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Nutrient Composition of Locally Available Browses Consumed by Matschie's Tree Kangaroos (*Dendrolagus matchiei*) in Six North American Zoological Facilities

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Authors' contributions

This work was carried out in collaboration among all authors. Author ESD designed the study, wrote the sample collection and handling protocol, performed the statistical analyses, and drafted the manuscript. Authors MB and LD contributed with sample submission, data interpretations, funding and literature acquisition. All authors read and approved the final manuscript.

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ABSTRACT

Locally collected browses (n=17 spp.) consumed by Matschie's tree kangaroos (*Dendrolagus matschiei*) in 6 North American zoological institutions were analyzed for comparison with native plants eaten by this species in Papua New Guinea to evaluate dietary suitability. Primary nutrients including crude protein and fat, fiber, starch, non-fiber carbohydrates, and ash were determined using standard analytical methods for forages. Macrominerals calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na) and sulfur (S), as well as trace elements copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn) were quantified in leaf (n=18), flower (n=1), twig (n=9) and bark (n=6) samples. Tannin content was estimated through the bovine serum albumin methodology. On a dry matter basis (DMB) , foods averaged (\pm SD) moderate protein (12 \pm 5%) and soluble carbohydrate (27 \pm 12%) content, along with low starch (1 \pm 1%) and crude fat (3 \pm 2%) values, and moderate to high values in fiber fractions (neutral

detergent fiber 49 \pm 15%, acid detergent fiber 33 \pm 13%, lignin 11 \pm 5%). Macromineral concentrations (Ca 2.2 \pm 1.6%, P 0.2 \pm 0.1%, Mg 0.3 \pm 0.2%, K 1.5 \pm 0.6%, Na 0.03 \pm 0.04%, S 1.2 \pm 1%) and select trace minerals were within anticipated ranges (Cu 11 \pm 5 mg/kg, Mo 1 \pm 1 mg/kg and Zn 33 \pm 18 mg/kg); exceptions Fe (122 \pm 11 mg/kg) and Mn (51 \pm 81 mg/kg) were considered on the high end of dietary adequacy for most herbivores. Leaves differed significantly from woody parts for all proximate nutrients, as well as K, S, Fe, and tannin content. Consumed in a 50:50 DMB ratio, locally available browses provide similar nutrient profiles as plants eaten by free-living tree kangaroos. Combined data provide information useful in establishing nutrient targets for dietary development, leading to improved health, welfare, and feeding management of tree kangaroo populations under human care.

Keywords: Browse; herbivore; marsupial; nutrition.

1. INTRODUCTION

A recent publication documented chemical composition of 24 spp. of foods eaten by the Matschie's endangered tree kangaroo (Dendrolagus matschiei) in Papua New Guinea, including ferns, shrubs, vines, orchids, herbaceous plants, and tree leaves [1]. These native forages were found to contain moderate levels of protein and soluble carbohydrates, moderate to high fiber levels, low crude fat and starch content, and generally expected mineral concentrations. Conversely, diets fed to the arboreal tree kangaroos in zoological institutions reportedly contain few browse plants, but rather ingredients containing high sugar and starch, with low fiber concentrations [2]. As a consequence, zoo-housed individuals can weigh up to 50% more than healthy, free-living tree kangaroos [3,4], a condition which may contribute to suboptimal reproduction observed in zoo populations through demographic assessment [5].

This study was undertaken to evaluate nutritional parameters in locally available browses fed to tree kangaroos in North American zoos as suitable substitutes for native foods.

2. MATERIALS AND METHODS

Preferred locally available browse species fed in six US zoological facilities were collected, separated into edible fractions (i.e. leaves, twigs and/or bark) as determined by caretaker feeding observations, and weighed both prior to and following air drying to determine fresh water content. Samples were coarsely ground using a coffee mill, and shipped to Dairy One Forage

Laboratory (Ithaca, NY, USA) where they were further ground to 1 mm particle size prior to analysis for crude protein, crude fat, starch, nonfiber carbohydrates (NFC) and fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin using standard analytical methods for forages. Ash content was determined via incineration of organic matter, and macrominerals calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na) and sulfur (S), as well as trace elements copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn) were quantified. For mineral analyses, samples were digested using a CEM Microwave Accelerated Reaction System (MARS6) with MarsXpress Temperature Control in 50-ml calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves (CEM, Mathews, NC, USA) and then analyzed by ICP using a Thermo iCAP 6300 Inductively Coupled Plasma Radial Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA USA). Subsamples (5 to10 g dry weight) were submitted to the Wildlife Nutrition Laboratory at Washington State University (Pullman, WA, USA) for tannin analysis utilizing the bovine serum albumin (BSA) precipitation method described by Martin and Martin [6].

3. RESULTS AND DISCUSSION

Chemical composition and tannin content from 34 samples representing 17 species of browses and plant parts consumed by tree kangaroos in select US zoos are found in Table 1. Mineral concentrations are displayed in Table 2, and mean (± SD) values in vegetative (leaves, flowers) portions are compared with woody segments (bark, twigs) in Table 3.

Scientific	Common		Sampling	Water	Crude Protein	ADF	NDF	Lignin	NFC	Starch	Crude Fat	Ash	Tannin mg BSA
Name	Name	Part	Location/Month	<		%		>					/mg
Acacia longifolia	Golden/coast wattle	Leaf	San Diego CA/Sep	18.7	12.1	26.9	35.1	15.1	39.2	0.4	3.0	10.7	0.0874
C C		Twig	0	19.7	5.2	52.4	70.6	12.8	18.3	0.2	1.7	4.2	0.0611
Bauhinia X blakeana	Hong Kong orchid	Leaf	Miami FL/Dec	49.0	16.1	31.0	51.4	10.3	14.9	1.2	5.2	12.4	0
Betula spp.	Birch	Leaf	Lincoln NE/Oct	60.9	15.6	21.3	36.9	9.5	32.2	0.4	7.7	7.6	0.0710
Buddleja spp.	Butterfly bush	Leaf	Seattle WA		15.7	16.1	23.7	5.8	48.4	0.6	4.0	8.3	0
	,	Flower			13.1	22.5	31.6	7.7	47.7	0.4	2.6	5.0	0
		Bark			6.9	46.6	57.6	22.3	28.0	1.3	2.5	4.9	0
Bursera simaruba	Gumbo limbo	Leaf	Miami FL/Dec	54.9	11.6	19.7	27.3	8.9	49.2	0.7	3.1	8.8	0.1340
		Twig		63.0	4.9	33.0	40.9	10.6	42.0	1.7	1.8	10.4	0.0845
		Bark		69.6	4.6	43.9	50.5	19.6	31.1	1.7	3.1	10.7	0.0637
Celtis occidentalis	Hackberry	Leaf	San Antonio TX/Nov		12.7	16.1	31.9	3.6	24.8	0.3	6.9	23.9	0
Cotoneaster spp.	Cotoneaster	Leaf	Seattle WA		13.9	28.1	39.7	8.7	35.7	1.0	3.8	7.0	0.0429
		Twig			6.0	48.2	63.4	8.1	25.3	1.2	1.8	3.5	0.0527
		Bark			5.3	42.1	52.8	12.7	35.5	0.1	2.1	4.3	0.0525
Ehretia anacua	Anacua	Leaf	San Antonio TX/Nov		14.1	18.5	30.1	4.0	28.7	1.7	2.4	24.7	0
Elaegnus pungens	Thorny olive	Leaf	Seattle WA		18.5	45.8	60.3	18.0	13.2	0.3	2.1	5.9	0
o , o	,	Bark			14.1	52.1	63.6	24.1	16.6	0.6	1.0	4.7	0.0299
Fagus grandifolia	Beech	Leaf	Providence RI	50.0	17.0	24.8	46.4	8.9	28.6	1.4	2.9	5.1	0.0649
		Twig		37.5	5.9	49.3	68.0	19.2	21.1	0.9	2.1	2.9	0.0247
Ficus benjamina	Ficus	Leaf	Miami FL/Dec	65.1	9.9	37.0	49.5	14.0	6.3	1.2	5.3	29.0	0
Ficus nitida	Jewel leaf ficus	Leaf	San Diego CA/Sep	4.0	7.9	27.5	38.9	11.3	36.6	1.1	2.3	14.3	0.0719
		Twig		18.0	6.8	45.2	59.1	14.8	25.4	0.8	1.7	7.0	0.0495
Grewia occidentalis	Crossberry	Leaf	San Diego CA/Sep	28.0	19.3	19.1	45.3	4.9	18.8	0.7	5.0	11.5	0.0427
	, , , , , , , , , , , , , , , , , , ,	Twig		37.0	9.5	47.4	76.3	9.0	5.9	<0.1	0.9	7.3	0
Morus spp.	Mulberry	Leaf	Lincoln NE/Oct	66.4	22.3	14.2	23.1	3.3	30.8	0.6	4.1	19.8	0
)	Twig		47.3	7.6	50.5	65.7	14.7	17.2	1.3	2.2	7.3	0
		Bark		49.0	7.7	41.5	50.3	10.3	28.1	5.5	4.4	9.5	0
Morus spp.	Mulberry	Leaf	Providence RI	65.4	24.3	14.2	28.1	3.1	34.0	0.9	3.2	10.4	0
)	Twig		52.4	8.3	53.0	70.3	12.4	15.2	1.4	1.7	4.6	0
Phyllostachys spp.	Bamboo	Leaf	Providence RI	51.5	18.3	26.3	63.2	3.6	9.7	0.4	3.1	5.7	0
Ulmus parvifolia	Chinese elm	Leaf	Lincoln NE/Oct	52.9	18.7	19.7	42.1	5.6	13.7	0.7	4.6	20.9	0.0167
		Twig		45.6	10.1	40.4	55.0	14.1	24.1	2.4	2.2	8.6	0
		Bark		48.6	9.1	38.7	65.4	13.4	14.1	5.6	2.3	9.2	0.0215
<i>Xylosma</i> spp.	Xylosma	Leaf	San Antonio TX/Nov		11.0	24.1	33.9	9.1	43.3	0.8	2.4	9.3	0

Table 1. Chemical composition of locally harvested browses eaten by Matschie's tree kangaroos (Dendrolagus matschiei) in North American zoological institutions. Data (except water) on a dry matter basis

Abbreviations: ADF = acid detergent fiber; NDF = neutral detergent fiber; NFC = non-fiber carbohydrates

Scientific	Common	Part	Sampling	Ca	Р	Mg	K	Na	S	Cu	Fe	Mn	Мо	Zn
Name	Name		Location/Month	<	%			<mg kg<="" th=""><th colspan="2">></th></mg>			>			
Acacia longifolia	Golden/coast wattle	Leaf	San Diego CA/Sep	2.97	0.27	0.33	1.09	0.11	0.37	13	52	43	0.9	27
-		Twig	- .	0.87	0.19	0.16	0.97	0.085	0.1	8	66	11	3	20
Bauhinia X blakeana	Hong Kong orchid	Leaf	Miami FL/Dec	4.33	0.16	0.35	0.75	0.003	0.24	7	56	21	0.2	30
Betula spp.	Birch	Leaf	Lincoln NE/Oct	1.76	0.22	0.29	1.73	0.004	0.2	4	118	48	0.3	45
Buddleja spp.	Butterfly bush	Leaf	Seattle WA	0.55	0.4	0.13	2.1	0.009	0.09	21	60	20	0.5	48
	-	Flower		0.73	0.14	0.09	1.57	0.01	0.18	12	83	25	1.7	17
		Bark		0.75	0.45	0.19	1.57	0.008	0.22	28	188	26	1.3	40
Bursera simaruba	Gumbo limbo	Leaf	Miami FL/Dec	1.56	0.2	0.31	1.23	0.118	0.67	7	37	11	3.7	24
		Twig		2.84	0.24	0.4	1.28	0.143	0.17	9	12	10	1.3	38
		Bark		2.33	0.27	0.23	2.52	0.031	0.19	12	29	10	0.7	28
Celtis occidentalis	Hackberry	Leaf	San Antonio TX/Nov	6.26	0.14	0.48	1.56	< 0.001	0.21	9	110	29	0.7	17
Cotoneaster spp.	Cotoneaster	Leaf	Seattle WA	1.85	0.2	0.23	1.32	<0.001	0.17	10	82	41	0.6	39
		Twig		0.64	0.17	0.11	1.17	0.007	0.1	9	38	13	0.7	24
		Bark		1.05	0.16	0.18	1.08	0.006	0.06	8	35	19	0.7	30
Ehretia anacua	Anacua	Leaf	San Antonio TX/Nov	6.98	0.12	1.08	1.48	0.003	0.4	10	119	28	1.5	26
Elaegnus pungens	Thorny olive	Leaf	Seattle WA	1.22	0.18	0.12	1.58	0.005	0.23	9	78	95	0.9	31
0 / 0	,	Bark		2.44	0.34	0.21	1.23	0.005	0.33	18	139	88	3.6	47
Fagus grandifolia	Beech	Leaf	Providence RI	0.69	0.17	0.14	0.83	0.02	0.17	12	202	449	0.2	49
		Twig		0.92	0.14	0.08	0.28	0.017	0.06	12	96	217	0.4	101
Ficus benjamina	Ficus	Leaf	Miami FL/Dec	5.17	0.11	0.39	2.2	0.024	0.17	6	42	9	<0.1	33
Ficus nitida	Jewel leaf ficus	Leaf	San Diego CA/Dec	3.53	0.13	0.35	2.21	0.036	0.17	8	277	27	1	13
		Twig		1.85	0.25	0.31	1.52	0.055	0.09	14	60	15	3.3	16
Grewia occidentalis	Crossberry	Leaf	San Diego CA/Sep	2.37	0.29	0.42	2.52	0.028	0.34	14	570	97	2.3	56
		Twig		1.1	0.35	0.25	2.33	0.066	0.16	9	47	30	1.6	22
Morus spp.	Mulberry	Leaf	Lincoln NE/Oct	3.75	0.4	0.37	2.2	0.003	0.29	10	227	59	2.4	35
		Twig		1.68	0.31	0.17	1.25	0.002	0.1	9	110	24	1.2	32
		Bark		2.49	0.22	0.16	0.8	0.002	0.1	13	162	18	0.7	8
Morus spp.	Mulberry	Leaf	Providence RI	1.8	0.38	0.23	2.78	0.004	0.26	8	127	86	1.1	56
	· ···· · · · · · · · · · · · · · · · ·	Twig		0.8	0.34	0.1	1.5	< 0.001	0.1	9	61	38	0.4	42
Phyllostachys spp.	Bamboo	Leaf	Providence RI	0.38	0.19	0.25	1.75	0.004	0.21	13	381	39	1	24
Ulmus parvifolia	Chinese elm	Leaf	Lincoln NE/Oct	3.08	0.28	0.22	1.67	0.002	0.21	8	179	28	0.4	13
		Twig		2.24	0.23	0.22	1.37	0.005	0.13	8	130	29	0.3	45
		Bark		2.45	0.16	0.2	1.01	0.01	0.10	8	93	7	0.2	20
Xylosma spp.	Xylosma	Leaf	San Antonio TX/Nov	2.68	0.15	0.27	1.49	0.017	0.21	10	66	33	0.5	18

Table 2. Mineral composition of locally harvested browses eaten by Matschie's tree kangaroos (*Dendrolagus matschiei*) in North American zoological institutions. Data presented on a dry matter basis

Abbreviations: Ca = calcium, P = phosphorus, Mg = magnesium, Na = sodium, S= sulfur, Cu = copper, Fe = iron, Mn = manganese, Mo = molybedenum, Zn = zinc

Table 3. Comparison of chemical composition in vegetative (leaves, flowers) compared with woody (twigs, bark) portions of locally sourced browses eaten by tree kangaroos
(Dendrolagus matschiei) in six US zoos. Data (except water) dry matter basis

	Water	Crude Protein	ADF	NDF	Lignin	NFC	Starch	Crude Fat	Ash	Tannins	
	%	%	%	%	%	%	%	%	%	mg BSA/mg	
Mean values in vegetative portions of local browses	47.2	15.4	23.8	38.9	8.2	29.3	0.8	3.9	12.6	0.03	
SD; n=19 samples	19.9	4.2	8.0	11.5	4.3	13.5	0.4	1.6	7.4	0.04	
Mean values in woody fractions of browses consumed	44.3	7.5	45.6	60.6	14.5	23.2	1.8	2.1	6.6	0.03	
SD; n= 15 samples	15.8	2.5	5.7	9.5	4.8	9.2	1.7	0.8	2.6	0.02	
P significance of paired samples; n=11; tannins (n=9 leaf - twig pairs)		<.001	<.001	<.001	**	*	*	***	**	**	
Aean values in native browses from Papua New Guinea	75.6	10.9	39.3	51.8	15.1	26.5	0.9	3.2	7.6	n/a	
SD; n=26 samples from 24 spp. ¹	10.1	4	10	12.9	6.1	8.4	0.9	1.9	4		
	Са	Р	ĸ	Mg	Na	S	Cu	Fe	Mn	Мо	Zn
	<	%%				<>					
Aean minerals in vegetative portions of local browses	2.7	0.2	1.7	0.3	0.02	0.3	10.1	150.8	62.5	1.1	31.6
SD; n=19 samples	1.9	0.1	0.5	0.2	0.04	0.1	3.7	135.8	97.1	0.9	13.9
Mean minerals in woody fractions of browses consumed	1.6	0.3	1.3	0.2	0.02	0.1	11.6	84.4	37.0	1.3	34.2
SD; n=15 samples	0.8	0.1	0.6	0.1	0.04	0.1	5.4	52.4	53.6	1.1	21.7
Significance of paired samples; n=11			*			**		*			
Mean values in native browses from Papua New Guinea	1.1	0.2	1.8	0.3	0.02	n/a	11.9	47.5	268.3	n/a	33.9
SD; n=26 samples from 24 spp. ¹	1.0	0.1	0.9	0.2	0.0	n/a	12.7	26.0	225.2	n/a	17.7

Abbreviations: ADF = acid detergent fiber; NDF = neutral detergent fiber; NFC = non-fiber carbohydrates; Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, S= sulfur, Cu = copper, Fe = iron, Mn = manganese, Mo = molybdenum, Zn = zinc; n/a = not analyzed. (* P=.05; ** P=.001); ¹Dierenfeld et al. 2020

Leaves (n=18) and the single flower sample analyzed contained moderate protein levels, varying 3-fold (8 to 24% of dry matter (DM)), whereas woody fractions (twigs and bark) contained about half those levels (~5 to 14% of DM); differences were highly significant (P <.001) in the 11 samples with paired vegetative: woody fractions. Similarly, crude fat ranged almost 4-fold from low to moderate (2 to 8% of DM in leaves, and 1 to 4% in woody parts (P =.001; n=11). All starch values in leaves, regardless of origin, were exceptionally low at <1.5% of DM: starch content in two bark samples analyzed at ~5.5% starch, but in general values were also <1.7% of DM, nonetheless differed significantly (P = .05; n=10) between paired portions of the same samples/species. Plant cell wall constituents (hemicellulose, cellulose and lignin) concentrations within the NDF of leaves were moderate (20 to 60% of DM) and, not unexpectedly, higher in woody fractions (41 to 76%; P <. 001 for NDF and ADF, P =.004 for lignin; n=11 paired samples); nonetheless, lignification index (lignin as a proportion of NDF) was the same between plant parts, averaging ~22% of NDF fiber. NFC (P = .03) and ash (P =.005) values also differed significantly between leaf and woody fractions.

Tannin results revealed that fully 65% of the North American browse samples contained no or very low tannin concentrations (<0.03 mg BSA/mg forage); 30% of native browse samples contained notable (>0.05 mg BSA/mg forage; 7 samples, 21%) or high levels (>0.075 mg BSA/mg forage; 3 samples, 9% of samples submitted). Although no statistical differences were seen in tannin concentrations measured in paired samples comparing leaves with bark from the same species (P = .38; n=6), nor between paired bark or twig samples from the same (*P*=.12; n=3), twigs species contained significantly less tannin (P = .008) than paired leaf samples (n=9) from US browses.

Regarding minerals, only K (P = .05), S (P = .01) and Fe (P = .05) concentrations differed significantly between the vegetative and woody fractions analyzed as paired samples (n=11), with leafy portions always displaying higher values.

According to reports from caretakers submitting browse samples, animals always consume leaves, but only sometimes eat bark or twigs from the various browses offered. Composition of native plants/portions eaten by tree kangaroos in Papua New Guinea [1] appears intermediate to values measured in leafy compared to woody fractions of this array of shrubs and trees fed in North American zoological institutions, with a ratio of ~50:50 leaves to twig/bark fractions closely matching the proximate and fiber content of forages consumed in field habitats. Obtaining a more accurate estimate of actual intakes and digestibility of the various plant portions would allow us to better calculate nutrient contributions of browses in managed feeding programs for tree kangaroos.

The low starch, high fiber nutritional profiles represented by these locally available browses may indeed provide suitable moderately digestible forage substrates for the foregutfermenting tree kangaroo [2], and contribute to optimal body condition and digestive physiology. A low fiber lignification index, as found in these browses, suggests substantial fermentation potential, given a proper microbial environment and adequate residence time in the digestive tract [7]. The further high fiber, low fat and starch content of browses would also tend to support, rather than inhibit, growth of beneficial cellulolytic gut microbes as is seen in other foregutfermenting herbivores [8,9]. Nonetheless. detailed aspects of digestion, fermentation, passage, and microbiology in response to different diets remain to be further examined in marsupial herbivores.

It may also prove useful to investigate aspects of dietary tannins in native browses for comparison with local alternatives; large salivary glands have been reported in tree kangaroos, a morphological feature often associated with adaptation to tannin-containing diets [10]. Although higher tannin levels are anticipated to possibly impact palatability, mineral or protein bioavailability, as well as digestibility [11], no effect has been reported with inclusion of quebracho tannins in diets of a browsing macropodid marsupial (foregut fermenter) or two species of hindgutfermenting arboreal folivorous marsupials [12], suggesting inherent adaptations. Nonetheless, potential health and/or intake behavioral implications of these observations remain to be determined for tree kangaroos, and they clearly do not seem to avoid concentrations measured in this study.

Although minerals quantified in browses are considered to be within expected ranges to meet known maintenance mineral requirements of domestic herbivores, elevated calcium and iron concentrations were found in locally sourced browses, and particularly high and variable (30 to 40-fold) levels of manganese were recorded in both native and locally sourced browses. Neither mineral nutrition nor status has been investigated widely in tree kangaroos, nor have specific mineral imbalances been reported as health issues [2,4]. Interactions between Ca and Fe, as well as impacts of dietary fiber on mineral bioavailability, may be of future interest for the species, particularly if higher fiber diets are implemented in captive populations. Although trace mineral status (in particular, Cu and Fe), has been anecdotally suggested as linked with coat quality and coloration in zoo individuals, no supportive evidence is found in published literature. High dietary P and interactions with Ca can interfere with Fe uptake, and Mn directly impacts uptake of both Cu and Fe through competition for absorption binding sites [13], but the significance of elevated dietary Mn levels for tree kangaroo health, if any, is unknown at this time.

Correlations of specific nutrients or anti-nutrients with palatability rankings of various browses can be further examined to optimize welfare and enrichment opportunities for tree kangaroos, as can regional and seasonal differences in composition and preferences. The current study provides initial comparison with native browses eaten by tree kangaroos, and confirms that locally sourced plants can provide suitable nutrient profiles.

4. CONCLUSION

Information obtained to date on browse composition provides useful guidelines for lowering calorie content of managed tree kangaroo diets, particularly through reducing starch and fat, and increasing fiber content of Future research monitoring seasonal diets. selection of browses - possibly including threshold levels of tannins that may impact intake behaviors - and time budget allocation for foraging on these items, may help to define detailed annual differences in diet composition and nutritional status of this species. Projects studying fecal microbiome of free ranging and captive populations may also be useful for future diet and health assessment. Overall, the results contribute to science-based diet recommendations for improved feeding of tree kangaroos.

Increased feeding of locally-available browses, including both leafy and woody fractions, will best duplicate nutrient profiles of native forages, and may improve health and reproduction, as well as lower obesity rates reported for this species in captivity. Targeted agroforestry practices and harvesting of suitable browses for use in herbivore diets should be encouraged to increase quality ingredient supplies going forward.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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