	0.1
Sweet gum	1.5	0.1	0.1	0.3	0.0	0.1	56	40	9	666	0.2	0.0
Thorny elaeagnus	0.3	0.1	0.1	0.9	0.0	0.2	96	12	9	348	0.2	0.1
Tulip poplar	1.3	0.1	0.2	0.5	0.0	0.1	45	25	6	180	0.2	0.0
Wax myrtle	0.7	0.1	0.1	0.3	0.0	0.1	79	33	6	574	0.3	0.1
Spring LS means[d]	**0.9 ± 0.1**	**0.2 ± 0.0**	**0.1^{*} ± 0.0**	**1.3 ± 0.1**	**0.0 ± 0.0**	**0.2 ± 0.0**	**301 ± 22**	**28 ± 2**	**6 ± 0**	**248 ± 26**	**0.7 ± 0.1**	**0.1 ± 0.0**
Spring total range	**0.2–1.5**	**0.0–0.4**	**0.0–0.2**	**0.2–3.8**	**0.0**	**0.1–0.4**	**36–2420**	**10–168**	**4–10**	**14–706**	**0.1–2.6**	**0.0–0.2**
Mixed sp. bamboo	0.2	0.2	0.1	1.0	0.0	0.2	179	22	8	93	0.4	0.0
Sweet gum	0.9	0.1	0.2	0.6	0.0	0.1	73	50	7	281	0.3	0.1
Tulip poplar	0.9	0.1	0.2	0.7	0.0	0.1	29	18	6	233	0.4	0.0
Summer LS means	**0.8 ± 0.1**	**0.2 ± 0.0**	**0.2^{**} ± 0.0**	**1.4 ± 0.1**	**0.0 ± 0.0**	**0.2 ± 0.0**	**245 ± 27**	**27 ± 2**	**7 ± 0**	**214 ± 32**	**0.7 ± 0.1**	**0.0 ± 0.0**
Summer total range	**0.2–1.8**	**0.1–0.4**	**0.1–0.3**	**0.3–2.7**	**0.0**	**0.1–0.5**	**29–1940**	**15–147**	**5–11**	**20–744**	**0.1–1.6**	**0.0–0.1**
Sweet gum	1.1	0.1	0.2	0.5	0.0	0.1	42	54	10	654	0.2	0.2
Thorny elaeagnus	0.5	0.1	0.1	1.0	0.0	0.2	115	27	8	81	0.2	0.1
Tulip poplar	1.1	0.1	0.2	0.6	0.0	0.1	58	29	9	178	0.5	0.1
Autumn LS means	**0.9 ± 0.1**	**0.2 ± 0.0**	**$0.2^{*,**}$ ± 0.0**	**1.0 ± 0.1**	**0.0 ± 0.0**	**0.2 ± 0.0**	**282 ± 25**	**31 ± 2**	**6 ± 0**	**226 ± 31**	**0.5 ± 0.1**	**0.1 ± 0.0**
Autumn total range	**0.3–1.9**	**0.1–0.4**	**0.1–0.3**	**0.5–5.5**	**0.0**	**0.1–0.4**	**42–1855**	**8–54**	**3–13**	**29–654**	**0.1–2.2**	**0.0–0.2**
Mixed sp. bamboo	0.2	0.1	0.07	0.7	0.0	0.1	121	26	5	97	0.4	0.1
Sweet gum	1.1	0.1	0.14	0.3	0.0	0.1	29	36	9	243	0.1	0.0
Thorny elaeagnus	0.6	0.1	0.12	0.9	0.0	0.2	145	20	9	150	0.5	0.1
Wax myrtle	0.9	0.0	0.14	0.3	0.0	0.1	65	41	8	428	0.1	0.1
Winter LS means	**0.9 ± 0.1**	**0.2 ± 0.0**	**$0.2^{*,**}$ ± 0.0**	**1.1 ± 0.1**	**0.0 ± 0.0**	**0.2 ± 0.0**	**311 ± 33**	**30 ± 3**	**6 ± 1**	**201 ± 40**	**0.5 ± 0.1**	**0.1 ± 0.0**
Winter total range	**0.2–1.4**	**0.0–0.3**	**0.1–0.2**	**0.3–2.4**	**0.0–0.1**	**0.1–0.4**	**29–2483**	**18–41**	**4–9**	**30–598**	**0.1–2.1**	**0.0–0.2**

[a]Seasons are defined as: Spring (March–May), Summer (June–August), Autumn (September–November), Winter (December–February).
[b]Differing superscripts (*,**) in seasonal least squares mean columns are significantly different at (p = .05).
[c]Seasonal least square means and ranges include all sampled browse species, hay, and pasture.
[d]Spring samples include spring 2016 and spring 2017.

larger branches could be used to meet foraging behavioral needs without providing excessive energy.

Proximate analysis of feed items found that DM was the only value to differ among seasons. Winter had the highest DM percentages of all four seasons. The range for DM (16.0–91.6%) was a wider range than free-ranging values outlined by Ullrey et al. (1997) (30–45%) and Dierenfeld (2006) (40–60%). Winter samples of thorny elaeagnus and wax myrtle, two regularly fed winter browse species, contained greater than 20% lignin. This increased level of lignin in winter should be considered as high levels of lignin have negative effects on energy digestibility (Moore & Jung, 2001). The inclusion of these two species in the diet should be monitored to reduce negative DE effects.

Dietary intake levels of minerals were outside reference ranges in several instances. Sodium levels were low for nearly all feed items, making Na a mineral of concern. The low level of Na would decrease more if pellets are excluded, with or without additional browse (Test Diet 2 and 3). Selenium was very low in the current diet and both simulated diet scenarios (Ullrey et al., 1997). Elephants were noted to practice geophagy on exhibit. This practice could be an instinctual method of consuming more Na and other minerals (Dierenfeld, 2006). The water these animals consume contains 23 mg/L of Na, this is within the public water range (5–370 mg/L) collected by the Environmental Protection Agency (U.S. Environmental Protection Agency Office of Water, 2003). This is another source of Na in the diet. Selenium was present in the water at less than 0.005 mg/L making it an unlikely source of Se. Future research into dietary minerals for elephants would benefit from analyzing soil samples to fill gaps in NPDI data.

Nearly all browse species were high (>100 mg/kg) in Fe or Mn during at least one season. Iron was consistently very high (>1,000 mg/kg) in pasture grass samples. The high levels of Fe could be attributed to some soil contamination in the samples during collection although every attempt was made to limit this concern. Because of observed geophagy practices, this high value may be reflective of what the animals are consuming. Arundo, the sixth most commonly fed browse species, as well as mixed species bamboo contained >100 mg/kg Fe in both spring and summer. While

TABLE 7 Means, standard deviations (SD), minimums, and maximums for α-tocopherol and carotenoid concentrations ($n = 26$), vitamin D concentrations ($n = 25$), and fatty acids ($n = 54$) in non-produce feed (browse, hay items, and pasture) fed from February 2016 to April 2017 to six African elephants (*Loxodonta africana*) at the NC Zoo

Nutrient	Mean ± SD	Range
α-Tocopherol	2.0 ± 1.3	0.6–5.3
Lutein	20.4 ± 14.8	0.7–58.9
Zeaxanthin	3.9 ± 2.4	0.3–9.5
β-Cryptoxanthin	2.0 ± 1.0	0.3–4.5
β-Carotene	7.5 ± 8.4	0.3–33.0
α-Carotene	1.8 ± 1.6	0.3–6.8
Ergocalciferol (D_2)	0.0 ± 0.0	0.0–0.0
Cholecalciferol (D_3)	0.0 ± 0.0	0.0–0.0
Lauric acid (12:0)	0.7 ± 1.2	0.0–5.6
Myristic acid (14:0)	0.9 ± 0.7	0.0–3.9
Pentadecylic acid (15:0)	0.3 ± 0.4	0.0–1.4
Palmitic acid (16:0)	19.6 ± 4.9	8.0–28.8
Linoleic acid (18:2n6)	15.7 ± 5.1	6.9–30.2
α-Linolenic acid (18:3n3)	26.1 ± 9.9	7.9–54.8
Other	15.4 ± 6.8	4.2–38.1
Total saturate	30.7 ± 6.7	16.3–44.4
Total PUFA	41.9 ± 8.7	23.3–65.1
Total omega-3 (n3)	26.1 ± 9.9	7.9–54.8
Total omega-6 (n6)	15.8 ± 5.1	6.9–30.3
n6/n3	0.8 ± 0.5	0.2–2.8

nonheme iron in plants is less-well absorbed, there are variations in the ability to absorb iron from different species due to tannin content (Clauss et al., 2007; Ems & Huecker, 2019). Excessive Fe is known to competitively inhibit Zn uptake in many mammals leading to Zn deficiency (Schmidt, 1989). Hyperkeratosis, a common skin concern in elephants, has been attributed to Zn deficiencies (Schmidt, 1989). Fe consumption should be monitored in pachyderm species managed at the NC Zoo and Southeastern institutions. Mn was consistently high (>200 mg/kg) in sweet gum across seasons. These Mn values far exceed the recommended intake level in African elephants (40 mg/kg) (Ullrey et al., 1997) but did not exceed the maximum tolerance level in horses (1,000 mg/kg) (National Research Council, 2007). While Mn is not well studied in elephants and considered one of the least toxic minerals for horses, excesses may inhibit P absorption and thus knowing the levels in feed sources may be important (Schryver, Hintz, & Lowe, 1971). A noteworthy increase in Mo was seen in summer pasture grass. While no information is available on recommended intake levels of Mo in African elephants, it is understood that ruminant species can suffer from molybdenosis, a Cu deficiency disease (Erdman, Ebens, & Case, 1978). Molybdenosis can have negative effects on reproduction and general health in ruminants, including antelope species (Mbatha, Lane, Lander, Tordiffe, & Corr, 2012). These elephants are housed next door to an exhibit that holds several ruminant antelope species thus monitoring Mo levels is beneficial.

Dietary vitamin D_2 and D_3 were not significantly different in summer versus winter, but numerically D_2 levels were higher in summer than winter. The pelleted feed was the only considerable source of D_3 in the diet but is fed at a very low (<5%) inclusion rate of the total diet. Most herbivorous animals consume a majority of vitamin D as vitamin D_2 or ergocalciferol, which can be converted to vitamin D_3 in the animal's body (Combs & McClung, 2017). Currently, there are no recorded values of recommended vitamin D_2 intakes for African elephants but compared to recommended intake values of vitamin D_3, the current diet is low and would have nearly non-existent levels in the diet without the pellet (Ullrey et al., 1997). Vitamin D deficiencies have been noted in animals housed for long periods without access to sunlight (Du Toit & Mikota, 2006). Based on this observation, it is assumed that African elephants can synthesize some vitamin D from direct sunlight. Vitamin D_3 is critical for Ca metabolism and maintaining bone health, which has become a concern in elephant calves displaying symptoms of metabolic bone disease (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Miller, Chen, Holick, Mikota, & Dierenfeld, 2009). Monitoring vitamin D intake with potential supplementation in winter months with less sunlight and dietary vitamin D available is recommended. Additional research into vitamin D metabolism in African elephants and geographical location differences would be of benefit to nutritional management.

Vitamin E, a group of eight tocopherols and tocotrienols, is an antioxidant often associated with Se and polyunsaturated fatty acids (PUFAs) and may also play a role in immune function (Jurgens, 1988; Kenny, 2001; Machlin, 1984; McDonald, Edwards, & Greenhalgh, 1988). The only form of vitamin E that has a described function and is considered “active” is α-tocopherol (Jurgens, 1988; Kenny, 2001; Machlin, 1984; McDonald et al., 1988). In elephants and other animals, vitamin E deficiencies have negative effects on the muscular, nervous, circulatory, and reproductive systems and have been linked to some human managed elephant deaths (Dierenfeld & Dolensek, 1988; Dierenfeld & Ranglack, 1994; Savage et al., 1999). Concentrations of dietary α-tocopherol differed significantly between summer and winter. As expected in a concentrated pellet, Mazuri® Hay Enhancer™ contained the highest levels of α-tocopherol out of all feed items. Owing to the low levels contained in browse, hay and other enrichment feed items, elephant diets without a fortified pellet are not recommended. The primary role of carotenoids in mammals is to act as precursors for vitamin A or retinol (Lietz, Oxley, Boesch-Saadatmandi, & Kobayashi, 2012; Schweigert, 1998). The equine carotenoid metabolism is not well understood, but the ability to convert dietary carotenoids into retinol in grazing horses is poor (Greiwe-Crandell et al., 1997). No data is currently available on dietary recommendations for carotenoids in elephants. It should be noted that our plant samples were dried in a drying oven before analyses. There is literature that suggests this process may artificially lower the analytical results of some nutrients compared to freeze drying (Roshanak, Rahimmalek,

TABLE 8 Vitamin D, α-tocopherol, and carotenoid concentration (mg/kg) analyses of five most common browse species fed to six NC Zoo African elephants (*Loxodonta africana*) in summer 2016 and winter 2016 with seasonal least square (LS) means and standard deviations[a,b]

Feed Item	Ergocalciferol (D_2)[c]	Cholecalciferol (D_3)[c]	α-tocopherol	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	α-Carotene
Mixed sp. bamboo	<0.0005	<0.0005	1.2	9.2	2.2	1.2	3.7	1.1
Sweet gum	0.01	<0.0005	1.5	14.8	3.0	1.4	6.5	1.4
Tulip poplar	0.01	<0.0005	1.9	15.0	2.8	1.6	4.6	1.4
Summer LS mean[d]	**0.02 ± 0.02**	**0.00 ± 0.00**	**2.8* ± 0.1**	**23.7 ± 4.4**	**4.2 ± 0.7**	**2.3 ± 0.2**	**9.8 ± 1.9**	**2.4 ± 0.3**
Summer total range	**< 0.0005–0.04**	**<0.0005**	**1.2–4.4**	**5.0–58.9**	**1.5–7.9**	**1.1–4.5**	**1.3–33.0**	**1.0–6.8**
Mixed sp. bamboo	0.00	<0.0005	0.9	17.4	3.2	1.7	5.0	1.2
Sweet gum	< 0.0005	<0.0005	0.6	1.2	0.6	0.3	0.4	0.3
Thorny elaeagnus	0.01	<0.0005	1.3	16.9	2.6	1.7	4.5	1.2
Wax myrtle	0.01	0.00	1.5	14.5	3.4	1.2	5.3	1.1
Winter LS Mean	**0.01 ± 0.02**	**0.00 ± 0.00**	**1.1** ± 0.2**	**16.2 ± 5.8**	**3.4 ± 1.0**	**1.6 ± 0.3**	**3.8 ± 2.5**	**0.9 ± 0.5**
Winter total range	**<0.0005–0.04**	**<0.0005–0.001**	**0.6–5.3**	**0.7–31.4**	**0.3–9.5**	**0.3–3.6**	**0.3–16.0**	**0.3–3.4**

[a]Seasons are defined as: Summer (June–August) and Winter (December–February).
[b]Differing superscripts (*,**) in seasonal least squares mean columns are significantly different at ($p = .05$).
[c]Values listed as <.0005 were below the detection level and were converted to zeros for all statistical analysis.
[d]Summer browse does not contain detectable levels of D_3.

& Goli, 2016), However, other available references are not consistent and much of the comparative historic data used a drying oven and therefore we believe our results are appropriate for comparison (Chuyen et al., 2016; Saini et al., 2014).

Fatty acids are critical for the maintenance of cellular membranes, regulation of cellular functions, immunity, blood flow regulation, and reproduction (Clauss et al., 2003). In human managed exotic species, n6:n3 dietary imbalances have been noted when compared to free-ranging counterparts. These imbalances are associated with the higher levels of n6 fatty acids present in human managed diets while free-ranging diets contain more n3 fatty acids (Clauss et al., 2003; Cordain et al., 2002; Davidson, 1998; French et al., 2000; Koizumi, Suzuki, & Kaneko, 1991). Additionally, decreased levels of PUFAs have been noted in human managed diets (Clauss et al., 2003; Crawford, 1968; Dierenfeld, 2006). While there was no significant difference across seasons for the n6:n3 ratio, visually total n6 fatty acid were lower than n3 fatty acids in browse species. The n6:n3 imbalances and decreased PUFA levels have been attributed to the skin and vascular diseases seen in human-managed African elephants (Clauss et al., 2003). Prior research with free-ranging elephants has found evidence to suggest higher levels of browse reduces the risk of arterial disease because of the higher levels of PUFAs and n:3 fatty acids compared to grasslands (Clauss et al., 2003; McCullagh & Lewis, 1967; McCullagh, 1969a; McCullagh, 1969b; McCullagh, 1975; McCullagh, 1972; McCullagh, 1973). This information further supports the idea that inclusion of browse in the diet may more closely mimic the fatty acids in free-ranging diets and be beneficial to human-managed African elephant health. Linoleic acid and α-linolenic acid cannot be synthesized in the body and male African elephants with deficiencies have previously shown poor spermatozoa development (Dierenfeld, 2006; Macdonald, Rogers, Morris, & Cupps, 1984; Marzouki & Coniglio, 1982). Concentrations of α-linolenic acid had the widest range (7.9–54.8%) of all the fatty acids analyzed. Dietary fatty acid data from this study is novel to exotic animal nutrition and can provide additional information on critical diet information. Because of the lack of information on required dietary fatty acid concentrations and dietary concentrations of free-ranging browse species, research into dietary fatty acids should be pursued.

From formulated total diet calculations for Diet 2, it was found that excluding nutritionally fortified pellets with no change to the amount of browse consumed would lead to decreases in required dietary minerals, vitamin D, and vitamin E. If the current amount of browse offered was doubled (Diet 3), elephant nutrient recommendations for various minerals (Mg, Cu, Na, and Se) and vitamins D and E would still not be fulfilled in absence of the pellet. The removal of pellet from elephant diets in the Southeast USA would require strict management of the species and amounts of browse provided as well as additional vitamin E supplements to meet current recommended intakes (Dierenfeld, 2006; Miller et al., 2009; Species360 Zoological Information Management System, 2017).

To increase foraging behavior, Stoinski et al. (2000) replaced a portion of hay in the diet of African elephants with approximately

TABLE 9 Seasonal fatty acid analyses (%) of the five most common browse species fed to six NC Zoo African elephants (*Loxodonta africana*) from spring 2016 to spring 2017 with seasonal least square (LS) means and standard deviations[a,b,c]

Feed Item	Lauric acid (12:0)	Myristic acid (14:0)	Pentadecylic acid (15:0)	Palmitic acid (16:0)	Linoleic acid (18:2n6)	α-Linolenic acid (18:3n3)	Other[e]	Total Saturate	Total PUFA	Total n3	Total n6	n6/n3 Ratio
Golden bamboo	0.0	2.0	0.4	19	15	24	21	27	40	24	15	0.6
Thorny elaeagnus	0.0	0.8	0.5	15	10	32	16	28	41	32	10	0.3
Wax myrtle	0.0	0.5	0.0	27	28	17	8	33	45	17	28	1.7
Spring LS means[d]	**$0.1^{*} \pm 0.4$**	**$1.0^{*} \pm 0.2$**	**$0.2^{*} \pm 0.1$**	**$19^{*,**} \pm 1$**	**17 ± 2**	**$28^{*} \pm 2$**	**$14^{*,**} \pm 1$**	**$28^{*} \pm 1$**	**$46^{*} \pm 2$**	**$29^{*} \pm 2$**	**17 ± 2**	**0.8 ± 0.2**
Spring total range	**0.0–0.2**	**0.4–2.0**	**0.0–0.6**	**8–27**	**10–28**	**9–55**	**8–37**	**16–41**	**28–65**	**10–55**	**10–28**	**0.2–1.9**
Mixed sp. bamboo	0.0	0.0	0.0	22	25	15	16	28	40	15	25	1.6
Sweet gum	0.0	0.0	1.0	27	16	29	6	35	45	29	16	0.5
Tulip poplar	0.0	0.6	0.9	18	21	24	13	31	45	24	21	0.9
Summer LS means	**$0.0^{*} \pm 0.4$**	**$0.2^{**} \pm 0.1$**	**$0.7^{**} \pm 0.1$**	**$22^{*} \pm 1$**	**15 ± 1**	**$27^{*,**} \pm 2$**	**$11^{*} \pm 1$**	**$33^{**,***} \pm 1$**	**$42^{*,**} \pm 1$**	**$28^{*,**} \pm 2$**	**15 ± 1**	**0.6 ± 0.1**
Summer total range	**0.0–1.6**	**0.0–0.9**	**0.0–1.4**	**9–29**	**9–25**	**8–45**	**4–38**	**23–44**	**25–59**	**8–45**	**9–25**	**0.3–2.2**
Sweet gum	0.6	2.2	0.0	24	18	18	20	33	36	18	18	1.0
Thorny elaeagnus	0.6	1.1	0.7	15	7	33	14	30	39	33	7	0.2
Tulip poplar	0.8	1.1	0.2	14	28	23	14	24	51	23	28	1.2
Autumn LS means	**$0.8^{*,**} \pm 0.3$**	**$1.5^{*} \pm 0.1$**	**$0.1^{*} \pm 0.1$**	**$21^{*} \pm 1$**	**17 ± 1**	**$20^{**} \pm 2$**	**$18^{**} \pm 1$**	**$34^{**} \pm 1$**	**$37^{**} \pm 1$**	**$20^{*} \pm 2$**	**17 ± 1**	**0.9 ± 0.1**
Autumn total range	**0.2–5.2**	**0.9–3.9**	**0.0–0.7**	**14–27**	**7–28**	**15–38**	**11–25**	**24–43**	**27–51**	**15–38**	**7–28**	**0.2–1.2**
Mixed sp. bamboo	0.5	1.2	0.5	18	14	33	18	25	48	33	15	0.5
Sweet gum	1.1	0.6	0.0	17	30	11	20	29	41	11	30	2.8
Thorny elaeagnus	0.4	1.0	0.5	13	11	36	15	25	47	36	11	0.3
Wax myrtle	0.6	1.0	0.0	21	17	24	15	29	42	24	17	0.7
Winter LS means	**$1.7^{**} \pm 0.4$**	**$1.1^{*} \pm 0.2$**	**$0.1^{*} \pm 0.1$**	**$16^{**} \pm 1$**	**15 ± 2**	**$29^{**} \pm 2$**	**$17^{**} \pm 0$**	**$28^{**} \pm 0$**	**$44^{*} \pm 2$**	**$29^{*,**} \pm 2$**	**17 ± 1**	**0.8 ± 0.2**
Winter total range	**0.3–5.6**	**0.6–1.7**	**0.0–0.5**	**10–21**	**11–30**	**8–40**	**15–29**	**20–44**	**23–52**	**8–40**	**11–30**	**0.3–2.8**

[a]Seasons are defined as: Spring (March–May), summer (June–August), autumn (September–November), winter (December–February).
[b]Differing superscripts (*,**) in seasonal least squares mean columns are significantly different at ($p = .05$).
[c]Seasonal least square means and ranges include all sampled browse species.
[d]Spring samples include spring 2016 and spring 2017.
[e]Includes fatty acids that are not known; can include odd and/or branched chain fatty acids.

TABLE 10 Dietary breakdowns of four formulated NC Zoo African elephant (*Loxodonta africana*) diets with nutrient ranges in summer 2016 compared to reference values

Overall diet proportions (DMB)	Diet 1[a]	Diet 2[b]	Diet 3[c]		
Hay, %	73.5–80.2	76.7–83.1	73.0–78.8		
Browse, %	4.8–5.3	5.1–5.5	9.6–10.4		
Pasture, %	6.9–13.7	7.2–14.3	6.8–13.6		
Pellet, %	3.5–4.1	0.0	0.0		
Enrichment/other, %	3.8–4.1	4.0–4.3	3.8–4.1		
Daily intake, % BW	1.5–3.0	1.5–2.9	1.5–3.1		
Nutrients (DMB)				**Ele. Rec.[d]**	**Horse Rec.[e]**
CP, %	11.3–12.0	10.9–11.6	10.7–11.3	8.0–10.0	8.0–10.7
ADF, %	34.2–38.4	34.4–34.7	34.8–35.1	30.0	NA
Lignin, %	3.7–3.8	3.9	4.3–4.4	NA	
Starch, %	1.3	1.3	1.3	NA	
NFC, %	13.7–14.0	14.3–14.6	15.0–15.3	NA	
CF, %	3.2–3.3	3.2	3.2	NA	
Ash, %	9.0–9.6	9.0–9.6	8.8–9.4	NA	
Linoleic acid, %	15.2–15.4	15.7–15.8	15.8–15.9	NA	
Linolenic acid, %	28.7–29.6	29.7–30.8	29.4–30.5	NA	
Ca, %	0.6	0.5	0.6	0.3	0.3
P, %	0.2–0.3	0.3	0.3	0.2	0.2
Na, %	0.04–0.05	0.01	0.01	0.10	0.20
K, %	2.0	2.0	1.9	0.4	0.4
Mg, %	0.2	0.2	0.2	0.1	0.1
Fe, mg/kg	299–458	289–453	278–435	50	60
Cu, mg/kg	10.3–11.3	6.3–6.6	6.3–6.6	10.0	14.0
Mn, mg/kg	68–69	65	80–81	40	59
Zn, mg/kg	45–50	24–25	24–25	40	59
Se, mg/kg	0.12–0.13	0.04–0.05	0.04–0.05	0.20	0.15
S, %	0.2	0.2	0.2	0.2[f]	NA
Vit D_3, IU/kg	494–583	0	0	800	489
Vit E, IU/kg	78–91	4	4	100	75

[a]Current diet including pasturepasture.
[b]Diet excluding Hay Enhancer™.
[c]Diet excluding Hay Enhancer™ with doubled browse.
[d]Ullrey et al. (1997).
[e]National Research Council (2007).
[f]Williams et al. (2015).

equal parts DM of browse. His results showed an increase in time spent eating and a decrease in inactivity of the animals while on exhibit. Based on results from Stoinski et al. (2000), reducing the amount of hay in conjunction with increased browse is recommended to increase foraging behavior during the day while avoiding exceeding energy requirements.

Increasing the amount of browse does have limitations; it would require more time and labor to cut and collect browse and could be unsustainable for most institutions that strictly utilize woodland on property. Outside of time and labor, collected browse can appear to be a larger amount because of the bushy nature compared to physical weights. Diets are formulated with specific weight inclusions thus estimating browse inclusion by number of branches or bushiness of cut browse may not provide adequate weight. For these reasons, browse management may require additional staff to exclusively cut browse for a sizable portion of the day if browse is included in multiple species diets. Even with a specified team to cut browse, there may not be enough large, sustainable trees available for approved browse species on site. Delivery of browse from outside sources may be considered but would need to be vetted for pesticides and herbicides. In addition, for zoos considering use of browse, education on browse feeding, identification and training will be needed.

TABLE 11 Dietary breakdowns of four formulated NC Zoo African elephant (*Loxodonta africana*) diets with nutrient ranges in winter 2017 compared to reference values

Overall diet proportions (DMB)	Diet 1[a]	Diet 2[b]	Diet 3[c]		
Hay, %	70.3–78.3	73.1–81.0	69.5–76.6		
Browse, %	5.0–5.6	5.2–5.8	9.9–10.9		
Pasture, %	8.9–17.3	9.2–18.0	8.7–17.1		
Pellet, %	3.3–3.9	0.0	0.0		
Enrichment/other, %	3.5–3.9	3.7–4.1	3.5–3.9		
Daily intake, % BW	1.6–3.1	1.5–3.0	1.6–3.1		
Nutrients (DMB)				**Ele. rec.[d]**	**Horse rec.[e]**
CP, %	11.1–12.0	10.8–11.6	10.6–11.4	8.0–10.0	8.0–10.7
ADF, %	32.3–32.4	32.4–32.5	33.7–33.9	30.0	NA
Lignin, %	3.9	4.0–4.1	4.8	NA	
Starch, %	0.9–1.0	0.9–1.0	1.0–1.1	NA	
NFC, %	18.4–19.4	19.2–20.1	19.0–19.8	NA	
CF, %	2.7–2.8	2.6–2.7	2.6–2.7	NA	
Ash, %	7.4–8.4	7.4–8.4	7.3–8.2	NA	
Linoleic acid, %	12.3–12.7	12.7–13.1	12.9–13.2	NA	
Linolenic acid, %	33.0–34.6	34.1–35.9	35.5–33.7	NA	
Ca, %	0.4	0.3	0.3–0.4	0.3	0.3
P, %	0.2	0.2	0.2	0.2	0.2
Na, %	0.04	0.01	0.01	0.10	0.20
K, %	1.6–1.7	1.6–1.7	1.39–1.42	0.4	0.4
Mg, %	0.2	0.2	0.1–0.2	0.1	0.1
Fe, mg/kg	311–511	302–508	291–488	50	60
Cu, mg/kg	10.2–11.1	6.4–6.8	6.4–6.7	10.0	14.0
Mn, mg/kg	72–73	70	54–66	40	59
Zn, mg/kg	43–48	23–24	77–79	40	59
Se, mg/kg	0.10	0.03–0.04	0.04	0.20	0.15
S, %	0.2	0.1–0.2	0.1–0.2	0.2[f]	NA
Vit D_3, IU/kg	467–500	1	2	800	489
Vit E, IU/kg	72–83	2	2	100	75

[a]Current diet including pasture.
[b]Diet excluding Hay Enhancer™.
[c]Diet excluding Hay Enhancer™ with doubled browse.
[d]Ullrey et al. (1997).
[e]National Research Council (2007).
[f]Williams et al. (2015).

5 | CONCLUSION

The NPDI data presented is both novel and provides a more complete picture of a representative Southeastern zoo elephant diet. Nutritional analyses from browse species recorded and grasses in this study will contribute to a NC Zoo browse database as well as a developing online browse database for all seasons. This will enable both the NC Zoo and other zoos within the United States to better incorporate browse into all appropriate animal diets, and ensure nutrient intakes are based on current knowledge. However, given variation in browse species between season and region, it may be important to compare local samples with findings from this study. This allows for better management of elephants and other exotic herbivore species well-being within human care facilities. Total diet calculations determined within this project do not currently support the exclusion of pellets from African elephant diets.

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