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The composition of ruffed lemur (*Varecia* spp.) diets in six UK zoological collections, with reference to the problems of obesity and iron storage disease

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Abstract

The formulation and provision of appropriate diets for zoo animals is important in ensuring the continued health of populations. Inappropriate diets can lead to a number of nutritional deficiencies and increase the risk of disease and obesity. Ruffed lemurs (*Varecia* spp.) are the most intensely frugivorous of extant lemur species. Captive animals are often fed a diet which may not accurately reflect the composition of the wild diet. As such, the species is prone to obesity and can suffer from nutrition-related diseases. Here the historical diets of several populations of ruffed lemurs across UK zoological collections are described, highlighting differences in nutritional content with a focus on the problems of obesity and iron storage disease. Dietary data were collected from six zoological institutions. Comparative calculations were conducted to investigate differences in the amount of metabolisable energy, carbohydrates, sugar and iron provisioned per individual per day, between institutions. The composition of ruffed lemur diets, and the amount of food offered, differed between institutions. Metabolisable energy exceeded suggested maintenance energy requirements at all institutions. One population was found to be obese, and two institutions reported mortalities where excessive iron accumulation and iron storage disease (ISD) was observed. Reducing the relative proportion of sugar-rich fruit, removing food items high in iron and limiting daily iron to 2 mg per individual may be an effective means of decreasing the prevalence of obesity and ISD in the captive population.

Introduction

The provision of an appropriate and considered diet is important in ensuring the continued health of populations ex situ (Hile 2004; Donadeo et al. 2016). Designing diets for animals in captivity is often difficult, given the variety of potential factors (e.g. gastrointestinal physiology, wild diet composition, foraging behaviour and/or dental morphology), many of which remain unquantified or poorly understood for several exotic species (sensu Fidgett and Plowman 2009). Indeed, the provision of inappropriate diets can lead to a variety of ailments as a result of nutrient deficiencies, including reproductive disorders (Tubbs et al. 2012) and increased incidence of disease (e.g. Clauss and Paglia 2012), obesity (D'Eath et al. 2009) and mortality (Hawn 2005).

Ruffed lemurs (*Varecia* spp.) are the largest extant lemurid species (Mittermeier et al. 2010). They are endemic to the eastern rainforests of Madagascar, from the Masoala Peninsula in the north to the Vangaindrana Farafangana region in the south (Mittermeier et al. 2010) and are classified as critically endangered in the IUCN Red List of Threatened Species (Andriaholinirina et al. 2014). Ruffed lemurs are considered to be the most intensely frugivorous of the extant lemurs. Wild diets consist of between 74% and 92% fruit (Britt 2000; Vasey 2002), with the remainder comprising young leaves, flowers and nectar (White 1989; Rigamonti 1993; Britt 2000; Vasey 2003). Despite an overt preference for fruit, however, free-ranging ruffed lemurs are adaptable in their feeding habits; at least 132 different plant species have been recorded in the diet in the wild (Morland 1991; Vasey 2000). The quantity of non-fruit food items consumed varies seasonally and depends on local availability (Britt 2000).

Obesity is a common problem for many primate species housed in zoological collections (Schwitzer and Kaumanns

2001; Videan et al. 2007). Excessive accumulation of adipose tissue (i.e. body fat) has been shown to be a contributory factor in the development of heart disease, diabetes, cancer and reproductive issues (Goodchild and Schwitzer 2008; Register and Clarkson 2009). The problem is often compounded by inactivity and the onset of lethargy, which reduces energy expenditure and facilitates continued weight gain (Goodchild and Schwitzer 2008). Zoo lemurs exhibit very different behaviours to their wild counterparts. For example, blue-eyed black lemurs (*Eulemur flavifrons*) were found to spend 12–14% of their time foraging and feeding in captivity, compared to 32% in the wild (Schwitzer et al. 2006). Moreover, diets in captivity, even for ostensibly folivorous species, are often dominated by fruit (Plowman 2013). Commercially produced fruit is substantially different from that found in the wild, being higher in sugar content and metabolisable energy, and lower in fibre, protein, minerals and vitamins (Goodchild and Schwitzer 2008; Solman 2009; Plowman 2013). The nutritional profile and physiological impact of a diet in captivity may, therefore, be very different to that of the diet in the wild (Fidgett and Plowman 2009). In the wild, ruffed lemurs weigh about 3.3 kg (females) to 3.6 kg (males; Vasey 2003). Compared to their wild counterparts, animals in captivity can be prone to obesity, with some European zoo populations averaging as much as 4.3 kg (Schwitzer and Kaumanns 2001).

Iron storage disease (ISD), or hemochromatosis, is another diet-related complication for zoo lemurs. While iron is an essential trace element, appropriate dietary quantities are unknown for the majority of species (Beard 2001). Threshold levels, that is, the

Table 1. The availability of plants in the enclosures of five populations of ruffed lemurs (*Varecia* spp.) housed in UK zoological collections. Year-round=growing in the outdoor enclosure; seasonal=provisioned when available. No browse was made available to animals in Z2.

Species		Availability	Z1	Z3	Z4	Z5	Z6
Bamboo	*Phyllostachus* sp.	Year-round		✓			
Bramble	*Rubus fruticosus*	Year-round		✓			
Buddleia	*Buddleia davidii*	Year-round		✓			
Chasun palm	*Trachycarpus fortunei*	Year-round		✓			
European beech	*Fagussylvatica*	Year-round					
Grasses	*Poaceae* sp.	Year-round	✓	✓	✓	✓	✓
Hazel	*Corylus avellana*	Seasonal				✓	
Horse chestnut	*Aesculus hippocastanum*	Year-round		✓			
Palm bamboo	*Sasa palmatta*	Year-round		✓			
Red-barked dogwood	*Cornus alba*	Year-round		✓			
Silver poplar	*Populus alba*	Seasonal				✓	
Sycamore maple	*Acer pseudoplatanus*	Year-round		✓			
Willow	*Salix* sp.	Seasonal				✓	✓

Table 2a. Weight of food (g, DM), metabolisable energy (ME; Kcal), carbohydrates (CH; g), sugar (g) and iron (Fe; mg) provided per individual per day for ruffed lemurs at three zoological institutions (Z1–Z3) in the UK in 2008. Data are sorted by category (Cat: F=fruit; V=vegetable; O=other) and alphabetically by food type.

		Z1					Z2					Z3				
Food Type	Cat	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)
Apple	F	33.95	87.44	19.89	19.89	0.15	8.83	22.74	5.17	5.17	0.04	23.36	60.18	13.69	13.69	0.11
Banana	F	63.98	98.33	24.64	21.97	0.33	140.03	215.22	53.94	48.09	0.72	128.51	197.52	49.50	44.14	0.66
Cherry	F						10.67	16.36	3.92	3.92	0.07					
Citrus	F						8.29	10.27	2.40	2.40	0.02					
Grapes	F						27.74	91.51	22.67	22.67	0.32					
Kiwi	F						2.18	3.29	0.71	0.70	0.02					
Mango	F						9.20	11.92	2.95	2.89	0.15	61.95	80.25	19.85	19.43	0.99
Orange	F						2.38	6.59	1.50	1.50	0.02					
Peach	F						9.54	15.74	3.63	3.63	0.19					
Pear	F	41.04	65.43	16.80	16.80	0.21						14.16	22.58	5.80	5.80	0.07
Plum	F						2.29	3.92	0.96	0.96	0.04					

Table 2a (continued). Weight of food (g, DM), metabolisable energy (ME; Kcal), carbohydrates (CH; g), sugar (g) and iron (Fe; mg) provided per individual per day for ruffed lemurs at three zoological institutions (Z1–Z3) in the UK in 2008. Data are sorted by category (Cat: F=fruit; V=vegetable; O=other) and alphabetically by food type.

		Z1					Z2					Z3				
Food Type	Cat	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)
Pomegranate	F											8.67	9.22	2.13	2.13	0.13
Strawberry	F						0.88	3.13	0.64	0.64	0.03					
Tomato	F	0.91	2.36	0.51	0.51	0.04	1.48	3.84	0.82	0.82	0.07					
Broccoli	V						4.48	12.91	1.21	0.72	0.40					
Carrot	V	12.01	34.32	7.35	6.86	0.34	1.20	3.43	0.73	0.69	0.03	5.12	14.62	3.13	2.92	0.14
Celery	V						2.12	1.32	0.17	0.17	0.08					
Chicory	V											2.98	5.76	1.47	0.37	0.21
Courgette	V															
Cucumber	V											0.89	3.58	0.31	0.31	0.08
Lettuce	V	2.58	7.27	0.92	0.92	0.09	0.81	2.28	0.29	0.29	0.03					
Parsnip	V						1.64	5.07	0.99	0.45	0.05	4.95	15.31	2.99	1.36	0.14
Peas	V															
Pepper	V						0.52	1.61	0.33	0.32	0.03					
Potato	V	7.54	33.78	8.08	0.37	0.13	3.55	15.92	3.81	0.17	0.06					
Snap peas	V						2.22	7.28	1.07	0.79	0.17					
Sweet potato	V						2.42	7.36	1.80	0.48	0.06					
Sweetcorn	V											55.32	36.08	4.81	1.20	0.41
Bread	O		71.61	13.86	0.92	0.79										
Egg	O						2.73									
Low Fe nuts	O												55.57	1.23	0.61	0.06
Mashed potato	O							54.54	6.38	2.83	0.50	12.86				
Peanut	O											13.40	80.57	1.79	0.89	0.36
Trio Munch[1]	O	19.40														
Total		181.41	400.54	92.05	68.25	2.08	245.19	516.25	116.08	100.30	3.10	332.18	581.23	106.69	92.85	3.35

[1] Special Diet Services (SDS), UK.

level(s) above which iron may have a toxic effect, vary between organisms and species. Lemurs have been observed to accumulate excess iron when fed on diets containing less than 300 mg/kg dry matter (DM) (Spelman et al. 1989). Excessive iron accumulation can lead to toxic effects such as lesions in the liver, hepatocellular adenoma, carcinoma, necrosis and death (Crawshaw et al. 1995; Andrews et al. 2005; Olsen et al. 2006). ISD has been described in several lemur species, including ruffed, ring-tailed (*Lemur catta*), black (*Eulemur macaco*), brown (*E. fulvus*) and crowned lemurs (*E. coronatus*) and Coquerel's sifaka (*Propithecus coquereli;* Spelman et al. 1989; Wood et al. 2003; Glenn et al. 2006; Clauss and Paglia 2012). While it has been suggested that the incidence of excessive iron accumulation in lemurs has been exaggerated (Glenn et al. 2006), ISD remains a concern for zoological institutions housing these species.

To improve husbandry, Donadeo et al. (2016) highlighted a clear need for additional data on the nutritional composition of lemur diets so that species-specific guidelines can be developed. Here, this knowledge gap is addressed by collating and describing the historical diets of several populations of black-and-white ruffed lemurs (*Varecia variegata*, Gray 1863) housed in zoos in the UK, and investigating differences in nutritional content with relevance and reference to the problems of obesity and ISD.

Methods

Data collection

Dietary data were collected from six UK zoological institutions (abbreviated as Z1–Z6 from here on) during July and August 2008 with the support of the British and Irish Association of Zoos and Aquaria (BIAZA). Zoos were chosen to maximise population sample size, and with consideration for the project's financial and temporal constraints. Each institution provided four sequential days of data, which consisted of provisioned weights of individual food types (e.g. apples, carrots) and which were assumed to be representative of the core diet provided throughout the year. Normal husbandry procedures were maintained throughout the study period; no experimental changes were made to the normal feeding routines. Animals in all institutions were kept in indoor enclosures overnight and were allowed access to larger outdoor enclosures during the day time. Food was provided in both indoor and outdoor areas; keepers at all institutions reported that all food items were consumed, with little waste. Outdoor enclosures contained trees, branches, climbing frames, ropes and/or other objects to varying degrees, thus facilitating the species' arboreal habits. Although browse may be included in lemur diets at some institutions and several enclosures contained living vegetation (Table 1), keepers reported that the animals in the focal collections rarely consumed foliage.

Nutritional composition calculations

Nutritional data (metabolisable energy [ME; kcal/100g]; carbohydrates [CH; g/100g]; sugar [g/100g]; iron [Fe; mg/100g]; all in dry matter) were extracted from McCance and Widdowson's 'composition of foods integrated dataset' (Finglas et al. 2015). Nutritional information for supplemental foods produced by Mazuri, Kasper Faunafood, and SDS were obtained from relevant product information sheets. Non-structural (i.e. readily digestible) carbohydrates were estimated as: 100% minus crude fat, crude protein, Neutral Detergent Fibre (NDF) and ash (all in % dry matter). It was not possible to quantify the proportion of each food item consumed by individual animals. Furthermore, Z3 maintained a polytypic collection consisting of eight ruffed lemurs, two red lemurs (*Eulemur rufus*) and three red-bellied lemurs (*E. rubriventer*), and each institution housed a different number of individuals (n=2–13). Mean food weight and, hence, nutrient

Table 2b. Weight of food (g, DM), metabolisable energy (ME; Kcal), carbohydrates (CH; g), sugar (g) and iron (Fe; mg) provided per individual per day for ruffed lemurs at three zoological institutions (Z4–Z6) in the UK in 2008. Data are sorted by category (Cat: F=fruit; V=vegetable; O=other) and alphabetically by food type.

		Z4					Z5					Z6				
Food Type	Cat	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)
Apple	F	19.80	51.00	11.60	11.60	0.09	12.75	32.83	7.47	7.47	0.06	28.71	73.95	16.82	16.82	0.13
Banana	F	34.58	53.16	13.32	11.88	0.18	15.48	23.79	5.96	5.32	0.08	105.40	162.00	40.60	36.20	0.54
Blackberries	F						0.38	0.63	0.13	0.13	0.02					
Dates	F											13.76	43.40	10.96	10.96	0.11
Grapes	F	7.39	24.38	6.04	6.04	0.09	0.74	2.44	0.60	0.60	0.01					
Kiwi	F	4.87	7.35	1.59	1.56	0.05										
Melon	F	9.88	6.00	1.38	1.38	0.06						39.50	24.00	5.50	5.50	0.23
Nectarine	F						2.74	5.25	1.18	1.18	0.05					
Orange	F											7.96	22.05	5.02	5.02	0.07
Peach	F											2.00	3.30	0.76	0.76	0.04
Pear	F	23.81	37.95	9.75	9.75	0.12	2.42	3.85	0.99	0.99	0.01	15.53	24.75	6.36	6.36	0.08

Table 2b (continued). Weight of food (g, DM), metabolisable energy (ME; Kcal), carbohydrates (CH; g), sugar (g) and iron (Fe; mg) provided per individual per day for ruffed lemurs at three zoological institutions (Z4–Z6) in the UK in 2008. Data are sorted by category (Cat: F=fruit; V=vegetable; O=other) and alphabetically by food type.

		Z4					Z5					Z6				
Food Type	Cat	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)	Weight (g)	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)
Plum	F											2.11	3.60	0.88	0.88	0.04
Strawberry	F	2.63	9.38	1.91	1.91	0.08										
Sultanas	F						3.71	12.03	3.04	3.04	0.10					
Tomato	F											3.58	9.28	1.99	1.99	0.16
Aubergine	V						0.98	2.06	0.30	0.28	0.04					
Broccoli	V	0.74	2.13	0.20	0.12	0.07										
Cabbage	V						2.34	5.69	0.96	0.96	0.10					
Carrot	V	11.20	32.00	6.85	6.40	0.32	1.40	4.00	0.86	0.80	0.04	11.20	32.00	6.85	6.40	0.32
Celery	V	6.72	4.20	0.54	0.54	0.24	1.19	0.74	0.10	0.10	0.04	2.52	1.58	0.20	0.20	0.09
Chicory	V															
Courgette	V						2.05	5.85	0.59	0.55	0.26	1.73	4.95	0.50	0.47	0.22
Cucumber	V	1.30	5.21	0.45	0.45	0.11										
Leek	V	2.07	4.95	0.65	0.50	0.25										
Lettuce	V	1.94	5.47	0.70	0.70	0.06	1.46	4.13	0.53	0.53	0.05					
Peas	V						1.43	4.67	0.64	0.13	0.16					
Pepper	V	1.33	4.13	0.83	0.81	0.08	4.22	13.06	2.64	2.55	0.26					
Sweetcorn	V	6.90	4.50	0.60	0.15	0.05	12.08	7.88	1.05	0.26	0.09					
Bread	O	2.84	5.56	1.08	0.07	0.06		113.38	21.95	1.46	1.25		124.78	24.15	1.61	1.38
Egg	O		28.60			0.39										
Leaf-eater pellet[1]	O						47.48	14.03	19.31		0.24					
Low Fe nuts	O						7.54									
Panda cake[1]	O	11.25	4.35	5.60		0.04										
Primate pellet[2]	O	0.28						3.30	4.12	1.17	0.01					
SA37[3]	O		0.00			0.00						27.00				
Trio Munch[4]	O	1.51					30.72					33.81	8.05	11.47	1.69	0.09
Total		151.01	290.29	63.07	53.82	2.33	151.08	259.61	72.39	27.51	2.87	294.80	537.67	132.05	94.85	3.48

[1]Mazuri Zoo Foods, Witham, Essex, UK; [2]Kasper Faunafood, Woerden, NL; [3]Intervet, Worksop, UK; [4]Special Diet Services (SDS), UK.

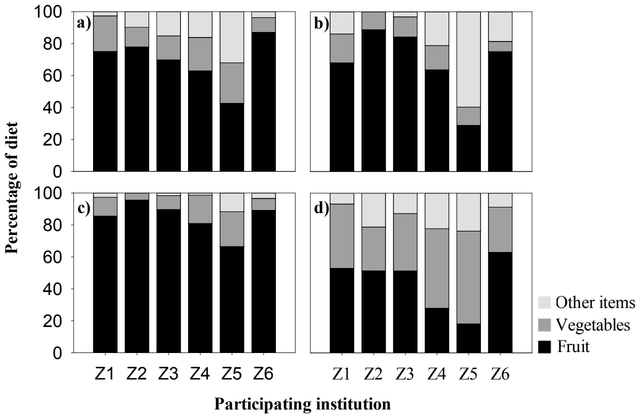

Figure 1. Percentage of (a) metabolisable energy (ME; Kcal), (b) carbohydrates (CH; g), (c) sugar (g), and (d) iron (Fe; mg) accounted for by fruit, vegetables and other food items over 4 days in the diets of ruffed lemurs in six UK zoological collections (Z1–Z6).

content, were calculated per lemur, per day, at each institution (hereafter referred to as 'per individual'). Lemur weights were obtained post hoc from the zoo aquarium animal management software, ZIMS. Weights were only available for three institutions; with the exception of Z4, data did not represent all animals in the collection: Z1 (n=4 of 5); Z3 (n=2 of 14); Z4 (n=4 of 4). Weights for Z3 reported herein refer to ruffed lemurs only.

Statistical analyses
Initial analyses revealed that residuals from one-way ANOVAs were not normally distributed and variances were not evenly distributed. Therefore, non-parametric Kruskal–Wallace tests were used with post-hoc Dunn tests to investigate significant differences, across collections, of the following: mean weight (DM) of provisioned diets per individual; ME content; CH content; sugar content; and Fe. Statistical analyses were carried out using R version 3.4.1 (R Core Team 2018).

Results

A total of 44 different food items were provided to ruffed lemurs over the course of the study (Table 2). Of these, only three items were given by all institutions on all days. Fruits accounted for the greatest proportion of the diet in five of the six institutions (51%–78%); vegetables accounted for 44% in Z4 and 47% in Z5. The quantity (weight, DM) of food provided per individual varied significantly between institutions (χ^2=24.81, df=5, P<0.0001; Table 3). This variation was largely accounted for by observed differences in the amount of fruit provided by each institution (χ^2=15.62, df=5, P=0.008). The amount of vegetation provided to each population also varied significantly (χ^2=13.05, df=5, P=0.023). Post-hoc tests described inter-institutional differences between several collections, for all three metrics (Table 3). There were no significant differences in provisioned weights of other food items.

There were significant differences in ME content between institutions (χ^2=14.48, df=5, P=0.013), for example, Z2 relative to Z5 (P=0.011), Z3 relative to Z4 (P=0.045) and Z5 (P=0.009; Table 4). Daily individual ME ranged from 189.52 (±27.17; Z5) to 547.66 (±5.76; Z3; Table 2) kcal/d. Fruit was the primary source (>60%) of ME in four institutions; other dietary items accounted for 32% of ME in Z5, with fruit accounting for 43%. Vegetables accounted for between 9%–25% of ME (Figure 1).

The amount of CH provisioned per individual differed significantly between institutions (χ^2=16.22, df=5, P=0.006). Post-hoc tests showed that Z6 (118.42±18.39 g/d) differed significantly from Z5 (67.03±18.32 g/d; P=0.013; Table 4). Fruit accounted for between 64%–89% of CH in Z1–Z4 and 75% in Z6, but only 29% in Z5 where other dietary items accounted for 60%. Vegetables accounted for between 6%–18% of CH (Figure 1).

There was a significant difference in the amount of dietary sugars provided per individual, between institutions (χ^2=21.33, df=5, P<0.0001), for example, Z2 (97.72±19.12 g/d) relative to Z5 (27.17±6.85 g/d; P=0.012), and Z3 (93.38±2.61 g/d) to Z5

Table 3. Weight of all provisioned food, fruit, vegetables and other dietary items (g; dry matter) provided to ruffed lemurs at six zoological collections (Z1–Z6) in the UK across 4 days in 2008. Values are given per individual per day; n=total number of animals in the collection. Uppercase letters indicate post-hoc Tukey test results, where A>a, B>b, C>c, at P≤0.05.

Institution	n	Weight	Fruit	Vegetable	Other
Z1	5	181.41 ± 28.83	139.88 ± 25.49	22.13 ± 7.46	19.40 ± 12.97
Z2	8	245.19 ± 47.06	223.50 ± 42.41^{A}	18.96 ± 5.97^{a}	2.73 ± 5.45^{a}
Z3	13	303.20 ± 58.25^{A}	193.55 ± 75.10	52.46 ± 25.23^{A}	17.50 ± 15.16
Z4	4	151.01 ± 22.62^{a}	102.94 ± 6.87	32.20 ± 12.35	15.87 ± 29.43
Z5	2	151.08 ± 44.76^{a}	38.21 ± 3.68^{a}	27.14 ± 22.54	85.74 ± 23.97^{A}
Z6	5	294.80 ± 14.72^{A}	218.54 ± 39.14^{A}	15.45 ± 10.55^{a}	60.81 ± 25.57^{A}

(P=0.029) and Z6 (34.78±7.74 g/d) relative to Z5 (P=0.018; Table 4). Fruit accounted for between 66%–96% of dietary sugar. Vegetables contributed 18% in Z4 and 22% in Z5 (Figure 1).

Animals at Z1 weighed an average of 3.77 kg (±0.47 kg; n=4), those at Z4 weighed an average of 3.48 kg (±0.45 kg), while animals at Z3 weighed an average of 4.45 kg (±0.28 kg). Using a threshold value of 4.274 kg to determine obesity (sensu Terranova and Coffman 1997), animals in Z3 were considered obese.

Calculated individual Fe did not generally differ significantly across institutions. However, post-hoc analyses showed that Z3 (3.49±0.14 mg/d) differed significantly from Z5 (1.78±0.31 g/d; Table 4). Fruit accounted for 18%–63% of dietary iron. Vegetables accounted for 27%–58%, and other dietary items for 7%–24% (Figure 1). Two institutions provided veterinary post-mortem reports describing excessive accumulation of Fe and the onset or presence of ISD. Z3 provided three reports, the oldest from 2002, the most recent from 2007, and Z4 provided one report from 2006. No reports specifically excluded ISD and no veterinary reports were provided by the other institutions.

Discussion

Few studies have investigated the diet of ruffed lemurs in captivity (but see: White 1989; Morland 1991; Rigamonti 1993; Britt 2000; Vasey 2003; Donadeo et al. 2016). The purpose of the present study was to quantify the amount of food provisioned to ruffed lemurs, and their basic nutritional profiles (i.e. metabolisable energy, carbohydrates, sugars and iron). Quantification of these fundamental parameters is essential in informing the development of appropriate species-specific diets, particularly given the vulnerability of captive lemurs to obesity (Schwitzer and Kaumanns 2001). As is typical for studies across several collections, there was significant variation between participating institutions in all aspects. This, combined with the observation of obesity in one institution and historical records of pathologically relevant stored iron from two institutions, highlights the lack of consistency and species-specific knowledge employed when formulating dietary guidelines for ruffed lemurs.

The data presented herein were derived from an undergraduate research project that sought to investigate dietary and retained iron via non-invasive faecal analyses. Unsuccessful attempts were made to collect historical body weight and contemporary dietary data from participating institutions in 2015–2016 to enable investigation of changes in both aspects between 2008 and the present day. The inferential potential of the present study is, therefore, limited. Furthermore, it was not possible to quantify the food intake of individual animals, hence the use of average values for food as-fed throughout. It is highly likely that the amount of food consumed varied considerably between individuals, as social hierarchy is known to impact food intake in some lemur species (e.g. *Lemur catta*; Rasamimanana 1999). Ruffed lemurs exhibit a dynamic social structure (Vasey 2006), which adds further complexity in a social provisioning setting and would require significant manipulation to quantify individual intake. Furthermore, individuals may vary in their requirements for, and/or ability to utilise, nutrients such as carbohydrates and sugars. Despite these limitations, the data and results represent a valuable addition to the literature, clearly demonstrating that zoo diets for ruffed lemurs are highly variable, and potentially contribute to illness and mortality of individual animals. The study also facilitates information exchange across institutions and provides base data for future meta-analyses.

Ruffed lemurs may be susceptible to overfeeding in captivity where there is a lack of seasonal variation in climate and food supply (Schwitzer and Kaumanns 2001). In a recent study, Donadeo et al. (2016) found that the diet of ruffed lemurs in the US was entirely unlike that of their wild counterparts, also with little consistency between institutions. Certainly, there is cause for concern given that levels of metabolisable energy at five participating institutions exceeded the suggested maintenance energy requirement of 249.3 kcal/d, as suggested by Schwitzer and Kaumanns (2001). However, the National Research Council (2003) provides little in the way of guidance with regards to the composition of ruffed lemur diets, with almost all recommendations being broadly applied to all nonhuman primates. Indeed, most captive primate diets are comprised of at least 50% fruit and vegetables (Kaumanns et al. 2000); the lemur diets described herein were no exception, and almost all were dominated by fruits. Commercially grown fruit often contains a substantial amount of non-structural carbohydrates (e.g. sugar), high levels of which are known to cause health problems in captive primates (e.g. Kuhar et al. 2013). This contrasts with fruits found

Table 4. Metabolisable energy (ME; Kcal), carbohydrates (CH; g), sugar (g) and iron (Fe; mg) contained in the diets of ruffed lemurs at six zoological collections (Z1–Z6) in the UK across 4 days in 2008. Values are given per individual per day; n=total number of animals in the collection. Uppercase letters indicate post-hoc Dunn test results, where A>a, B>b, C>c at P≤0.05.

Institution	n	ME (Kcal)	CH (g)	Sugar (g)	Fe (mg)
Z1	5	337.78 ± 21.75	90.81 ± 12.05	69.18 ± 13.03	1.38 ± 0.005
Z2	8	519.22 ± 71.12^{A}	111.82 ± 29.01	97.72 ± 19.12^{A}	3.28 ± 1.01
Z3	13	547.66 ± 5.76^{B}	108.03 ± 2.18	93.38 ± 2.61^{B}	3.49 ± 0.14^{A}
Z4	4	300.34 ± 74.45^{b}	71.53 ± 25.47	54.50 ± 6.41^{a}	2.37 ± 0.73
Z5	2	189.52 ± $27.17^{a,b}$	67.03 ± 18.32^{a}	28.17 ± $6.85^{a,b}$	1.78 ± 0.31^{a}
Z6	5	420.57 ± 33.19	118.42 ± 18.39^{A}	94.78 ± 7.74^{B}	2.21 ± 0.27

in the wild diet, which have lower energy content (Goodchild and Schwitzer 2008). Fruits provided the greatest proportion of metabolisable energy, carbohydrates and sugars in the current study. It would be reasonable, therefore, to omit food items with high sugar content from ruffed lemur diets in captivity, e.g. sultanas (69.4 g/100 g), dates (31.3 g/100 g), banana (18.1 g/100 g) and mango (13.8 g/100 g). Reducing the availability of sugar can have additional welfare benefits. For example, an average 25% reduction in non-structural carbohydrates and an increase in fibre resulted in decreased aggression and increased foraging across four lemur species, including ruffed lemur (Britt et al. 2015). The only zoo with obese lemurs was Z3, where the provisioned diet contained the most metabolisable energy (547.66±5.76 kcal/day) and the second-most sugar (93.38±2.61 g/day) of all the zoos in the study. However, lemurs at Z3 were not fed items with particularly high sugar content. It is likely, therefore, that the quantity of food provided (332.18±7.16 g/day, the highest in the study) was the main contributory factor.

Spelman and colleagues (1989) suggested that the susceptibility of lemurs to ISD is indicative of specific adaptations to a wild diet high in iron-chelating agents. Diets in captivity are low in secondary plant polyphenols (such as tannins) and high in ascorbic acid, which may be the main cause of excessive iron accumulation (Gonzalez et al. 1984; Spelman et al. 1989), as absorption is inhibited by the former and enhanced by the latter (Yip and Dallman 1996). Indeed, the diets of many free-ranging lemur species contain high levels of tannins (Jolly 1966; Tattersall 1982). Conversely, the diets of zoo animals often contain low levels of tannins, a situation which may be problematic for those species that have evolved to rely upon them (Clauss 2003). Diets high in iron, and without the iron-chelating components of diets in the wild, may be particularly problematic for monogastric browsers such as ruffed lemurs. As such, adjustments to reduce dietary iron for the species in captivity are likely to be beneficial (Wood et al. 2003). The observations of iron storage-related issues from two institutions add to the existing evidence regarding ISD in captive lemurs. Notably, no necropsy reports stating the absence of ISD-related findings were communicated during our study. It should be noted that iron is highly variable in its abundance and availability (Henry and Miller 1995) and the dietary data presented in this study represent a temporally-limited sample. Moreover, mortalities occurred at least 1 year before this study, and zoo diets are subject to ongoing review and manipulation. Indeed, Z3 began a series of dietary trials focused on lemurs several months after the conclusion of this study. It is therefore not possible to relate iron-related mortality directly to the diets described herein. Nevertheless, given the potential risks of diets rich in iron and taking the reported mortalities and diets into account, foods with high iron content, such as peas (2.8 mg/100 g), egg (2.4 mg/100 g), sultanas (2.2 mg/100 g) and broccoli (1.1 mg/100 g) should be avoided and dietary iron should not exceed 2 mg per individual per day.

Dietary adjustments are often necessary to deal with obesity and ISD. Any such changes should be responsive to the issue at hand but should also consider the composition of the diet and other influencing factors (e.g. seasonality, weight, time spent foraging) in the wild. Dietary changes can also have other beneficial effects, beyond mitigating disease. For example, animals may respond more favourably to naturalistic diets, resulting in welfare benefits (e.g. Cabana and Plowman 2014). The diets of captive populations are, however, limited by resources available to the host institution(s). Nevertheless, institutions should strive to provide lemurs with as close an approximation of the wild diet, including accounting for feeding strategy and seasonal variability, as possible. Given the degree of variation in diet, and the evidence of ruffed lemur obesity and excess iron accumulation presented herein, the development of species-specific diets is an important aim. Reformulation of diets to reduce the relative abundance of fruits, particularly those high in metabolisable energy and non-structural carbohydrates, and the removal of food items rich in iron may prove effective in decreasing the prevalence of obesity and ISD in the captive population.

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