NUTRIENT COMPOSITION OF FOODSTUFFS AVAILABLE TO WILD LEMURS LIVING IN THE ANALAMAZAOTRA SPECIAL RESERVE, EASTERN MADAGASCAR, AND A SURVEY OF DIETS FED TO CAPTIVE BLACK-AND-WHITE RUFFED LEMURS AT UNITED STATES ZOOLOGICAL INSTITUTIONS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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ABSTRACT

Lemurs are a group of primates endemic to Madagascar, an island off the southeastern coast of Africa. The International Union for Conservation of Nature (IUCN) lists 39 of the 93 recognized species of lemur in Threatened categories, including the Endangered diademed sifaka (*Propithecus diadema*) and the Critically Endangered black-and-white ruffed lemur (*Varecia variegata*). Human activities on the island, such as agriculture, logging, and hunting of lemurs for bushmeat, are significant stresses on lemur populations. Conservation efforts for lemurs include Species Survival Plan® Programs (SSP) for some species. Diets for animals in zoological parks often are based on the nutrient requirements of domestic animals or closely related exotic animals that have a history in captivity. In order to more fully understand the nutrient requirements of a species, studies of foraging behavior in the wild may be combined with nutrient analyses of the items selected in the wild. Nutrient composition of captive diets also must be determined to assess the impact of ingredient inclusion on captive lemur health, especially obesity and diabetes that are common problems in captive populations.

Previous studies have investigated the nutrient composition of wild lemur diets, but the literature specific to *P. diadema* is limited. To obtain more data on the diets of wild *P. diadema*, twelve known groups of *P. diadema* in the Analamazaotra Special Reserve (ASR), Madagascar, were observed from October, 2008, to March, 2009. Samples from plants within the range occupied by the observed lemurs were collected, dried at 55°C, and secured in heat sealed bags for transport to Omaha's Henry Doorly Zoo and Aquarium (OHDZ). The Nutrition Department at OHDZ analyzed the samples for crude protein (CP) and gross energy (GE) and then stored them at 4°C until shipment to the University of Illinois (UI). At UI, 13 plant species, totaling 36 samples, were selected for further analysis, with both fruits (n = 15) and leaves (n = 21) analyzed for each species. Fat concentrations were determined via acid hydrolysis. Dietary fiber fractions were determined via three assays: total dietary fiber (TDF), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Nitrogen-free extract (NFE) was calculated to estimate digestible carbohydrate content. Leaves tended to be higher in CP compared with fruits. Total dietary fiber concentrations also were higher in leaves than in fruits. Conversely, leaves were lower in AHF and NFE than were fruits. The data from this study will improve understanding of the nutrient composition of dietary items available to wild lemurs in ASR.

Additionally, a survey was conducted to determine items used in diets fed to captive *V. variegata* at institutions in the United States listed in the International Species Information Systems (ISIS) registry. The survey identified the type and amount of diet items fed to captive *V. variegata*, and nutritional analysis software was used to estimate the chemical composition and gross energy content of captive diets. Data from 33 institutions that responded to the survey were compiled. The most commonly included items were bananas (31 of 33 institutions) and apples (29 of 33 institutions). A majority of institutions fed Marion Zoological's Leaf Eater biscuit (10 institutions), Mazuri's Leaf Eater biscuit (14 institutions), Mazuri's Primate Browse biscuit (10 institutions), or a combination of those biscuits. Estimated DM, OM, CP, fat, and TDF concentrations of captive diets ranged from 14.5% to 67.6% (DM basis, DMB), 93.1% to 97.2% DMB, 7.9% to 23.9% DMB, 2.0% to 6.5% DMB, and 10.1% to 28.1% DMB, respectively. In general, captive diets contained higher CP concentrations and lower fat and fiber concentrations than did wild diet items. Reducing the amount of fruit included in diets fed to captive *V. variegata*, and reformulating captive diets to more closely resemble wild diet items, could reduce the prevalence of obesity in captive *V. variegata*.

DEDICATION

This thesis is dedicated to my wife, Kristin Donadeo, who believes in me even when I doubt myself. Because of her, I try to be my best.

ACKNOWLEDGEMENTS

I thank my advisor, Dr. Kelly Swanson, for the opportunities to work on projects that would not have been available to me anywhere else. I also thank him for supporting me in my professional development.

I thank my committee members, Drs. George C. Fahey, Jr., Carl M. Parsons, and Cheryl L. Morris for their participation in my committee and valuable feedback on my project. I would also like to thank Dr. Morris for her help in designing the project.

I thank the comparative nutrition group for their support during my project. Although my project did not require the group effort needed for some of the projects in our lab, I appreciated having cohorts to commiserate with about the trials and tribulations of graduate school, classes, and laboratory work. I thank Maria Godoy for training me on the total dietary fiber assay, Alison Beloshapka for answering all the little questions I had about lab work, Katie Kerr for being my sounding board, and all three for being great friends outside of work, as well. I thank Laura Bauer for imparting as much of her laboratory knowledge to me as I could absorb, and for the discussions about beer. I thank Mariana Rossoni-Serao and her family for making me and my wife feel like family, too.

Finally, I thank my own family – my wife, Kristin, for being genuinely amazing and an inspirational superhero; my parents, Tom and Karen, for pushing me to be my best, but letting me know that I get to decide what that is; my friends in North Carolina for making me feel like I always have a home there; my grandparents, who gave me the work ethic to achieve everything I have thus far.

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Chapter 1

Introduction

Lemurs are a group of five extant families of primates in the suborder Strepsirrhini. All lemurs are endemic to Madagascar, a nearly 600,000 km^2 island off the southeastern coast of Africa, separated from the continent by the Mozambique Channel. The International Union for Conservation of Nature (IUCN) lists 93 species of lemur, of which 39 are in Threatened categories, including the Endangered diademed sifaka (*Propithecus diadema*) and the Critically Endangered black-and-white ruffed lemur (*Varecia variegata*) (IUCN, 2012). Human activities on the island, such as agriculture and logging, have diminished lemur habitat extensively while lemurs have also been hunted by locals as bushmeat.

Concern for the lemur population of Madagascar has led the Association of Zoos and Aquariums (AZA) to establish Species Survival Plan® Programs (SSP) for some species of lemur. This, of course, requires formulation of diets that will satisfy the nutrient requirements of the lemurs in captivity. Diets of captive animals in zoological parks often are based on the nutrient requirements of domestic animals thought to be most similar, or closely related exotic animals that have a history in captivity. Along with these two methods, observation of feeding choices in the wild may reveal dietary preferences that aid in captive diet formulation. A well-known example is that of the maned wolf (*Chrysocyon brachyurus*), which was originally fed as a strict carnivore, but later given diets formulated for domestic canids (Childs-Sanford & Angel, 2006). Field studies suggested that the maned wolf was omnivorous, going so far as to seek out wolf's fruit (*Solanum lycocarpum*) as part of its diet even when it was not readily available in the animal's preferred habitat (Motta-Junior et al., 1996; de Arruda Bueno & Motta-Junior, 2009).

As the only primates in Madagascar, lemurs have adaptively radiated to inhabit a wide range of ecological niches and exploit a variety of available food resources, although many species of lemur consume fruit as some portion of their diet (Ganzhorn, 1988). The aye-aye (*Daubentonia madagascariensis*) includes large amounts of nuts and wood-boring insects in its diet, and has specialized digits and incisors to locate and extract these diet items (Lhota et al., 2008). Sifakas (*Propithecus* spp.) are known for their largely folivorous diets that are accompanied by extensive hindgut specialization to process large amounts of dietary fiber (Campbell et al., 2000). The black-and-white ruffed lemur (*V. variegata*), as the most frugivorous member of the family Lemuridae, consumes a diet composed of up to 92% fruit (Britt, 2000), and has a simple hindgut with a rapid passage rate averaging 1.7 h to 2.7 h (Cabre-Vert & Feistner, 1995; Edwards & Ullrey, 1999).

In order to more fully understand the nutrient requirements of a species, studies of foraging behavior may be combined with nutrient analyses of items chosen by the animals in the wild. This information may lead to more appropriate diets that are beneficial to the health and well-being of animals kept in captivity. While previous studies have investigated the nutrient composition of wild lemur diets (Ganzhorn, 1988; Atsalis, 1999; Powzyk & Mowry, 2003; Curtis, 2004), the literature specific to *P. diadema* (Powzyk & Mowry, 2003) or *V. variegata* (Schmidt et al., 2010) is limited. In recognition of the need for more data on the diets of lemurs in the wild, this thesis seeks to measure the dry matter (DM), crude protein (CP), gross energy (GE), acid hydrolyzed fat, and fiber [neutral detergent fiber (NDF), acid detergent fiber (ADF), total dietary fiber (TDF), and crude fiber] concentrations of fruits and leaves of 13 plant species known to be available to *P. diadema* and *V. variegata* in the Analamazaotra Special Reserve, Madagascar. In addition, a survey of 58 institutions in the United States currently registered with

International Species Information Systems (ISIS) as holding *V. variegata* was conducted to determine the feed items used in diets fed to captive populations. As well as identifying the type and amount of diet items fed to captive *V. variegata*, Nutritionist Pro^{TM} software (Axxya Systems, Stafford, TX) was used to estimate the chemical composition and gross energy content of individual feed items and entire captive diets.

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Chapter 2

Literature Review

Wild Lemurs

Madagascar, home to the unique group of primates known as lemurs, is the world's fourth largest island (Tattersall & Sussman, 1975; Krause et al., 1997), and is located off the coast of Mozambique in the Indian Ocean (Fig. 2.1). Due to the isolation of Madagascar from continental Africa for at least 88 million years (Krause et al., 1997), lemurs evolved on the island without the influence of anthropoid primates until the arrival of humans around 1,500 to 2,000 years ago (Brockman et al., 1987). This isolation has allowed lemurs to retain some features that are thought to resemble ancestral primates (Junge et al., 2009), such as the wet nose, or rhinarium, that is found in many other mammal orders but is uncommon in primates. However, they also have developed some traits that separate them from most extant and extinct (fossil) primates, such as canines and incisors of the lower jaw that face forward, creating a dental comb that is used for grooming (Garbutt, 1999). The potential for lemurs to serve as models for early primates and answer questions related to biogeography and evolution, as well as their singular position in the biodiversity of Earth, should lead humans to conserve these unusual primates. While efforts are being made toward that end, there is still much improvement possible.

Conservation concerns: Since the time that humans established a presence on the island, 17 species of lemur are known to have gone extinct (Tattersall, 1982; Simons et al., 1995; Simons, 1997), and it is believed that this loss of species was due, at least in part, to human activities (Brockman et al., 1987). The stresses that killed off these subfossil lemurs are probably the same ones that affect existing lemur populations. Human-induced pressures include the hunting of extant lemurs for meat, deforestation through the use of *tavy* (slash-and-burn farming), and habitat fragmentation due to tree removal for fires and construction (Richard & Sussman, 1975). Hunting is a considerable stress on many lemur species, leading to local extinction of several species in multiple locations throughout the island (Irwin et al., 2005; Lehman et al., 2005), while deforestation and fragmentation are detrimental to all of the animals in the affected forests. Frugivores may be at greater overall risk of population declines from these problems, though. The black-and-white ruffed lemur (*Varecia variegata*), a highly frugivorous species (Dew & Wright, 1998; Britt, 2000), is known as a favored prey of hunters in the Makira Forest (Golden, 2009). Additionally, fragmentation of habitat leads to changes in food availability (Irwin, 2008a) that require changes in feeding habits if the species is to survive. Frugivores are more susceptible to the effects of fragmentation because fruit-producing trees are valuable as human resources (Medley, 1993).

In an attempt to maintain viable habitat for the extraordinary wildlife of the island, and prevent the further extinction of lemurs, Madagascar National Parks manage the reserves and parks that are set aside as protected areas. Analamazaotra Special Reserve (ASR; S18°48'56.1", E048°25'11.2") is one of these protected areas. This reserve is in the Eastern Region of Madagascar (Fig. 2.2), which is characterized by forests, relatively constant temperatures, and higher humidity compared to the drier Western Region that has more variable temperatures and contains not only forest but brush and desert-like habitats (Sussman, 1999). The ASR has a mean temperature of around 25°C (77°F) versus a mean temperature of approximately 22°C (72°F) in the southern part of the island, though the eastern part of the island is generally cooler than the west (Tattersall & Sussman, 1975). There are two important seasons on the island: the period from May to October is generally considered to be the dry season, while the period from

November to April is referred to as the wet season. A short transition period exists during the change from one season to another. Variations in fruit and flower production in the eastern rain forest do not seem to be under the influence of these seasons, and instead exhibit an extended periodicity of greater than one year (Overdorff, 1996). Analamazaotra Special Reserve has an area of 810 hectares (Day et al., 2009) and is home to populations of several lemur species. The diademed sifaka (*Propithecus diadema*) and *V. variegata* are endemic to the Eastern Region of the island and ASR, but were extirpated from the reserve in the 1970s, largely due to hunting activities of humans (Day et al., 2009). A reintroduction program has since brought both species back to ASR, with 27 diademed sifakas and 7 black-and-white ruffed lemurs (of the subspecies *V. variegata variegata*), translocated from other locations on the island to ASR between January, 2006 and July, 2007 (Day et al., 2009).

Ecology: Black-and-white ruffed lemurs were first considered to be the same species as the red ruffed lemur (*Varecia rubra*, formerly *V. variegata rubra*), and there was little distinction made in early studies of their ecology (Tattersall, 1982; Sussman, 1999). In fact, both species likely have similar ecology and physiology (Tattersall, 1982; Vasey, 2003), and data on one species can therefore be relevant to the other. Today, the red ruffed lemur and black-and-white ruffed lemur have both been elevated to species level, but the black-and-white ruffed lemur is now divided into three subspecies (*V. v. subcincta*, *V. v. editorum*, and *V. v. variegata*) (IUCN, 2012). The range of *V. variegata* habitat extends throughout the humid forests of eastern Madagascar, bordered on the north by the Anove River with no well-defined southern boundary, *V. v. subcincta* having the northernmost range of the three subspecies and *V. v. editorum* the furthest south (Tattersall, 1982; IUCN, 2012). Black-and-white ruffed lemurs in general are primarily diurnal and known to prefer the high canopy, where they move about easily and frequently use suspensory postures to feed, sometimes hanging from only their back feet (Tattersall, 1982; Sussman, 1999; 2002). Their primary social group is the bonded pair, but they participate in fission-fusion social organization with groups usually ranging in size from five to 31 individuals, depending on the time of day and the activity (Morland, 1991; 1993a; Junge et al., 2009). Despite the relatively stable weather patterns of eastern Madagascar, black-and-white ruffed lemurs exhibit some behavioral changes that are synchronized with the season, increasing sunning activity and feeding bouts in the cooler wet season, and breeding from May to July (Morland, 1993a; 1993b; Britt, 2000). They can be considered litter-bearing, as they commonly give birth to more than one offspring per reproductive event, with as many as five young born to one female after a gestation of 98 to 102 days (Junge et al., 2009). They are unique among day-active lemurs for leaving their offspring in a nest while the parents seek food (Morland, 1990; Vasey, 2003), requiring the young to reach maturity quickly, at around 18 months of age (Foerg, 1982).

Like *V. variegata*, the diademed sifaka's taxonomy has shifted over the years. They were previously known as *P. diadema diadema*, one of several subspecies (Tattersall, 1982; Sussman, 1999), but have since been elevated to species status and are now properly referred to as *P. diadema* (IUCN, 2012). Their range has some overlap with *V. variegata,* as they are also found in humid forests in the Eastern Region, but the southern border is formed by the Mangoro and Onive Rivers, and they are bounded on the north by the Mananara River (Tattersall, 1982; IUCN, 2012). Sifakas are primarily active during the day (Sussman, 1999), which makes the presence of a tapetum lucidum an unusual feature. This structure is used to reflect light back from the choroid to the retina to improve night vision, but it seems to be present in all of the diurnal lemurs (Tattersall, 1982). Marking territory with scent glands is another common feature of day-active lemurs, though the scent glands are located at various points on the body depending on the species (Junge et al., 2009). *P. diadema* have been observed to be more focused on scent-marking than some species such as the indri (*Indri indri*) (Tattersall, 1982). As is common among sifakas and indris, *P. diadema* move through the trees using an upright leaping motion that can carry them in excess of 10 m at a time (Tattersall, 1982; Sussman, 2002). Social groups for *P. diadema* are usually made of two to six individuals (Irwin, 2008b).

Diets: There are many similarities between the black-and-white ruffed lemur and the diademed sifaka, but among the most prominent differences are their feeding habits. As opposed to *V. variegata*, which has been observed to consume a diet of up to 92% fruit (Britt, 2000), *P. diadema* is considered largely folivorous. Despite this classification, the sifaka's diet varies seasonally, with the greatest consumption of fruits and seeds during the wet season, greater intake of buds and flowers found in the dry season, and the highest consumption of leaves occurring in the change of seasons (Irwin, 2008a). According to Ganzhorn (1988), niche specialization becomes more apparent when chemical composition of foods is considered. Folivores are adapted to be better at detoxifying secondary plant compounds (Junge et al., 2009), allowing them to consume foods higher in antinutritional factors, such as tannins. Morphological differences in the masticatory anatomy and the digestive tract contribute further to niche partitioning by allowing lemurs to exploit different resources based on other aspects of chemical composition, such as concentrations of fiber and protein (Ganzhorn, 1988; Campbell et al., 2000). As frugivores, ruffed lemurs have a preference for ripe fruits (Sussman, 2002). Their gut is short (162.5 cm), approximately 4.5 times the length of their body (Fig. 2.3), as compared to the 15.5:1 gut:body length ratio of the folivorous Coquerel's sifaka (*Propithecus coquereli*) (Campbell et al., 2000). Passage rate in ruffed lemurs is rapid (1.5 to 3.9 h transit time), whereas sifakas have much longer transit times (24.6 h; Cabre-Vert & Feistner, 1995; Edwards & Ullrey, 1999; Campbell et al., 2004). This indicates that *V. variegata* likely rely on simple sugars or fats from fruit as their primary energy source, because food passes quickly through their simple guts. The slower passage rate and more distinctly sacculated cecum of sifakas (Fig. 2.4) are adaptations that allow them greater utilization of fiber. In addition to physiological adaptations to diet, other physical and behavioral qualities, such as variation in activity patterns, prevalence of leaves in the diet, food species targeted, body weight, social organization, and vertical habitat separation serve to minimize competition for food among lemur species (Ganzhorn, 1988).

Despite the minimization of food competition, Dew and Wright (1998) observed that four lemur species, including *P. diadema* and *V. variegata*, present in the same location (Ranomafana National Park, Madagascar) ate many of the same fruits during the end of the dry season and the shift into the wet season. This is logical even with the focus on niche separation, because lemurs tend to seek foods containing greater concentrations of available protein, as demonstrated by Ganzhorn (1988) and as reported in folivorous primates other than *P. diadema* (Clutton-Brock & Harvey, 1977; Hladik, 1979). Because the inaccessibility of Madagascar has led to the development of a unique assemblage of vegetation, with 161 endemic tree genera found on the island (Wright et al., 2011), and a dearth of seed dispersing species from taxonomic orders other than primates (Dewar, 1984; Fleming et al., 1987; Wright, 1997), lemurs are the primary seed dispersers of the island (Wright & Martin, 1995; Britt, 2000; Wright et al., 2005). Both *V. variegata* and *P. diadema* are among the largest lemurs in their ecological niches, weighing around 3,600 g and 6,500 g, respectively (Glander & Powzyk, 1995; Terranova & Coffman, 1997), and both are known to consume some of the same fruit species. Therefore, some overlap in the size of fruits chosen by both species may be expected. While this may be true for smaller fruits, it is likely not true for larger fruits, as Perry and Hartstone-Rose (2010) observed in their study of maximum ingested food size (V_b) in captive lemurs of 17 different species housed at the Duke Lemur Center (DLC). Red ruffed lemurs in that study consistently had a larger V_b than the diademed sifaka. *V. variegata* are also known to swallow whole fruits from plants that have seeds of >30 mm in diameter, a trait that is unknown in the other species observed by Dew & Wright (1998). Additionally, it has been shown that vine and tree seeds are not chewed by *Varecia*, exiting the alimentary canal nearly unaltered except for the chemical digestion of the seed coat, thereby increasing the ability of the seeds to germinate, whereas diademed sifakas are seed predators that crush most of the seeds they consume (Dew & Wright, 1998), making *V. variegata* in particular a valuable seed disperser.

There are currently few published reports on the chemical composition of wild lemur diet items (Ganzhorn, 1988; Atsalis, 1999; Powzyk & Mowry, 2003; Curtis, 2004), and even fewer specific to *V. variegata* (Schmidt et al., 2010) or *P. diadema* (Powzyk & Mowry, 2003). However, studies on anthropoid primates eating similar diets may provide insight into how and what lemurs eat in the wild. Given that food availability and composition vary temporally due to effects of both biotic (e.g., plant production, consumption of plants by other species) and abiotic (e.g., rainfall, temperature) factors, animals are required to adapt their feeding strategies to the given resources at a specific time. However, it has been reported that gorillas and cercopithicine monkeys can consume different species and parts of plants than their conspecifics at separate locations and still consume comparable nutrient concentrations (Conklin-Brittain et al., 1998; Twinomugisha et al., 2006; Rothman et al., 2007). If this relationship also holds true across seasons, it is likely that comparisons of the chemical composition of diets of a single species can be made between studies conducted at different locations and different times of the year, even if there is inconsistency in the specific items being consumed. It also has been reported that diets having high concentrations of easily extractable macronutrients are preferred by vertebrates of small to medium body sizes (Milton, 1987), likely because of their higher relative metabolism compared to larger homeotherms. Simple sugars and other non-structural carbohydrates are accessible macronutrients present in high concentrations in fruits and are readily oxidized for energy. This is probably a driving factor in selection of foods by vertebrate frugivores (Ungar, 1995; Remis, 2002), and explains their predilection for ripe fruits, as free sugars become more available from starches with ripening (Prasanna et al., 2007). These changes are the source of some of the difficulty in making cross-study comparisons, though, as they contribute to variation in proximate analysis results for the same fruits over time (Prasanna et al., 2007). Even with their high concentrations of available sugars, fruits are less digestible than leaves when the seeds are consumed along with the pulp because the seed portion is less digestible than the fiber portion of leaves (Rothman et al., 2008). Additionally, fruit contains a lower protein concentration than leaves (Rode & Robbins, 2000). The protein:fiber ratio is thought to be highly relevant to the food choices of primates (Milton, 1979) and, more specifically, the ratio of protein to ADF may be a determining factor in the ability of a given location to sustain folivorous primate populations (Oates et al., 1990; Ganzhorn, 1992; Chapman et al., 2002). However, primates have relatively low protein requirements due to their slow growth rates, typically late age of maturity, and production of milk with low macronutrient concentrations (Oftedal, 1992). A high ratio of sugar to fiber in ripe fruits may serve to increase the protein:ADF ratio to a level capable of sustaining primate metabolism. Lemurs may be further suited to survive on lower quality (i.e., lower protein, higher fiber) diets because of their low basal metabolic rates (Junge et al., 2009).

Captive Management

In addition to *in situ* conservation programs such as wildlife reserves and re-introduction efforts, zoological institutions are contributing to conservation through captive breeding programs (Association of Zoos and Aquariums, 2011) for species such as *V. variegata*. Success with captive housing of *P. diadema* has so far been limited. Only one captive subject is known from the literature (Campbell et al., 2001; Perry & Hartstone-Rose, 2010), and as of March 20, 2013, no institutions registered with the International Species Information System (ISIS, Eagan, MN) house *P. diadema*. Therefore, data on their captive habits are lacking. Captive breeding of *V. variegata*, on the other hand, had its earliest success with the first birth in captivity of a thriving black-and-white ruffed lemur in 1969 at the San Diego Zoo (Brockman et al., 1987). By the end of 1985, the International Studbook of the Ruffed Lemur reported 358 *V. v. variegata* in captivity (Brockman, 1986), and there were 833 *V. variegata* in captivity as of August 2012, according to ISIS. Mating in captive black-and-white ruffed lemurs housed in North America occurs from October to February (Junge et al., 2009), nearly the opposite time of year as wild breeding. The reversed breeding cycles may be explained either by differences in light or temperature cycles between the southern (Madagascar) and northern (United States) hemispheres. Early captive diets were formulated without the benefit of knowing the chemical composition of wild diets, and information on wild diets was mostly inferred from studies of dental morphology (Brockman et al., 1987).

Weight management: Even though a lack of data pertaining to wild diets still exists today, captive *V. variegata* regularly live to be 20 to 30 years of age (Crawford et al., 2005; Junge et al., 2009), though they are subject to a suite of problems that appear to be nutritionally related. A better understanding of wild diets could potentially ameliorate issues such as obesity, which is widespread in captive *V. variegata* (Terranova & Coffman, 1997; Schwitzer & Kaumanns, 2001), although underweight lemurs are also known in captivity. In one study, 30% to 50% of captive lemurs were reported as being obese, despite the fact that the mean captive weight (3,524 g for *V. variegata*) did not differ from the mean wild weight (3,600 g) within the same species (Terranova & Coffman, 1997). As a countermeasure to development of obesity, Junge et al. (2009) recommended limit-feeding all domestic produce and providing most of the calories with nutritionally-complete commercial products (i.e., biscuits and chows). Because of the high concentrations of starches and sugars present in commercially-available fruits and vegetables, it was suggested that they be used sparingly, mainly for enrichment and training. Their suggestion was to provide a diet at a rate of 2.0% to 2.5% of a lemur's ideal body weight (dry matter basis), with 80% to 85% of the diet being composed of biscuits and the remainder provided as produce. Junge et al. (2009) recommended using the mean body weights from Terranova and Coffman (1997) as the ideal weight (3,600 g) for an individual black-and-white ruffed lemur and suggested that animals weighing more than 4,724 g were obese and in a state when nutritional intervention was required. These dietary recommendations would require close monitoring of the dietary fiber concentrations because it is known that *V. variegata* will lose weight on diets containing high fiber (30% acid detergent fiber) (Edwards & Ullrey, 1999). In contrast, insufficient dietary fiber leads to loose stools, reduced satiety, and blood glucose fluctuations (Edwards & Ullrey, 1999; Junge et al., 2009). Promoting foraging activity in captivity has the potential to increase overall activity, leading to potential weight loss in obese animals, and can be achieved by hiding food in the enclosure or requiring manipulation of objects (e.g., boxes, phone books) to obtain food. It would also act as enrichment by encouraging wild-type behaviors (Britt, 2000).

Diabetes: Obesity is a contributing factor to the development of diabetes, at least in ring-tailed lemurs (*Lemur catta*) (Kuhar et al., 2013). Diabetes has been reported in multiple lemur species (Walzer 1999; Dutton et al., 2003; Kuhar et al., 2013), with ring-tailed lemurs being the most commonly diagnosed prosimians and a majority of diabetic primates being female (Kuhar et al., 2013). Measurement of blood glucose is an inadequate method of diabetes diagnosis in lemurs because stress-induced hyperglycemia is common (Junge et al., 2009). The preferred diagnostics for diabetes in lemurs include serum fructosamine, glycosolated hemoglobin, and the insulin:glucose ratio (Walzer, 1999; Dutton et al., 2003). Because all of these methods require a blood sample, diagnosis can be difficult if the animal is untrained or unwilling to participate in blood draws. Diagnosis can, therefore, be made using non-invasive means such as observation of sticky enclosure floors or urinary glucose readings, both reliant on the presence of hyperglycosuria (Kuhar et al., 2013). Recommendations for managing diabetes in lemurs include reducing consumption of starches and simple sugars, an accompanying increase in dietary protein, fat, and fiber, and feeding multiple, small meals throughout the day to help maintain steady blood glucose concentrations (Junge et al., 2009).

Iron storage disease: A condition of great concern in captive lemurs that is potentially influenced by diet is iron storage disease (ISD), which is marked by excessive storage of iron in the body with or without associated tissue damage. Liver damage (e.g., fibrosis, hepatocellular necrosis) and excessive iron concentrations have been observed in captive lemurs during necropsy (Lowenstine & Munson, 1999; Dorrestein et al., 2000; Smith, 2000). The spleen and duodenum are most often affected secondary to the liver (Gonzales et al., 1984; Benirschke et al., 1985; Spelman et al., 1989). Organ damage is caused by the build-up of toxic free-radicals in the tissues from redox reactions catalyzed by the ferrous (Fe^{2+}) ion (Crichton et al., 2002). In general, non-ruminants that consume a browse diet in the wild have been found to be more likely to suffer from ISD when fed a captive diet (Clauss et al., 2002). Hemosiderosis, the excess storage of hemosiderin in tissues without accompanying damage, also appears to be a common problem in captive lemurs (Gonzales et al., 1984; Benirschke et al., 1985; Spelman et al., 1989; Williams et al., 2008). The problem is thought to develop over time in captivity, as high iron loads have not been demonstrated at birth in captivity (Wood et al., 2003), nor in adult wild-caught lemurs (Gonzales et al., 1984; Spelman et al., 1989). It is believed that the diets of captive lemurs contribute to ISD, especially diets that were high in citrus and low in tannins. Citrus has been demonstrated to enhance iron absorption in humans, and iron storage in humans is apparently enhanced by fruit intake in general (Ballot et al., 1987; Fleming et al., 2002). Because all lemurs can synthesize vitamin C (Nakajima et al., 1969), limiting it in the diet by eliminating or reducing citrus is unlikely to result in harm. Therefore, limiting citrus is a common practice when developing captive lemur diets. Conversely, tannins, which appear in high concentrations of many wild lemur diets (Tattersall, 1982), are iron chelators that bind the mineral in the small intestine and decrease its absorption from the diet. These secondary plant compounds are likely present in low concentrations in captive diets containing large amounts of commercially-available produce, as they produce a bitter taste that is generally found unacceptable to humans. However, evidence is lacking in support of adding tea or beans, both known to contain tannins, or of adding tannins directly, in order to reduce iron absorption in captive lemurs (Junge et al., 2009). Existing ISD-related damage cannot be reversed by lowering systemic iron (Wood et al., 2003). Prevention, however, may be possible by feeding a well-balanced, plant-based diet containing species-appropriate dietary fiber concentrations to prevent excess iron absorption and storage (Junge et al., 2009).

The relationship between ISD and diet specific to lemurs is still unclear, however. Crawford et al. (2009) examined 10 different commercial diet components, and serum concentrations of minerals and fat-soluble vitamins in lemurs eating them at 21 institutions. No relationship between dietary vitamin C or iron concentrations and serum iron concentrations was observed in captive *V. variegata*. Serum copper concentrations also were measured, and compared to dietary copper concentrations in the commercial diets, with no correlation being observed. It also has been reported that circulating copper concentrations were higher in captive *V. variegata* when compared to wild lemurs of other species, including *V. rubra* (Dutton et al., 2003; 2008; Junge & Louis, 2005; 2007). In fact, serum copper concentrations in captive *V. variegata* were reported to be above expected ranges for all mammals (McDowell, 1992). Given the similar pathology of copper toxicosis and ISD, with hemolytic anemia and hepatopathy concomitant with both diseases (Crawford et al., 2005; 2009), and the competition between iron and copper for absorption and transport, these two minerals should be considered and measured in tandem in any further studies on ISD in lemurs. Because other trace minerals, such as manganese, zinc, and calcium, also have been reported to inhibit iron absorption in humans (Rossander-Hultén et al., 1991; Roughead et al., 2005; Olivares et al., 2007), further study focused on dietary mineral concentrations of lemur diets is needed to understand ISD and similar diseases.

Diets: Reports on captive lemur diets are so far limited (Brockman et al., 1987; Spelman et al., 1989; Crawford et al., 2009), and current studies have not investigated the macronutrient content of the diets. The San Diego Zoo's diet for *V. variegata* was previously described (Brockman et al., 1987), but without any measurements of the complete diet or individual ingredients. Dietary ingredients that could have high concentrations of tannins, ascorbic acid, or iron were investigated by Spelman et al. (1989), in the context of their effects on hemosiderosis. However, this study did not provide any analysis of the chemical composition of these ingredients, instead focusing on signs of hemosiderosis observed during captive lemur necropsies. Mineral and fat-soluble vitamin concentrations in diets of captive *V. variegata* at 20 institutions in the US were determined by Crawford et al. (2009), though no data was reported on macronutrient concentrations. Captive lemur diets should be characterized for both ingredient inclusion and chemical composition, in order to determine the effects of specific ingredients on lemur health. This will allow institutions to make adjustments to their diets that could alleviate diseases observed in captivity.

Thesis Objective

The objective of this thesis was three-fold. First, we intended to determine the chemical composition of fruits and leaves available to lemurs in the Analamazaotra Special Reserve, Madagascar. Peer-reviewed literature currently does not report these data, which will contribute to the overall knowledge of wild lemur diets. Second, we aimed to survey zoological institutions in the United States to determine the ingredient composition of diets fed to black-and-white ruffed lemurs. To determine if the variability in ingredient composition affected nutrient content, the third objective was to use nutritional analysis software to approximate the chemical composition of the diets provided by the zoological institutions.

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Figures

Figure 2.1: Map of Madagascar (as indicated with red marker) in relation to Africa. (image from Google Earth)

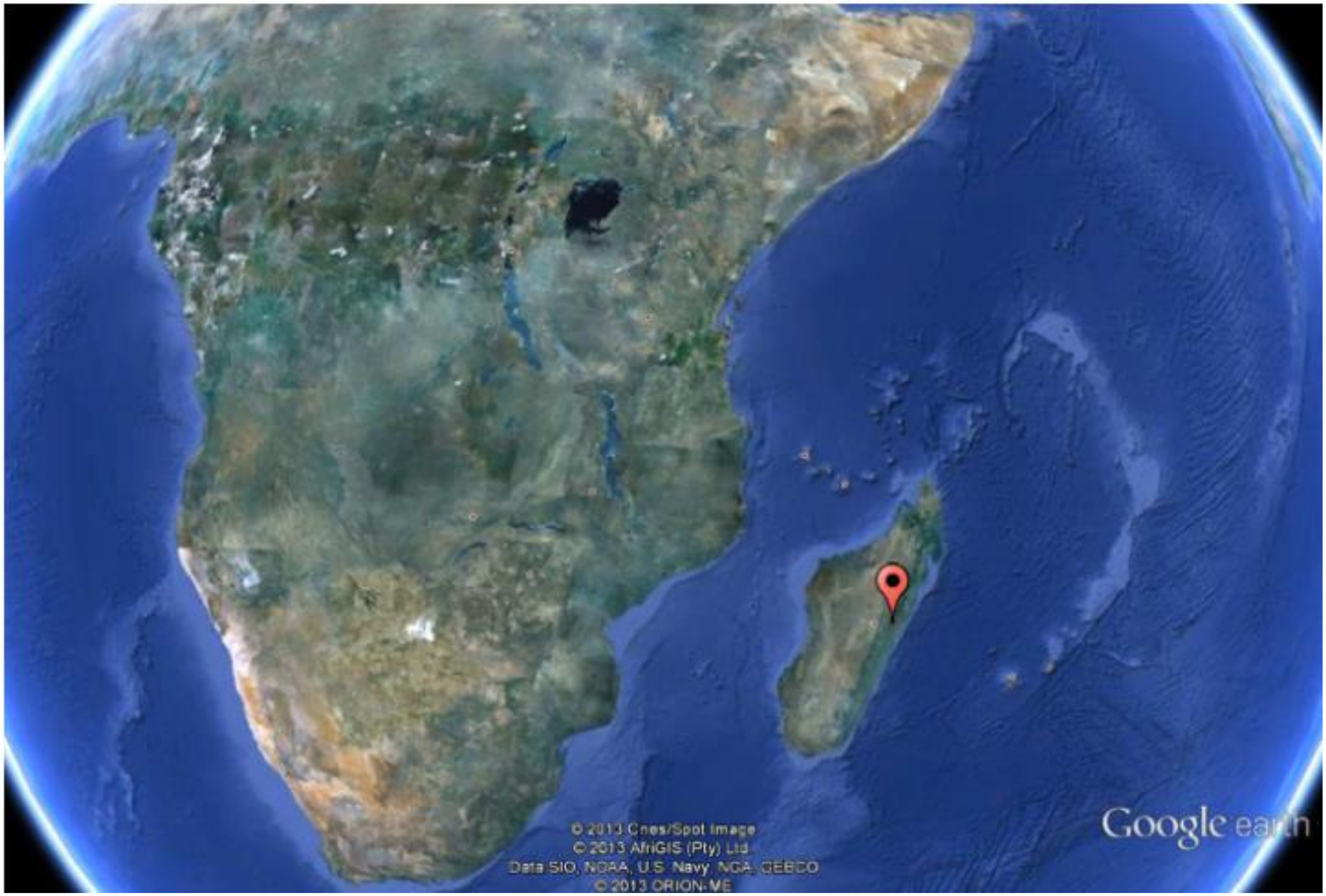


Figure 2.2: Map of Analamazaotra Special Reserve, Madagascar (as indicated by coordinate marker). (image from Google Earth)

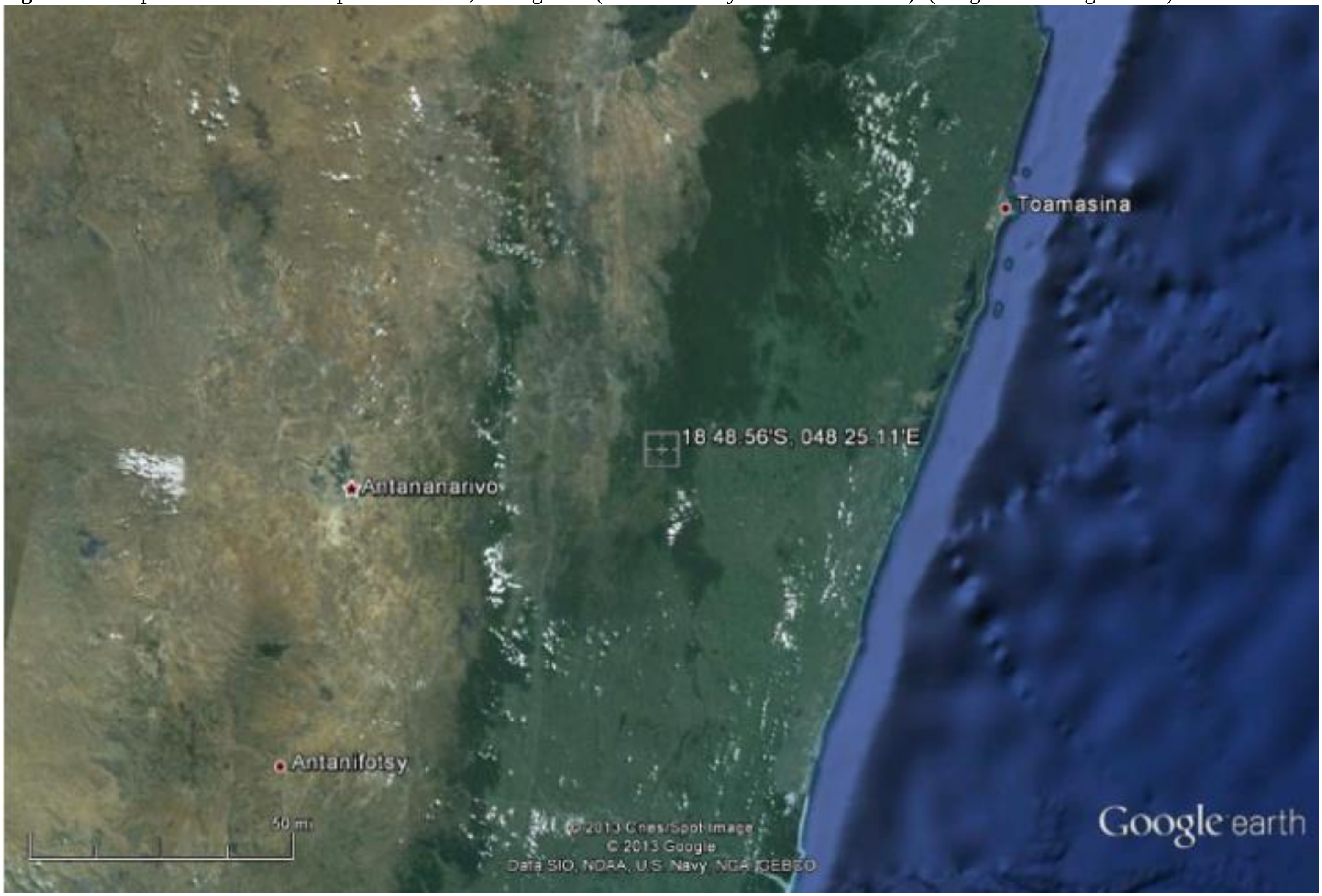


Figure 2.3: Gastrointestinal tract of a red ruffed lemur (*Varecia rubra*), a close relative of *V. variegata*. Scale equals 1cm. (image from Campbell et al., 2000)

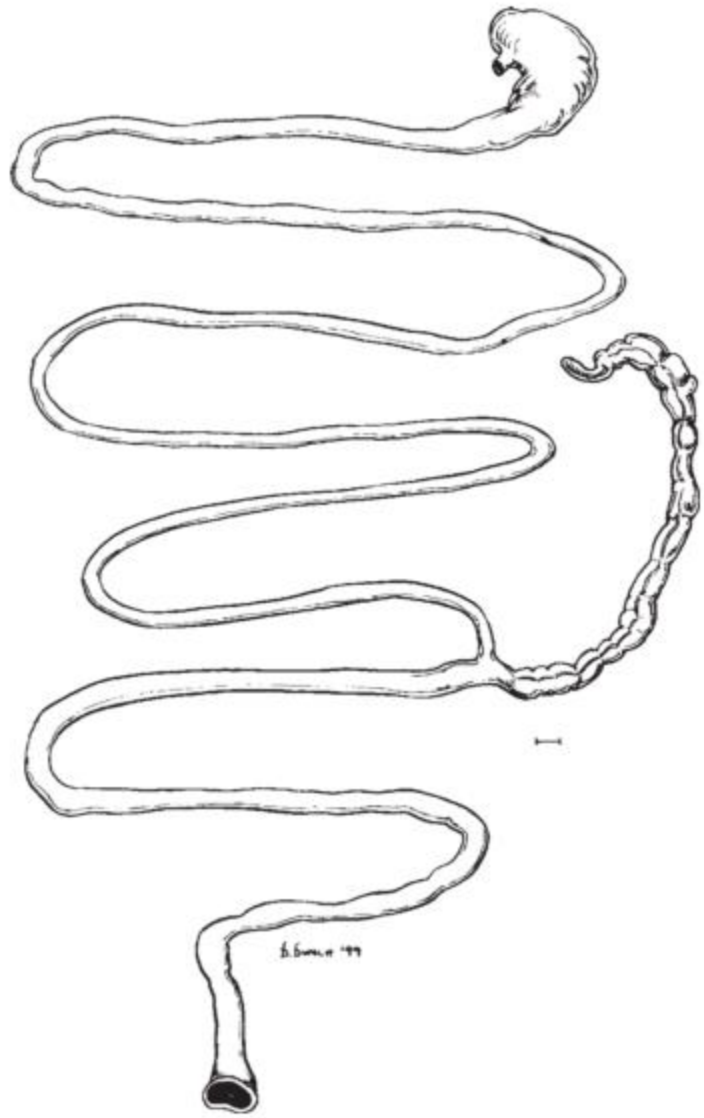
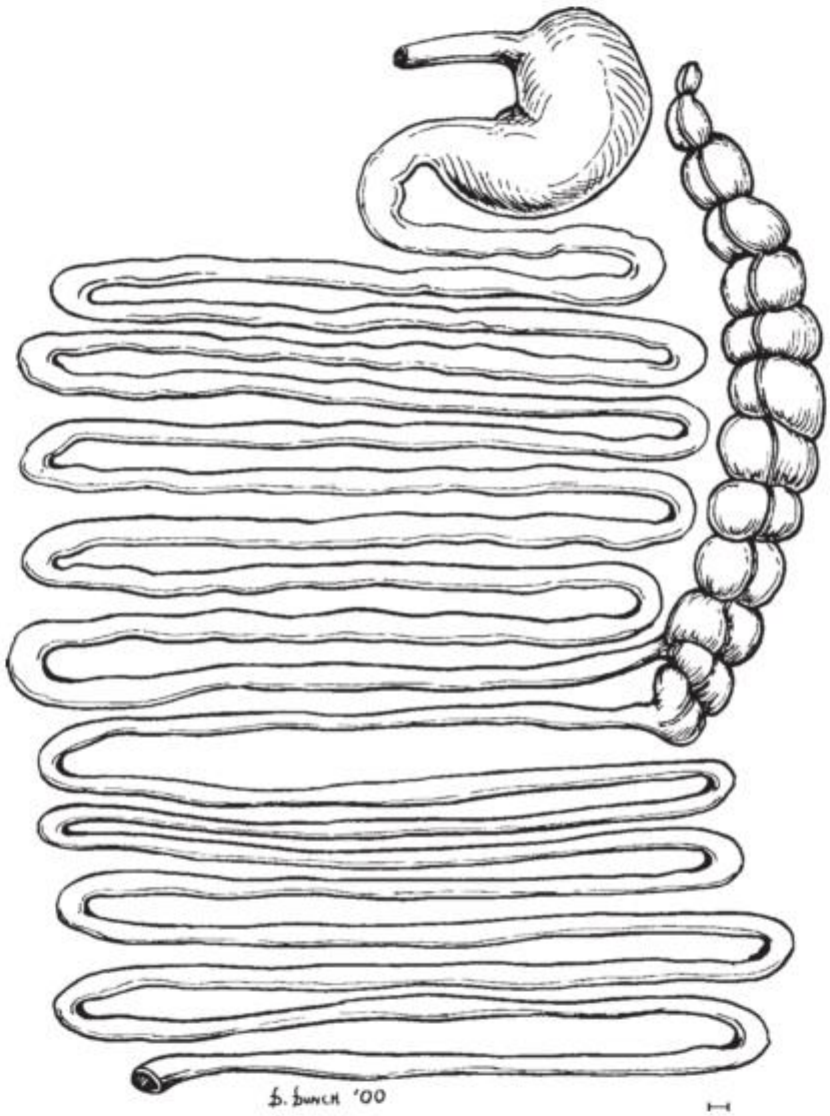


Figure 2.4: Gastrointestinal tract of a Coquerel's sifaka (*Propithecus coquereli*), a relative of *P. diadema*. Scale equals 1 cm. (image from Campbell et al., 2000)



Chapter 3

Nutrient composition of fruits and leaves available to wild lemurs living in the Analamazaotra Special Reserve, eastern Madagascar

Abstract

The objective of this study was to determine the nutrient concentrations of selected fruits and leaves in the Analamazaotra Special Reserve in eastern Madagascar. Twelve known groups of diademed sifakas (*Propithecus diadema*), comprised of 1 to 4 individuals, were observed from October, 2008, to March, 2009, for food selection preferences. Samples were collected from plants, dried at 55°C, and secured in heat sealed bags for transport to Omaha's Henry Doorly Zoo and Aquarium (OHDZ). The Nutrition Department at OHDZ analyzed the samples for crude protein (CP) concentrations and gross energy (GE). Samples then were stored at 4°C until shipment to the University of Illinois (UI). At UI, 13 plant species, totaling 36 samples, were selected for analysis. Both fruits (n = 15) and leaves (n = 21) were analyzed for each species. Fat concentrations were determined via acid hydrolysis. Dietary fiber fractions of the samples were determined using the total dietary fiber (TDF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) assays. Nitrogen-free extract (NFE) was calculated as an estimate of digestible carbohydrate content. Leaves tended to be higher in CP concentrations than fruits. Total dietary fiber concentrations were higher in leaves than in fruits, while leaves were lower in AHF and NFE than fruits. Concentrations of TDF in fruits and leaves consumed by *P. diadema* during the observation period were lower than in fruits and leaves that were not consumed. However, the ratio of CP:ADF was not different between food and non-food items. Ratios of macronutrients to estimated metabolizable energy were not different between food and non-food items, either. The

data from this study will improve understanding of the nutrient composition of dietary items available to wild lemurs in ASR, and may be beneficial for improvement of captive lemur diets.

Introduction

There are few reports in the literature on the nutrient composition of wild lemur diets (Ganzhorn, 1988; 1992; Atsalis, 1999; Powzyk & Mowry, 2003; Curtis, 2004; Schmidt et al., 2010). Furthermore, each of the existing reports is limited in the number of plants analyzed, the types of assays used, or the species of lemur studied. Therefore, further information is needed on the chemical composition of plants consumed by lemurs throughout Madagascar.

Omaha's Henry Doorly Zoo and Aquarium (OHDZ) is involved in an ongoing reintroduction project for diademed sifakas (*Propithecus diadema*) in the Analamazaotra Special Reserve (ASR), Madagascar, making this species and location of particular interest for dietary studies. Only one existing study has analyzed nutrient composition of diets for wild *P. diadema* (Powzyk & Mowry, 2003), and only two studies of the chemical composition of plants consumed by lemurs in ASR (Ganzhorn, 1988; 1992). Currently, no studies provide data on the total dietary fiber (TDF) concentrations of wild lemur diets, although this should be an important consideration for species that rely heavily on hindgut fermentation, such as sifakas (Campbell et al., 2000).

The dietary preferences of wild lemurs, combined with chemical composition data of those items, can help to improve both *in situ* and captive conservation efforts. Knowing the items that wild lemurs choose to eat can aid in the preservation of trees necessary for the lemurs' survival. The nutrient composition of the selected food items is not necessarily indicative of the nutrient requirements of the lemurs, but may reveal what nutrient concentrations allow the animals to thrive and (or) reproduce effectively. Lastly, understanding the chemical composition of available food sources for wild lemurs can allow formulation of captive diets that more closely represent these wild diets, which may assist in alleviating health issues specific to captive lemurs (e.g., obesity).

The objective of this study was to analyze fruits and leaves from the ASR, using proximate analysis techniques, as well as TDF. Items were separated into two categories based on whether or not *P. diadema* in ASR chose to consume them during a six month observation period in late 2008 and early 2009. Wild diet items consumed by *P. diadema* were expected to be high in fiber, with leaves containing higher protein concentrations than fruits. In addition, consumed plants were expected to contain higher protein concentrations than those that *P. diadema* did not consume.

Materials and Methods

Sample collection: Twelve family groups of *P. diadema* were observed by field biologists in the ASR, Madagascar, between the months of October, 2008, and March, 2009, for foraging and feeding behaviors. Day et al. (2009) describe the animals and study site. Leaf and fruit samples were collected from plants present within the ranges of these groups for proximate analysis.

Proximate analyses: Following collection, 15 fruit samples and 21 leaf samples were weighed, dried in a 55°C oven for 5 days, and weighed back to determine the loss of moisture (AOAC, 1975). After drying, samples were placed in bags, which then were heat-sealed for shipment to the Nutrition Department at OHDZ where they were ground on either a Retsch Grindomix GM 200 (Retsch Inc., Newtown, PA) or a Wiley Mill 3383-L10 (Thomas Scientific, Swedesboro, NJ) with a 20 mesh screen. A subsample was further dried at 105°C to determine the absolute dry matter (DM). A subsample of each item also was ashed to determine organic matter (OM) concentrations (AOAC, 1975). Gross energy (GE) was estimated using a LECO AC500 bomb calorimeter (LECO Corp., St. Joseph, MI) and crude protein (CP) concentrations were measured with a LECO FP528 nitrogen/protein determinator (AOAC, 2006). Samples then were stored at 4°C until being shipped to the University of Illinois for further analyses.

At the University of Illinois, acid-hydrolyzed fat (AHF) content of the samples was determined according to the American Association of Cereal Chemists (1983). Crude fiber (CF) concentrations were estimated for a subset of samples according to Schneider & Flatt (1975), but not continued for all samples due to the high variability of the assay. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed non-sequentially for all samples in order to improve the precision of the ADF assay (Goering & Van Soest, 1970; Van Soest et al., 1991). Total dietary fiber content was evaluated for all samples, including both soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) fractions for the fruit samples based on procedures described by Prosky et al. (1984; 1992). All assays were performed in duplicate, with assays re-run for replicates that varied from each other by more than 5% if total concentration was greater than 10%, or if variance was more than 10% if total concentration was less than 10%. Insoluble dietary fiber and SDF assays were not performed on leaf samples because of the expectation of high concentrations of insoluble fiber in these samples. Nitrogen-free extract (NFE) was calculated to represent the amount of digestible carbohydrate present in the samples, with the exception that TDF values were used in place of CF values.

Calculations and charts: Absolute DM was calculated by multiplying the 57°C DM concentration from the initial moisture loss from drying in Madagascar by the DM concentration from drying at OHDZ (105°C). Estimated metabolizable energy (ME) was calculated using Atwater factors of 4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for carbohydrates (NFE).

Charts were created using Excel 2010 (Microsoft, Redmond, WA), with ternary plots utilizing the TRI-PLOT spreadsheet design from Graham and Midgley (2000). In the ternary plots, each side of the triangle is an axis representing a macronutrient. The closer a point appears to the corner labeled by a macronutrient, the larger the percentage of ME represented by that macronutrient.

Results

The chemical composition of the analyzed Madagascan plant samples is listed in Table 3.1. Dry matter concentrations of all fruits ranged from 20.1% to 50.4% (median = 35.1%), whereas all leaves had DM concentrations ranging from 17.8% to 54.1% (median = 35.7%). Concentrations of OM of all fruits ranged from 94.0% to 98.1% (DM basis; DMB; median = 96.6% of DM), and from 88.7% to 97.2% DMB (median = 94.2% DMB) in all leaves. Fruit CP concentrations (range = 4.8% to 15.1% DMB; median = 8.0% DMB) tended to be lower than that of leaves (range = 5.5% to 19.9% DMB; median = 10.1% DMB). Median CP concentrations of consumed fruits and leaves were 7.4% and 10.1% (DMB), respectively. Leaves (range = 1.5% to 11.1% DMB; median = 3.7% DMB) had numerically lower AHF concentrations than fruits (range = 3.1% to 23.1% DMB; median = 7.9% DMB). As would be expected, NFE concentrations were numerically higher in fruits (range = 8.9% to 49.4% DMB; median = 22.4% DMB) than leaves (range = 6.2% to 27.7% DMB; median = 19.1% DMB). Also as expected, TDF concentrations were numerically higher in leaves (range = 46.9% to 78.9% DMB; median = 59.1% DMB) than in fruits (range = 18.7% to 72.4% DMB; median = 58.0% DMB), though median values were similar.

Fruit and leaf TDF, IDF, SDF, NDF, ADF, and CF concentrations are presented in Table 3.2. Concentrations of TDF were numerically lower than NDF in 11 of the 36 analyzed samples, while the remaining 25 samples had numerically greater concentrations of TDF than NDF (range = 27.5% to 79.8% DMB; median = 56.7% DMB). Neutral detergent fiber concentrations for consumed fruits ranged from 27.5% to 66.2% DMB (median = 57.0% DMB), and for consumed leaves ranged from 32.3% to 72.5% DMB (median = 60.9% DMB). Acid detergent fiber concentrations of consumed fruits ranged from 9.8% to 48.3% DMB (median = 23.4% DMB), and for consumed leaves ranged from 25.8% to 57.9% DMB (median = 49.8% DMB). Crude fiber concentrations were always lower than either TDF or NDF concentrations and showed little correlation with either of those measures.

Figure 3.1 is a scatter plot chart of the CP:ADF ratio of all 36 samples, and demonstrates that there was considerable overlap of this ratio in the plant parts chosen for consumption by *P. diadema* ("wild food") and those not selected by *P. diadema* ("wild non-food") during the observation period. Figure 3.2 is a scatter plot chart of the CP:TDF ratio of all 36 samples, and demonstrates that similar overlap still is present among food items and non-food items using this ratio. Little distinction is observed among food items and non-food items on the basis of the ratios of CP:estimated ME (Fig. 3.3), AHF:estimated ME (Fig. 3.4), NFE:estimated ME (Fig. 3.5), or TDF:estimated ME (Fig. 3.6).

The percentage of metabolizable energy (ME) derived from CP, AHF, and NFE were examined in the analyzed Madagascan plant samples using a ternary plot (Fig. 3.7). This relationship showed that approximately half of the non-food items (n = 8) were clustered in a range that contained approximately 10% to 30% CP, 25% to 40% AHF, and 40% to 55% NFE. The consumed items showed no noticeable clustering in their CP:AHF:NFE ratios. However, none of the items chosen as food had the same macronutrient pattern observed in the non-food items.

Discussion

Chemical composition data of wild lemur diets available in the literature are limited (Ganzhorn, 1988; 1992; Atsalis, 1999; Powzyk & Mowry, 2003; Curtis, 2004; Schmidt et al., 2010), with only one published study on the chemical composition of diets of wild *P. diadema* (Powzyk & Mowry, 2003). Furthermore, few studies have been published with data specific to chemical composition of plants in the ASR (Ganzhorn, 1988; 1992) and none have included TDF analysis of wild lemur diet items. With ongoing reintroduction efforts at ASR, it is important to know the chemical composition of available food sources. This also allows comparison of chemical composition of food sources between ASR and other reserves for a better understanding of the dietary needs and challenges of wild lemurs. Lastly, it helps to build an understanding of those plants crucial to the survival of lemurs based on the chemical composition of their food selection choices.

Powzyk & Mowry (2003) analyzed a small set of diet items preferred by *P. diadema* and the related folivore, the indri (*Indri indri*), in the 10,000 hectare Mantadia National Park, Madagascar. Observations of these lemurs were made over 19 mo in both wet and dry seasons. Mantadia's southern border is just north of ASR's northern border, and the two reserves used to be part of a continuous forest. The Powzyk & Mowry study included analysis of CP, NDF, and ADF of 2 fruits and 3 leaves consumed by *P. diadema*, and 9 leaves consumed by *I. indri*. In that study, fruits consumed by *P. diadema* had a median CP concentration of 9.6%, close to the 7.4% median CP of consumed fruits in our study. However, the median CP concentration of the immature leaves consumed by *P. diadema* in Mantadia was 21.0%, and *I. indri* consumed leaves with a median CP concentration of 10.7% (Powzyk & Mowry, 2003). Intake of CP by *P. diadema* in that study was considerably higher than the 10.1% median CP concentration of leaves from our study, while intake of CP by *I. indri* was similar to that found in leaves from our study. Fruits consumed by *P. diadema* in Mantadia had a median of 42.2% NDF and 30.6% ADF, as compared to 40.5% NDF and 22.1% ADF for consumed fruits from our study. The leaves consumed by *P. diadema* in Powzyk and Mowry (2003) had median concentrations of 61.3% NDF and 46.2% ADF, while *I. indri* consumed leaves with median concentrations of 58.3% NDF and 53.2% ADF. In our study, *P. diadema* consumed leaves with median concentrations of NDF and ADF of 49.6% and 46.6%, respectively. While NDF consumed by *P. diadema* in our study was lower than that reported in lemurs from Mantadia, the difference was greater for leaves than for fruits. Conversely, *P. diadema* at ASR ate fruits with a much lower median ADF concentration than those sampled at Mantadia, and leaves with a similar median ADF concentration to those consumed by *P. diadema* in Mantadia. Perhaps *P. diadema* at ASR are able to consume diets with NDF and ADF concentrations similar to that found in diets of their conspecifics at Mantadia by consuming a larger proportion of fruits than *P. diadema* do in Mantadia. However, more study of food selection by *P. diadema* in ASR is needed to determine if their preferences reflect these differences, as well.

The CP:ADF ratio has been hypothesized to be a primary determinant in the selection of foods by primates (Milton, 1979), with sites with higher CP:ADF better able to support folivorous primate biomass (Oates et al., 1990; Chapman et al., 2002). Although this has been reported in folivorous lemurs (e.g., *Propithecus tattersalli* and *Propithecus diadema edwardsi*), including those in the ASR (Ganzhorn, 1992), our data suggest that it does not appear to hold true for *P. diadema* in the ASR. Among the 36 Madagascan plant parts analyzed in this study, 21 were known to be consumed by *P. diadema* during the observational study. All 36 samples are presented as the ratio of CP:ADF in Figure 3.1. No clear distinction can be made between the food and non-food items on this chart. Therefore, additional aspects of the chemical composition of the Madagascan plant parts were examined to determine if any were indicative of the food selection preferences of *P. diadema* in the ASR.

Acid detergent fiber is an important determinant of diet quality for herbivores, and is composed of a portion of the insoluble and largely non-fermentable portions of cell walls (i.e., cellulose and lignin), while NDF captures these compounds and a portion of the hemicelluloses from the cell wall. Total dietary fiber captures all components of the cell wall (i.e., lignin, cellulose, and hemicelluloses), as well as some of the fermentable non-structural components of the plant cell (e.g., pectins and β-glucans) and is, therefore, a better measure of fiber intake and the fermentable energy available in plant parts. It is therefore unexpected that some samples had lower concentrations of TDF than of NDF. This is likely due to the TDF assay degrading more of the macronutrients in the sample than did the NDF assay, meaning that the NDF assay measured some portion of the protein, fat, or carbohydrate that should not have been present.

Propithecus diadema rely heavily on fermentation to process their diets and have hindgut adaptations to increase their fermentative capacity (Campbell et al., 2000). When the CP:TDF ratio of the Madagascan plant samples was examined, however, there was still a noticeable overlap of items consumed by *P. diadema* and those not chosen during the observation period (Figure 3.2). If the CP:fiber ratio of foods in the environment is similar, additional factors, such as macronutrient concentrations or the relationship between a given macronutrient and available energy may also influence food choices of *P. diadema*. It is also possible that the observed group of *P. diadema* consumes more of these plants than was observed during the study period.

Further exploration of this hypothesis was carried out by examining the relationship between estimated ME and CP (Fig. 3.3), AHF (Fig. 3.4), NFE (Fig. 3.5), and TDF (Fig. 3.6) in the Madagascan plant samples. The ratios of these chemical components to ME also demonstrated little difference between food and non-food items. Items that were chosen as food by *P. diadema* commonly had similar estimated ME to items that were not consumed, when viewed on the basis of any of the three macronutrients as well as TDF. Some items with lower estimated ME were consumed in favor of items that had greater estimated ME. The consumed items with lower estimated ME may have been chosen due to greater availability of these plant parts at the time of consumption. Alternatively, they may have had greater overall digestibility than some of the items that were not consumed, possibly due to the presence of antinutritional factors or indigestible seeds in the unconsumed items.

Currently, the literature does not contain any TDF data for wild lemur diets. While ADF measures only the cellulose and lignin present in a sample, NDF measures the lignin, cellulose, and most of the hemicelloses. These detergent fiber assays are well-designed to measure their intended fiber fractions, and are still used frequently in nutrition laboratories. Crude fiber is more limited than NDF, and less accurate than either of the detergent fiber assays or TDF, and only captures a portion of each of the cell wall structural components (i.e., lignin, cellulose, and the hemicelluloses). However, CF is still the required assay for reporting fiber on feed labels. We analyzed both NDF and ADF in order to allow comparison of our results with previous studies. Crude fiber analysis was also attempted for some samples, but proved to be too variable to justify continued pursuit with the small amounts of sample available for some plants. Total dietary fiber was analyzed to provide the most accurate value for potentially fermentable substrates present in the plant parts.

It is important to use the proper fiber assay when analyzing feeds for animals. The inaccuracy of the CF assay is recognized by animal scientists, and some producers of lemur chows already list the NDF and ADF concentrations of their products on their specification sheets. For animals that rely on hindgut fermentation to digest a significant portion of their food (e.g., *P. diadema*), it is desirable to have a better measure of available fermentative substrate in a feed, and TDF should, therefore, be used. Likewise, TDF should be used when analyzing fiber content of wild diet items, not only to allow comparison of wild and captive diets but to have a better understanding of the fiber present in wild diet items.

While the Madagascan plant parts analyzed here were observed being eaten by *P. diadema*, it should be noted that other species in the ASR, including *V. variegata* have access to the same food sources. It has been shown in other reserves that sympatric populations of lemurs select many of the same fruits (Dew & Wright, 1998). In addition, although *V. variegata* has been shown to be almost completely frugivorous, leaves constitute a portion of their wild diet (Britt, 2000). It is likely, however, that *V. variegata* would not consume as wide a variety of leaves as *P. diadema*. Therefore, the analyzed plant parts may also be eaten by *V. variegata* in the ASR, despite the fact that only one analyzed fruit was reported to be consumed by *V. variegata* during the period of plant sample collection.

In conclusion, fruits and leaves consumed by *P. diadema* in ASR were high in TDF, though they were slightly lower in TDF than fruits and leaves that were not consumed during the observation period. Leaves consumed by *P. diadema* had higher CP content than consumed fruits, as hypothesized. While this study provides more data on the chemical composition of food items consumed by wild lemurs, it also highlights the continued need for additional studies of lemur diets. Specifically, data on the food preferences of wild lemurs need to be collected in tandem with data on the chemical composition of those plants. This will allow researchers to estimate the nutrient intake of wild lemurs, and while this is not necessarily the same as the nutrient requirements of these lemurs, it may indicate which plant species are necessary to wild lemur survival, and provide insights as to improvements that can be made in captive diets.

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Tables and figures

Table 3.1: Analyzed chemical composition, gross energy (GE), and estimated metabolizable energy (ME) content of Madagascan plant samples.

			Dry matter basis							As-is basis				
Plant species	**Part**	**DM,[1] %**	**OM, %**	**CP, %**	**AHF, %**	**TDF, %**	**NFE, %**	**GE, kcal/g**	**ME, kcal/g**	**CP, %**	**AHF, %**	**TDF, %**	**NFE, %**	**ME, kcal/g**
Food items														
Camellia thea 1	fruit	47.2	97.3	9.4	19.7	18.7	49.4	5.30	4.13	4.4	9.3	8.8	23.3	1.95
Camellia thea 2	fruit	50.4	98.1	8.2	9.5	61.0	19.4	5.32	1.96	3.9	4.5	28.8	9.2	0.00
Camellia thea 3	fruit	22.2	97.8	10.0	9.0	63.9	14.9	5.37	1.81	4.7	4.3	30.2	7.0	0.40
Erythroxylum sphaeranthum	fruit	30.3	94.0	15.1	3.1	55.6	20.3	4.41	1.69	7.1	1.5	26.3	9.6	0.51
Mammea aff. punctata	fruit	35.1	95.8	5.6	23.1	33.7	33.4	5.65	3.64	2.6	10.9	15.9	15.8	1.28
Mammea bongo	fruit	20.1	95.7	6.6	7.0	40.6	41.5	5.34	2.55	3.1	3.3	19.2	19.6	0.51
Psidium cattleianum	fruit	36.8	94.2	4.8	5.6	59.0	24.8	5.06	1.69	2.3	2.6	27.8	11.7	0.62
Syzygium emirnense	fruit	23.8	97.7	5.1	9.7	57.4	25.5	4.89	2.10	2.4	4.6	27.1	12.0	0.50
Abrahamia nitida	leaf	38.9	94.4	7.2	3.2	65.6	18.3	5.10	1.31	3.4	1.5	31.0	8.6	0.51
Erythroxylum capitatum 1	leaf	53.5	96.3	10.7	1.6	58.3	25.7	5.29	1.60	5.1	0.7	27.5	12.1	0.86
Erythroxylum capitatum 2	leaf	34.5	92.8	11.5	1.4	63.2	16.7	5.14	1.26	5.4	0.7	29.8	7.9	0.43
Erythroxylum sphaeranthum 1	leaf	54.1	90.4	12.1	2.1	55.6	20.6	4.91	1.50	5.7	1.0	26.2	9.7	0.81
Erythroxylum sphaeranthum 2	leaf	45.8	92.1	10.8	1.7	59.9	19.7	4.95	1.37	5.1	0.8	28.3	9.3	0.63
Erythroxylum sphaeranthum 3	leaf	41.4	94.2	10.1	1.6	61.3	21.2	5.07	1.39	4.8	0.8	28.9	10.0	0.58
Mammea aff. punctata 1	leaf	47.2	93.8	8.1	11.1	46.9	27.7	5.44	2.43	3.8	5.2	22.2	13.1	1.15
Mammea aff. punctata 2	leaf	32.3	93.1	7.7	10.8	54.5	20.0	5.24	2.08	3.6	5.1	25.7	9.5	0.67
Mammea aff. punctata 3	leaf	29.9	95.0	8.0	9.4	57.4	20.2	5.45	1.97	3.8	4.4	27.1	9.6	0.59
Mammea bongo	leaf	35.7	97.2	8.5	5.9	69.4	13.4	5.82	1.41	4.0	2.8	32.8	6.3	0.50
Olax madagascariensis 1	leaf	17.8	88.7	19.9	5.9	51.8	11.3	5.04	1.77	9.4	2.8	24.4	5.3	0.31
Olax madagascariensis 2	leaf	26.5	91.8	19.2	6.5	59.1	7.0	5.21	1.63	9.1	3.1	27.9	3.3	0.43
Syzygium emirnense	leaf	40.7	95.7	8.7	4.9	70.0	12.1	5.37	1.27	4.1	2.3	33.0	5.7	0.52

Table 3.1 (cont.)

Plant species	Part	DM,[1] %	Dry matter basis: OM, %	CP, %	AHF, %	TDF, %	NFE, %	GE, kcal/g	ME, kcal/g	As-is basis: CP, %	AHF, %	TDF, %	NFE, %	ME, kcal/g
Non-food items														
Abrahamia nitida	fruit	45.6	96.6	4.8	3.9	45.0	42.8	4.87	2.26	2.3	1.9	21.2	20.2	1.03
Cryptocarya crassifolia	fruit	42.8	97.2	6.0	5.7	72.4	13.1	5.68	1.27	2.8	2.7	34.2	6.2	0.54
Dichapetalum leucosia	fruit	20.5	96.6	10.5	5.6	58.0	22.4	5.09	1.82	5.0	2.6	27.4	10.6	0.37
Erythroxylum capitatum	fruit	31.7	96.8	10.6	7.2	58.3	20.6	5.44	1.90	5.0	3.4	27.5	9.7	0.60
Harungana madagascariensis	fruit	36.2	96.1	8.0	12.0	67.2	8.9	5.66	1.76	3.8	5.7	31.7	4.2	0.64
Olax madagascariensis	fruit	40.9	95.6	14.8	9.9	41.1	29.8	5.11	2.67	7.0	4.7	19.4	14.1	1.09
Tinopsis aff. apiculata	fruit	26.0	97.3	7.9	7.9	59.3	22.2	5.40	1.91	3.7	3.7	28.0	10.5	0.50
Camellia thea	leaf	30.8	93.1	18.9	3.4	55.8	15.0	5.45	1.66	8.9	1.6	26.3	7.1	0.51
Cryptocarya crassifolia	leaf	46.4	95.6	7.0	2.9	66.6	19.1	5.60	1.31	3.3	1.4	31.4	9.0	0.61
Dichapetalum leucosia	leaf	43.8	92.6	12.6	2.7	71.0	6.2	4.60	1.00	6.0	1.3	33.5	2.9	0.44
Harungana madagascariensis 1	leaf	28.6	95.0	12.1	7.0	55.3	20.7	5.60	1.94	5.7	3.3	26.1	9.8	0.55
Harungana madagascariensis 2	leaf	22.3	94.8	9.8	8.6	56.2	20.3	5.73	1.97	4.6	4.0	26.5	9.6	0.44
Harungana madagascariensis 3	leaf	27.7	94.7	5.5	4.4	66.8	18.0	5.17	1.34	2.6	2.1	31.5	8.5	0.37
Psidium cattleianum	leaf	31.7	93.1	11.9	3.7	57.3	20.1	4.71	1.62	5.6	1.8	27.0	9.5	0.51
Tinopsis aff. apiculata	leaf	42.6	97.0	9.1	2.7	78.9	6.4	5.47	0.86	4.3	1.3	37.2	3.0	0.37

[1]DM = dry matter, OM = organic matter, CP = crude protein, AHF = acid hydrolyzed fat, TDF = total dietary fiber, NFE = nitrogen-free extract.

Table 3.2: Analyzed dietary fiber concentrations (as % of dry matter) of Madagascan plant samples.

Plant species	Part	TDF[1]	IDF	SDF	NDF	ADF	CF
Food items							
Camellia thea 1	fruit	18.7	18.4	0.3	27.5	9.8	.
Camellia thea 2	fruit	61.0	60.1	0.9	66.2	39.7	.
Camellia thea 3	fruit	63.9	61.5	2.4	63.6	45.3	.
Erythroxylum sphaeranthum	fruit	55.6	54.0	1.6	54.5	21.9	.
Mammea aff. punctata	fruit	33.7	30.8	3.0	31.3	24.9	.
Mammea bongo	fruit	40.6	32.7	7.9	36.2	15.8	12.5
Psidium cattleianum	fruit	59.0	59.0	0.0	59.6	48.3	.
Syzygium emirnense	fruit	57.4	55.3	2.1	59.5	19.4	.
Abrahamia nitida	leaf	65.6	.	.	61.5	49.8	29.0
Erythroxylum capitatum 1	leaf	58.3	.	.	56.0	57.9	.
Erythroxylum capitatum 2	leaf	63.2	.	.	65.1	55.9	.
Erythroxylum sphaeranthum 1	leaf	55.6	.	.	53.2	52.4	.
Erythroxylum sphaeranthum 2	leaf	59.9	.	.	60.9	53.0	28.6
Erythroxylum sphaeranthum 3	leaf	61.3	.	.	60.9	54.8	.
Mammea aff. punctata 1	leaf	46.9	.	.	32.3	25.8	27.1
Mammea aff. punctata 2	leaf	54.5	.	.	40.1	29.9	28.0
Mammea aff. punctata 3	leaf	57.4	.	.	44.2	37.1	28.9
Mammea bongo	leaf	69.4	.	.	66.8	57.1	43.8
Olax madagascariensis 1	leaf	51.8	.	.	46.1	30.2	.
Olax madagascariensis 2	leaf	59.1	.	.	61.9	51.2	18.6
Syzygium emirnense	leaf	70.0	.	.	72.5	48.1	.
Non-food items							
Abrahamia nitida	fruit	45.0	41.1	3.8	40.5	29.9	.
Cryptocarya crassifolia	fruit	72.4	70.8	1.6	72.4	56.1	.
Dichapetalum leucosia	fruit	58.0	57.0	1.1	57.3	42.6	.
Erythroxylum capitatum	fruit	58.3	58.3	0.0	63.5	36.6	.
Harungana madagascariensis	fruit	67.2	63.2	4.0	61.7	53.1	.
Olax madagascariensis	fruit	41.1	41.1	0.0	37.3	22.1	.
Tinopsis aff. apiculata	fruit	59.4	56.8	2.5	62.2	35.7	.
Camellia thea	leaf	55.8	.	.	40.1	33.1	.
Cryptocarya crassifolia	leaf	66.6	.	.	53.5	52.6	.
Dichapetalum leucosia	leaf	71.0	.	.	67.2	43.3	.
Harungana madagascariensis 1	leaf	55.3	.	.	47.6	39.2	.
Harungana madagascariensis 2	leaf	56.2	.	.	47.2	39.6	.
Harungana madagascariensis 3	leaf	66.8	.	.	54.5	50.5	.
Psidium cattleianum	leaf	57.3	.	.	43.1	45.2	.
Tinopsis aff. apiculata	leaf	78.9	.	.	79.8	65.9	.

[1]TDF = total dietary fiber, IDF = insoluble dietary fiber, SDF = soluble dietary fiber, NDF = neutral detergent fiber, ADF = acid detergent fiber, CF = crude fiber.

Figure 3.1: Crude protein (CP):acid detergent fiber (ADF) ratios in Madagascan plant samples.

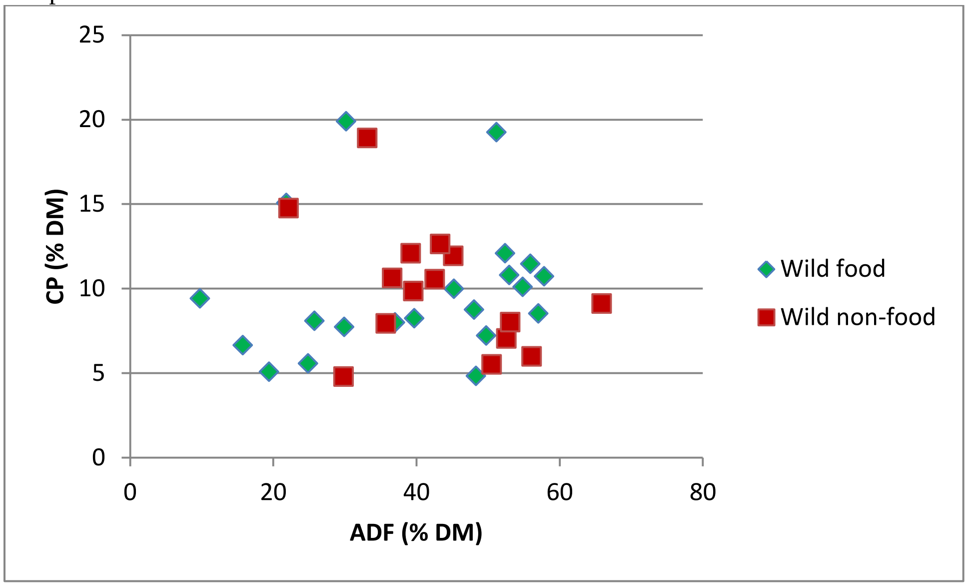


Figure 3.2: Crude protein (CP):total dietary fiber (TDF) ratios in Madagascan plant samples.

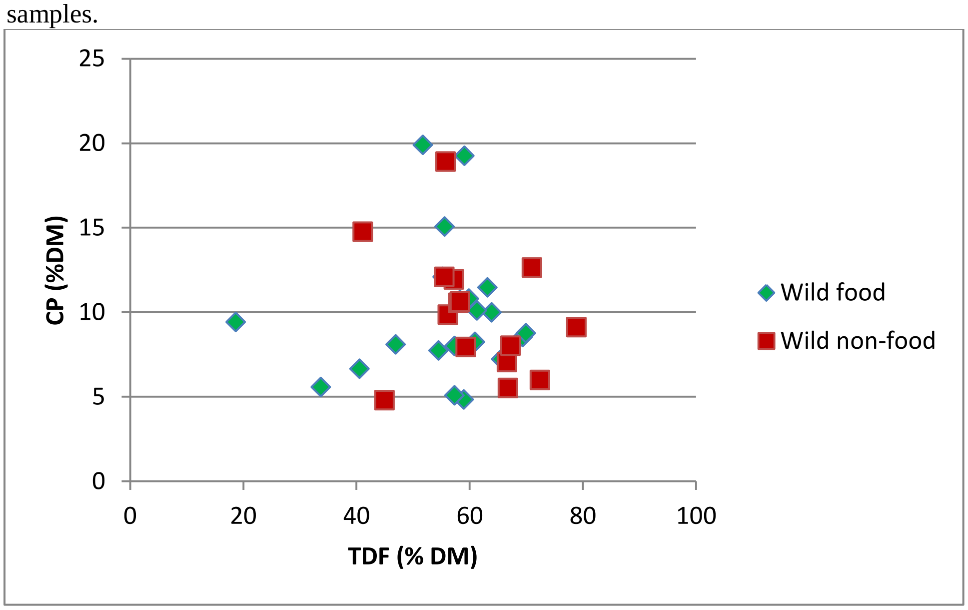


Figure 3.3: Crude protein (CP):estimated metabolizable energy (ME) ratios in Madagascan plant samples.

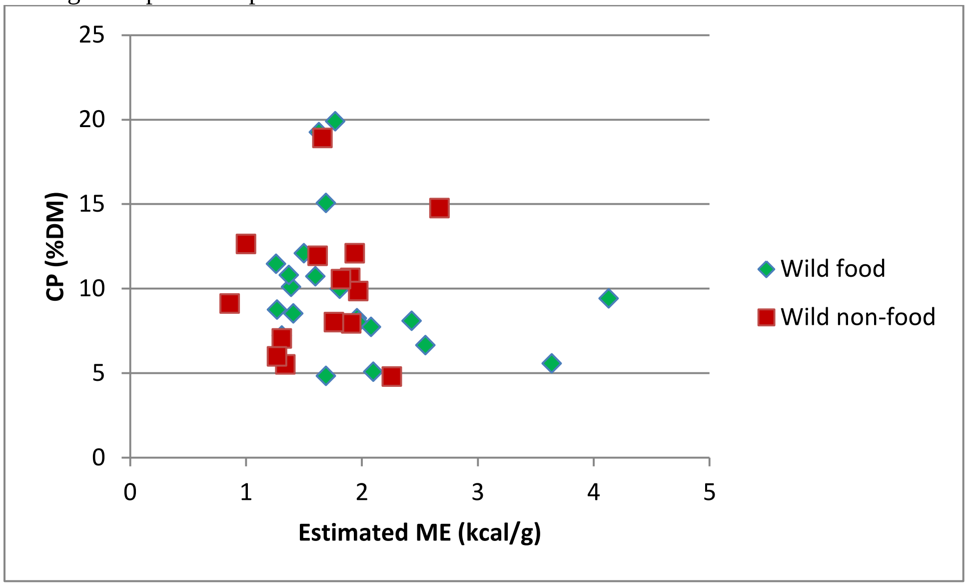


Figure 3.4: Acid hydrolyzed fat (AHF):estimated metabolizable energy (ME) ratios in Madagascan plant samples.

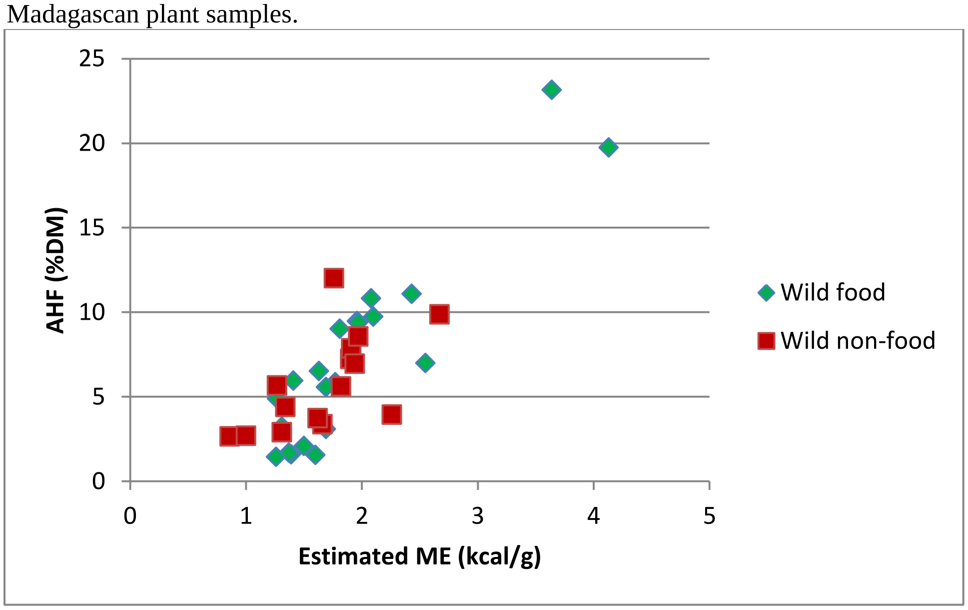


Figure 3.5: Nitrogen-free extract (NFE):estimated metabolizable energy (ME) in Madagascan plant samples.

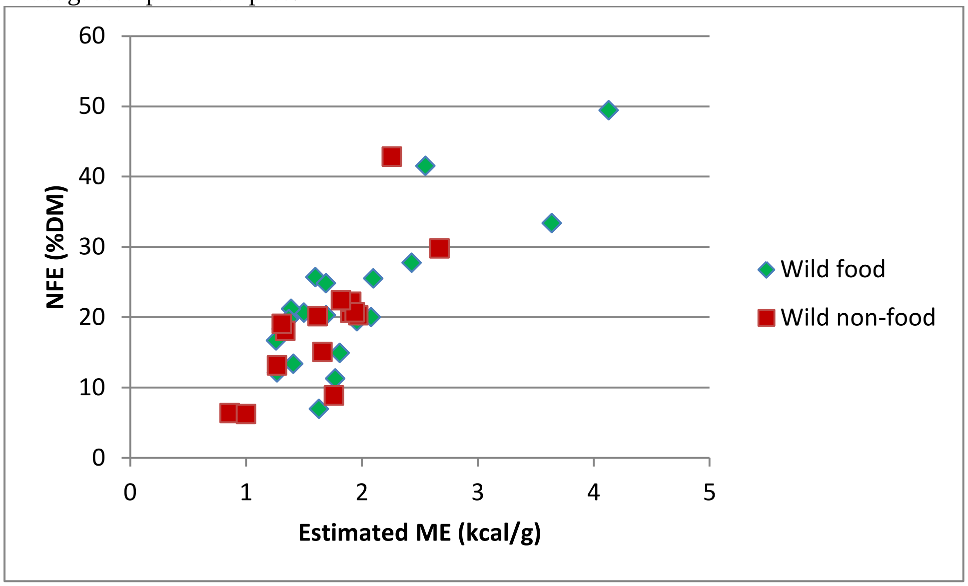


Figure 3.6: Total dietary fiber (TDF):estimated metabolizable energy (ME) in Madagascan plant samples.

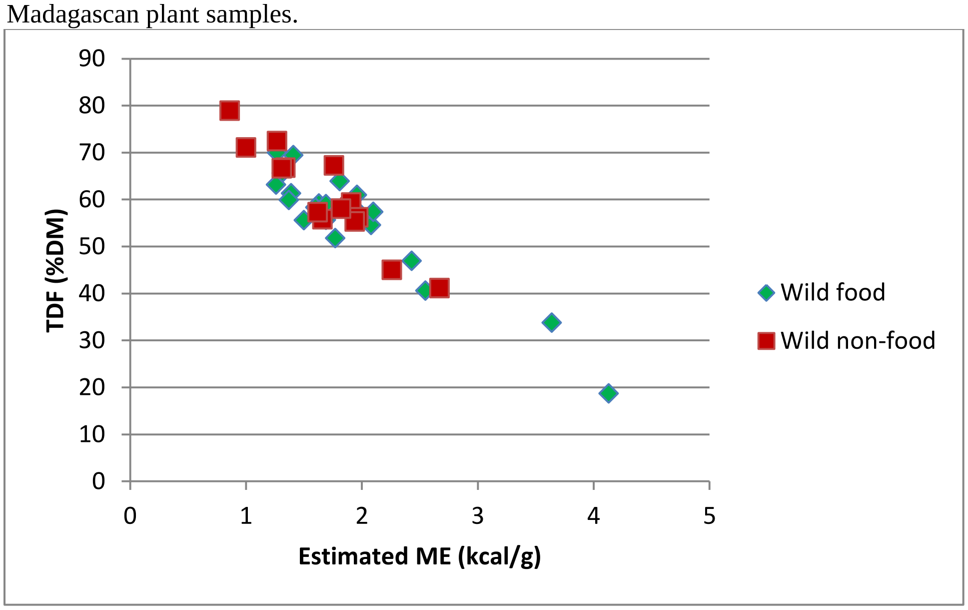
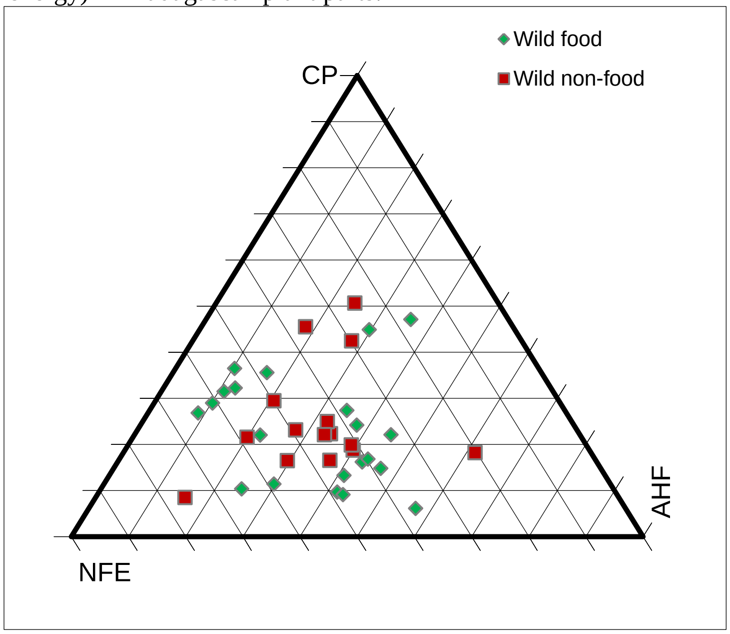


Figure 3.7: Ternary plot of crude protein (CP), acid hydrolyzed fat (AHF), and nitrogen-free extract (NFE) (as % metabolizable energy) in Madagascan plant parts.



Chapter 4

Survey of diets fed to captive black-and-white ruffed lemurs (*Varecia variegata*) in US zoological institutions

Abstract

Data on captive diets for black-and-white ruffed lemurs (*Varecia variegata*) are limited, especially as regards the specific food items used and their inclusion amounts. These factors, along with chemical composition of the diets, must be known in order to determine the effects of ingredient inclusion on health problems seen in captive lemurs, such as weight concerns and diabetes. Our objectives were to determine the ingredient composition and nutrient composition of diets for captive *V. variegata*. We used SurveyMonkey.com to create a survey and gather this information from 56 US zoological institutions registered with the International Species Information System (ISIS). The link for the survey was sent out via the Prosimian Taxon Advisory Group listserv and direct email to request participation in the study. Data from 33 institutions that responded to the survey are presented here. The most commonly included items were bananas (31 of 33 institutions) and apples (29 of 33 institutions). A majority of institutions fed either Marion Zoological's Leaf Eater biscuit (10 institutions), Mazuri's Leaf Eater biscuit (14 institutions), or Mazuri's Primate Browse biscuit (10 institutions). Browse was fed by 27 institutions, but 9 of those reported little to no consumption of browse offered. Nutritionist Pro™ was used to estimate the chemical composition of the captive diets, including dry matter (DM), organic matter (OM), crude protein (CP), fat, and total dietary fiber (TDF). Dry matter, OM, CP, fat, and TDF concentrations of captive diets ranged from 14.5% to 67.6%, 93.1% to 97.2% DM basis (DMB), 7.9% to 23.9% DMB, 2.0% to 6.5% DMB, and 10.1% to 28.1% DMB,

respectively. We also calculated nitrogen-free extract (NFE), a measure of digestible carbohydrate content, which ranged from 38.9% to 74.4% DMB. Compared to plant parts from Madagascar, captive diets were estimated to have higher CP and NFE, and lower fat and fiber concentrations. Reducing the amount of fruit included in captive diets for black-and-white ruffed lemurs would decrease digestible carbohydrate content and increase fiber content of these diets, which could reduce the prevalence of obesity and diabetes in captive *V. variegata*.

Introduction

Black-and-white ruffed lemurs (*Varecia variegata*) are frugivorous primates native to the island of Madagascar. Wild populations of *V. variegata* are considered Critically Endangered, with declining populations due to many anthropogenic stresses (IUCN, 2012). With more than 833 *V. variegata* housed in zoological institutions worldwide, and at least 58 US institutions keeping them (International Species Information System [ISIS], Eagan, MN), investigation of diets for captive *V. variegata* is needed. In order to ensure the health of captive populations, both as educational tools for zoo visitors and as potential population sources for reintroduction efforts, the chemical composition of diets being fed to them must be well characterized. Currently, few reports on captive diets for black-and-white ruffed lemurs are present in the literature (Brockman et al., 1987; Spelman et al., 1989; Crawford et al., 2009). There are no reports on the as-fed chemical composition of captive lemur diets, and little is known about the wild diets or nutrient requirements of lemurs, giving nutritionists little data to support nutrient recommendations specific to lemurs (NRC, 2003). Institutions housing lemurs are limited in the resources they can consult to develop diets. Existing studies have not examined diets across multiple institutions, nor have they determined the macronutrient content of the diets. Therefore, more data are needed on the dietary ingredients, and the concomitant nutrients, being fed to captive *V. variegata*.

Comparison of captive *V. variegata* diets to those consumed by their wild conspecifics and National Research Council (NRC) recommendations is also important, as it may lead to improvements in captive lemur health. While the chemical composition of diets consumed by wild lemurs is not necessarily representative of their nutritional requirements, it provides information about the diets that allow them to thrive. Additionally, obesity and diabetes are observed in captive animals, both of which are thought to be brought on by conditions encountered in captivity that are different from those encountered by wild conspecifics, such as diet alterations and reduced activity (Terranova & Coffman, 1997; Wagner et al., 2006; Kuhar et al., 2013). Data on the chemical composition of diets consumed by wild and captive *V. variegata* are limited (Brockman et al., 1987; Spelman et al., 1989; Crawford et al., 2009; Schmidt et al., 2010) so more research in both areas is necessary.

The first objective of this study was to determine the ingredient composition of diets fed to captive *V. variegata* in US zoological institutions. These diets were expected to vary by institution, based on the fact that individuals of different expertise (e.g., nutritionists, veterinarians, zoo keepers) and experience would be responsible for formulating them. Another objective of the study was to use nutritional analysis software to estimate the macronutrient composition of the captive diets.

Materials and Methods

Diet Survey: To determine what is being fed to *V. variegata* in US zoos, a survey was created using the website SurveyMonkey.com (SurveyMonkey.com, Palo Alto, CA). Survey questions were created to reflect descriptive elements of the diet and obviate any concerns related to psychometrics. Fifty-eight institutions housing *V. variegata* were registered with ISIS as of August 6, 2012. Because the Bronx Children's Zoo and the Bronx Zoo/Wildlife

Conservation Society (WCS) were listed as two separate institutions in the ISIS database, and the Tallahassee Museum of History and Natural Science had lemurs on loan from the Brevard Zoo that were returned at the end of September 2012, the actual number of possible survey respondents was 56. Of the 35 institutions that responded to the survey, 33 provided adequate information to be used for analysis: Akron Zoological Park, Audubon Nature Institute, Austin Savanna/Texas Disposal Systems Exotic Game Ranch, Baton Rouge Zoo, Binghamton Zoo, Brevard Zoo, Bronx Zoo/WCS, Capron Park Zoo, Cincinnati Zoo & Botanical Garden, Dallas Zoo, Detroit Zoological Society, Dickerson Park Zoo, Duke Lemur Center, Ellen Trout Zoo, Happy Hollow Zoo, Hattiesburg Zoo, Houston Zoo Inc., Little Rock Zoo, Louisville Zoo, Omaha's Henry Doorly Zoo and Aquarium, Peoria Zoo, Philadelphia Zoo, Pittsburgh Zoo & PPG Aquarium, Safari West, Saint Louis Zoo, San Francisco Zoo, Santa Ana Zoo at Prentice Park, Santa Barbara Zoo, Sedgwick County Zoo, Seneca Park Zoo, Topeka Zoo, Utah's Hogle Zoo, and Zoo Atlanta.

Respondents reported their institution name, approximate total diet weight (in grams) fed per lemur per day, food items, and the proportion of each item fed. Inclusion of items in the diet were reported on a percent weight basis, with ranges of 5 percentage units for items included at less than 30% of the diet (e.g., < 5%, 5% to 10%) and ranges of 10 percentage units for items included at greater than 30% of the diet (e.g., 30% to 40%, 40% to 50%). The Nutritionist Pro™ software package (Axxya Systems, Stafford, TX) then was used to estimate the chemical composition of the captive diets, including dry matter (DM), organic matter (OM), crude protein (CP), fat, and total dietary fiber (TDF).

Because survey data were reported in ranges of dietary percentages, captive diet estimates required converting ranges to single representative numbers. Allowing for a 5%

variance around 100% (i.e., from 95% to 105%), each diet was standardized according to a set of predetermined rules. After each rule was applied, if a balance remained outside of the allowable variance, the next rule was applied (part ‘a’ if the balance was > 105%, or part ‘b’ if the balance was < 95%) until the diet was within the acceptable range. This was achieved by first changing all values reported as < 5% to 1%, and all items reported as ≥5% to the mid-range value (e.g., 10% to 15% range becomes 12.5%). Then, either (a) items reported as ≥5% were rounded down to the bottom of their original range (e.g., 10% to 15% range becomes 10%), or (b) items reported as ≥5% were rounded up to the top of their original range (e.g., 10% to 15% range becomes 15%). Next, either (a) all items reported as <5% were decreased by 0.5% (i.e., all 1% values become 0.5%), or (b) all items reported as <5% were increased by 0.5% (i.e., all 1% values become 1.5%). Finally, any remaining balance was divided equally among all items reported as ≥ 5% of the diet, with the amount to be added to each item rounded to the nearest tenth of a percent. These percentages then were multiplied by the total diet weight to approximate a daily value (in grams) for each item fed. This also served to represent items in the diet that were fed on a rotation or on an occasional basis rather than fed daily.

Calculations and charts: Nitrogen-free extract (NFE), a representation of the digestible carbohydrate in a food, was calculated for captive diets as 100% of DM minus CP, fat, TDF, and ash. Estimated gross energy (GE) was calculated by multiplying CP by 5.65 kcal/g, fat by 9.5 kcal/g, TDF by 4.2 kcal/g, and NFE by 4.2 kcal/g. Estimated metabolizable energy (ME) was calculated using Atwater factors of 4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for NFE. Charts were created using Excel 2010 (Microsoft, Redmond, WA), with ternary plots utilizing the TRI-PLOT spreadsheet design from Graham and Midgley (2000).

Results

Estimated chemical composition data and energy content expressed on a DM basis (DMB) and on an as-fed basis and reported diet weights for captive black-and-white ruffed lemur diets are listed in Table 4.1. Dietary ingredients and summarized estimated chemical composition of all diets are presented in the Appendix, along with the guaranteed analysis, ingredients, and available proximate analysis data for the three most commonly fed biscuits. The as-fed weight of reported diets ranged from 132 g per animal • day^{-1} to 955 g per animal • day^{-1} (median = 517 g per animal • day^{-1}), with DM weights ranging from 45.3 g per animal • day^{-1} to 251.3 g per animal • day^{-1} (median = 169.8 g per animal • day^{-1}). Although all reported institutions included some type of commercially-produced complete diet product (i.e., chow) and 31 of the 33 respondents included biscuits in their diet formulas, the proportion of diet provided varied greatly. For instance, at Institution 17, Marion Zoological's Leaf Eater biscuits (Marion Zoological, Plymouth, MN) provided approximately 70% of as-fed diet weight (92.6% of diet, DMB). Similarly, inclusion of Mazuri's Leaf-Eater biscuits (PMI Nutrition International, St. Louis, MO) also was high, reported at up to 40% to 50% of the as-fed diet weight (83.8% of diet, DMB) at Institution 23. Neither of these biscuits was used by more than half of the respondents, however (n = 14 for Mazuri Leaf-Eater; n = 10 for Marion Zoological Leaf Eater). The most commonly offered diet items were bananas and apples, used by 31 and 29 out of the 33 respondents, respectively. Apples were the fruit items fed in the highest percentage, with a maximum inclusion of 40% of as-fed diet weight (18.6% of diet, DMB) at Institution 5. Sweet potatoes and carrots were the most commonly offered vegetables, used by 27 and 26 institutions out of the 33 respondents, respectively. These vegetables were included in the highest amounts, with up to 25% of each in the diet expressed on an as-fed basis. Carrots accounted for 14.7%

(DMB) of the diet at Institution 6. Sweet potatoes accounted for 15.8% (DMB) of the diet at Institution 25. By far, the most commonly offered item in the greens category was Romaine lettuce, which was included in the diets of 22 reported institutions. Romaine lettuce was used at the highest rate of any greens, at 25% to 30% of as-fed diet weight (4.3% of diet, DMB) at Institution 16. After Romaine lettuce, spinach and cabbage were the greens offered in the highest amounts, contributing up to 15% of as-fed diet weight in some diets. Cabbage made up 5.4% (DMB) of the diet at Institution 6. Spinach was offered at 2.8% (DMB) of the diet at Institution 11. Nearly one-third of reported institutions (n = 10) fed oranges to their collection of *V. variegata* in amounts up to 15% of the diet (3.6% to 7.3% of diet, DMB).

Of the reporting institutions, 17 indicated that they provided browse at less than 5% of the as-fed diet weight, 7 included it at 5% to 10% of the as-fed diet weight, and 3 offered it at 10% to 15% of the as-fed diet weight. The remaining 6 institutions could not quantify the amount of browse offered, as it was offered too infrequently, only offered as plants growing in the animals' enclosures, or not weighed separately. Approximately one quarter of the respondents (n = 9) specifically stated that browse was only offered as enrichment or that there was little to no consumption of browse by the lemurs.

Estimated median DM of captive diets was 34.4% (range = 14.5% to 67.6%; mean = 35.1%). Estimated median OM of captive diets was 94.4% (DMB) (range = 93.1% to 97.2%; mean = 94.5%). Estimated median CP concentration of captive diets was 17.0% (DMB) (range = 7.9% to 23.9%; mean = 16.0%), and was higher than the median CP concentration of fruits from the Analamazaotra Special Reserve (ASR) in Madagascar (8.01%, DMB). Estimated median dietary fat concentration was 4.7% (DMB) (range = 2.0% to 6.5%; mean = 4.4%), and was lower than median fat concentration of ASR fruits (7.86%, DMB). Estimated median TDF concentration was 20.0% (DMB) (range = 10.1% to 28.1%; mean = 19.8%), and was lower than the median TDF concentration of Madagascan fruits (58.04%, DMB). Median neutral detergent fiber (NDF) concentration in reported lemur chows was 20.6% (DMB) (range = 8.4% to 33.1%; mean = 20.9%). Median acid detergent fiber (ADF) concentration in reported lemur chows was 11.5% (DMB) (range = 2.8% to 21.9%; mean = 11.4%). Estimated median digestible carbohydrate content, represented by NFE, was 52.7% (DMB) (range = 38.9% to 74.4%; mean = 54.2%), and was higher than the median NFE concentration of fruits from ASR (22.39%, DMB). Median estimated GE was 4.5 kcal/g DM (range = 4.3 to 4.6 kcal/g DM; mean = 4.4 kcal/g/DM). Median estimated ME was 3.2 kcal/g DM (range = 2.9 to 3.7 kcal/g DM; mean = 3.2 kcal/g DM).

Figure 4.1 is a scatter plot chart of the CP:ADF ratio for reported lemur chows and the mean CP and ADF concentrations of plant parts consumed by wild *V. variegata* as reported in Schmidt et al. (2010). The CP:ADF ratio of the lemur chows placed them in a cluster that was outside of the mean CP:ADF ratios found in the wild plant parts.

Captive diets were plotted on a ternary chart of their relative proportions of CP, fat, and NFE for comparison to wild fruits from ASR, Madagascar, which were analyzed at the University of Illinois (Fig. 4.2). Unlike the analyzed Madagascan samples, the captive diets showed an obvious clustering, based mostly on their low fat content. Surprisingly, and perhaps more importantly, the captive diets showed no overlap with the Madagascan fruits on the ternary plot.

Discussion

Few studies have investigated the diets of captive *V. variegata* (Brockman et al., 1987; Spelman et al., 1989; Crawford et al., 2009). The studies available in the literature have not described the macronutrient content of the diets. Brockman et al. (1987) described the diet being fed at that time to *V. variegata* at the San Diego Zoo, but did not provide measurements of individual ingredients. Spelman et al. (1989) described signs of hemosiderosis in necropsies of captive lemurs of several species, and diet items with potentially high concentrations of tannins, ascorbic acid, or iron that could affect this disease. Crawford et al. (2009) was limited to concentrations of minerals and fat-soluble vitamins in diets of captive *V. variegata* at 20 institutions in the US. Optimization of husbandry practices for captive exotic animals is dependent on providing diets that meet the needs of the animals, and this requires understanding the chemical composition of captive diets. The nutrient composition of captive diets also must be known in order for institutions housing *V. variegata* to evaluate ingredient inclusion and any impacts on lemur health.

Captive diets reviewed in this study varied in ingredient composition, as was expected. Our assumption was that a standard set of nutrient recommendations would be used to formulate diets, though it would need to be from a group of primates other than lemurs, due to the lack of data on lemur nutrient requirements. However, the estimated chemical composition of captive diets varied to a greater degree than was anticipated, indicating that there was no agreement among institutions as to which primate nutrient requirements should be applied for *V. variegata*, and that diet ingredients were chosen based on the food preferences of the zoos. This supports the idea that there is a need to determine better estimates of the nutrient requirements of *V. variegata*, and lemurs in general, in order to improve their captive diets.

Crude protein concentrations in captive *V. variegata* diets fell mostly within the range of 15% to 22% (DMB) suggested by the NRC for adequate nutrition of post-weaning non-human primates. However, 12 of the reported diets had CP concentrations lower than the NRC's minimum recommendation, ranging from 7.9% to 13.7% (DMB). This could present a problem, especially during life stages with higher amino acid needs. Given the dilute nature of primate milk (Oftedal, 1992), lemur offspring nursing from dams fed diets with CP at the higher end of this range (i.e., 13.7%, DMB) may not experience amino acid deficiency, although mothers may have an increased risk of protein deficiency during gestation and lactation. Diets at the lower end of this CP range (i.e., 7.9%, DMB) are more likely to cause amino acid deficiency regardless of life stage, if the NRC's recommendations are relevant for lemurs. Protein deficiency in non-human primates has similar outcomes as it does in humans, meaning that nursing offspring would experience problems such as stunted growth and kwashiorkor. Protein-deficient adults would exhibit edema, weight loss, and slowed healing, among other possible signs.

Excessive protein also could present problems for lemurs. Riopelle et al. (1975) reported that rhesus macaques (*Macaca mulatta*) fed a diet with protein concentrations of 4 g/kg BW had smaller offspring, based on skeletal measurements, than those born to mothers fed 2 g/kg BW, although no differences in fetal mortality were observed. Twenty-five of the 33 reported institutions in our study fed protein in excess of 4 g/kg BW based on the ideal weight of captive *V. variegata* (3,600 g) from Terranova and Coffman (1997), with two institutions feeding protein at approximately three times this amount. This may be negatively affecting the size of any offspring born in captivity. Continually producing small offspring in captivity could also impact reintroduction programs through effects on adult size, or if the reproductive canals of captive female *V. variegata* adapt to birthing smaller offspring.

The NRC's minimal dietary fiber recommendation to promote gastrointestinal health in post-weaning lemurs is 20% NDF and 10% ADF, but with no distinction made for species having different feeding strategies (e.g., folivore, frugivore, etc.) (NRC, 2003). Neither NDF nor

ADF data were available from the software package used for analysis of captive diets; therefore, a direct comparison of captive diets to this recommendation data was not possible. However, if we assume that the TDF concentration is similar to or higher than the NDF concentration of these diets, then more than half (n = 17) of the reported diets failed to meet this minimum recommendation. The short gastrointestinal transit time of *V. variegata* (1.5 to 3.9 h) and their relatively simple gastrointestinal tracts mean that they are unlikely to utilize the less fermentable substrates from consumed plants, though hemicelluloses and non-structural carbohydrates appear to be fermented to some degree (Cabre-Vert & Feistner, 1995; Edwards & Ullrey, 1999; Campbell et al., 2004). Because TDF is a more accurate measure of substrates available to *V. variegata* for fermentation, captive lemur diet analysis in the future should include this method of fiber analysis.

Schmidt et al. (2010) analyzed plant samples from Madagascar in their study of *V. variegata* diets in the Betampona Natural Reserve (BNR). In that study, the authors analyzed fruits (n = 84), leaves (n = 34), and flowers (n = 4) observed to be eaten by *V. variegata* from December, 1999 to December, 2000. Fruit makes up the largest part of the diet of wild *V. variegata*, making up approximately 92% of their diet in BNR (Britt, 2000), but it is worth examining the composition of each of the plant parts analyzed, as all were consumed by *V. variegata* during the study period. Mean CP concentrations were 7.1% (DMB) for fruits, 15.2% (DMB) for leaves, and 7.9% (DMB) for flowers, all of which were lower than the mean CP concentration for diets reported in our study. Conversely, mean fat concentrations reported in BNR (11.2%, DMB, for fruits, 6.0%, DMB, for leaves, and 8.7%, DMB, for flowers) were all higher than the mean fat concentration of diets in our study, with Madagascan flowers having nearly twice as much fat as captive diets and Madagascan fruits having almost three times as much fat compared to captive diets. Neutral detergent fiber data for captive diets were not provided by Nutritionist Pro™, but relying on the previous assumption that TDF should be higher than NDF, we can compare data from these two assays. In samples from Schmidt et al. (2010), mean NDF concentrations were 39.6% (DMB) for fruits, 48.4% (DMB) for leaves, and 44.2% (DMB) for flowers. This is more than twice as high as the mean TDF concentration of captive diets, which means that captive black-and-white ruffed lemurs are consuming much less fiber than their wild conspecifics. Data on ADF concentrations in lemur chows listed in respondent diets were obtained from zoo nutritionists and(or) the manufacturers of the lemur chows. Mean ADF concentrations from Schmidt et al. (2010) were 30.1% (DMB) for fruits, 32.4% (DMB) for leaves, and 31.7% (DMB) for flowers, nearly three times the mean ADF concentration of lemur chows reported in our study. Comparisons could not be made to complete diets, as only detergent fiber assays were used by Schmidt et al. (2010). Reformulating captive diets to simulate the chemical composition of wild plant parts would require increasing the fiber and fat contents of captive diets, which are changes suggested to manage diabetes in captive lemurs (Junge et al., 2009).

Captive diets also were compared to the fruits from ASR that were analyzed in Chapter 3. The median CP concentration of captive diets was more than twice that of the analyzed fruits, whereas the median fat concentration of captive diets was nearly half what was seen in the Madagascan fruits. The median TDF concentration in the wild fruits was nearly 3-fold that of the captive diets, and only one Madagascan fruit with a very low TDF concentration (< 19%) fell within the range of TDF concentrations for captive diets. The median concentration of NFE in wild fruits was less than half that of reported captive diets. The median ME content of captive diets was 50% higher than that of analyzed wild fruits, with all of the reported institutions feeding diets that had greater ME than the median ME found in Madagascan fruits. All reported institutions fed diets with higher ME than most (n = 13) of the Madagascan fruits, and only the two fruits with high fat content (> 19%) had ME within the range of captive diets. Fruits sampled from ASR and those from Schmidt et al. (2010) both had lower CP concentrations, higher fat concentrations, and higher fiber concentrations than were found in captive diets, despite the fact that the Schmidt samples were collected in a separate reserve. This indicates that wild *V. variegata* are encountering potential dietary items of similar nutrient concentrations, regardless of their location in Madagascar, supporting the idea that diet is a contributing factor to the differences seen in captive versus wild *V. variegata* (e.g., obesity and diabetes), and that altering captive diets to resemble wild diets could potentially alleviate these problems.

Captive diets and analyzed Madagascan fruits from ASR were plotted as their relative proportions of CP, fat, and NFE on a ternary plot (Fig. 4.2), which revealed that captive diets form a cluster on the chart that is almost entirely outside of the area represented by wild samples. This indicates that lemurs consuming the reported captive diets would not be able to achieve the same macronutrient pattern as lemurs in ASR. Current literature does not provide adequate data to examine this relationship, because NFE for the captive diets had to be based on TDF data. There is also insufficient data to determine if this relationship has any relevance to lemur nutritional and health status. It would be valuable to analyze more Madagascan plants, especially those consumed by wild *V. variegata*, to determine what macronutrient pattern is normally consumed in the wild. These data could then be compared to diets eaten by *V. variegata* in captivity to determine if any wild diets share the same macronutrient proportions as captive diets.

The markedly lower fat and fiber concentrations of captive diets as compared to wild plants of Madagascar are reflective of the chemical composition of domestic fruits. The fruits fed to captive *V. variegata* are obviously not the same as the fruits of Madagascar, and the classification of these lemurs as frugivores in the wild should not lead to the conclusion that fruits preferred by humans must be included in the captive diet. It may be best to restrict fruits to use as enrichment items and formulate diets that are closer in chemical composition to Madagscan plants, which also will reduce the amount of simple sugars in captive diets. Simple sugar content must also be considered in the formulation of lemur chows, as carboydrates must be included in extruded diets to hold the product together. However, given that chows used by reporting institutions had starch content as low as 7.0% (Mazuri Primate L/S Banana, PMI Nutrition International, St. Louis, MO), it should be possible to make a product that fits in a low NFE diet. Excess calories combined with a lower energy expenditure in captivity will lead to deposition of adipose tissue, leading to obesity as observed in captive lemurs (Terranova & Coffman, 1997), which can then contribute to development of diabetes. Zoos housing *V. variegata* should evaluate the fat and simple sugar content of their diets, and consider reducing ME content if obesity is an issue in their collection.

None of the chows listed were formulated specifically for lemurs, with some intended for monkeys and others for folivorous primates. A positive relationship exists between the CP:ADF ratio and the biomass of some folivorous primates within a forest (Oates et al., 1990; Chapman et al., 2002). It may be that the CP:ADF ratio is even more important in food selection by *V. variegata*, because ADF would have a greater antinutritional impact for frugivores than it would for folivores. Specifically, it should be noted that *V. variegata* have been shown to lose weight on a diet containing 30% ADF, though no weight loss was observed on diets containing 15% ADF (Edwards & Ullrey, 1999). Acid detergent fiber represents the insoluble and mostly non-fermentable cell wall components, cellulose and lignin, which would not be well utilized in the gastrointestinal tract of *V. variegata*. Frugivores have little fermentative capacity, and ADF would be expected to make digesta move faster through the gut and limit time for absorption of nutrients from other, more digestible components of the diet. The CP:ADF ratios of lemur chows in captive diets all lie outside the mean CP:ADF ratios of plant parts consumed by wild *V. variegata*, with lower CP and higher ADF found in the Madagascan plant parts (Fig. 4.1). The higher ADF content of Madagscan plant parts contradicts the expectation for frugivores to consume low fiber diets, and these plant parts have higher mean ADF concentrations than the diets that led to weight loss when fed by Edwards & Ullrey (1999). It may be that some other aspect of the diet, such as greater digestibility of wild plant parts or a more concentrated source of energy in the form of the higher fat content of wild plant parts, allows wild *V. variegata* to thrive on diets with higher ADF concentrations than can be used in captivity. This supports the need for further analysis of fruits and leaves consumed specifically by wild *V. variegata* in order to understand how the chemical composition of their diets compares to the diets of captive conspecifics, as well as a need for determination of the digestibility of both wild and captive diets. In addition, characterizing and controlling the dietary fiber may be easier if lemur chow is used as the majority of the diet, allowing use of a fiber blend formulated for optimal health and nutrition of *V. variegata*.

Additional research is needed to better comprehend the nutritional requirements of wild and captive lemurs. Future studies on nutritional requirements should focus on a particular lemur species in order to provide the most relevant data. While data from this study cannot be used to determine the nutrient requirements of *V. variegata*, they can be used to identify differences between wild and captive diets that may lead to improvements in diets for captive *V. variegata* that will reduce the incidence of negative health outcomes like obesity and diabetes. Data from this study also can be used in assessing the influence of diet on health in captive *V. variegata* if institutions are willing to share medical data on their collections. A study with the specific aim of comparing health outcomes of *V. variegata* at institutions across the US and correlating those data with the chemical composition of the diets fed at each institution would be especially beneficial. In the meantime, reformulation of diets for captive *V. variegata* to mimic wild diet items may decrease the prevalence of obesity and diabetes in the captive population.

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Tables and figures

Table 4.1: Estimated chemical composition, estimated gross energy (GE), and estimated metabolizable energy (ME) of captive diets fed to black-and-white ruffed lemurs, *Varecia variegata*, in US zoological institutions.

				Dry matter basis (DMB)							As-is basis				
Institution number	Reported weight,[1] g	DMB weight, g	DM,[2] %	OM, %	CP, %	Fat, %	TDF, %	NFE, %	GE, kcal/g	ME, kcal/g	CP, %	Fat, %	TDF, %	NFE, %	ME, kcal/g
1	595	213	37.6	94.6	15.2	4.7	19.6	55.1	4.44	3.24	5.7	1.8	7.4	20.7	1.22
2	955	249	26.1	95.3	12.4	3.1	15.7	64.1	4.35	3.34	3.2	0.8	4.1	16.7	0.87
3	855	212	24.9	95.3	13.1	3.0	20.2	59.0	4.36	3.16	3.3	0.8	5.0	14.7	0.79
4	200	76	38.3	95.2	17.0	5.0	10.1	63.1	4.51	3.65	6.5	1.9	3.9	24.2	1.40
5	925	251	28.0	95.7	9.1	3.4	17.4	65.8	4.33	3.30	2.6	0.9	4.9	18.4	0.92
6	570	102	18.7	93.5	16.8	4.1	19.9	52.7	4.38	3.15	3.1	0.8	3.7	9.9	0.59
7	521	152	29.4	94.0	17.8	4.5	23.4	48.3	4.45	3.05	5.2	1.3	6.9	14.2	0.90
8	460	186	41.5	94.0	19.2	5.3	22.5	46.9	4.51	3.12	8.0	2.2	9.3	19.5	1.30
9	510	223	43.6	93.8	19.3	5.6	18.7	50.2	4.51	3.28	8.4	2.4	8.2	21.9	1.43
10	550	189	34.0	94.1	17.4	4.6	19.3	52.7	4.45	3.23	5.9	1.6	6.6	17.9	1.10
11	553	170	32.4	93.5	18.8	4.8	25.4	44.5	4.45	2.96	6.1	1.6	8.2	14.4	0.96
12[3]	514	191	39.1	95.0	13.5	4.8	16.8	60.0	4.44	3.37	5.3	1.9	6.6	23.5	1.32
13	395	75	19.0	95.7	8.2	2.0	13.0	72.5	4.25	3.41	1.6	0.4	2.5	13.8	0.65
14	400	91	22.8	93.5	17.8	4.6	20.7	50.4	4.43	3.14	4.1	1.0	4.7	11.5	0.72
15	800	116	14.5	94.8	11.7	3.1	15.6	64.4	4.31	3.32	1.7	0.4	2.3	9.3	0.48
16	132	45	35.4	94.3	17.3	5.0	28.1	43.9	4.48	2.90	6.1	1.8	10.0	15.5	1.03
17	205	133	66.4	93.3	23.9	6.5	21.8	41.1	4.61	3.19	15.9	4.3	14.5	27.3	2.12
18	470	162	34.4	94.2	17.1	5.1	21.1	51.0	4.48	3.18	5.9	1.8	7.2	17.5	1.09
19	340	90	26.4	94.7	13.7	3.5	20.0	57.5	4.36	3.16	3.6	0.9	5.3	15.2	0.83
20	596	233	39.1	94.3	18.8	4.6	22.5	48.4	4.48	3.10	7.4	1.8	8.8	19.0	1.21
21	375	158	43.1	94.6	18.8	5.3	14.5	56.0	4.53	3.47	8.1	2.3	6.3	24.1	1.50

Table 4.1 (cont.)

Institution number	Reported weight, g	DMB weight, g	DM, %	Dry matter basis OM, %	CP, %	Fat, %	TDF, %	NFE, %	GE, kcal/g	ME, kcal/g	As-is basis CP, %	Fat, %	TDF, %	NFE, %	ME, kcal/g
22	517	186	36.6	94.4	16.0	4.1	17.5	56.8	4.42	3.28	5.9	1.5	6.4	20.8	1.22
23	312	151	50.6	93.5	20.9	5.5	26.7	40.3	4.52	2.94	10.6	2.8	13.5	20.4	1.49
24	530	172	31.9	94.1	18.0	5.2	20.2	50.6	4.49	3.22	5.8	1.7	6.4	16.1	1.02
25	524	170	33.3	95.2	12.4	3.1	19.3	60.4	4.34	3.19	4.1	1.0	6.4	20.1	1.06
26	340	75	22.3	95.0	12.3	3.3	16.9	62.5	4.35	3.29	2.7	0.7	3.8	14.0	0.74
27	540	186	34.5	94.8	13.7	3.7	21.9	55.5	4.37	3.10	4.7	1.3	7.6	19.1	1.07
28	565	208	37.2	93.8	18.9	5.1	23.0	46.8	4.48	3.08	7.0	1.9	8.6	17.4	1.15
29	667	197	29.6	94.7	17.0	4.3	22.4	51.0	4.45	3.11	5.0	1.3	6.6	15.1	0.92
30	200	91	45.4	95.1	13.7	5.1	16.5	59.9	4.46	3.40	6.2	2.3	7.5	27.2	1.54
31	175	59	33.8	97.2	7.9	2.2	12.7	74.4	4.31	3.49	2.7	0.7	4.3	25.2	1.18
32	190	129	67.6	93.1	23.2	6.2	24.9	38.9	4.57	3.04	15.7	4.2	16.8	26.3	2.05
33	555	231	40.5	93.7	16.9	6.0	26.7	44.1	4.50	2.98	6.9	2.4	10.8	17.9	1.21

[1]Reported weight is amount fed per animal per day, as-fed.
[2]DM = dry matter, OM = organic matter, CP = crude protein, TDF = total dietary fiber, NFE = nitrogen-free extract.
[3]Institution 12 did not report an as-fed diet weight. The as-fed diet weight used here is the median of all other respondents' as-fed diet weights.

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Figure 4.1: Ratios of crude protein (CP):acid detergent fiber (ADF) in commercial lemur chows offered to *Varecia variegata* in captivity and wild lemur diet items (Schmidt et al., 2010).

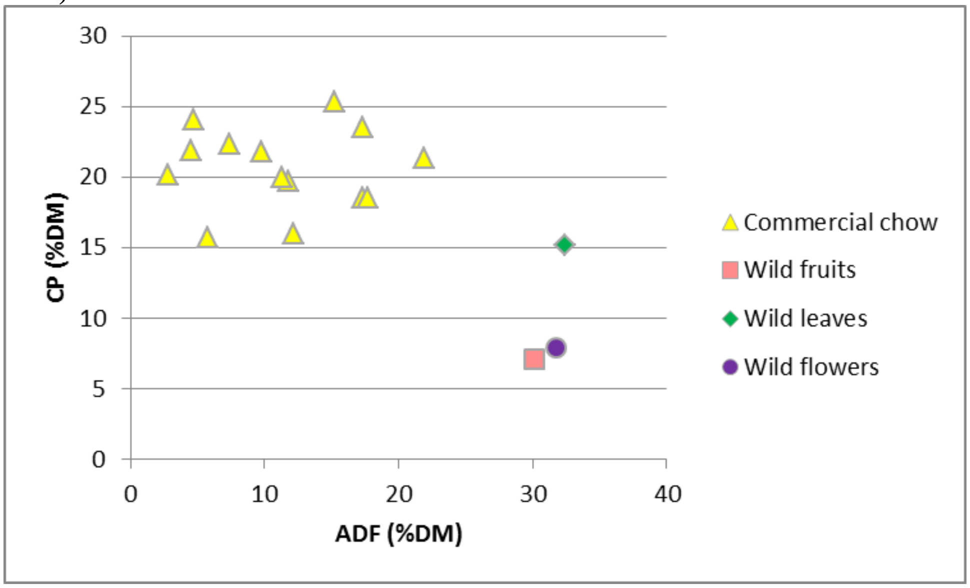
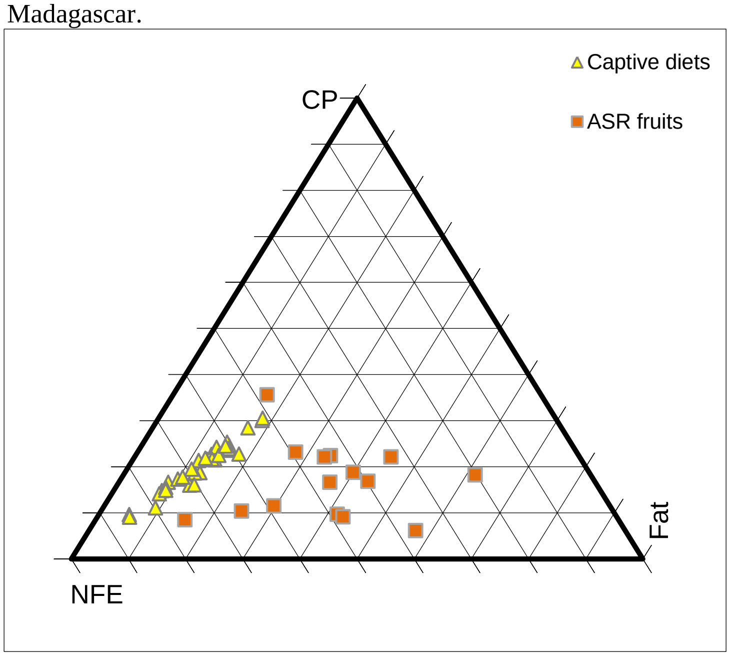


Figure 4.2: Ternary plot of crude protein (CP), fat, and nitrogen-free extract (NFE) (as % metabolizable energy) in complete captive diets and fruits from Analamazaotra Special Reserve, Madagascar.



Appendix

Dietary ingredient and estimated chemical composition of diets fed to *Varecia variegata* in US zoological institutions

Institution 1

Ingredient	% (as-is)
Fruit mix (apple, banana, honeydew melon, watermelon, mango, peach, plum, papaya, cherries, grapes, pear, cantaloupe)[1]	42.15
Vegetable mix (acorn squash, beets, broccoli, carrots, tomatoes, zucchini, cauliflower, cucumber, bell pepper, potato, yam, green beans, peas, corn)[2]	15.82
Mazuri Primate Browse Biscuit[3]	15.76
LabDiet Monkey Diet 5038[4]	15.76
Greens mix (cabbage, kale, mustard greens, spinach, Romaine lettuce, collard greens, turnip greens)[5]	10.50
Calculated composition	
Dry matter, %	37.56
	% of dry matter
Organic matter	94.58
Crude protein	15.16
Fat	4.73
Nitrogen-free extract	55.07
Total dietary fiber	19.62

[1]A combination of at least 3 of these fruits is fed daily. Types and amounts of fruit vary, but total percentage is constant.
[2]A combination of at least 3 of these vegetables is fed daily. Types and amounts of vegetable vary, but total percentage is constant.
[3]PMI Nutrition International, St. Louis, MO.
[4]PMI Nutrition International, St. Louis, MO.
[5]A combination of at least 3 of these greens is fed daily. Types and amounts of greens vary, but total percentage is constant.

Institution 2

Ingredient	% (as-is)
Banana	24.99
Apple, Medium	15.00
Yam, Baked or Boiled, Drained	15.00
Orange	9.99
Broccoli	9.99
Mazuri Primate Growth & Repro Biscuit[1]	9.99
Kale	5.00
Spinach, Chopped, Boiled, Drained	2.50
Lettuce, Romaine, Shredded	2.50
Papaya	0.39
Pear	0.39
Peach	0.39
Strawberries	0.39
Mango	0.39
Kiwi Fruit, Green	0.39
Plum	0.39
Cantaloupe	0.39
Honeydew Melon	0.39
Watermelon	0.39
Blueberries	0.39
Blackberries	0.39
Raspberries	0.39
Calculated composition	
Dry matter, %	26.09
	% of dry matter
Organic matter	95.34
Crude protein	12.39
Fat	3.15
Nitrogen-free extract	64.09
Total dietary fiber	15.73

[1]PMI Nutrition International, St. Louis, MO.

Institution 3

Ingredient	% (as-is)
Vegetables, Mixed, Frozen	23.85
Apple, Medium	18.83
Banana	18.83
Carrots	13.83
Potatoes, Sweet	13.83
Mazuri Leaf-Eater Biscuit[1]	8.81
Mango	0.50
Papaya	0.50
Pear	0.50
Pineapple	0.13
Raisins, Seedless	0.13
Grapes, Red or Green	0.13
Cranberries, Dried, Sweetened	0.13
Calculated composition	
Dry matter, %	24.89
	% of dry matter
Organic matter	95.34
Crude protein	13.14
Fat	3.04
Nitrogen-free extract	58.99
Total dietary fiber	20.16

[1]PMI Nutrition International, St. Louis, MO.

Institution 4

Ingredient	% (as-is)
ZuPreem Primate Diet - Dry[1]	28.51
Banana	18.47
Figs	6.73
Grapes, Red or Green	6.73
Apple, Medium	3.41
Pear	3.41
Plum	3.41
Peach	3.41
Watermelon	3.41
Broccoli	3.41
Carrots	3.41
Cabbage, Shredded	3.41
Lettuce, Romaine, Shredded	3.41
Green Beans	3.41
Potatoes, Sweet	3.41
Celery, Stalk	0.50
Beets	0.50
Corn, Yellow, Sweet, On the Cob	0.50
Peas, Green	0.50
Calculated composition	
Dry matter, %	38.31
	% of dry matter
Organic matter	95.16
Crude protein	17.00
Fat	5.01
Nitrogen-free extract	63.08
Total dietary fiber	10.06

[1]Premium Nutritional Products, Inc., Overland Park, KS.

Institution 5

Ingredient	% (as-is)
Apple, Medium	36.08
Banana	28.35
Potatoes, Sweet	12.88
Mazuri Primate Maintenance Biscuit[1]	12.88
Carrots	7.73
Grapes, Red or Green	1.04
Lettuce, Romaine, Shredded	1.04
Calculated composition	
Dry matter, %	28.01
	% of dry matter
Organic matter	95.70
Crude protein	9.11
Fat	3.38
Nitrogen-free extract	65.76
Total dietary fiber	17.45

[1]PMI Nutrition International, St. Louis, MO.

Institution 6

Ingredient	% (as-is)
Carrots	23.43
Cabbage, Shredded	13.02
Potatoes, Sweet	13.02
Squash, Summer, All Varieties	13.02
Celery, Stalk	7.82
Broccoli	7.82
Corn, Yellow, Sweet, On the Cob	7.82
Marion Zoological Leaf Eater Biscuit[1]	7.82
Apple, Medium	1.04
Banana	1.04
Mango	1.04
Papaya	1.04
Honeydew Melon	1.04
Grapes, Red or Green	1.04
Calculated composition	
Dry matter, %	18.71
	% of dry matter
Organic matter	93.46
Crude protein	16.78
Fat	4.07
Nitrogen-free extract	52.71
Total dietary fiber	19.90

[1]Marion Zoological, Plymouth, MN.

Institution 7

Ingredient	% (as-is)
Mazuri Leaf-Eater Biscuit[1]	20.20
Squash, Summer, Zucchini	15.16
Banana	10.10
Potatoes, Sweet	10.10
Orange	5.06
Apple, Medium	5.06
Lettuce, Romaine, Shredded	5.06
Grapes, Red or Green	4.05
Watermelon	1.01
Blueberries	1.01
Honeydew Melon	1.01
Mango	1.01
Papaya	1.01
Peach	1.01
Pear	1.01
Plum	1.01
Raspberries	1.01
Strawberries	1.01
Blackberries	1.01
Broccoli	1.01
Carrots	1.01
Celery, Stalk	1.01
Onions, Chopped	1.01
Pumpkin	1.01
Tomatoes, Red	1.01
Potatoes, White, Flesh and Skin	1.01
Cantaloupe	1.01
Kale	1.01
Kiwi Fruit, Green	1.01
Corn, Yellow, Sweet, On the Cob	1.01
Green Beans	1.01
Peas, Green	1.01
Turnips	1.01
Calculated composition	
Dry matter, %	29.43
	% of dry matter
Organic matter	94.01
Crude protein	17.78
Fat	4.54
Nitrogen-free extract	48.28

Institution 7 (cont.)

Total dietary fiber	23.41

[1]PMI Nutrition International, St. Louis, MO.

Institution 8

Ingredient	% (as-is)
Apple, Medium	20.50
Marion Zoological Leaf Eater Biscuit[1]	20.50
Mazuri Primate Browse Biscuit[2]	15.38
Blueberries	10.25
Carrots	10.25
Orange	1.54
Banana	1.54
Tomatoes, Red	1.54
Celery, Stalk	1.54
Broccoli	1.54
Potatoes, White, Flesh and Skin	1.54
Lettuce, Looseleaf	1.54
Lettuce, Romaine, Shredded	1.54
Cabbage, Shredded	1.54
Kale	1.54
Corn, Yellow, Sweet, On the Cob	1.54
Green Beans	1.54
Spinach, Chopped, Raw	1.54
Grapes, Red or Green	0.78
Grapefruit, Pink or Red	0.78
Beets	0.78
Yam	0.78
Calculated composition	
Dry matter, %	41.47
	% of dry matter
Organic matter	93.98
Crude protein	19.22
Fat	5.32
Nitrogen-free extract	46.92
Total dietary fiber	22.52

[1]Marion Zoological, Plymouth, MN.
[2]PMI Nutrition International, St. Louis, MO.

Institution 9

Ingredient	% (as-is)
Mazuri Leaf-Eater Biscuit[1]	11.78
LabDiet Monkey Diet[2]	11.78
ZuPreem Primate Diet - Dry[3]	11.78
ZuPreem Primate Diet - Canned[4]	11.78
Apple, Medium	7.18
Banana	7.18
Broccoli	2.19
Carrots	2.19
Cauliflower, Chopped	2.19
Celery, Stalk	2.19
Pumpkin	2.19
Squash, Summer, Zucchini	2.19
Lettuce, Looseleaf	2.19
Kale	2.19
Mustard Greens	2.19
Lettuce, Romaine, Shredded	2.19
Endive or Escarole, Chopped	2.19
Green Beans	2.19
Potatoes, Sweet	2.19
Squash, Summer, All Varieties	2.19
Spinach, Chopped, Raw	2.19
Blueberries	0.51
Honeydew Melon	0.51
Papaya	0.51
Pear	0.51
Peach	0.51
Plum	0.51
Raspberries	0.51
Watermelon	0.51
Cantaloupe	0.51
Kiwi Fruit, Green	0.51
Blackberries	0.14
Cherries, Sweet	0.14
Figs	0.14
Fruit Cocktail, Canned in Juice	0.14
Calculated composition	
Dry matter, %	43.65
	% of dry matter
Organic matter	93.78
Crude protein	19.32

Institution 9 (cont.)

Fat	5.57
Nitrogen-free extract	50.20
Total dietary fiber	18.69

[1]PMI Nutrition International, St. Louis, MO.
[2]PMI Nutrition International, St. Louis, MO.
[3]Premium Nutritional Products, Inc., Overland Park, KS.
[4]Premium Nutritional Products, Inc., Overland Park, KS.

Institution 10

Ingredient	% (as-is)
Vegetables (broccoli, brussels sprouts, carrots, cauliflower, corn, cucumber, eggplant, mushrooms, bell pepper, sweet potato, potato, radish, zucchini, tomato, turnips)[1]	33.83
Banana	18.85
Marion Zoological Leaf Eater Biscuit[2]	18.85
Additional fruit (apricots, blueberries, cantaloupe, kiwi, mango, papaya, peach, pear, plum, pomegranate, prunes, raspberries, strawberries, watermelon)[3]	17.99
Greens (collards, dandelion greens, Romaine lettuce, mustard greens, spinach, turnip greens)[4]	7.49
Mazuri Primate Browse Biscuit[5]	1.50
Mazuri Primate Maintenance Biscuit[6]	1.50
Calculated composition	
Dry matter, %	34.03
	% of dry matter
Organic matter	94.12
Crude protein	17.44
Fat	4.65
Nitrogen-free extract	52.74
Total dietary fiber	19.28

[1]Equal parts of 5 different vegetables daily. Sweet potato fed most often.
[2] Marion Zoological, Plymouth, MN.
[3]Equal parts of 3 different fruits daily.
[4]One type offered per day, rotated daily.
[5]PMI Nutrition International, St. Louis, MO.
[6]PMI Nutrition International, St. Louis, MO.

Institution 11

Ingredient	% (as-is)
Mazuri Leaf-Eater Biscuit[1]	15.80
Spinach, Chopped, Raw	10.54
Mazuri Primate Browse Biscuit[2]	10.54
Apple, Medium	5.26
Banana	5.26
Orange	5.26
Pear	5.26
Papaya	5.26
Green Beans, Boiled, Drained	5.26
Celery, Stalk	5.26
Squash, Summer, All Varieties, Sliced, Boiled, Drained	5.26
Broccoli, Chopped, Boiled, Drained	5.26
Carrots, Sliced, Boiled, Drained	5.26
Potatoes, Sweet, Boiled, Mashed	5.26
Lettuce, Butterhead (Boston or Bibb)	1.75
Watercress	1.75
Lettuce, Red Leaf	1.75
Calculated composition	
Dry matter, %	32.36
	% of dry matter
Organic matter	93.47
Crude protein	18.75
Fat	4.82
Nitrogen-free extract	44.47
Total dietary fiber	25.43

[1]PMI Nutrition International, St. Louis, MO.
[2]PMI Nutrition International, St. Louis, MO.

Institution 12[1]

Ingredient	% (as-is)
LabDiet Monkey Diet 5038[2,3]	31.61
Apple, Medium	10.61
Banana	10.61
Orange	10.61
Carrots	10.61
Lettuce, Romaine, Shredded[3]	10.61
Potatoes, Sweet	10.61
Corn, Yellow, Sweet, On the Cob	1.57
Green Beans	1.57
Peas, Green	1.57
Calculated composition	
Dry matter, %	39.14
	% of dry matter
Organic matter	94.98
Crude protein	13.47
Fat	4.77
Nitrogen-free extract	59.99
Total dietary fiber	16.76

[1]No as-fed diet weight was reported by Institution 12. The as-fed diet weight used for calculations was the median of all other respondents' as-fed diet weights.
[2]PMI Nutrition International, St. Louis, MO.
[3]Not fed on Thursdays.

Institution 13

Ingredient	% (as-is)
Banana	17.93
Grapes, Red or Green	12.93
Kale	12.93
Orange	12.93
Papaya	12.93
Pear	12.93
Potatoes, Sweet	12.93
Apple, Medium	1.50
Lettuce, Iceberg	1.50
Mazuri Geriatric Gel Diet[1]	1.50
Calculated composition	
Dry matter, %	18.97
	% of dry matter
Organic matter	95.71
Crude protein	8.21
Fat	2.02
Nitrogen-free extract	72.50
Total dietary fiber	12.99

[1]PMI Nutrition International, St. Louis, MO.

Institution 14

Ingredient	% (as-is)
Endive or Escarole, Chopped	13.37
Marion Zoological Leaf Eater Biscuit[1]	13.37
Pineapple	8.92
Apple, Medium	8.34
Orange	8.34
Carrots	8.34
Squash, Summer, Zucchini	8.34
Cantaloupe	8.34
Lettuce, Romaine, Shredded	8.34
Potatoes, Sweet	8.34
Grapes, Red or Green	4.45
Spinach, Chopped, Boiled, Drained	0.50
Corn, Yellow, Sweet, On the Cob	0.50
Mazuri Primate Browse Biscuit[2]	0.50
Calculated composition	
Dry matter, %	22.77
	% of dry matter
Organic matter	93.45
Crude protein	17.82
Fat	4.60
Nitrogen-free extract	50.38
Total dietary fiber	20.65

[1]Marion Zoological, Plymouth, MN.
[2]PMI Nutrition International, St. Louis, MO.

Institution 15

Ingredient	% (as-is)
Watermelon	11.50
Banana	6.50
Orange	6.50
Honeydew Melon	6.50
Pepper, Bell or Sweet, Green	6.50
Carrots	6.50
Pumpkin	6.50
Squash, Summer, Zucchini	6.50
Lettuce, Romaine, Shredded	6.50
Cantaloupe	6.50
Corn, Yellow, Sweet, On the Cob	6.50
Potatoes, Sweet	6.50
Squash, Summer, All Varieties	6.50
Kiwi Fruit, Green	6.50
Mazuri Leaf-Eater Biscuit[1]	1.50
Mazuri Primate Growth & Repro Biscuit[2]	0.75
LabDiet Hi-Fiber Primate Biscuit[3]	0.75
Apple, Medium	0.50
Pineapple	0.50
Calculated composition	
Dry matter, %	14.50
	% of dry matter
Organic matter	94.79
Crude protein	11.75
Fat	3.08
Nitrogen-free extract	64.38
Total dietary fiber	15.58

[1]PMI Nutrition International, St. Louis, MO.
[2]PMI Nutrition International, St. Louis, MO.
[3]PMI Nutrition International, St. Louis, MO.

Institution 16

Ingredient	% (as-is)
Lettuce, Romaine, Shredded	28.38
Mazuri Primate L/S Sticks Banana[1]	28.38
Apple, Medium	12.90
Banana	12.90
Carrots	7.74
Sweet Potato, Steamed	7.74
Lettuce, Iceberg	1.02
Enrichment items (dried cranberries, blueberries, strawberries, watermelon, grapes, mixed dried fruit)[2]	0.94
Calculated composition	
Dry matter, %	35.41
	% of dry matter
Organic matter	94.30
Crude protein	17.34
Fat	5.00
Nitrogen-free extract	43.85
Total dietary fiber	28.11

[1]PMI Nutrition International, St. Louis, MO.
[2]Offered occasionally.

Institution 17

Ingredient	% (as-is)
Marion Zoological Leaf Eater Biscuit[1]	66.72
Lettuce, Looseleaf	7.71
Potatoes, Sweet	7.71
Apple, Medium	2.55
Grapes, Red or Green	2.55
Banana	2.55
Plum	2.55
Pear	2.55
Peach	2.55
Honeydew Melon	0.85
Cantaloupe	0.85
Watermelon	0.85
Calculated composition	
Dry matter, %	66.42
	% of dry matter
Organic matter	93.27
Crude protein	23.92
Fat	6.51
Nitrogen-free extract	41.06
Total dietary fiber	21.78

[1]Marion Zoological, Plymouth, MN.

Institution 18

Ingredient	% (as-is)
Vegetable mix 1 (carrots, broccoli, asparagus, yam, celery, cucumber)[1]	33.18
Mazuri Primate Browse Biscuit[2]	20.76
Vegetable mix 2 (canned green beans, canned corn, canned peas)[3]	17.39
Banana	10.79
Fruit mix 1 (watermelon, cantaloupe, honeydew melon, pineapple, grapes)[4]	6.05
Mazuri New World Primate Biscuit[5]	5.80
Fruit mix 2 (apples, pears)[6]	3.02
Tomatoes, Red	1.51
Lettuce, Romaine, Shredded	1.51
Calculated composition	
Dry matter, %	34.39
	% of dry matter
Organic matter	94.21
Crude protein	17.08
Fat	5.10
Nitrogen-free extract	50.95
Total dietary fiber	21.08

[1]Equal parts of each.
[2]PMI Nutrition International, St. Louis, MO.
[3]Equal parts of each.
[4]In the ratio 4 parts watermelon:4 parts cantaloupe:4 parts honeydew melon:2 parts pineapple:1 part grapes.
[5]PMI Nutrition International, St. Louis, MO.
[6]Equal parts of each.

Institution 19

Ingredient	% (as-is)
Banana	18.15
Apple, Medium	13.14
Grapes, Red or Green	13.14
Carrots	13.14
Squash, Summer, Zucchini	13.14
Potatoes, Sweet	13.14
Mazuri Leaf-Eater Biscuit[1]	13.14
Pear	1.50
Lettuce, Romaine, Shredded	1.50
Calculated composition	
Dry matter, %	26.37
	% of dry matter
Organic matter	94.68
Crude protein	13.72
Fat	3.51
Nitrogen-free extract	57.48
Total dietary fiber	19.98

[1]PMI Nutrition International, St. Louis, MO.

Institution 20

Ingredient	% (as-is)
Mazuri Leaf-Eater Biscuit[1]	26.46
Banana	16.43
Grapes, Red or Green	11.42
Broccoli	11.42
Corn, Yellow, Sweet, On the Cob	11.42
Peas, Green	11.42
Potatoes, Sweet	11.42
Calculated composition	
Dry matter, %	39.13
	% of dry matter
Organic matter	94.33
Crude protein	18.79
Fat	4.60
Nitrogen-free extract	48.43
Total dietary fiber	22.51

[1]PMI Nutrition International, St. Louis, MO.

Institution 21

Ingredient	% (as-is)
ZuPreem Primate Diet - Dry[1]	25.66
Banana	10.26
Potatoes, Sweet	10.26
Mazuri Leaf-Eater Biscuit[2]	10.26
Apple, Medium	5.14
Orange	5.14
Carrots	5.14
Lettuce, Romaine, Shredded	5.14
Blueberries	1.53
Honeydew Melon	1.53
Mango	1.53
Papaya	1.53
Peach	1.53
Pear	1.53
Plum	1.53
Raspberries	1.53
Strawberries	1.53
Watermelon	1.53
Broccoli	1.53
Cantaloupe	1.53
Corn, Yellow, Sweet, On the Cob	1.53
Green Beans	1.53
Peas, Green	1.53
Calculated composition	
Dry matter, %	43.09
	% of dry matter
Organic matter	94.65
Crude protein	18.80
Fat	5.35
Nitrogen-free extract	56.00
Total dietary fiber	14.51

[1]Premium Nutritional Products, Inc., Overland Park, KS.
[2]PMI Nutrition International, St. Louis, MO.

Institution 22

Ingredient	% (as-is)
Banana	20.39
ZuPreem Primate Diet - Canned[1]	20.39
Grapes, Red or Green	15.30
Potatoes, Sweet	10.20
Bread, White	10.20
Mazuri Leaf-Eater Biscuit[2]	10.20
Apple, Medium	1.03
Pear	1.03
Carrots	1.03
Onions, Chopped	1.03
Broccoli	1.03
Corn, Yellow, Sweet, Boiled, Drained	1.03
Lettuce, Romaine, Shredded	1.03
Kale	1.03
Endive or Escarole, Chopped	1.03
Kiwi Fruit, Green	1.03
Green Beans	1.03
Potatoes, White, Flesh and Skin	1.03
Collards	1.03
Calculated composition	
Dry matter, %	36.60
	% of dry matter
Organic matter	94.39
Crude protein	16.01
Fat	4.14
Nitrogen-free extract	56.78
Total dietary fiber	17.46

[1]Premium Nutritional Products, Inc., Overland Park, KS.
[2]PMI Nutrition International, St. Louis, MO.

Institution 23

Ingredient	% (as-is)
Mazuri Leaf-Eater Biscuit[1]	47.16
Apple, Medium	7.87
Banana	7.87
Orange	7.87
Carrots	7.87
Potatoes, Sweet	7.87
Vegetable chop mix (broccoli, bell pepper, tomato, cauliflower, zucchini, green beans)[2]	6.25
Fruit chop mix (mango, watermelon, cantaloupe)[3]	3.13
Mustard Greens	1.04
Cabbage, Bok Choy or White Mustard	1.04
Collards	1.04
Basil, Fresh	0.34
Rosemary, Fresh	0.34
Thyme, Fresh	0.34
Calculated composition	
Dry matter, %	50.64
	% of dry matter
Organic matter	93.45
Crude protein	20.91
Fat	5.47
Nitrogen-free extract	40.33
Total dietary fiber	26.73

[1]PMI Nutrition International, St. Louis, MO.
[2]Equal parts of each.
[3]Equal parts of each.

Institution 24

Ingredient	% (as-is)
Apple, Medium	14.70
Banana	14.70
Carrots	14.70
Corn, Yellow, Sweet, On the Cob	14.70
Kale	9.80
Marion Zoological Leaf Eater Biscuit[1]	9.80
Mazuri Leaf-Eater Biscuit[2]	9.80
Peach	0.98
Pear	0.98
Plum	0.98
Cauliflower, Chopped	0.98
Broccoli	0.98
Cucumber	0.98
Lettuce, Romaine, Shredded	0.98
Cantaloupe	0.98
Green Beans	0.98
Squash, Summer, All Varieties	0.98
Spinach, Chopped, Raw	0.98
Cranberries, Dried, Sweetened	0.17
Chips, Banana, Dried	0.17
Strawberries, Unsweetened, Frozen	0.17
Raspberries, Frozen	0.17
Blackberries, Unsweetened, Frozen	0.17
Blueberries, Unsweetened, Frozen	0.17
Calculated composition	
Dry matter, %	31.87
	% of dry matter
Organic matter	94.06
Crude protein	18.05
Fat	5.21
Nitrogen-free extract	50.62
Total dietary fiber	20.18

[1]Marion Zoological, Plymouth, MN.
[2]PMI Nutrition International, St. Louis, MO.

Institution 25

Ingredient	% (as-is)
Potatoes, Sweet	23.09
Apple, Medium	17.96
Mazuri Primate Browse Biscuit[1]	17.96
Orange	12.83
Banana	7.70
Celery, Stalk	7.70
Grapes, Red or Green	3.86
Raisins, Seedless	3.86
Broccoli	1.02
Pepper, Bell or Sweet, Green	1.02
Lettuce, Romaine, Shredded	1.02
Marion Zoological Leaf Eater Biscuit[2]	1.02
Kale	0.20
Spinach, Chopped, Boiled, Drained	0.20
Mustard Greens	0.20
Turnip Greens, Chopped, Boiled, Drained	0.20
Cabbage, Shredded	0.20
Calculated composition	
Dry matter, %	33.26
	% of dry matter
Organic matter	95.21
Crude protein	12.37
Fat	3.13
Nitrogen-free extract	60.44
Total dietary fiber	19.26

[1]PMI Nutrition International, St. Louis, MO.
[2]Marion Zoological, Plymouth, MN.

Institution 26

Ingredient	% (as-is)
Banana	27.96
Additional fruit (mango, apple, kiwi, strawberries, cantaloupe, blueberries, prunes, figs, cranberries)[1]	26.30
Vegetable mix (bell pepper, carrots, sweet potatoes)[2]	22.87
Greens rotation (greenleaf lettuce, Romaine lettuce)[3]	15.25
Marion Zoological Leaf Eater Biscuit[4]	7.62
Calculated composition	
Dry matter, %	22.34
	% of dry matter
Organic matter	95.05
Crude protein	12.27
Fat	3.32
Nitrogen-free extract	62.53
Total dietary fiber	16.93

[1]Offered in a ratio of approximately 25 parts mango: 25 parts apple:25 parts kiwi:3 parts strawberries:3 parts cantaloupe:1 part blueberries:1 part prunes:1 part figs:1 part cranberries.
[2]Rotated daily.
[3]Rotated based on availability.
[4]Marion Zoological, Plymouth, MN.

Institution 27

Ingredient	% (as-is)
Mazuri Primate Browse Biscuit[1]	23.93
Orange	18.92
Apple, Medium	13.92
Banana	13.92
Carrots	13.92
Potatoes, Sweet	13.92
Lettuce, Romaine, Shredded	0.37
Kale	0.37
Collards	0.37
Spinach, Chopped, Raw	0.37
Calculated composition	
Dry matter, %	34.48
	% of dry matter
Organic matter	94.77
Crude protein	13.74
Fat	3.66
Nitrogen-free extract	55.45
Total dietary fiber	21.90

[1]PMI Nutrition International, St. Louis, MO.

Institution 28

Ingredient	% (as-is)
Biscuit rotation (Marion Zoological Leaf Eater Biscuit[1], Mazuri Leaf-Eater Biscuit[2], Mazuri Primate Browse Biscuit[3])[4]	30.43
Vegetable rotation (cucumber, beets, celery, bell pepper, onion, broccoli, carrots, corn, green beans, sweet potato, turnips, potato, summer squash)[5]	26.94
Banana	20.28
Greens rotation (Romaine lettuce, cabbage, kale, iceberg lettuce)[6]	14.72
Additional fruit (apple, pear, orange, honeydew melon, cantaloupe, pineapple, mango, plum, peach, strawberries, blueberries, kiwi)[7]	7.63
Calculated composition	
Dry matter, %	37.24
	% of dry matter
Organic matter	93.77
Crude protein	18.85
Fat	5.06
Nitrogen-free extract	46.81
Total dietary fiber	23.04

[1]Marion Zoological, Plymouth, MN.
[2]PMI Nutrition International, St. Louis, MO.
[3]PMI Nutrition International, St. Louis, MO.
[4]Biscuit type is rotated on a daily basis.
[5]Two types of vegetables offered on a daily rotation.
[6]Greens type is rotated on a daily basis.
[7]One or two types of fruits offered on a daily rotation.

Institution 29

Ingredient	% (as-is)
Blueberries	18.32
Peas, Green	18.32
Mazuri Primate Browse Biscuit[1]	18.32
Apple, Medium	1.50
Banana	1.50
Papaya	1.50
Watermelon	1.50
Plum	1.50
Peach	1.50
Pear	1.50
Strawberries	1.50
Mango	1.50
Broccoli	1.50
Carrots	1.50
Pumpkin	1.50
Tomatoes, Red	1.50
Squash, Summer, Zucchini	1.50
Cantaloupe	1.50
Cabbage, Bok Choy or White Mustard	1.50
Cabbage, Shredded	1.50
Lettuce, Looseleaf	1.50
Kale	1.50
Lettuce, Romaine, Shredded	1.50
Collards	1.50
Green Beans	1.50
Potatoes, Sweet	1.50
Squash, Summer, All Varieties	1.50
Spinach, Chopped, Raw	1.50
Kiwi Fruit, Green	1.50
Lettuce, Iceberg	1.50
Mazuri Monkey Crunch[2]	1.50
Lettuce, Red Leaf	0.75
Cabbage, Red, Shredded	0.75
Figs	0.26
Grapes, Red or Green	0.26
Currants, Red and White	0.26
Tamarind	0.26
Nectarine	0.26
Coconut, Pieces	0.26
<u>Calculated composition</u>	
Dry matter, %	29.60

Institution 29 (cont.)

	% of dry matter
Organic matter	94.69
Crude protein	17.01
Fat	4.34
Nitrogen-free extract	50.99
Total dietary fiber	22.36

[1]PMI Nutrition International, St. Louis, MO.
[2]PMI Nutrition International, St. Louis, MO.

Institution 30

Ingredient	% (as-is)
LabDiet Monkey Diet 5038[1]	40.00
Apple, Medium	10.00
Banana	10.00
Pear	10.00
Grapes, Red or Green	3.35
Pineapple	3.35
Blackberries	3.00
Honeydew Melon	1.00
Watermelon	1.00
Peach	1.00
Plum	1.00
Orange	1.00
Mango	1.00
Broccoli	1.00
Carrots	1.00
Cauliflower, Chopped	1.00
Celery, Stalk	1.00
Cucumber	1.00
Cantaloupe	1.00
Cabbage, Shredded	1.00
Kale	1.00
Kiwi Fruit, Green	1.00
Corn, Yellow, Sweet, On the Cob	1.00
Green Beans	1.00
Potatoes, Sweet	1.00
Squash, Summer, All Varieties	1.00
Collards	1.00
Blueberries	0.15
Strawberries	0.15
Calculated composition	
Dry matter, %	45.42
	% of dry matter
Organic matter	95.12
Crude protein	13.67
Fat	5.10
Nitrogen-free extract	59.87
Total dietary fiber	16.49

[1]PMI Nutrition International, St. Louis, MO.

Institution 31

Ingredient	% (as-is)
Apple, Medium	11.58
Banana	11.58
Pear	11.58
Peach	6.62
Plum	6.62
Figs, Dried	6.62
Dates, Dried	6.62
Grapes, Red or Green	6.62
Corn, Yellow, Sweet, On the Cob	6.62
Mazuri Leaf-Eater Biscuit[1]	6.62
Raisins, Seedless	2.05
Cherries, Sweet	2.05
Raspberries	1.48
Blueberries	1.48
Apricots	1.48
Nectarine	1.48
Tamarind	1.48
Cranberries, Dried, Sweetened	1.48
Celery, Stalk	1.48
Lettuce, Romaine, Shredded	1.48
Mango, Dried	1.48
Applesauce, Unsweetened, Canned	1.48
Calculated composition	
Dry matter, %	33.83
	% of dry matter
Organic matter	97.17
Crude protein	7.87
Fat	2.21
Nitrogen-free extract	74.43
Total dietary fiber	12.66

[1]PMI Nutrition International, St. Louis, MO.

Institution 32[1]

Ingredient	% (as-is)
Marion Zoological Leaf Eater Biscuit[2]	34.21
Mazuri Leaf-Eater Biscuit[3]	34.21
Potatoes, Sweet	14.27
Carrots	14.27
Kale	1.52
Chicory Greens	1.52
Calculated composition	
Dry matter, %	67.60
	% of dry matter
Organic matter	93.13
Crude protein	23.15
Fat	6.16
Nitrogen-free extract	38.93
Total dietary fiber	24.89

[1]This diet was formulated for geriatric animals.
[2]Marion Zoological, Plymouth, MN.
[3]PMI Nutrition International, St. Louis, MO.

Institution 33

Ingredient	% (as-is)
Mazuri Primate High Fiber Sticks[1]	34.15
Banana	17.07
Spinach, Chopped, Boiled, Drained	6.71
Cabbage, Bok Choy	6.71
Kale	6.71
Lettuce, Romaine, Shredded	6.71
Yam	3.66
Potatoes, Sweet	3.66
Carrots	3.66
Pepper, Bell or Sweet, Green	3.66
Broccoli	3.66
Squash, Summer, All Varieties	3.66
<u>Calculated composition</u>	
Dry matter, %	40.53
	<u>% of dry matter</u>
Organic matter	93.67
Crude protein	16.94
Fat	5.96
Nitrogen-free extract	44.08
Total dietary fiber	26.68

[1]PMI Nutrition International, St. Louis, MO.

Guaranteed analysis, ingredients, and proximate analysis data for the three most frequently fed lemur chows

Marion Leaf Eater Food

Guaranteed analysis

Nutrient	As-fed, %	Dry matter basis, %
Crude protein (min)	23.0	25.6
Crude fat (min)	6.5	7.2
Crude fiber (max)	10.0	11.1
Moisture (max)	10.0	.
Ash (max)	7.0	7.8

Ingredients: Soybean meal, corn gluten meal, soybean hulls, sugar beet pulp, corn hominy feed, sucrose, yellow corn, dehydrated alfalfa meal, soybean oil (stabilized), flaxseed oil, dicalcium phosphate, apple fiber, calcium carbonate, sodium chloride, L-lysine, vitamin C 2-polyphosphate, propionic acid, choline chloride, zinc sulfate monohydrate, ferrous sulfate monohydrate, manganese sulfate monohydrate, nicotinic acid, D-calcium pantothenate, cupric sulfate pentahydrate, menadione dimethylpyrimidinol bisulfite (vitamin K), riboflavin supplement, thiamin monoitrate, ethylene diamine, dihydriodide, sodium selenite, D-biotin, folic acid, pyridoxine HCl (vitamin B-6), cyanocobalamin (vitamin B-12), stabilized retinyl palminate (vitamin A), D-activated animal sterol (vitamin D-3), DL-alpha tocopheryl acetate (vitamin E), FDC #40.

Proximate analysis

Nutrient	As-fed, %	Dry matter basis, %
Dry matter[1]	92.2	.
Crude protein[1]	23.3	25.3
Fat[2]	6.4	7.1
Crude fiber[3]	9.5	10.4
Neutral detergent fiber[4]	20.7	22.4
Acid detergent fiber[4]	14.0	15.2
Ash[5]	6.2	6.8

[1]Mean of values provided by the San Diego Zoo, Fort Wayne Children's Zoo, Fort Worth Zoo (all analyzed by Dairy One, Inc., Ithaca, NY), and Brookfield Zoo (analyzed by Midwest Laboratories, Inc., Omaha, NE).
[2]Acid hydrolyzed fat value provided by Brookfield Zoo (analyzed by Midwest Laboratories, Inc., Omaha, NE).
[3]Mean of values provided by the Fort Worth Zoo (analyzed by Dairy One, Inc., Ithaca, NY), and Brookfield Zoo (analyzed by Midwest Laboratories, Inc., Omaha, NE).
[4]Mean of values provided by the San Diego Zoo, Fort Wayne Children's Zoo, and Fort Worth Zoo (all analyzed by Dairy One, Inc., Ithaca, NY).
[5]Mean of values provided by the San Diego Zoo, Fort Worth Zoo (both analyzed by Dairy One, Inc., Ithaca, NY), and Brookfield Zoo (analyzed by Midwest Laboratories, Inc., Omaha, NE).

Mazuri Leaf-Eater Diet Mini-Biscuit

Guaranteed analysis

Nutrient	As-fed, %	Dry matter basis, %
Crude protein (min)	23.0	25.6
Crude fat (min)	5.0	5.6
Crude fiber (max)	14.0	15.6
Moisture (max)[1]	10.0	.
Ash (max)	8.0	8.9

Ingredients: Dehulled soybean meal, ground soybean hulls, ground corn, corn gluten meal, dried beet pulp, ground oats, dried apple pomace, soybean oil, dehydrated alfalfa meal, dicalcium phosphate, calcium carbonate, flaxseed, brewers dried yeast, salt, L-ascorbyl-2-polyphosphate, DL-methionine, pyridoxine hydrochloride, choline chloride, folic acid, vitamin A acetate, cholecalciferol, D-alpha-tocopheryl acetate, calcium pantothenate, ferrous sulfate, menadione sodium bisulfite complex, biotin, nicotinic acid, thiamine mononitrate, vitamin B-12 supplement, riboflavin, zinc oxide, natural mixed tocopherols (a preservative), citric acid, ascorbic acid, manganous oxide, rosemary extract, lecithin, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate, sodium selenite.

Proximate analysis

Nutrient	As-fed, %	Dry matter basis, %
Dry matter[1]	90.0	.
Crude protein[1]	21.2	23.5
Crude fat[1]	5.6	6.2
Crude fiber[1]	12.6	14.0
Neutral detergent fiber[1]	25.8	28.7
Acid detergent fiber[1]	15.6	17.4
Ash[1]	6.2	6.9

[1]Analyzed by PMI Nutrition International, St. Louis, MO.

Mazuri Primate Browse Biscuit

Guaranteed analysis

Nutrient	As-fed, %	Dry matter basis, %
Crude protein (min)	18.0	20.2
Crude fat (min)	3.0	3.4
Crude fiber (max)	16.0	18.0
Moisture (max)[1]	11.0	.
Ash (max)	8.0	9.0

Ingredients: Ground corn, dehulled soybean meal, corn gluten meal, sucrose, ground aspen, dried beet pulp, powdered cellulose, dried apple pomace, fructose, calcium carbonate, soybean oil, flaxseed, sodium hexametaphosphate (DentaGuard), dicalcium phosphate, brewers dried yeast potassium chloride, L-lysine, natural orange oil (preserved with propylene glycol and butylated hydroxyanisole), salt, taurine, zinc proteinate, choline chloride, DL-methionine, manganese proteinate, L-ascorbyl-2-polyphosphate (stabilized vitamin C), cholecalciferol (vitamin D3), mixed tocopherols (a natural preservative), ascorbic acid, citric acid, rosemary extract, lecithin, vitamin A acetate, beta-carotene, calcium pantothenase, D-alpha tocopheryl acetate (natural source of vitamin E), menadione sodium bisulfite complex (vitamin K), copper proteinate, iron proteinate, vitamin B-12 supplement, niacin, magnesium oxide, plant protein products, pyridoxine hydrochloride, folic acid, riboflavin, thiamin mononitrate, cobalt proteinate, ethylenediamine dihydriodide, biotin, sodium selenite.

Proximate analysis

Nutrient	As-fed, %	Dry matter basis, %
Dry matter[1]	90.0	.
Crude protein[1]	16.7	18.5
Crude fat[1]	4.7	5.2
Crude fiber[1]	13.5	15.0
Neutral detergent fiber[1]	23.2	25.8
Acid detergent fiber[1]	15.6	17.3
Ash[1]	5.4	6.0

[1]Analyzed by PMI Nutrition International, St. Louis, MO.