ANALYSIS OF NUTRIENTS, MOISTURE LOSS, AND VITAMIN STABILITY IN PRIMATE BROWSE HARVESTED AT THE SAN DIEGO ZOO

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Abstract

The objective of this study was to obtain nutrient profiles along with insight on rates of moisture and vitamin loss in browse harvested at San Diego Zoo Global (SDZG). Ten species of browse commonly offered to colobines were manually separated into leaf and bark components for analysis at a commercial laboratory. Findings indicate that a variety of browse species and dietary items are essential to meet nutrient requirements. *Morus alba* was utilized for the water retention and vitamin stability trials. Browse that was presented upright or upside down without water for 24 h lost 12-14% of the original leaf moisture compared to browse presented upright in water for 24 h, which only lost 4% of the original leaf moisture (difference numerical, not statistically significant, $P > 0.05$). Large variance in the data suggests that the study evaluating water loss should be repeated. Refrigerating the *M. alba* browse at 4°C maintained the α-tocopherol concentration (270 μg/g DM) for 172 h post-harvest, and β-carotene concentration (225 μg/g DM) was maintained for 100 h before decreasing 23% after 172 h post-harvest (difference numerical, not statistically significant, $P > 0.05$). Alpha-tocopherol and β-carotene remained relatively stable; subsequent studies on vitamin stability could incorporate longer storage time of *M. alba*.

Introduction

Three projects performed during the summer of 2014 included: browse components and nutrient assessment, browse water retention, and vitamin stability in browse offered to primates. Nutrient profiles of browse offer information to improve dietary formulation. Browse composition is especially important to consider when it constitutes a significant portion of an animal's diet. In order to collect data regarding browse offered to leaf-eating primates, 10 species of browse plants grown at San Diego Zoo Global (SDZG) were analyzed. One of these species, white mulberry (*Morus alba*), was used to study water retention and vitamin stability.

Mulberry is a deciduous plant that grows quickly in various climates and is utilized as feed for insects and animals (Kandylis et al., 2009). To explore the effect that presentation methods may have on rate of moisture loss, *M. alba* was subjected to three different treatments and monitored for water retention. Moisture retention over 24 h was examined to improve presentation for increased consumption. Wild colobines have demonstrated the ability to select leaves based on moisture content (Mckey et al., 1981; Mowry et al., 1996), which may affect palatability and receptivity among primates (Laska et al., 2000).

Additionally, mulberry leaves are valued for nutritive and antioxidant components, including α-tocopherol and β-carotene (Yen et al., 1996). In response to the installation of a cooler dedicated to primate browse at the San Diego Zoo (SDZ), the stability of α-tocopherol, and β-carotene concentrations in *M. alba* leaves was determined over 172 h (7 d). Alpha-tocopherol is the most bioavailable form of vitamin E, and β-carotene is the precursor to vitamin A (Robbins et al., 1994).

Materials and Methods

Nutrient Profiling

The browse species analyzed included orchid tree (*Bauhinia galpinii*), magenta lily pilly (*Eugenia peniculata*), weeping fig (*Ficus benjamina*), Chinese banyan (*Ficus microcarpa*), crossberry (*Grewia occidentalis*), Hawaiian hibiscus (*Hibiscus rosa sinensis*), white mulberry (*Morus alba*), cape honeysuckle (*Tecomaria capensis*), rosewood (*Tipuana tipu)*, and garden nasturtium (*Tropaeolum majus*). All samples were harvested around 0500 and stored in a large, outdoor cooler (4°C) until 0900 retrieval. Branches (46 cm) were stored by species in bundles of 10 branches and kept dry. Upon retrieval, the browse was transported to the Nutrition Laboratory where whole branch bundle weights were obtained.

Each portion of the plant was manually removed. Leaves and flowers were removed by the stem base, and bark was stripped from the wood. All components of the deconstructed browse were dried separately for a minimum of 48 h in a 55°C forced-air drying oven to gravimetrically determine moisture content. Dried specimens were ground through a 2.0 mm screen (Thomas Wiley® Mill, Thomas Scientific, Swedesboro, NJ 08085). Plant proportions were calculated based on weight.

Ground bark and leaf subsamples weighing 65 g or greater were submitted for proximate analysis (Dairy One, Ithaca, New York 14850). Nutrients analyzed included crude protein, crude fat, detergent fiber, starch, total ash, and select trace minerals. Due to limited sample size, only *T. majus* flowers were analyzed. An increased branch sample size would yield more floral material

Moisture Loss

M. alba branches were harvested from SDZG for use in the moisture loss and vitamin stability study. Harvest and delivery of the bundles to the primate browse cooler occurred between 0500 and 0900. The bundles were retrieved at approximately 0900.

M. alba, commonly offered to primates at the SDZ, was evaluated for water retention when presented in three different methods, using a control and positive control. The methods of presentations were control, initial leaf DM; upright; upside down; upright in water; and a positive control, stored upright in a 4°C cooler for 24 h. Fifteen branches of *M. alba* (46 cm) were received each morning for four consecutive days (four replicates). Bundles of three branches were randomly assigned to one of the presentation treatments: control, deconstructed upon retrieval; upright with no water source; upside down with no water source; upright in an enclosed PVC pipe containing water; and upright in a cooler with no water source. The control was processed upon retrieval at 0900 by manually removing the leaf from the stem at the base of the node. The remaining branch bundles were secured onto a fence in correspondence to the assigned treatment using zip ties. All bundles were secured within 61 cm (24 in) of one another with moderate exposure to both sunlight and shade. Each bundle was removed from treatment conditions and processed after 24 h of exposure.

Dry matter was obtained gravimetrically after a minimum of 48 h in a 55°C forced-air drying oven. Changes in moisture content by treatment were analyzed using single factor ANOVA (MS Excel®) with means separated by Tukey-Kramer test (Gill, 1987). Means were considered significantly different when $P < 0.05$.

Vitamin Stability

Branches (91 cm) were harvested between 0500 and 0900, and stored without water in a 4°C cooler until scheduled processing date. On three separate days (replicates) the stability study was started by randomly assigning 25 branches to 5 sampling times. Five branches were sampled at 4, 28, 52, 100, and 172 h post-harvest.

Leaves were manually separated from branches and stored in a -20°C freezer until samples were transported under dry ice to be freeze dried (Flexi-Dry MP™, SP Industries, Stone Ridge, NY 12484; DuoSeal® Vacuum Pump 1402, Welch-Ilmvac, Niles, IL 60714). The maximum length of time from collection and storage to freeze drying was 30 d. Leaves were dried for a minimum of 48 h, or until a constant weight was obtained. Freeze-drying was selected over air drying due to reported antioxidant activity sensitivity to temperature (Katsube et al., 2009).

Dried leaves were ground (Thomas Wiley® Mill, Thomas Scientific, Swedesboro, NJ 08085) through a 2.0 mm screen. Samples were sent to a commercial laboratory (Michigan State University DCPAH, East Lansing, MI 48910-8104) for α-tocopherol and β-carotene analysis. Changes in vitamin concentration were analyzed using single factor ANOVA (MS Excel®) with means separated by Tukey-Kramer test (Gill, 1987). Means were considered significantly different when $P < 0.05$.

Results

Nutrient Profiling

Browse branches (76.7 g) contained 54.5% leaves, 15.8% bark, 28.6% wood, and 1.3% flowers (Table 1). *T. tipu* was not included in the averages since total leaf weights were not recorded and *T. majus* was not included because distinct vines were not separated. In general, the browse leaves had greater protein (15.4%) and fat (4.4%) and lower ADF (20.2%), NDF (34.6%) and lignin (6.9%) than bark (protein, 7.2%; fat, 2.3%; ADF, 38.2%; NDF, 49.2%; lignin, 9.0%) (Table 2). The exceptions are the leaves from *T. tipu* that had greater NDF than the bark and the leaves of *T. capensis* that had greater lignin than bark.

Calcium content of *Ficus* spp. leaves and bark were greater (3.1-4.8%) than the other browse species (Table 3). *H. rosa sinensis* leaves had the highest P content (0.8%). *G. occidentalis*, *H.rosa sinensis*, *M. alba*, *T. capensis* and *T. majus* had leaves, bark or both that had K content greater than 2.4%. *H. rosa sinensis* (leaves) and *T. majus* (leaves and vines) were high in Na (> 1.2%). The S content of the *T. majus* components were over 1% and the *T. majus* leaves had the highest Cl content (1.7%). *F. microcarpa* (leaves and bark) and H. *rosa sinensis* (leaves) had greater Fe content (> 477 ppm) than the other browse species. *T. majus* leaves had more than twice the Zn content (137 ppm) compared to the other browse species leaves. *T. capensis* had the greatest Mn content (67 ppm).

Moisture Loss

There were no statistical differences ($P > 0.05$) observed between browse-presentation treatment groups (Table 4). Browse that was presented upright or upside down for 24 h lost 12-14% of the original leaf moisture compared to the 4% moisture loss in leaves from browse that was presented upright in water for 24 h. The large variance suggests that the study should be repeated.

Vitamin Stability

The concentration of α-tocopherol (270 µg/g DM) in *M. alba* leaves did not decrease ($P > 0.05$) following storage up to 172 h in a 4 °C cooler (Table 5). Although not statistically significant ($P>0.10$), β-carotene concentration (225 µg/g DM) was stable for 100 h post-harvest in the cooler and then decreased 23% after 172 h post-harvest.

Discussion

Nutrient Profiling

Food items, which are infrequently analyzed, may be unaccounted for in diet formulation or inaccurately represented in current diets. The species included in this study were chosen due to a combination of limited published data and to obtain current analyses for browse species grown at SDZG and fed to colobines.

Leaves in addition to bark were analyzed to account for foraging behavior observed in SDZG primates, in which the bark is often stripped and consumed. Because woody portions of these plants are seldom ingested, wood was not analyzed. Seasonal comparison of the browse could capture nutrient variation throughout the year.

Comparing the nonhuman primate requirements (NRC, 2003) to the data in Table 2 and 3, no single species of browse would solely meet the requirements. By providing a varied diet along with a commercial primate biscuit, nutrient requirements can be met.

While these analyses provide specific data regarding browse grown at SDZG, this information can be extrapolated for use in other facilities. Data representing the nutrient content of these species can aide in diet formulation.

Moisture Loss

Colobines, such as Asian langurs, rely on fruits and vegetation as a primary source of water, rarely opting for free water (Harris, 1970). This would cause speculation that primates may choose to select foods containing higher water content. Moisture content of browse may also affect palatability among primates (Jildmalm et al., 2008). Leaves containing higher levels of moisture are associated with younger plants, which are characterized by an abundance of nutrients and lower fiber in comparison to a mature plant (Mowry et al., 1996). Wild colobus monkeys show selectivity for young plant leaves, only consuming mature plant leaves when resources are scarce (Mckey et al., 1981; Mowry et al., 1996). Additionally, wild ring-tailed lemurs displayed preference for foliage with higher water content, leading to speculation that primates may favor plant material based on moisture content (Mertl-Millhollen et al., 2003). Several studies, however, have demonstrated no or negative correlation for primate preference among fruits and leaves with high water content, possibly due to less nutrient density (Jildmalm et al., 2008; Laska et al., 2000). While this preference was not examined in this study, identifying the type of browse presentation that will improve utilization will help reduce food waste at SDZ. A follow-up trial repeating the procedure to reduce variance in combination with examining preference could be explored.

Vitamin Stability

Motivation for this study stems from findings in which α-tocopherol was stable in *Eucalyptus sideroxylon* leaves for 141 h post-harvest when refrigerated (Desai and Schlegel, 2011). The only loss of nutrients in *E. sideroxylon* with refrigeration was a loss of non-fiber carbohydrates (NFC). The current study suggests that during a 172 h period, no significant change was detectable in the measured antioxidant activity of *M. alba* leaves. A study with a longer cooler storage time would be required to follow the trend of decreased α-tocopherol or β-carotene concentration beyond 172 h (7 d). One study supports that no significant change in antioxidant activity occurs in refrigerated mulberry leaf extract until 60 days of storage (Arabshahi-Delouee and Urooj, 2007). Nonhuman primates require 8,000 IU vitamin A/kg DM (14.4 μg β-carotene/g) and 100 mg/kg (100 μg/g) vitamin E (NRC 2003). Sampled *M. alba* leaves contain adequate concentrations of α-tocopherol and β-carotene up to 172 h post harvest. Mulberry stems were not examined in this study, but can be a good source of antioxidants that could be considered for future work (Syvacy and Sokmen, 2004). Additionally, a non-refrigerated negative control could be used to demonstrate the benefits of refrigeration.

Findings emphasize that diets including browse should be varied to achieve nutrient requirements. *M. alba* presentation methods tested did not demonstrate significant differences of water loss, but the resulting large variance suggests that the study should be repeated. During a 172 h period, α-tocopherol and β-carotene concentration in *M. alba* leaves remained stable.

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Table 1. Primate browse component weights per branch and the calculated percentage of total branch weight (as-fed basis).

	Leaves		Bark		Wood		Flower	
Species	Wt per branch, g	% of branch weight	Wt per branch, g	% of branch weight	Wt per branch, g	% of branch weight	Wt per branch, g	% of branch weight
Bauhinia galpinii	47.7	45.8	15.3	14.7	40.9	39.3	0.2	0.2
Eugenia peniculata	79.7	58.2	9.9	7.3	47.1	34.4	0.1	0.1
Ficus benjamina	122.3	55.1	27.8	12.5	71.8	32.4	0.1	0.03
Ficus microcarpa	152.0	50.8	40.1	13.4	104.8	35.0	2.4	0.8
Grewia occidentalis	40.3	53.6	14.4	19.1	17.4	23.1	3.1	4.2
Hibiscus rosa sinensis	43.6	41.4	26.2	24.9	33.6	31.9	2.0	1.9
Morus alba	82.9	63.0	18.6	14.1	30.0	22.8	NP[1]	NP
Tecomaria capensis	45.3	68.4	13.3	20.0	6.6	9.9	1.1	1.6
Tipuana tipu	DNR[2]	DNR	258.3	DNR	469.7	DNR	0.4	DNR
Tropaeolum majus	29.3	16.5	142.5[3]	80.1	NP	NP	6.1	3.4

[1] Plant component not present.

[2] Data not recorded.

[3] Refers to vines.

Table 2. Nutrient composition of ten species of primate browse.

Species	Part	DM, %[1]	Crude Protein, %[1]	ADF, %[1]	NDF, %[1]	Lignin, %[1]	Crude Fat, %[1]	NFC[1, 2], %	ESC[1, 3], %
Nonhuman Primates[4]	-	NR[5]	15-22	5-15	10-30	NR	NR	NR	NR
Bauhinia galpinii	Leaves	46.9	15.3	16.3	24.2	4.6	2.9	50.9	10.4
	Bark	52.5	5.9	38.2	45.5	7.4	1.8	39.4	8.2
Eugenia peniculata	Leaves	28.6	10.1	19.3	25.4	6.1	4.8	55.8	6.6
	Bark	31.7	5.6	30.2	38.1	9.2	2.5	46.2	10.3
Ficus benjamina	Leaves	40.4	10.6	28.2	40.8	10.3	4.4	29.7	9.2
	Bark	39.3	6.3	49.5	60.7	13.8	2.3	17.1	4.0
Ficus microcarpa	Leaves	37.7	8.0	24.7	34.1	8.2	4.3	34.4	5.8
	Bark	43.1	3.8	30.1	37.2	9.1	2.6	40.6	9.3
Grewia occidentalis	Leaves	35.0	18.6	17.6	42.0	4.2	3.2	26.4	6.4
	Bark	38.5	8.0	39.0	66.0	6.9	1.4	15.8	6.6
Hibiscus rosa sinensis	Leaves	27.8	18.3	16.2	40.9	4.8	6.8	19.4	4.9
	Bark	27.8	7.0	51.6	65.0	6.4	1.6	15.1	4.8
Morus alba	Leaves	32.1	17.9	13.3	24.0	2.8	5.2	40.7	10.4
	Bark	30.7	8.8	34.3	41.3	7.0	4.2	37.6	7.2
Tecomaria capensis	Leaves	34.3	19.3	22.7	37.9	12.1	5.2	29.9	8.4
	Bark	36.7	8.7	36.0	49.1	9.0	2.0	33.2	11.5
Tipuana tipu	Leaves	35.1[6]	20.9	23.7	42.2	9.0	3.0	26.4	9.0
	Bark	43.9	10.5	34.8	39.7	12.1	2.1	40.3	10.5
Tropaeolum majus	Leaves	15.6	29.4	10.8	21.4	2.5	9.2	25.3	7.7
	Vines	7.7	15.5	32.7	38.1	5.1	2.5	24.3	11.1
	Flowers	14.4	26.0	11.4	20.1	2.3	7.7	36.9	11.9

[1] All nutrients, except for DM, reported on a dry matter basis. [2] Non-fiber carbohydrates calculated as 100% - (CP% + NDF% + Fat% + Ash%). [3] Ethanol soluble carbohydrates (simple sugars). [4] Published nonhuman primate requirements (NRC 2003). [5] No requirement established. [6] In the original dataset, the dry matter content could not be calculated due to missing data. The leaf DM listed is from unpublished data (San Diego Zoo Global).

Table 3. Macromineral and micromineral content (DM basis) of ten species of primate browse.

Species	Part	Ash, %	Ca, %	P, %	Mg, %	K, %	Na, %	S, %	Cl, %	Fe, ppm	Zn, ppm	Cu, ppm	Mn, ppm	Mo, ppm
Nonhuman Primates[1]	-	NR[2]	0.8	0.6	0.1	0.4	0.2	NR	0.2	100	100	20	20	NR
Bauhinia galpinii	Leaves	6.7	1.5	0.3	0.2	1.0	0.02	0.2	0.2	171.0	41.0	8.0	34.0	0.6
	Bark	7.4	1.7	0.5	0.1	1.4	0.01	0.1	0.4	63.0	59.0	7.0	18.0	1.2
Eugenia peniculata	Leaves	3.9	0.4	0.1	0.2	0.8	0.30	0.2	0.1	201.0	14.0	7.0	25.0	0.3
	Bark	7.6	1.4	0.1	0.4	1.2	0.31	0.1	0.2	99.0	13.0	10.0	18.0	0.0
Ficus benjamina	Leaves	14.6	3.4	0.1	0.3	1.4	0.03	0.2	0.1	157.0	16.0	5.0	29.0	0.2
	Bark	13.5	3.1	0.2	0.2	1.5	0.03	0.1	0.1	131.0	14.0	11.0	15.0	3.0
Ficus microcarpa	Leaves	19.1	4.8	0.1	0.5	1.5	0.10	0.2	0.3	569.0	31.0	7.0	36.0	0.2
	Bark	15.9	4.2	0.3	0.2	1.2	0.05	0.1	0.4	602.0	55.0	10.0	20.0	1.1
Grewia occidentalis	Leaves	9.8	1.7	0.3	0.3	2.4	0.02	0.3	0.7	195.0	68.0	15.0	46.0	1.0
	Bark	8.9	1.2	0.2	0.3	2.4	0.03	0.2	0.8	76.0	40.0	10.0	23.0	0.7
Hibiscus rosa sinensis	Leaves	14.6	2.2	0.8	0.5	1.3	1.35	0.7	0.5	477.0	63.0	12.0	39.0	0.8
	Bark	11.3	1.5	0.5	0.2	2.8	0.54	0.3	0.9	62.0	27.0	13.0	16.0	0.1
Morus alba	Leaves	12.2	1.9	0.6	0.4	2.4	0.01	0.2	0.3	209.0	27.0	5.0	36.0	2.8
	Bark	8.2	1.3	0.3	0.3	1.8	0.01	0.1	0.1	70.0	19.0	4.0	30.0	0.9
Tecomaria capensis	Leaves	7.7	1.1	0.3	0.3	1.3	0.54	0.2	0.8	212.0	36.0	9.0	67.0	1.5
	Bark	7.1	0.5	0.2	0.2	2.3	0.29	0.1	1.0	116.0	46.0	23.0	33.0	0.9
Tipuana tipu	Leaves	7.5	1.6	0.2	0.5	1.0	0.03	0.2	0.6	259.0	37.0	11.0	30.0	1.3
	Bark	7.5	1.9	0.2	0.3	1.0	0.02	0.2	0.4	147.0	48.0	13.0	12.0	1.4
Tropaeolum majus	Leaves	14.7	1.9	0.5	0.6	2.2	1.20	1.1	1.7	278.0	137.0	10.0	28.0	1.5
	Vines	19.5	1.5	0.4	0.5	4.8	1.52	1.1	0.8	121.0	63.0	6.0	6.0	1.1
	Flowers	9.2	0.4	0.7	0.3	3.1	0.15	1.0	0.3	129.0	80.0	10.0	18.0	0.0

[1] Published nonhuman primate requirements (NRC 2003).

[2] No requirement established.

Table 4. Moisture content of *Morus alba* leaves 24 h after browse presentation treatment.

	Treatment					
	Control	Upright	Upside down	Upright in water	Refrigerated	SEM[1]
Moisture, % as-fed	69.08	59.15	60.89	66.24	66.98	3.29

[1] Standard error of the means.

Table 5. Alpha-tocopherol and β-carotene concentration stability of refrigerated *Morus alba* leaves following harvest and refrigeration up to 172 h.

Vitamin (DM basis)	Hours post-harvest					
	4[1]	28	52	100	172	SEM[2]
α-tocopherol (μg/g)	251.0	272.67	286.6	262.7	276.1	33.83
β-carotene (μg/g)	230.9	214.9	231.2	224.8	176.7	19.71

[1] Initial nutrient concentration post-harvest prior to refrigeration.

[2] Standard error of the means.