

Nutritional Analyses and Intervention in the
Captive Woolly Monkey
(Lagothrix lagotricha)

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Nutritional Analyses and Intervention in the Captive Woolly Monkey

(Lagothrix lagotricha)

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ABSTRACT. *Ange-van Heugten, K. 2008. Nutritional Analyses and Intervention in the Captive Woolly Monkey (Lagothrix lagotricha).*

Woolly monkeys (*Lagothrix spp.*) are a threatened species in the wild and are extremely difficult to breed and successfully maintain in captivity. The majority of health complications in woolly monkeys (WM) may be of nutritional origin. The objectives of this thesis were to: 1) determine the current status of the captive WM, 2) isolate potential nutritional causes for primary disorders in captive WM, and 3) investigate the effects that diet nutrients have on WM serum chemistry and cortisol concentrations. Our studies showed that the number of captive WM have decreased by 11% in the past 16 years. The number of institutions holding WM decreased and the birth to death ratio is 0.65 compared to 1.26 for their close relative the spider monkey (SM) (*Ateles spp.*). Lack of genetic diversity in captive WM also may negatively influence their success. Serum chemistry from 30 WM housed at two zoos were similar to previously reported concentrations for howler (*Aloutta sp.*) and SM; however, serum glucose was above the baseline range compared to humans and SM. Fasting concentrations of glucose, insulin, fructosamine, glycated hemoglobin, circulating lipids and urinary glucose were within normal ranges in six WM with known hypertension problems compared to other monkeys and humans. Potential stressors, such as unnatural diet, can contribute to the low success of endangered primates *via* noted health abnormalities. Fecal and salivary cortisol concentrations in WM and SM, at multiple zoological institutions showed that zoos with the highest dietary total carbohydrates, total sugars, glucose and fruit content had the highest cortisol. Supplementation of WM and SM diets with inulin-type fructans numerically decreased fecal cortisol after 4 weeks of supplementation, primarily in SM. The lifespan and reproductive success of captive primates will improve if stressors and negative effects of nutrition on the health status can be reduced and dietary nutrients can be optimized.

PREFACE

Throughout my life there has never been a moment when I considered a career that did not involve animals in some capacity. Therefore, my natural fit entering college was into the animal science program. After completing my Masters concentrating in swine nutrition in December 1998, I decided to accept a residency position in zoological nutrition at The Brookfield Zoo. I grew up working on our family farm and had always worked around livestock and companion animals, thus I was excited to branch out in order to learn more about exotic animals. My residency position became a two year associate nutrition position and I quickly learned that there were many basic areas of research needed in the exotic animal nutrition arena.

My supervisor at The Brookfield Zoo, Dr. Sue Crissey, asked me to work with her on a project to propose low salt items for hypertensive woolly monkeys at an American zoological institution. This was my first exposure to this New World primate that would ultimately control most of my spare time for the next decade. Dr. Crissey and I often spoke about my idea to pursue a PhD working with woolly monkeys but this potential endeavor was put on hold as I started a full time faculty position teaching at North Carolina State University in January 2001. I followed my new job with zoo nutrition consulting opportunities with Dr. Crissey and my marriage to Dr. Eric van Heugten. Unfortunately, during this time period Dr. Crissey was diagnosed with ovarian cancer to which she lost the battle in late 2002.

In August of 2002 I had a chance encounter with Dr. Walter Jansen. He inspired me to, once again, consider the plight of the woolly monkey and pursue a PhD. After much soul searching, I applied and was accepted into the external PhD program at Wageningen University. I had previously met Dr. and Mrs. Verstegen at animal science conferences and I was honored to have Martin agree to be my advisor. I will not lie and pretend that my pursuit

of a PhD was an easy task. I, of course, did not make the process any easier as I continued to work full time, had three children (Ryan in 2003, Connor in 2005, and Cailey in 2007) and try to be a wife that my husband still wanted to continue to live with!

This PhD thesis would not be possible without the help of several people and companies. I would first and foremost like to thank my husband Dr. Eric van Heugten. Without him, not only would I have never met Dr. Martin Verstegen, but I would have never had the courage and will to finish this PhD. His confidence in my ability to finish this degree was there even when I no longer had it. His nutritional expertise and insight has been invaluable. Eric also helped give me the best three things in the world; my children. They make every day special, regardless of the tribulations of research and writing. They are the best motivation one could ask for. I also sincerely thank my parents (Mr. and Mrs. Dempsy Ange, Jr), brother (Dr. Dempsy Ange, III), and grandmother (Mrs. Mamie Nobles) for their unwavering support not just during my PhD but throughout everything I have ever tried. My closest friends and family (you each know who you are) also have been vital to my success. You listened to my complaints and understood when I frequently changed plans, offered babysitting services, designed the cover and artwork in this dissertation, and were strong positive influences in my life. I am lucky to know so many wonderful people.

Dr. Martin Verstegen has made my pursuit of a PhD a wonderful experience. I can only hope to one day provide the guidance and support to my students that he has provided to me. His rapid response anytime I needed something, and his sincere optimism and encouragements were invaluable. Martin and Mariet do not perceive Martin's students as a job, but as an extension of their family and that is simply amazing. I am also very thankful to Dr. Peter Ferket for his willingness to be my co-promoter, his advice, and time as he travels with me to defend this degree. Not everyone would take on a student studying a monkey they had never heard of.

For their research assistance and insight I also sincerely thank Guido Bosch and Saskia Timmer. They have both shown a tremendous interest in zoological nutrition and have helped collect samples and information that was vital to this thesis. Guido, in particular, went above expectations as a colleague to help with all the international challenges. Other individuals vital to the research and its interpretation include: Roy & Cece Burns, Joost van den Borne, Jackie Hooley, Warner Jens, Jacqueline Ruijs, Christina Rush, Michael Stoskopf, Hans Swarts, Jan Vermeer and Scott Whisnant. The staff at the following zoological institutions were also invaluable: Gladys Porter Zoo, TX, USA; Henry Doorly Zoo, NE, USA; Highwater Farms, NC, USA; La Vallee des Singes, France; Little Mans Zoo, NC, USA; Little Rock Zoo, AR, USA; Louisville Zoo, KY, USA; The Monkey Sanctuary, England, UK, and Twycross Zoo, England, UK.

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I also thank North Carolina State University and the Department of Animal Science for the support I have received while completing my PhD. In particular, my department head, Dr. Roger McCraw, has provided both inspiration and flexibility with my full time teaching position. I realize that I am quite lucky to have been able to enjoy my career and my personal goals simultaneously.

Without a doubt, this work was produced by the joint support and efforts of many.

For that, I thank all of you.

Kimberly Ange-van Heugten

Table of Contents		Page
Abstract		
Preface		
Chapter 1	General Introduction	1
Chapter 2	Nutritional and Health Status of Woolly Monkeys - A review	9
Chapter 3	Sixteen Year Review of the Life Expectancy of the Worldwide Captive Woolly Monkey (<i>Lagothrix lagotricha ssp.</i>) Population with Spider Monkey (<i>Ateles spp.</i>) Comparative Data	33
Chapter 4	Serum Chemistry Concentrations of Captive Woolly Monkeys (<i>Lagothrix lagotricha</i>)	47
Chapter 5	Evaluation of diabetes determinants in woolly monkeys (<i>Lagothrix lagotricha</i>)	71
Chapter 6	Diet Effects on Fecal and Salivary Cortisol Concentrations in Woolly (<i>Lagothrix lagotricha ssp.</i>) and Spider Monkeys (<i>Ateles spp.</i>)	99
Chapter 7	Effect of Dietary Inulin-Type Fructans on Fecal Dry Matter and Fecal Cortisol in Spider and Woolly Monkeys	125
Chapter 8	General Discussion	149
Summary		163
Samenvatting		171
Curriculum Vitae and Publications of the Author		179
Notes		191

Chapter 1:

General Introduction

The first zoo opened in ancient Egypt in approximately 1400BC and zoos have since multiplied across the world to entertain and educate the general public (Hanson 2002). The first zoos were designed for display of unique animals and conservation was not a priority because most animals were easily replaced. As free-ranging animal populations became more scarce, conservation slowly became a concern, and today conservation programs are an integral part of zoo management. As part of this management, proper nutrition of captive animals became a high priority. However, it is very difficult to determine nutritional requirements for many species even though literature exists for their domesticated relatives. This is a matter of considerable concern because species that are endangered in the wild are likely to become extinct if our society cannot manage to properly feed and breed them in captivity.

Research with captive exotic animals is complicated because many species are scarce in both captivity and in the wild, and all situations that may increase their stress or be perceived as threats are highly limited by holding institutions. Moreover, many of these holding institutions do not allow blood or other biological samples to be taken. Investigators are rarely allowed to change the animal's environment or diets as experimental treatments. Many traditional animal research methods call for animals to be isolated or separated into age or gender specific groups and this can very stressful for wild animals. If collection of samples is allowed, transporting them among locations increases the risk of spreading zoonotic disease. This means specific required permits are needed before endangered animals or their biological samples can be transported. Evidently, it is challenging to determine the nutritional requirements and diet related health concerns affecting many species kept in zoological institutions.

The woolly monkey (*Lagothrix lagotricha*) is a species that is difficult to manage and feed in captivity. They are a large South American primate that taxonomically belongs to the

family Cebidae and the genus *Lagothrix*. Woolly monkeys have a body length of 508 to 686 mm (excluding the prehensile tail) and they weigh 5.5 to 10.8 kg (Nowak, 1999). Although their exact taxonomy is somewhat disputed (Di Fiore and Campbell, 2007), there are two species of this primate, *L. lagotricha* and *L. flavicauda*. *L. lagotricha* is the only one kept in captivity and has four subspecies, *L. l. cana*, *L. l. lagotricha*, *L.l. lugens*, and *L. l. poeppigii* (Nowak, 1999; CITES, 2007). Both species and all subspecies are endangered in the wild to varying degrees and only approximately 80 woolly monkeys are reported to live in captivity worldwide (CITES, 2007). These non-human primates have been kept as pets, laboratory animals, and zoological exhibits. Captive breeding does not result in a sufficient number of animals. Attaining a long (normal) lifespan for these animals in most captive locations throughout the world has had very limited success. It has been suggested that captive woolly monkeys do not survive as well as similar non human primate species. The majority of the health complications in these monkeys are probably nutrition- or diet-related. This conclusion being deduced from the nature of their problems being similar to those of diabetes mellitus and hypertension in other primates. In particular, it is known that elevated levels of lipids, sodium and sugars in humans can increase the prevalence of Type II diabetes or hypertension (Lagua and Claudio, 2004). These two diseases are closely related to other conditions reported to negatively affect woolly monkeys, including congestive heart failure, renal failure, reproduction problems and immunosuppression (Ange-van Heugten et al., 2008; Giddens et al., 1997; Miller et al., 1995). As with humans, these woolly monkey health conditions are often more pronounced during pregnancy and may result in very large, difficult to deliver, infants (Lloyd et al., 1995). All of these health problems have contributed to lowering the captive life span and seriously decreasing the captive population of woolly monkeys. Therefore, it is vital to better understand the nutritional needs and associated disorders within

this species in order to determine whether the captive health problems can be prevented by feeding a nutritionally balanced diet.

To better understand the woolly monkey nutritional needs, it is critical to first understand the free-ranging population of this species. Compared to other primates, they spend a large amount of their time eating and moving in pursuit of food (approximately 60 to 75 %) (Dew, 2005; Di Fiore and Rodman, 2001, Di Fiore, 2004). They are primarily frugivores, consuming fruits that are characterized by tough outer shells. Compared to their close relative, the spider monkey (Di Fiore and Campbell, 2007; Ford and Davis 1992), they tend to eat fruits that are lower in fat and higher in both sugar and water (Dew, 2005). Woolly monkeys prefer harder fruits, consume more vegetative matter, forage in larger groups, consume from more plant species and never forage from the ground as compared to spider monkeys (Dew, 2005). It is likely that captive woolly monkey populations are fed diets that do not provide enough non-starch polysaccharides or crude fibers, being that fruits and vegetables cultivated by humans typically contain more water and sugar and less fiber and protein than their wild ancestors (Milton, 1999). It is likely that woolly monkeys are fed diets similar to their close relative the spider monkey which may be inappropriate.

Considerable diet variation within the wild population of woolly monkeys exists even among the four subspecies and across seasons (Defler and Defler, 1996; Stevenson et al, 1998). Depending on resources available, woolly monkeys are reported to spend large amounts of time preying on insects and small animals. Due to these predation habits being higher than most other new world primates, the protein requirement for this species is of particular interest. While many researchers have attempted to follow woolly monkeys in the wild to ascertain their natural behavior and endangered status, no one has yet collected their naturally consumed diet items for laboratory nutritional analyses. Thus, feeding this primate in captivity has proven to be a challenge. The captive diet also has not previously been

researched in detail and therefore woolly monkey nutritional needs are poorly understood. However, due to their susceptibility to diseases typically associated with nutrition, it appears that woolly monkeys are unique both with regard to their nutritional requirements and associated nutritional disorders.

The current research had two primary objectives. The first one was to determine whether a nutritional cause for the primary disorders afflicting captive woolly monkeys could be isolated *via* historical research. The second primary objective was to investigate the effects that carbohydrates (sugar and non starch polysaccharides) and lipids have on woolly monkey serum nutritional profiles, serum diabetic determinants, and stress hormone (cortisol) concentrations. The research to isolate these objectives was multifactorial:

- 1) An all inclusive review of the literature on this species was conducted. Particular emphasis was placed on literature regarding nutrition (both from free-ranging and captive animals) as well as captive management practices and health disorders.

- 2) Captive data on the life and death histories of woolly monkey was compiled for the previous 15+ years. This was conducted to determine if the species is in fact unsuccessful in captivity. A portion of this compilation also was to isolate woolly monkey medical pathologies related to all the recorded deaths of this primate in captivity. Finally, the historical captive success rate of woolly monkeys was compared to their closest living relative, the spider monkey (*Ateles spp.*).

- 3) All available serum chemistry reports from woolly monkeys were acquired and analyzed in order to publish normal values and then compare these values to similar species. This comparison was an attempt to isolate health and / or nutritional abnormalities that could potentially help to determine why the woolly monkey is less successful in captivity than many of its closest relatives.

4) Woolly monkeys are often suspected to have Type II diabetes. Thus, this disease was investigated within a captive population of woolly monkeys to determine if the species is predisposed to it.

5) It was researched whether the stress measure cortisol could be successfully taken and analyzed from spider monkeys via feces and saliva samples. Once these samples were taken and analyzed, they were compared to diet and management practices to determine their possible effects on cortisol. Spider monkeys were used due to their close genetic relationship to woolly monkeys and because they are quite numerous in zoological institutions. Therefore it was much easier and efficient to first research spider monkeys before deciding if examining woolly monkeys would be worth the expense and research time.

6) When the different diets provided by separate zoos appeared to alter cortisol measures for spider monkeys, the diet for three zoos housing woolly monkeys was also compared to fecal cortisol concentrations.

7) The non starch polysaccharide inulin was added to spider monkey and woolly monkey diets to determine if fecal dry matter percentages and cortisol hormone concentrations could be decreased over time.

Fulfilling the overall objective of determining whether a nutritional cause for the problems associated with captive woolly monkey welfare is essential to their survival in captivity. The research reported in the following dissertation explains many nutritional issues facing the woolly monkey and it also shows that despite numerous limitations, exotic animal research can be a critical tool to enhance the survival of captive populations of animals.

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Chapter 2:

Nutritional and Health Status of Woolly Monkeys - A Review

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ABSTRACT

Woolly monkeys (*Lagothrix lagotricha* and *L. flavicauda*) are threatened species in the wild and in captivity. Numerous zoological institutions have historically kept *Lagothrix lagotricha* spp., but only a few of them have succeeded in breeding populations. Therefore the majority of institutions that formerly kept *Lagothrix lagotricha* are no longer able or willing to do so. Captive populations of the species have frequent health problems, most significantly hypertension and related disorders. Researchers have conducted free-ranging dietary and behavior studies with respect to woolly monkeys, but have established no concrete link between diet or nutrients and captive health problems. The available literature we discuss indicates that researchers need to examine the link further. It is critical to the survival of the primates to be able to keep a breeding population in captivity owing to the increasing natural pressures such as deforestation and hunting. Therefore, better understanding of the captive and free-ranging behavior and health parameters of the species is vital to ensure their survival and to maintain forest health and diversity. Researchers need to conduct large-scale research studies comparing the health and complete diet of individuals in the wild and captivity to resolve health problems facing the species in captivity.

Keywords: Diet, Health, Hypertension, Primate, Woolly monkey

INTRODUCTION

Woolly monkeys (*Lagothrix* spp.) are one of the largest South American primates, with reported dimensions of 508–686 mm in head-to-body length and 5.5–10.8 kg in body mass (Nowak, 1999). There are 2 species: *Lagothrix lagotricha* and *L. flavicauda* (cf. *Oreonax flavicauda*). *Lagothrix lagotricha* has 4 subspecies: *L. l. cana*, *L. l. lagotricha*, *L. l. lugens*, and *L. l. poeppigii*—inhabiting the eastern Andes in Columbia, the Rio Tapajos, and the Mato Grosso in central Brazil, and eastern Peru and Ecuador. *Lagothrix flavicauda* resides primarily in the eastern slope of Cordillera Central in northern Perú (Nowak, 1999).

Woolly monkeys are threatened species, with *Lagothrix flavicauda* listed as critically endangered by the World Conservation Union (IUCN, 2006), as endangered by the United States Department of Interior (USDI, 2006), and on Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2006). *Lagothrix lagotricha* is listed on Appendix 2 of CITES, and of the 4 subspecies, *L. l. lugens* is considered vulnerable by IUCN (IUCN, 2006; Nowak, 1999). CITES, IUCN, and USDI have repeatedly changed the status of the woolly monkeys taxa on their scales of endangerment. Research is needed to identify the specific reasons why they are threatened with extinction. In some South American countries the threat is more severe than in others; for example, a Peruvian census in 1998 did not include woolly monkeys because they were rarely seen (Bennett *et al.*, 2001). Researchers have reported that woolly monkeys have a low r_{\max} (intrinsic rate of natural increase). A low r_{\max} correlates highly with the likelihood of specific extinction (Bodmer *et al.*, 1997).

Woolly monkeys are often a source of food and commerce (Peres, 1991; Shephard, 2002). *Lagothrix* has a large span between offspring and reproduce for a shorter period of their lives compared to many non ape primates (Mooney and Lee, 1999; Nishimura *et al.*, 1992). Further, *Lagothrix* have a comparatively large body size and low tolerance to habitat

destruction, and are thus especially vulnerable. The inevitable introduction of firearms into this area in the 21st century to hunt woolly monkeys makes them a threatened species (Peres, 1991; Shephard, 2002).

The situation for *Lagothrix* in captivity is thus precarious. In an attempt to conserve the species, numerous zoos have tried to develop and to maintain breeding populations. However, most institutions have not been able to replenish their own populations successfully. Very few institutions are currently able or willing to house *Lagothrix*. The most commonly reported causes of death in captivity include pregnancy complications and hypertension-related conditions. In fact, the European Endangered Species Program (EEP) showed that the captive woolly monkey population was reduced by 16% in 1 yr (1998). Owing to the limitations of importing wild animals and the problems associated with breeding, woolly monkey survivability in the wild and in captivity is of utmost concern. According to the last count, there were 107 captive woolly monkeys worldwide (www.ISIS.org; EEP, 2003). It is also important to note that approximately half of the individuals currently in captivity are subspecific hybrids and therefore some of the subspecific information relevant in the wild may not be valid in captive populations.

GENERAL BEHAVIOR *VERSUS* THAT OF OTHER NONHUMAN PRIMATES

Researchers have studied *Lagothrix lagotricha* ssp. in their natural habitats, since initial reports by Bates (1863). Historically, they have been harder to maintain in captivity than closely related spider monkeys are. Woolly monkeys are unique in that dominant males are very friendly toward subordinate males in their groups and all individuals have a chance to mate (Defler, 1995). In addition, males are excellent protectors: they accompany their mates after the birth of young and follow them and the offspring everywhere. They are tolerant of young monkeys and occasionally carry infants on their backs (Defler, 1995).

Female woolly monkeys with dependent infants are more efficient foragers than adult males are, and females do not necessarily sexually prefer the largest dominant males (Stevenson, 2002). Woolly monkeys live as solitary males or in solitary male groups but never as solitary females or in female-only groups (Di Fiore, 2002). Populations of wild woolly monkeys are genetically very diverse. They may have social patterns more similar to those of chimpanzees than of other ateline primates (Di Fiore, 2002).

Woolly monkeys, squirrel monkeys, and capuchins forage together. Typically numerous birds, deer, and peccaries follow them through the forest and eat fruits and leaves that the monkeys leave behind. Therefore, woolly monkeys are a vital part of the ecosystem and efforts to conserve them are essential for their own well-being and of their South American ecosystem. Woolly monkeys share many of their habitats with the following primate species: *Saquinus fuscicollis* (saddleback tamarin), *Saimiri boliviensis* (Bolivian squirrel monkey), *Pithecia monachus* (monk saki), *Cebus albifrons* (white-fronted capuchin), *Cebus apella* (brown capuchin), and *Alouatta seniculus* (howler).

Woolly monkey group sizes vary drastically but range from 10 to 49 individuals at the Tinigua National Park in Columbia and in Amazonian Ecuador (Dew, 2005; Stevenson and Castellanos, 1999). Stevenson and Castellanos (1999) showed that woolly monkey groups that are either very large or very small may be less successful in foraging. Very large groups have to travel more kilometers per day to obtain their food, and very small groups may travel more as a defense mechanism. Woolly monkeys prefer to rest and travel in the middle to upper canopy of the forest. They do not reside as high as many other primate species, e.g., spider monkeys (*Ateles belzebuth belzebuth*), do (Dew, 2005). Woolly monkeys seldom venture to the ground.

DIETS

Free-ranging subjects

Compared to other primates, woolly monkeys spend a large amount of time eating and moving in pursuit of food (*ca.* 60–75%) (Dew, 2005; Di Fiore, 2004; Di Fiore and Rodman, 2001). They typically cover 2 km/d moving and foraging (Defler and Defler, 1996; Dew, 2005). One acre of tropical rain forest typically has >200 species of trees, and because the woolly monkeys consume fruits from the majority of them as they travel through the forest they are one of the most important links to maintain forest diversity (Defler, 1995). Fruits consumed by woolly monkey are typically characterized by tough leathery outer shells with 1 or 2 large seeds in the middle with small fleshy parts adhering to each seed. For monkeys to eat the fleshy part they also must consume the seeds or spend time picking them out. Their vernacular name is macaco barrigudo (barrel or belly monkey) because after feeding on the ripe fruits and seeds their stomachs distend so that they appear to be pregnant (Bates, 1863; Defler, 1995).

Defler and Defler (1996) studied the diet of the wild *Lagothrix lagotricha lagotricha* in southeastern Colombia in an undisturbed rain forest at Estacion Biologica Caparu (EBC). They reported daily food consumption of 23 woolly monkeys in a single troop for *ca.* 2400 h between January 1985 and September 1987. Defler and Defler (1996) recorded a total of 2243 woolly monkey foraging bouts and identified the food items in 1719 cases. The monkeys consumed fleshy fruits as 78.9% of their diets, leaves (primarily new growth) as 11.4%, seeds as 4.3%, invertebrates (termites and katydids) as 4.9%, bark as 0.3%, flowers as 0.1%, and tendrils as 0.1%. The majority of the fruits consumed were very tough (could not be easily opened by human hands) and had seeds that the monkeys seemed to swallow completely. The fruits comprised 183 plant species, with 44% of them from the Moraceae, Sapotaceae, and Leguminosae. Sapotaceae was the most consumed. *Chrysophyllum amazonicum* and

Manilkara amazonica were the 2 most consumed fruit species and they each accounted for $\geq 5\%$ of the fruit total. There was considerable variation in diets of woolly monkey among months and years. They consumed fruits of *Iriartea ventricosa* and immature seeds of *Micrandra spruceana* when there was little else to eat.

In a 1-yr feeding behavior study in Amazonian Ecuador, Dew (2005) reported results similar to those of DeFler and DeFler (1996). However, the woolly monkeys in Ecuador ate more insects (6%), and 12% of the total diet items were unknown because their foraging behavior made exact recordings impossible for the researchers. Though unconfirmed, much of the unknown percentage was probably insects and prey items. Di Fiore (2003) had previously reported that woolly monkeys there seemed to forage and travel to locations where higher densities of prey live. Dew (2005) also reported that the woolly monkeys selected food items by discriminatively picking items for seeds that they wanted to swallow. Dew's research showed that although woolly and spider monkeys often live in the same regions, the woolly monkeys eat different diets than those of closely related spider monkeys. They tended to eat fruits that were lower in fat and higher in both sugar and water than the spider monkeys at the same location. Woolly monkeys prefer harder fruits, consume more vegetative matter, spend more of their time eating, forage in larger groups, and never forage from the ground versus spider monkeys. The woolly monkeys did not drink water in the year-long study whereas the spider monkeys did. The information is important for captive feeding of woolly monkeys and shows that though 2 species may be very closely related, their dietary needs may differ substantially.

Researchers believe that the 4 woolly monkey subspecies play a vital role in seed dispersal and increased germination for the forest plants on which they feed (DeFler and DeFler, 1996; Dew, 2005; Stevenson *et al.*, 2002; Yumota *et al.*, 1999). Di Fiore (2004) described the diet and feeding ecology of a population of *Lagothrix lagotricha poeppigii* in Yasuní National

Park, Ecuador in the western Amazon Basin. The main constituent of the diet was ripe fruits, varying between 64% and 89% of the monthly diet. Fruits, including exudates, comprised an average of 76.2% of the diet; immature seeds, leaves, and other nonreproductive plant parts comprised 10.6%, flowers 3.6% and animal prey 9.6% of the diet. The 3123 fruit feeding records in the study represent a minimum of 147 plants belonging to ≥ 80 different genera and 45 different families. The top 3 genera—*Inga* (Fabaceae), *Ficus* (Moraceae), and *Spondias* (Anacardiaceae)—each accounted for $>5\%$ of all feeding records.

Yumota *et al.* (1999) showed that woolly monkeys were especially important with regard to seed dispersal because they ate primarily fruit and also traveled in a larger area during the day than howlers did (17.2 ha vs. 69.8 ha, respectively). The average gastrointestinal retention time for the seeds they consumed was 2.0–6.1 h. The researchers studied retention times in the field and therefore used feeding bouts and the appearance of seeds in the feces to determine retention times instead of standard markers. Yumota *et al.* (1999) also showed that woolly monkeys in the La Macarena Forest in Columbia divided their foraging time as follows: fruit, 81.5%; leaves, 4.4%; and insects (primarily ants: *Crematogaster* spp. and *Eciton* sp., 14.1%).

Defler and Defler (1996) reported that 3 woolly monkey subspecies—*Lagothrix lagotricha cana*, *L. l. poeppiggi*, and *L. l. lugens*—similarly consume the majority of their diets as fruits (77 %, 73.6 %, and 60 %, respectively). The Anacardiaceae, Arecaceae, Fabaceae, Leguminosae, Moraceae, and Sapotaceae provide the most important food sources for woolly monkeys regardless of their subspecies or test site (Defler and Defler, 1996; Dew, 2005; Di Fiore, 2004, Iwanaga and Ferrari, 2001, Peres, 1994). The test sites for the 4 subspecies differ greatly with respect to rainfall, food availability, and soil quality. Though woolly monkey subspecies appear to have foraging preferences, they are commonly reported to sample items from numerous species. In fact, Peres (1994) reported that in 11 mo a large (39–41 members) group of *Lagothrix lagotricha cana* ate from 225 species of plants. During

times of very low fruit availability members of the group seemed to prefer young seeds, young foliage, and exudates of mature seed pods of *Parkia* instead of insects (Peres, 1994).

Research varies widely with regard to the amount of time that woolly monkeys spend trying to catch animal prey (Di Fiore, 2003; Di Fiore and Rodman, 2001). Depending on the study, woolly monkeys have reportedly spent between 0.1 and 36.2% of their time either trying to capture or consuming animal prey (Defler and Defler, 1996; Di Fiore and Rodman, 2001; Peres, 1994, Stevenson *et al.*, 1994, 1998). Researchers have suggested that some woolly monkey subspecies preferentially spend more time trying to catch animal prey when there is an abundance of fruit available. The time allows them to store fat reserves for enhanced survival when food is less available. *Lagothrix lagotricha cana* and *L. l. lagotricha* consume less animal matter than the other 2 subspecies, though they are larger (by 30–65%) than *L. l. lagotricha* and *L. l. lugens* (Di Fiore and Rodman, 2001). Larger individuals may be less able to catch large enough quantities of insects to fulfill their protein requirements. Overall, it seems that woolly monkeys (regardless of location or subspecies) preferentially consume ripe fruits and that the majority of the differences in their foraging habits are likely a response to habitat quality, available food sources, and body size. When food sources are scarce, woolly monkeys do not forage over large ranges. Instead they appear to forage longer in certain areas and therefore conserve energy that they would otherwise need to move around to more trees (Di Fiore, 2001).

Researchers have infrequently noted that woolly monkeys chase frogs and larger animals or are very interested in bird nests (Stearns *et al.*, 1988). Captives have preyed on birds. It may be an opportunistic way for them to eat additional calories in the wild, though it occurs at such a low level that it is not easily noticed (Stearns *et al.*, 1988).

Defler and Defler (1996) reported that the majority of consumed fruits were yellow-to-orange. The finding is surprising because they are 75% dichromatic—unable to use color

vision—and perhaps color in their captive diet may be an important yet often ignored concept (Jacobs and Deegan, 2001). Finally, researchers have suggested that some primates ingest the seeds of some fruits to rid their gastrointestinal systems of parasites. However, Stevenson *et al.* (2005) did not report this during woolly monkey observations in Columbia

Captive subjects

Owing to the health problems and low reproductive success of captive woolly monkeys, their captive diet is a major concern. Wilteveen *et al.* (1999) measured the captive diet of one of the largest captive population of woolly monkeys; it appeared sufficient in all essential nutrients versus the 1978 National Research Council nutritional requirements for New World monkeys. The diet did not appear to be a risk factor for hypertension when compared to human standards (Wilteveen *et al.*, 1999). However, our own studies reviewing captive diets for them indicated that there are few dietary similarities between institutions and that most institutional diets do not meet the current National Research Council estimated requirements for New World primates (Ange-van Heugten *et al.*, 2007; Timmer, 2006; Timmer and Ange-van Heugten, 2006; Timmer *et al.*, 2005). In addition, many institutions in our research overfeed their subjects and offer huge daily variations of fruits and vegetables while potentially underfeeding them insects. Thus, the fat and sugars offered widely differ in both type and quantity. Large-scale studies of captive and wild populations are needed for comparisons of nutrient supply and possible natural supplements to the diet that may be missing in captivity as well as comparisons with the updated New World monkey nutrient requirements.

HEALTH PROBLEMS IN CAPTIVITY

The captive management of *Lagothrix lagotricha* spp. is a major concern. There is a breeding program but it is in peril owing to the shrinking number of captive individuals, poor reproductive performance, and high infant mortality. Many females have a reduced body mass during pregnancy and often abort without obvious reasons. Offspring frequently die at a young age. Body sizes of the young woolly monkeys are considered of normal size. Müller and Heldstab (1989) found that a high prevalence of stillbirth, premature birth, and abortion coincided with a high prevalence of renal disease in mothers. A healthy pregnancy is difficult to maintain because of the decrease in body mass and because of the high prevalence of abortions (Mooney and Lee, 1999). Thus, individuals do not eat sufficient quantities of nutrients to maintain or increase body mass during pregnancy. The severe complications that may arise in breeding individuals are often similar to those of diabetes mellitus and hypertension in humans and other primates and ultimately include congestive heart failure, renal failure, and aneurysm (Giddens *et al.*, 1987; Miller *et al.*, 1995). The complications are often pronounced during pregnancy and also may result in very large, difficult to deliver infants (Lloyd *et al.*, 1995). They can ultimately result in arteriosclerotic changes in the placenta that lead to nutrient and metabolic insufficiencies with severe consequences for the fetus. Hypertension is said to be of multifactorial origin. Stress may play a role, along with obesity and genetic predisposition (Debyser, 1995). All of the factors may be associated with the shrinking number of captive woolly monkeys.

Hypertension-specific health problems

Captive woolly monkeys have a high prevalence of primary systemic hypertension: a blood pressure of >140 (systolic)/90 mm Hg (diastolic; Giddens *et al.*, 1987; Miller *et al.*, 1995; Muller *et al.*, 1989; Wagner, 1984). Hypertension in woolly monkeys appears to be a

multifactorial disease, and affected captive primates may die from congestive heart failure, renal failure, and aneurysms. Some of the underlying causes of the disease in woolly monkeys may be age, obesity caused by restricted physical activity, unnatural captive diets, gender, and psychological and physical stress including pregnancy (Muller *et al.*, 1989). Giddens *et al.* (1987) diagnosed hypertension in woolly monkeys via a direct measure: arteriolar puncture while anesthetized with ketamine, of systolic and diastolic blood pressure in 15 captive apparently healthy woolly monkeys (systolic and diastolic blood pressure measures from 194 mm Hg and 136 mm Hg, respectively) Giddens *et al.* (1987) also showed that systolic and diastolic blood pressures of the captive woolly monkeys were significantly higher than those of captive baboons (*Papio cynocephalus*), pigtailed macaques (*Macaca nemestrina*), and crab-eating macaques (*Macaca fascicularis*). Another group of 17 apparently healthy woolly monkeys had elevated pressures (176 mm Hg and 116 mm Hg, respectively) while anesthetized (Miller *et al.*, 1995; Wagner *et al.*, 1984).

Arteriolar nephrosclerosis is the first lesion associated with hypertension in woolly monkeys (Giddens, 1987; Miller *et al.*, 1995). The most common causes of death in captive woolly monkeys are congestive heart failure, renal diseases and failure, and cardiovascular failures (Brown *et al.*, 2000; Giddens, 1987; Henderson *et al.*, 1970; Miller *et al.*, 1995). Giddens *et al.* (1987) showed that direct arterial blood pressure in woolly monkeys increases by 16 mm Hg per kg of additional body mass for systolic pressure and 10 mm Hg per kg for diastolic pressure. The correlation has led many investigators to believe that the increase in mass with age exacerbates hypertension in woolly monkeys. However, young woolly monkeys that have healthy body masses have also been diagnosed as hypertensive.

Female woolly monkeys (80%) are more likely than males (20%) to become hypertensive (Muller *et al.*, 1989). There are reports of atherosclerosis in woolly monkeys, though they are more scarce than reports of hypertension (Giddens *et al.*, 1987; Henderson *et al.*, 1970). It is

not currently known whether woolly monkeys develop hypertension in the wild or if it is only a problem in captivity. It is very important to study hypertension-related problems in both captive and wild individuals. The survival of the species is dependent on further research.

Successful treatment of woolly monkeys with hypertension is still needed. Poor treatment success may be due to the late diagnosis of the condition. Oral furosemide and β -blockers have not been as effective in treating hypertension in woolly monkeys as they are in humans. Antihypertensive therapy with diltiazem given simultaneously with furosemide has reduced blood pressure in a woolly monkey (Miller *et al.*, 1995). Researchers believe that captive woolly monkeys are extremely stress sensitive and that making their enclosures more similar to their natural environments and providing a seasonal change in diet may help to decrease their hypertension. In support of the theory, fecal cortisol measurements are 10–30 times higher for captive woolly monkeys versus their free-ranging counterparts, though Ziegler (2001) did not report their behavior, gender, age, hypertension status, and social grouping.

Hepatitis health concerns

Woolly monkeys at the Louisville Zoo (13 of 16 individuals tested) were the first to be diagnosed with woolly monkey hepadnavirus (WMHBV, which is related to human hepatitis B) infections (Lanford *et al.*, 1998). Researchers have since studied and reported the virus in other captive woolly monkey populations though the association between WMHBV and potential health problems is not well understood.

Toxoplasmosis and related health concerns

Woolly monkeys are especially susceptible to the protozoan *Toxoplasma gondii* while Old World primates seem to be immune to it (Bouer *et al.*, 1999; Hessler *et al.*, 1971). In all captive situations, animal keepers should be very careful to limit the possible exposure of

woolly monkeys to the protozoa. For example, woolly monkey cages should be thoroughly cleaned and kept as far from felines as possible. Felines are the only animals known to pass *Toxoplasma gondii* oocysts in their feces. Therefore, feline care staff should not care for woolly monkeys at the same institution. Amebiasis, demodectic acariasis, *Klebsiella* infection, and salmonellosis are also especially problematic for captive woolly monkey populations (Peddle and Larson, 1971; Schiefer and Loew, 1978).

Bone formation health concerns

Rickets and osteomalacia have occurred in woolly monkeys. In fact, the condition is so well known that many clinicians refer to the condition as woolly monkey syndrome (NRC, 2003). Dew (2005) reported that Amazonian soil is very rich in calcium, and free-ranging diets are likely also to be high in phosphorus from the insects consumed. Therefore, it is vital to make sure that captive diets have adequate levels of calcium and phosphorus as well as proper calcium-to-phosphorus ratios. In addition, woolly monkeys should either have sunlight or proper artificial wavelength exposure to produce the vitamin D metabolites needed for normal bone mineralization as well as proper vitamin D₃ concentrations in their diets (NRC, 2003). It is not known if the bone formation problems noted in captivity are also problems in free-ranging populations. Research is critically needed in this area to ascertain if the problems are in captive populations only and, if so, how we can best prevent them from occurring.

REPRODUCTION FACTS AND CHALLENGES

Institutions have had difficulty in breeding captive woolly monkeys, and the individuals have not been able to replace themselves in sufficient numbers (Mooney and Lee, 1999). One study reports an infant mortality rate of 50% in captive woolly monkeys, with infant mortality increasing if the infant is a male or if it is the first pregnancy (Debyser, 1995). Debyser (1995)

also reported that there is increased risk of pregnancy complications if the social group is unstable and if the mother is captive born. Debyser (1995), Muller *et al.* (1985), and Ruedi and Heldstab (1980) have associated the high prevalence of woolly monkey stillbirths, premature births, and spontaneous abortions with renal disease and hypertension in the mothers. Hypertension is accompanied by arteriosclerotic changes in the placenta that lead to placental insufficiency and detrimental effects, possibly death, to the fetus (Debyser, 1995). Debyser (1995) and Ruedi and Heldstab (1980) have also linked dystocia, *Klebsiella* infections, septicemia, and meningitis to infant deaths in woolly monkeys.

Free-ranging woolly monkeys live in large heterosexual groups with *ca.* 10–49 individuals/group (Mooney and Lee, 1999; Stevenson and Castelanons, 2000). Members of both sexes are sexually mature between 4 and 5 yr of age and the females have a 21-d ovarian cycle. On average, wild females have their last offspring at 20 yr (Mooney and Lee, 1999; Robinson and Redford, 1986). The annual birth rate in free-ranging monkeys is reported as 0.29 or an interbirth interval of 37 mo (Nishimura, 2003; Robinson and Redford, 1986). The gestation period lasts 225 d, with infants born at *ca.* 10% of their adult mass (Mooney and Lee, 1999). Mothers typically nurse infants for 18–24 mo (Mooney and Lee, 1999). Captive woolly monkeys reach reproductive maturity later than their free-ranging counterparts do (averages of 6–7 yr vs. 4–5) and reproductive females have a mean life longevity of 13 yr versus a much longer life span for both free-ranging individuals and captive nonreproductive females (Timmer, 2006). Therefore, captive individuals that are able to reproduce have a much shorter life span.

ALTERNATIVE RESEARCH

The Apenheul Zoo in The Netherlands has used herb gardens to introduce Amazonian herbs from many plant families. They are useful for health problems related to high blood

pressure, liver deficiencies, and diarrhea (Vermeer, 1994a). Therefore they may also benefit woolly monkeys. Other zoological institutions have similarly incorporated feeding over-the-counter Amazonian herbs (Amazon Heart Support and Graviola, Raintree Nutrition, Inc., Carson City, NV, 89701) in hopes of decreasing hypertension and health problems in captive woolly monkeys (Ange-van Heugten *et al.*, 2007). There is no publication on medical effects of the supplements.

Several institutions holding woolly monkeys have instituted feeding schedules to deliver sugar loads gradually throughout the day to combat potential diabetic-like conditions (Vermeer, 1994b). Many institutions have taken woolly monkeys off exhibit to reduce stress and have stopped breeding in order not to sacrifice the current individuals to potential pregnancy-related health problems. The method is obviously problematic, and a short-term solution at best.

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Chapter 3:

Sixteen Year Review of the Life Expectancy of the Worldwide Captive Woolly Monkey (*Lagothrix lagotricha* sp.) Population with Spider Monkey (*Ateles* sp.) Comparative Data

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In Review

ABSTRACT

From preliminary data on woolly monkeys (*Lagothrix spp.*) it appears that they are difficult to successfully breed and maintain in captivity although captive life history data on this species is limited. This species is endangered in the wild and thus animal conservationist need to be aware of its potentially serious negative plight in captivity as well. Written survey reports, International Species Information System (ISIS) historical record analysis and woolly monkey specific zoological studbook record analysis were completed to gather data on the survivability of the captive woolly monkey from 1990 to 2005. Spider monkeys (*Ateles sp.*) are one of the closest living relatives of the woolly monkey. They are considered relatively successful in captivity and therefore the woolly monkey historical data was compared to this species from five representative institutions for further analysis. In the past 16 years, the total woolly monkey population has decreased by 11%. The number of institutions holding this species has decreased and the birth to death ratio is negatively inverted (0.65) compared to the spider monkey (1.26). Thus one woolly monkey death is reported for every 0.65 births and this is even more pronounced in the female population (0.47 birth to death ratio). New import regulations and the resulting decrease in captive monkeys along with the captive problems observed make woolly monkeys a priority for research. Attention is needed for this species to be viable in captivity and to ensure their free-ranging status is not elevated. Primary known causes of death in woolly monkeys included cardiovascular events, infant and maternal failures to thrive and the genetic relationship to reproductive success all need further examination with particular interest in nutritional aspects potentially involved.

Keywords: captive management, new world monkey, survivability

INTRODUCTION

Woolly monkeys are threatened in the wild with *L. lagotricha* listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2007). Therefore, trade must be controlled for this species to not be critically endangered (IUCN 2004; Nowak 1999). This research project was formulated in attempt to better understand the plight of woolly monkeys in captivity. Captive woolly monkeys have noted high prevalences of health problems including heart disease, hypertension and reproductive disorders that may have nutritional origins (Ange-van Heugten et al. 2008). Although never specifically compared and published for captive animals, woolly monkeys are historically considered less reproductively successful and healthy in captivity than their close relative spider monkey species (*Ateles sp.*) (Ford and Davis 1992; Strier 1992). Thus, the current research hypothesis was that the woolly monkey population is gradually declining in captivity and that it is performing more poorly than one of its closest relatives with regard to overall health, nutrition related disorders and reproduction problems.

ANIMALS AND METHODS

Monkey information was gathered by distributing written surveys and researching historical data from ISIS (International Species Information Systems) and the European species studbook for the following parameters: zoological institution, number of housed monkeys per year, gender, age, birth and death records and reasons for death. Information was collected from the years 1990 to 2005. All numbers reported per year are based on the total population as of December 31st of that year. Free-ranging woolly monkeys are currently separated into four species (*Lagothrix cana*, *Lagothrix lagotricha*, *Lagothrix lugens*, and *Lagothrix poeppigii*) (Di Fiore and Campbell 2007) or four subspecies by the same name (Nowak, 1999) depending on the source. Classifications have changed over the years and are

disputed, thus this research report and and most historical records analyzed make no distinction between the four species / subspecies or their hybrids. The five zoos reporting their spider monkey information for comparison held a total of three different species: *Ateles chamek*, *Ateles geoffroyi*, and *Ateles fusciceps*.

Surveys were distributed either via mail or in person to all institutions reported to hold woolly monkeys worldwide. Eight institutions in the United States holding spider monkeys were contacted and again given the survey either in person or via mail. Five of these institutions responded and were used for comparison. The current research project results were compiled with the information available via: 1) the returned surveys 2) ISIS historical records and 3) the woolly monkey studbook records. Therefore, data were available for woolly monkeys in the last 16 years.

RESULTS AND DISCUSSION

Woolly monkeys included in this research project were only from institutions that report to the species studbook, update their ISIS information, or were willing to respond to the written survey. Thus, some monkeys were likely unaccounted for, however, they are thought to represent only a relative small part of the worldwide woolly monkey population. While the current research is primarily concerned with the plight of woolly monkeys in captivity, data was also collected for comparison to their close relatives, the spider monkey (*Ateles spp.*). According to ISIS (2005), there were 1076 spider monkeys in captivity in 2005. This is over 10 times the woolly monkey population. For comparative purposes, the survey was completed by five of eight randomly selected United States institutions that hold large numbers of spider monkeys.

In the institutions who participated in the woolly monkey portion of the survey there were 86 confirmed captive woolly monkeys by the end of 2005 compared to 97 at the end of 1990.

Of the 20 worldwide institutions contacted via the survey for woolly monkeys, 14 of these still held woolly monkeys and responded to some portion of the survey. Four institutions indicated that they no longer had the species but did not elaborate and two did not respond at all. Over the period of 16 years, the woolly monkey population gradually decreased by 11% (Fig. 1). The results include data from 2005, however, this information is not entirely complete because the studbook was not complete for this year at last review and the surveys were returned before December 31st. The institutions housing woolly monkeys changed dramatically since 1990. In total, 38 different institutions housed this species in the 16 year study period. However, the total number of yearly holding institutions only decreased from 16 to 14. Only 15 of the 38 zoos reported woolly monkey births at their location, while 32 reported deaths. Of the zoos reporting more than two births, only one had more births than deaths (65 births and 56 deaths). The next best zoo with a breeding program had equal numbers of births and deaths (14 of each). The third best had 19 births and 25 deaths although they stopped breeding due to animal health concerns during the 16 year period. Many other locations also did not allow breeding although they held the species.

In total, 229 woolly deaths were recorded in 16 years. These numbers include some animals that were likely stillbirths but this was not clear from the records. In contrast, 148 monkeys were born. These numbers may also reflect some stillbirths erroneously reported as births. Over the whole period, the total number of deaths exceeds the number of births by 81 animals (148 births and 229 deaths) (Figure 1 and Table I). Less females were born than males (52 to 71) and more females than males died (110 to 96). This could indicate a potential for inbreeding concerns due to a small captive female population. Reproductive problems and failure to increase numbers via reproduction in captivity have previously been documented in this species (Debyser 1995; Muller et al. 1989; Ruedi and Heldstab 1980).

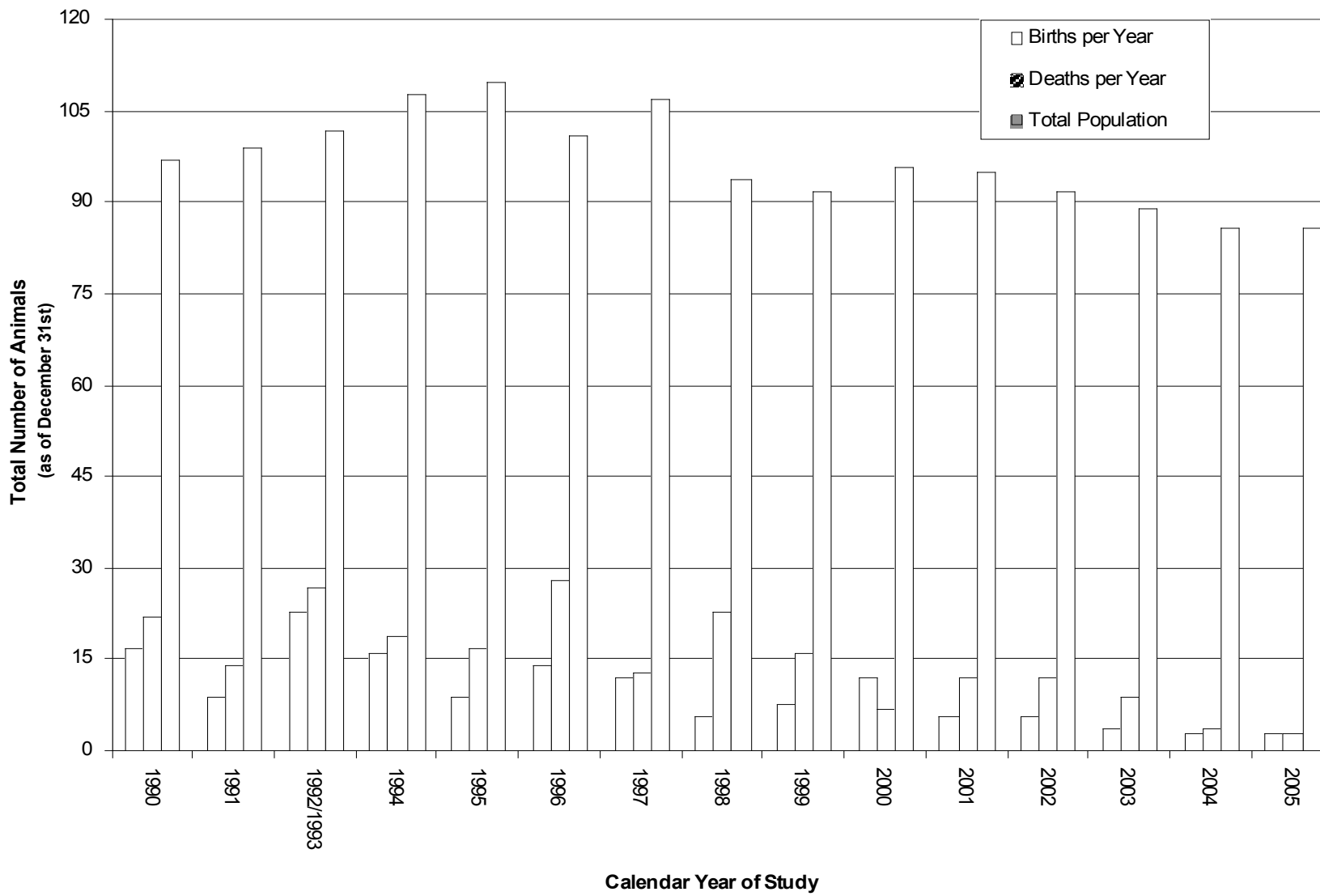


Figure 1. Total woolly monkey population including births and deaths per year in the period from 1990 to 2005

TABLE I. Overview of Total Births and Deaths and Birth to Death Ratios per Gender for Woolly and Spider Monkeys in the Period from 1990 to 2005

	Male	Female	Unknown sex	Total
Woolly Monkeys				
Total Births	71	52	25	148
Total Deaths	96	110	23	229
Birth to Death Ratio	0.74	0.47	1.09	0.65
Spider Monkeys				
Total Births	13	21	14	48
Total Deaths	10	14	14	38
Birth to Death Ratio	1.30	1.50	1.00	1.26

Table II provides a summary of deaths with reported causes, although for the majority of monkeys these causes were unknown or not investigated. Some animals may be included in more than one category because some causes of death were multifactorial. Failure to thrive was the most common listed reason for death. Failure to thrive is defined as any animal that is born and dies before its first birthday. Some of these likely include stillbirths and infants that died as a result of adults fighting over them. The second most common reason for death was heart and hypertension related complications and the third most common reason was bacterial and protozoan infections, including at least eight animal deaths from toxoplasmosis. The three top causes of death were previously documented (Ange-van Heugten et al. 2008; Gyimesi et al. 2006).

TABLE II. Woolly Monkey Reported Causes of Death from 1990 to 2005^a

Likely Causative Factor	Number of Animals
Anemia	1
Bacterial or protozoan infection	16
Failure to thrive ^b	65
Heart or hypertension related disease	18
Injury (fall or suspected fall)	5
Kidney disease	3
Liver disease (Hepatitis included)	11
Pregnancy complications	4

^aSome animals are listed by more than one factor. Many animals did not have necropsy results or have a list of possible causes that could be summarized appropriately.

^bFailure to thrive is defined as any animal that is born and dies before its first birthday.

The ages of the woolly monkeys that were alive by the end of 2005 were 0 to 36 years. The average age of the females was 15 ± 11.0 (mean \pm SD) years, and the average age of the males was 12 ± 7.4 years. The average age of death between 1990 and 2005 was 11 ± 8.1 years for females and 8 ± 7.6 years for males. It is clear that a few geriatric animals skewed the overall average age for the woolly monkeys. The oldest animal in this research was 36 years and the average age of the animals was 13.6 years indicating the animals die too young. Interestingly, the oldest females appear to be animals that were never bred or were less reproductively successful. Wild woolly monkeys are thought to mature sexually at 4 to 5 years for both males and females with female reproductive success until age 20 (Mooney and Lee 1999; Robinson and Redford 1986). The oldest female in the present study to have a baby was 27 years and the average age of giving birth was 12.7.

An important facet of the captive woolly monkey population studied in the survey was that most institutions stopped importing wild animals for breeding during the study period. Animal numbers were previously replenished from monkeys either captured in the wild or donated from the public. Removing this species from the wild is now more difficult due to tougher government restrictions and the potential negative impact of captivity. The number of woolly monkeys in captivity is theorized to continue to trend downward as reflected in Figure 1. Numerous animals in this 16 year period were either born in the wild or were public animal donations with unknown birth location. With these animals no longer being added, there is no doubt that this captive species is in jeopardy.

Data from the five responding spider monkey institutions showed that these institutions held 60 spider monkeys. An overview of the births and deaths in the period of 1990 to 2005 is given in Table I. In contrast to the woolly monkey, the population of spider monkeys at these institutions increased. The ages of the spider monkeys in this research, were between 0 and 32 years. The average age of the females was 15 ± 8.9 years, and the average age of the males was 9 ± 6.9 years old. The average age of death of females that died between 1990 and 2005 was 10 ± 9.9 years and for males it was 10 ± 10.7 years. In comparison with woolly monkeys, spider monkeys in this research had similar average ages and ages at death for females. The woolly monkey male's average age was higher yet their average age at death was lower. The reasons for this are unknown although spider monkeys have been previously recorded in zoological institutions at older ages than woolly monkeys (Nowak 1999).

Spider monkeys are more reproductively successful than woolly monkeys. Their birth to death ratio was 1.26 (1.26 live animals were born for each animal that died) while this is almost completely inverted for the woolly monkey that had a birth to death ratio of 0.65.

Being that they are so closely related and difficult to isolate from one another, the captive woolly monkey species were not separated in the historical records examined and therefore

the authors of this report also did not separate the species or isolate hybrids. Similarly, there were three spider monkey species analyzed. Although these three species are typically separated in captivity unlike the woolly monkeys, this should not confound the presented data being that these three spider monkey species are so closely related that some authors still consider them the same species of *Ateles paniscus* (Nowak 1999).

Due to the historical nature of the current research trial we were not able to compare captive housing situations or social groups between monkey species or among different housing institutions. While this information could potentially affect the results presented, the authors feel this would not change the outcome of the paper being that housing and social grouping vary considerably amongst all institutions regardless of species housed or institution location.

In summary, these retrospective data regarding the captive plight of the woolly monkey are alarming. It is known that this species is in danger in the wild and it appears that zoological institutions have limited success (CITES 2007; IUCN 2004; Nowak 1999). Only one institution has managed to breed several generations of the woolly monkey. This colony of animals seems to have provided the majority of the reproductive events analyzed either at this institution or when transferred to other facilities. Thus, lack of diversity in woolly monkey genetics may have a negative influence on reproductive success within this captive population. In order for the woolly monkey population to be successful in captivity, immediate action is needed. The following important possible research possibilities are proposed: 1) do woolly monkeys in the wild have distinctly lower reproductive rates; 2) do woolly monkeys have unique nutritional or housing specifications when compared to their close relatives; and 3) do woolly monkeys acquire metabolic conditions more easily than their close relatives and therefore their diets need to be more closely monitored. A

comprehensive follow-up study comparing housing, management and diet at multiple institutions that house woolly monkeys along with data from wild animals is vital.

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Chapter 4:

Serum Chemistry Concentrations of Captive Woolly Monkeys (*Lagothrix lagotricha*)

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ABSTRACT

Woolly monkeys (*Lagothrix* sp.) are threatened species and numerous zoos have failed to sustain successful populations. The most common causes of death in captive woolly monkeys are pregnancy and hypertension related. The objective of this retrospective study was to evaluate serum concentrations of a large number of captive woolly monkeys to establish baseline means and compare these concentrations to their closest related species to determine potential abnormalities. Serum analyses from 30 woolly monkeys housed at two institutions (Apenheul, The Netherlands and The Louisville Zoo, USA) over 12 years were collected. The statistical model included gender, age group (young, 0 to 4 yr of age; middle, 5 to 9 yr; and old, 10+ yr) and zoological institution. All panel result means were similar to previously reported concentrations for howler (*Alouatta* sp.) and spider monkeys (*Ateles* sp.) with the possible exception of alanine aminotransferase (ALT) and gamma-glutamyl-transferase (GGT) being higher while creatinine and P were lower. The serum glucose mean of 6.7 mmol/L is above the baseline range for humans and spider monkeys. Alkaline phosphatase, ALT, and Na were higher in females and Mg was higher in males ($P < 0.05$). Alkaline phosphatase, Mg, and P were highest ($P < 0.05$) and Ca and Na tended to be highest ($P < 0.10$) in the oldest animals. Ferritin tended to be highest ($P < 0.10$) in the oldest animals. Albumin, alkaline phosphatase, chloride, Na and total bilirubin were higher for Zoo A while GGT, glucose and lactate dehydrogenase were lower for Zoo A ($P < 0.05$). Areas of potential woolly monkey health risk were discussed. Future studies are needed to determine free-ranging serum concentrations to elucidate parameters that contain aberrant concentrations and decrease health status.

Key Words: captive animal health; non-human primate; baseline serum concentrations.

INTRODUCTION

The woolly monkey (*L. lagotricha* sp.) is a threatened species and one of its four known subspecies, *L. l. lugens* is considered especially vulnerable [IUCN, 2004; Nishimura et al., 1992]. Their threatened status is especially problematic because they are sought after as both a source of food and as a popular pet. These animals replenish their numbers slowly compared to other primate species and they are not tolerant of habitat destruction [Mooney and Lee, 1999; Nishimura et al., 1992]. To conserve the species, numerous zoos have attempted to house breeding populations. Many of these zoos have failed for various reasons. These reasons include the most common causes of death which are stillbirth, pregnancy complications and hypertension related conditions such as diabetes, congestive heart failure, and renal failure [Giddens et al., 1987; Miller et al., 1995]. It has been reported that over 16,000 woolly monkeys have been imported into the United States since 1960 for both public and private ownership and less than 8 animals reportedly remain in zoological institutions [Franceschini et al., 1997; ISIS, 2007].

The primary objectives of this retrospective study are: 1) to publish baseline serum concentrations for captive woolly monkeys; 2) compare serum concentration means by gender, age groups and zoological institution to potentially isolate differences; 3) compare overall serum concentration means to the closest relatives of the woolly monkey; and 4) potentially isolate abnormalities that may affect captive woolly monkey longevity.

MATERIALS AND METHODS

Animals

Data from serum chemistry analyses from 30 woolly monkeys housed at two institutions (Apenheul, Apeldoorn, The Netherlands and The Louisville Zoo, Kentucky, USA) were collected. These data consisted of blood samples taken over twelve years (1992 to early

2004). The blood samples were both from routine collections from apparently healthy animals and animals which were investigated for illness or pregnancy. Due to the different diagnostic procedures at the two institutions and the incomplete health history information for some animals, all animals were included in the data set unless their values were considered a statistical outlier as later described. Thus we were not able to group animals into sub category by health condition due to the small population size and variable diagnostics. All animals were housed in their usual exhibit areas and were fed diets that were considered nutritionally adequate by their institution as compared to published new world primate nutrient requirements [NRC, 1978; 2003]. However, exact diet items provided and diet nutrient analyses were not available for all twelve years of the data collection. Thus, serum concentration data could not be compared to diet. If possible, animals were fasted overnight prior to immobilization, and veterinary staff collected approximately 12 ml of femoral blood, drawn from the vein by palpating the artery, into appropriate analysis tubes. The protocol was performed according to the animal care guidelines of each institution.

Serum Concentrations

Serum concentrations measured included: albumin, alkaline phosphatase (ALP), alanine transferase (ALT), aspartate transferase (AST), calcium (Ca), chloride (Cl), total cholesterol, creatinine, ferritin, folic acid, gamma-glutamyl-transferase (GGT), glucose, lactate dehydrogenase (LDH), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), total protein, total bilirubin, triglycerides, and urea. The samples from The Louisville Zoo were measured by Antech Diagnostics (Alsip, Illinois, USA) using a Roche Hitachi 747 and 911 (Roche Diagnostics Corporation, Indianapolis, IN, USA) while the samples from Apenheul were measured by Medische Laboratoria Ziekenhuiscentrum, (Apeldoorn, The Netherlands) also using a Roche Hitachi 747.

Statistical Methods

Woolly monkeys were grouped into three age categories, which included a young group (0 to 4 yr of age), a middle group (5 to 9 yr), and an old group (10+ yr). Animals in the young group were those considered to be animals not sexually mature, while the old group was considered to be geriatric status animals. On average, captive reproductive females stop reproducing before the age of 11 yr and they died by about age 13 yr [Mooney and Lee, 1999]. Skewing the results due to one animal being represented more than one time within each age group was avoided as follows. Data from animals that were sampled more than once in each of the three age groups had their concentrations averaged together within age group, therefore each individual was represented only once per age group in all of the statistical analyses. For example, out of the 111 collections a maximum of 45 remained after means were calculated by animal within age group. The only exception was that the overall means were calculated as the mean of the individual means for each animal. Outlier analysis was performed using the univariate procedure of SAS. If a serum concentration was 3 times the standard deviation smaller or greater than the mean, the value was considered abnormal and was not used. Statistical analyses were performed using the General Linear Models procedure of SAS. The model included age group, gender, the calendar year the sample was taken and zoo. Least squares means \pm SEM were calculated.

RESULTS

The serum concentrations means of the woolly monkeys sampled in this study are presented in Table 1. Concentration means also are compared with published literature from their two closest related species (howler and spider monkeys) and humans in this table. The sample numbers for the published literature for the howler and spider monkeys as well as their living status (free-ranging or wild) are also presented in this table. Results of serum

comparisons by gender and age groupings are presented in Table 2 and statistical differences are shown by subscripts. Average age and numbers within each gender are shown. Serum concentrations between the two zoological institutions are shown in Table 3 and the statistical differences are presented by superscript. Per institution, average age and number within each gender are also shown. Serum concentration results per calendar year are not presented in tabular form because no differences by year (i.e. no changes over time) were noted. Potentially relevant serum concentration differences were noted in all three tables and are subsequently discussed.

DISCUSSION

Serum Comparison by Species

For the majority of serum concentrations, woolly monkey averages correspond well with their two closest related species, the howler (*Alouatta* sp.) and spider monkeys (*Ateles* sp.) [Crissey et al., 1999; Crissey and Ange, 2001; Crissey et al., 2003; Karesh et al., 1998; Vie et al., 1998]. The woolly monkey is thought to be most closely related to either the howler monkey or the spider monkey [Dunlap et al., 1985; Ford and Davis, 1992; Strier, 1992]. Although some captive management challenges exist with selected spider and howler subspecies, when compared to the woolly monkey, these species are successfully maintained and bred in captive institutions [ISIS, 2007].

Serum levels of ALT and GGT were elevated compared to the published baseline concentrations for various spider and howler monkeys subspecies. Woolly monkey serum AST and GGT were also elevated when compared to human average concentrations. Conversely, levels of creatinine are lower than howler and spider monkey published concentrations and woolly monkey P is lower than howler monkey concentrations. Research in humans has shown that elevated serum enzymes such as ALT, AST and GGT, are

associated with cardiovascular risk, including hypertension related disorders as noted in many captive woolly monkeys [Schindhelm et al., 2007; Whitfield et al., 2002]. Low concentrations of creatinine in serum have been associated with liver disease and low muscle mass [Kamath et al. 2001; Rahn, et al., 1999]. There are reported health problems within woolly monkey populations with regard to liver and muscle disorders (especially as related to pregnancy weight gain) [Ange-van Heugten et al., 2008; Lanford, 1998; Mooney and Lee, 1999].

Although P levels (1.5 mmol/L) were low compared to howler monkeys (2.5 mmol/L), we do not consider them a concern due to the normal Ca concentrations and the fact that they were close to spider monkey means [de Thoisy et al., 2001; Karesh et al., 1998; Vie et al., 1998]. The howler monkey P values are reflective of almost 190 red howlers individuals and all of which live in free-ranging conditions [de Thoisy et al., 2001; Vie et al., 1998]. Thus, the P differences shown within the current research, likely are reflective of higher P content in the Amazonian diet due to reported preferential insect consumption [Dew, 2005]. Serum P levels above 0.4 mmol/L may be associated with cardiovascular disease in humans and, therefore, a closer evaluation of P in woolly monkey serum may be warranted [Dhingra et al., 2007].

While howler and spider monkey published values were unavailable for comparison, serum ferritin in woolly monkeys (689 ± 204.0 pmol/L) was high compared to average human concentrations (45 to 450 pmol/L). Elevated ferritin in primates has been associated with numerous diseases including diabetes, hypertension, liver disease and atherosclerosis and is considered a general indicator of inflammation. Therefore the oldest woolly monkeys with the highest concentrations may have a higher risk for these diseases [Cutler, 1989; Williams et al., 2006].

Table 1. Woolly monkey serum concentration means compared to published concentrations for closely related species and humans.^{a,b,c}

Serum Parameter	Mean \pm SEM	Min and Max	Spider Monkey	Howler Monkey	Human Published
		Concentrations	Published Data	Published Data	Data
Albumin, g/L (n=24)	44 \pm 1.3	30 - 57	46 \pm 3.8 ⁵	29.5 ⁶	35 – 52
Alkaline phosphatase, U/L (n=26)	139 \pm 19.8	36 - 527	316 \pm 253.9 ⁵	129 – 366 ^{3,4,6}	30 – 120
Alanine aminotransferase, U/L (n=28)	42 \pm 4.5	19 - 126	8.2 \pm 2.6 ⁵	19 – 30 ^{3,4,6}	7 – 56
Aspartate aminotransferase, U/L (n=28)	80 \pm 9.3	34 - 249	37 \pm 8.1 ⁵	79 – 175 ^{3,4,6}	0 – 35
Calcium, mmol/L (n=13)	2.5 \pm 0.03	2.3 - 2.7	2.21 \pm 0.103 ⁵	2.6 ^{4,6}	2.1 – 2.6
Chloride, mmol/L (n=22)	101 \pm 1.3	91 - 113	99 \pm 3.2 ⁵	106 – 108 ^{4,6}	101 – 112
Total cholesterol, mmol/L (n=22)	3.4 \pm 0.20	1.9 - 5.6	3.1 - 5.63 ^{1,5}	2.7 - 3.2 ^{4,6}	< 5.2
Creatinine, μ mol/L (n=28)	76 \pm 4.8	44 - 148	117 \pm 18.6 ⁵	90 – 119 ^{3,4,6}	50 – 110
Ferritin, pmol/L (n=11)	689 \pm 204.0	100 - 1865	NA	NA	45 - 450
Folic acid, nmol/L (n=12)	34 \pm 1.7	22 - 43	NA	NA	> 7.3
Gamma glutamyl transferase, U/L (n=16)	63.5 \pm 10.2	25 - 176	54.4 ²	33.5 - 38.0 ^{4,6}	0-51
Glucose, mmol/L (n=25)	6.7 \pm 0.42	3.6 - 11.9	4.5 \pm 1.24 ⁵	5.9 - 6.4 ^{4,6}	3.9 – 6.1
Lactate dehydrogenase, U/L (n=18)	350 \pm 40.1	167 - 740	335 \pm 47.2 ⁵	305 – 1599 ^{3,4,6}	88 – 230
Magnesium, mmol/L (n=8) ^d	1.1 \pm 0.10	0.9 - 1.8	1.0 \pm 0.78 ⁵	1.4-1.5 ^{4,6}	0.7 – 1.1
Phosphorus, mmol/L (n=8)	1.5 \pm 0.10	1.1 - 2.0	1.7 \pm 0.35 ⁵	2.5-2.6 ^{4,6}	0.8 – 1.5

Potassium, mmol/L (n=22)	4.2 ± 0.14	2.8 – 5.5	3.8 ± 0.44 ⁵	5.1 - 5.3 ^{4,6}	3.5 – 5.0
Sodium, mmol/L (n=23)	138 ± 1.0	130 - 147	133 ± 3.1 ⁵	150 – 152 ^{4,6}	135 – 145
Total protein, g/L (n=18)	71 ± 2.0	60 - 94	85 ± 9.4 ⁵	63 – 91 ^{3,4,6}	60 – 84
Total bilirubin, µmol/L (n=17)	7.9 ± 1.1	2 - 16	2.2 ± 0.86 ⁵	15.1 - 15.2 ^{4,6}	0 - 17
Triglycerides, mmol/L (n=6)	0.6 ± 0.08	0.3 - 0.8	1.7 ± 0.3 ^{1,5}	0.9 - 1.4 ^{4,6}	0.5 – 1.7
Urea, mmol/L (n=28)	8.7 ± 1.2	1.7 - 34.9	0.9 ± 0.79 ⁵	4.9 - 8.6 ^{3,4,6}	4.0 – 8.2

^aAnimals with more than one sample within the data set had their mean calculated prior to calculating overall means (therefore no animal contributes more than once to this data set)

^bSuperscript numbers refer to literature cited as follows (the number of observations and animal location for each reference is indicated parenthetically): #1 (Crissey et al., 1999; n=7; captive); #2 (Crissey and Ange, 2001; n=8; captive); #3 (Crissey et al., 2003; n=6; free-ranging); #4 (de Thoisy et al., 2001; n=91; free-ranging); #5 (Karesh et al., 1998; n=6; free-ranging); #6 (Vie et al., 1998; n=68-91; free-ranging)

^cAll human reference data are as reported in Laguna and Claudio, 2004 except GGT and Total bilirubin from Medline Plus, 2008.

^dThe concentrations reported for magnesium in Ref #7 & Ref #33 were originally published as µmol/L, however, these concentrations were later corrected to be mmol/L.

Table 2. Woolly monkey serum concentration LSMeans \pm SEM separated by gender and age groupings.^a

	Animal Gender		Animal Age Group ^b		
	Male	Female	Young	Middle	Old
Average age, yr	8.0	7.0	2.9	6.9	12.7
Serum Parameter					
Albumin, g/L	44 \pm 1.4 (n=29)	47 \pm 2.6 (n=10)	46 \pm 2.1 (n=14)	48 \pm 2.1 (n=16)	43 \pm 2.6 (n=9)
Alkaline phosphatase, U/L	137 \pm 12.7 ^c (n=30)	187 \pm 21.3 ^d (n=13)	269 \pm 18.5 ^c (n=15)	101 \pm 17.4 ^d (n=18)	115 \pm 21.9 ^d (n=10)
Alanine aminotransferase, U/L	34 \pm 4.3 ^c (n=31)	55 \pm 7.0 ^d (n=14)	45 \pm 6.3 (n=15)	46 \pm 5.9 (n=19)	43 \pm 7.1 (n=11)
Aspartate aminotransferase, U/L	70 \pm 11.7 (n=29)	79 \pm 19.0 (n=14)	59 \pm 16.9 (n=15)	94 \pm 17.0 (n=17)	71 \pm 19.0 (n=11)
Calcium, mmol/L	2.4 \pm 0.05 (n=19)	2.5 \pm 0.07 (n=4)	2.6 \pm 0.06 ^e (n=8)	2.4 \pm 0.05 ^f (n=8)	2.5 \pm 0.06 ^{e,f} (n=7)
Chloride, mmol/L	102 \pm 1.2 (n=27)	105 \pm 2.0 (n=11)	104 \pm 1.7 (n=13)	104 \pm 1.7 (n=15)	103 \pm 2.0 (n=10)
Total cholesterol, mmol/L	3.4 \pm 0.19 (n=26)	3.4 \pm 0.31 (n=11)	3.4 \pm 0.26 (n=13)	3.2 \pm 0.26 (n=14)	3.6 \pm 0.30 (n=10)
Creatinine, μ mol/L	83 \pm 8.5 (n=31)	65 \pm 14.1 (n=14)	58 \pm 12.6 (n=15)	82 \pm 11.8 (n=19)	83 \pm 14.2 (n=11)
Ferritin, pmol/L	1146 \pm 272.8 (n=5)	613 \pm 218.0 (n=6)	333 \pm 209.2 ^e (n=6)	715 \pm 301.5 ^{e,f} (n=3)	1589 \pm 402.2 ^f (n=2)
Folic acid, nmol/L	38 \pm 2.6 (n=6)	32 \pm 1.9 (n=9)	34 \pm 2.3 (n=6)	31 \pm 2.3 (n=6)	40 \pm 3.6 (n=3)
Gamma glutamyl transferase, U/L	70 \pm 10.2 (n=19)	59 \pm 14.8 (n=8)	50 \pm 12.9 (n=9)	74 \pm 11.3 (n=10)	70 \pm 12.3 (n=8)
Glucose, mmol/L	5.9 \pm 0.48 (n=29)	6.8 \pm 0.78 (n=13)	6.2 \pm 0.68 (n=15)	6.8 \pm 0.64 (n=18)	6.0 \pm 0.83 (n=9)

Lactate dehydrogenase, U/L	286 ± 55.5 (n=14)	234 ± 74.1 (n=9)	276 ± 57.1 (n=11)	341 ± 72.2 (n=8)	164 ± 95.3 (n=4)
Magnesium, mmol/L	1.7 ± 0.06 ^c (n=9)	0.7 ± 0.15 ^d (n=1)	1.4 ± 0.08 ^e (n=3)	0.6 ± 0.13 ^d (n=2)	0.7 ± 0.10 ^d (n=5)
Phosphorous, mmol/L	1.5 ± 0.07 (n=7)	1.7 ± 0.31 (n=1)	1.9 ± 0.16 ^c (n=7)	1.6 ± 0.20 ^c (n=6)	1.3 ± 0.20 ^d (n=5)
Potassium, mmol/L	4.3 ± 0.16 (n=27)	3.9 ± 0.26 (n=11)	4.2 ± 0.22 (n=14)	3.8 ± 0.22 (n=15)	4.3 ± 0.26 (n=9)
Sodium, mmol/L	137 ± 0.9 ^c (n=28)	142 ± 1.5 ^d (n=11)	141 ± 1.2 ^e (n=14)	142 ± 1.2 ^e (n=15)	137 ± 1.4 ^f (n=10)
Total protein, g/L	72 ± 2.0 (n=2)	71 ± 4.5 (n=5)	72 ± 2.7 (n=12)	68 ± 3.3 (n=12)	75 ± 4.3 (n=6)
Total bilirubin, µmol/L	10.6 ± 1.66 (n=21)	7.6 ± 2.60 (n=7)	7.1 ± 2.23 (n=10)	12.3 ± 2.09 (n=9)	7.9 ± 2.09 (n=9)
Triglycerides, mmol/L	0.6 ± 0.07 (n=10)	0.3 ± 0.24 (n=1)	0.5 ± 0.13 (n=5)	0.5 ± 0.20 (n=2)	0.4 ± 0.17 (n=4)
Urea, mmol/L	8.9 ± 1.78 (n=21)	8.1 ± 2.94 (n=14)	6.6 ± 2.64 (n=15)	8.9 ± 2.46 (n=19)	10.1 ± 2.96 (n=11)

^aFor statistical purposes, animals with more than one blood sample within the age group categories had their mean calculated prior to calculating overall means for gender and age group categories (therefore no animal contributes more than one time to each age or gender category).

^bAnimals were assigned to one of three groups based on age (young from 0 to 4 years; middle from 5 to 9 years; old ≥ 10 years).

^{c,d}Means ± SEM within a row and main category without a common superscript are different (P < 0.05).

^{e,f}Means ± SEM within a row and main category without a common superscript are different (P < 0.10).

Table 3. Woolly monkey serum concentration LS Means separated into zoological institutions.^a

	Zoological Institution	
	Zoo A	Zoo B
Average age, yr	7.7	7.3
Serum Parameter		
Albumin, g/L	43 ± 1.7 ^b (n=21)	49 ± 2.2 ^c (n=18)
Alkaline phosphatase, U/L	119 ± 14.0 ^b (n=25)	204 ± 19.5 ^c (n=18)
Alanine aminotransferase, U/L	46 ± 4.6 (n=27)	43 ± 6.6 (n=18)
Aspartate aminotransferase, U/L	91 ± 12.3 ^d (n=27)	58 ± 18.4 ^e (n=16)
Calcium, mmol/L	2.5 ± 0.06 (n=5)	2.5 ± 0.05 (n=18)
Chloride, mmol/L	99 ± 1.4 ^b (n=20)	108 ± 1.8 ^c (n=18)
Total cholesterol, mmol/L	3.3 ± 0.21 (n=19)	3.6 ± 0.28 (n=18)
Creatinine, µmol/L	79 ± 9.2 (n=27)	69 ± 13.2 (n=18)
Ferritin, pmol/L	879 ± 175.3 (n=11)	NT ^f
Folic acid, nmol/L	35 ± 1.9 (n=15)	NT
Gamma glutamyl transferase, U/L	84 ± 12.0 ^b (n=9)	46 ± 12.0 ^c (n=18)
Glucose, mmol/L	7.4 ± 0.53 ^b (n=24)	5.3 ± 0.71 ^c (n=18)
Lactate dehydrogenase, U/L	368 ± 41.0 ^b (n=19)	153 ± 90.8 ^c (n=4)
Magnesium, mmol/L	NT	0.9 ± 0.09 (n=10)
Phosphorus, mmol/L	NT	1.6 ± 0.28 (n=18)
Potassium, mmol/L	4.2 ± 0.18 (n=20)	4.0 ± 0.23 (n=18)
Sodium, mmol/L	138 ± 0.97 ^b (n=21)	142 ± 1.29 ^c (n=18)
Total Protein, g/L	74 ± 2.9 (n=12)	69 ± 3.1 (n=18)
Total Billirubin, µmol/L	4.4 ± 1.96 ^b (n=10)	13.8 ± 2.08 ^c (n=18)
Triglycerides, mmol/L	NT	0.5 ± 0.13 (n=11)
Urea, mmol/L	9.7 ± 2.49 (n=27)	14.2 ± 12.94 (n=1)

^aFor statistical purposes, animals with more than one blood sample within the age group categories had their mean calculated prior to calculating overall means for zoological institution (therefore no animal contributes more than one time to each zoo).

^{b,c} Means ± SEM within a row and main category without a common superscript differ (P < 0.05)

^{d,e} Means ± SEM within a row and main category without a common superscript differ (P < 0.10)

^f Not tested

Research regarding serum ferritin and baseline levels is ongoing for most primate species and should be further evaluated for woolly monkeys before any diet changes related to this issue are conducted [Williams et al., 2006]. It could be argued that the population size for ferritin within this study (n=11) is too low for proper assessment of ferritin results particularly considering our inability to accurately identify health impaired animals in the cohorts.

Glucose concentrations for woolly monkeys in the current study (6.7 ± 0.42 mmol/L) were slightly higher than those published for spider monkeys and humans (4.5 to 6.1 mmol/L) [de Thoisy et al., 2001; Karesh et al., 1998; Laguna and Claudio, 2004; Vie et al., 1998]. This is a potential concern because diabetes has been viewed as a problem for woolly monkeys and glucose values above 5.6 mmol/L in humans are considered abnormal [Ange-van Heugten et al., 2007; Ange-van Heugten et al., 2008; Laguna and Claudio, 2004; Vermeer, 1994]. However, research by Ange-van Heugten et al., 2007 showed that a population of six woolly monkeys with known health problems that were examined in 2004 had no evidence of elevated diabetic blood determinants (serum glucose, fructosamine, or glycated hemoglobin). They showed an average serum glucose level of 3.9 mmol/L. In the retrospective study reported here, analysis of serum glucose by calendar year from which the samples were taken did not show a decrease in glucose concentrations over time in either zoo. It is still possible that recent changes in woolly monkey diets to limit sugars as reported by Ange-van Heugten et al., 2007 may obscure the magnitude of the potential glucose problem as detected in the current long term retrospective study. The retrospective data may have had delays in centrifugation that could slightly alter glucose and thus the differences noted may be clinically insignificant. Regardless, it appears that serum glucose concentrations in woolly monkeys need further research.

Average lactate dehydrogenase for woolly monkeys is high when compared to human concentrations but appears appropriate when compared to both howler and spider monkey published concentrations. Similarly, alkaline phosphatase appears slightly high when compared to humans but not when compared to howler or spider monkeys.

Serum urea concentrations for woolly monkeys (8.7 ± 1.2 mmol/L) are slightly high when compared to the published average concentrations for both howler monkeys (4.9 to 8.6 mmol/L) and humans (4.0 to 8.2 mmol/L). They are extremely elevated when compared to the spider monkey published average (0.9 ± 0.79 mmol/L). High serum urea concentrations can be indicative of renal disease and should thus be monitored.

In most species the previously described serum differences would be medically insignificant; however, considering the health problems of woolly monkeys, they might bear further investigation. There are some differences between serum concentrations for woolly monkeys in this study and the serum chemistry values reported in the ISIS (International Species Information Systems) database. Those differences can be explained by the more limited data available for ISIS which does not include the large Apenheul collection, nor are ISIS data reported in a way that minimizes the impact of multiple samples from individual animals.

Serum Concentration Comparisons by Gender and Age Groupings

The average age for males and females were similar (8.0 yr for males and 7.0 yr for females) and the average ages for each group were 2.9 yr for the young age group, 6.9 yr for the middle group and 12.7 yr for the oldest group. When compared by gender, ALP, ALT and Na were higher among females ($P < 0.05$). This does not agree with findings reported by Vie et al. (1998) that male howler monkeys tended to have higher concentrations of ALP, ALT, and Na. These measures can be naturally elevated during pregnancy, and without detailed

notation of gestation or lactation in records of female monkeys in this retrospective study, we cannot rule out this cause for the apparent elevation. It is important to note that although the female concentrations of ALP are higher, they were still within the baseline concentrations for spider and howler monkeys [Crissey et al., 2003; de Thoisy et al., 2001; Karesh et al., 1998; Vie et al., 1998]. In contrast, the female ALT concentrations (55.2 U/L) were above the published concentrations for spider and howler monkeys (8.2 – 30 U/L). The elevated ALP and ALT noted in females could be a potential concern for liver and heart disease and bears further investigation [Schindhelm et al., 2007; Sorbi et al., 2000].

The elevated Na levels suggested in females in this study could be partially due to diet and water consumption preferences or competition for preferred diet items. The monkeys at both zoos were fed ad libitum and reported given a large variety of diet items. The high Na levels may be a concern because elevated Na levels are associated with hypertension risk in humans [Meneely and Battarbee, 1976]. Because the mean age of both genders was similar, the Na differences noted are not expected to be a reflection of age within gender. No changes in Na related to diet changes over calendar year were detected.

Glucose concentrations were not statistically different between males (5.9 mmol/L) and females (6.8 mmol/L). However, the slightly higher female concentrations may be problematic because diabetic and hypertensive related birth complications are observed during captivity. While diabetes and hypertension have also been reported in males, these conditions have historically been more pronounced with pregnancy [Ange-van Heugten et al., 2008]. Interestingly, the middle age group had the highest glucose concentration (6.8 mmol/L vs. 6.0 to 6.2 mmol/L) though this was not statistically significant. These animals are the most important reproductively and thus these levels could be an issue for the species longevity.

The lower Mg concentrations in females compared to males (0.7 mmol/L vs. 1.7 mmol/L) could be partially due to diet item preferences and potential pregnancies within the population [Fleet and Cashman, 2001]. While chronically low concentrations of Mg have been linked to cardiovascular disease, osteoporosis, and preeclampsia, both male and female levels appear within baseline limits for spider and woolly monkeys [Fleet and Cashman, 2001].

ALP, Mg, and P differed statistically with respect to age group ($P < 0.05$) while Ca, Ferritin, and Na showed less clear trends towards differentiation between age groups ($P < 0.10$). ALP is expected to be higher in young animals because of bone growth (269 U/L) compared to the middle and old groups (101 to 115 U/L) [Takenaka et al, 1988; Trangerud et al, 2007]. The higher Mg levels in young animals (1.4 mmol/L) compared to the older animals (0.6 to 0.7 mmol/L) may be related to none of the younger animals having the potential to be pregnant during the study period, and dietary preferences. The young animals may be forced to eat less preferred items compared to more dominant older animals. The lower P levels (1.3 mmol/L) for the oldest woolly monkeys could be a result of decreased bone mass or lowered gastrointestinal P absorption seen with age in other species [Melton et al., 1997].

The older age group had greater ferritin levels (1589 pmol/L) compared to the younger (333 pmol/L) and middle (715 pmol/L). As previously discussed, the high ferritin values may need further exploration. Although not significantly different, the numerically higher serum creatinine noted for males and for the two older age groups is as expected due to the expected increased muscle mass for these groups (Latimer et al., 2003).

Serum Concentration Comparisons by Zoo

The average ages of monkeys in both zoos were similar (7.7 years for Zoo A and 7.3 years for Zoo B). Therefore, differences noted are not a reflection of age. Albumin, ALP, Cl, GGT, glucose, LDH, Na and total bilirubin concentrations differed by zoo ($P < 0.05$). These differences may be partially related to different laboratories conducting the analysis for the two zoos, different diet items reportedly fed in the separate institutions due to country specific food availability, and that different medications were occasionally used. Means for animals housed in both zoos, however, did not differ from the published baseline ranges for spider and howler monkeys for albumin, ALP, Cl, Na and total bilirubin. The elevated GGT levels from Zoo A compared to the baseline concentrations for the comparator species could be a potential concern as previously described. LDH concentrations for Zoo B were lower than the published baseline concentrations. This could be a reflection of the sample size for this analysis because of the population from Zoo A being much smaller ($n=4$ versus $n=19$) than for Zoo B. Woolly monkeys in Zoo A had a much higher glucose concentration than those in Zoo B (7.4 mmol/L versus 5.3 mmol/L). This study cannot determine whether or not this could be a reflection of diet because the exact diet at the time each blood sample was collected is not known.

Medications and Novelty of Research

The 30 woolly monkeys in this trial were given various medications over the course of the twelve years and they were all anesthetized prior to blood collections using ketamine hydrochloride injected intramuscularly (approximately 22 to 29 mg/kg body weight). Ketamine has been shown to decrease glucose in both sexes of cynomolgus monkeys (*Macaca fascicularis*) and decrease triacylglycerides and albumin, while increasing total cholesterol in males [Kim et al., 2005]. Therefore, it is feasible that the use of this drug may

have caused baseline concentrations of glucose, albumin, and triglycerides to be underestimated, and cholesterol to be overestimated. Several of the monkeys in the current research also were given veterinary recommended medications at some period during the twelve years measured. As previously discussed, we did not remove these animals from the data set because most medications were routine and not all records were complete enough to eliminate all animals that had any medication. The average housed captive woolly monkey is an animal that most consider in need of medications for survival during significant portions of their livelihood. Thus, the normally housed woolly monkey is often a medicated one. Animals were not separated by medical conditions due to the small population that could reliably be documented to meet this criterion. It should be noted that the monkeys in the comparative literature were typically sedated, primarily with ketamine, and most of the captive animals in the comparative literature were also given recommended medications.

CONCLUSIONS

1. Compared to howler and spider monkeys all measured results appeared within baseline ranges with the possible exception of ALT, creatinine, GGT and phosphorus. Glucose was high in woolly monkeys compared to humans and spider monkeys.
2. Differences noted statistically between age groups, sexes, and institutions could be due to variables not controlled or known in this study (diet, health status, etc.) and bear further investigation in prospective studies.
3. Research with free-ranging woolly monkeys is suggested in order to potentially improve the low success of maintaining captive populations of these endangered species in zoological institutions and maintaining their populations in the wild.

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Chapter 5:

Evaluation of Diabetes Determinants in Woolly Monkeys (*Lagothrix lagotricha*)

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ABSTRACT

Woolly monkeys (*Lagothrix lagotricha*) are a threatened species in the wild with limited successful management in captivity due to diagnosed hypertension and suspected diabetic conditions. Six woolly monkeys with known hypertension problems were tested to determine if diabetes mellitus and current daily diet are underlying links to health problems for the captive population of this species. Blood and urine were collected and serum was analyzed for fructosamine, glucose, glycated hemoglobin, insulin, triacylglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol while urine was tested for glucose concentrations. Diet disappearance was determined for three days prior to blood collection and nutrient content was calculated using Zoo Diet Analysis (ZDA) computer program. Serum analyses were within normal ranges (fructosamine (139 to 242 $\mu\text{mol/L}$), glucose (2.22 to 4.78 mmol/L), glycated hemoglobin (3.52 to 4.73 %), insulin (6.2 to 13.0 $\mu\text{U/ml}$), triacylglycerides (0.38 to 3.4 mmol/L), total cholesterol (2.5 – 5.1 mmol/L), HDL-cholesterol (0.4 to 1.6 mmol/L), and LDL-cholesterol (1.8 to 3.4 mmol/L)). Urine glucose concentrations were below the detection limit. Diets were not limiting in starch and total sugars and were similar in nonstarch polysaccharides. Potential dietary deficiencies were noted for vitamin A, vitamin D, calcium, phosphorus, and selenium. When compared to the available primate reference ranges, the results do not indicate problems with diabetes mellitus or with glucose metabolism and therefore they are not causes of the diagnosed hypertension. Further research to ascertain the true cause of health related problems and the role of dietary factors is needed.

Keywords: Diabetes, diet, fructosamine, glycated hemoglobin, lipids, and woolly monkey

INTRODUCTION

The woolly monkey (*Lagothrix lagotricha*) is a relatively large bodied species of new world primates that is considered threatened in their natural habitat (Nowak 1999). They are often sought after as a source of food or for trade in the pet industry (Peres 1991; Shephard 2002). This species has larger time spans between offspring and they are reproductive for a shorter period of their lives when compared to many non-ape primate species (Mooney and Lee 1999; Nishimura et al. 1992). Woolly monkeys have a low tolerance to habitat destruction and their large body size and the relatively new use of fire arms for hunting make them particularly vulnerable (Peres 1991; Shephard 2002).

To conserve this species, numerous zoos have attempted to house breeding populations. Most zoos, however, have not been able to successfully replenish their own populations. The most common reported causes of death in captive woolly monkeys are pregnancy complications and hypertension related conditions such as congestive heart failure, renal failure, and cardiovascular events (Giddens et al. 1987; Miller et al. 1995; Muller et al. 1989).

Woolly monkeys have been reported to suffer from diabetes mellitus (Type II) or a unique sugar intolerance that detrimentally affects their captive health (Vermeer 1994). Vermeer (1994) indicated that six woolly monkeys in The Netherlands suffered from sucrose intolerance and that a pregnant female suffered from glucose, sucrose and starch intolerance. In addition, several woolly monkeys in the United States and the United Kingdom have been treated by their institutional veterinarians for diabetic conditions since the early 1970's. Human and non-human primate diabetics reportedly have an increased risk of developing atherosclerosis (Goldberg 2001; Howard 1982). Therefore, one of the main causes of deaths in captive woolly monkeys (hypertension) may be associated with diabetes (Wagner et al. 1996). Diabetes mellitus in humans is thought to worsen with pregnancy and this is also suspected to be true for woolly monkeys. Thus diabetic conditions could be responsible for

the pregnancy complications in this species. Literature regarding diabetes in woolly monkeys is sparse. Therefore, the current experiment was conducted to evaluate nutritional and blood parameters of a captive woolly monkey population to determine whether hypertension conditions may be associated with diabetes mellitus. The objectives of this study were to ascertain whether diabetes is a true problem and to determine if the current diet could contribute to any blood parameters that deviated from normal.

We decided to examine fasting concentrations of several serum diabetic determinants. We evaluated: 1) fructosamine to indicate the concentration of blood glucose control over the past two or three weeks, 2) glucose as a real-time indicator that the body does not produce sufficient insulin, 3) glycated hemoglobin as a view of long term blood glucose status, which is six to eight weeks or for the lifespan of affiliated red blood cells, and 4) insulin to determine whether a high blood glucose concentration is the result of insulin problems. Circulating lipids (high density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), total cholesterol, & triacylglycerides) were also evaluated as potential diabetes causative factors. The lipids were also measured as indicators for potential risk of heart and vascular disease.

SUBJECTS AND METHODS

Animals: Six of only eight woolly monkeys held in United States zoological institutions were used in this research. The other two woolly monkeys were not available for the current study due to one being ill and one being located at a separate institution. These animals have routine health exams conducted by a veterinarian and therefore blood could be collected for the current study in addition to the routine veterinary procedures. Most worldwide institutions holding woolly monkeys do not conduct routine health exams that allow blood draws due to the potential stressors on a sensitive species. The woolly monkey ages ranged from 3 yr to 16

yr and all were considered sexually mature except the youngest animal, which was the female. Two of the six monkeys were full siblings; a third had the same mother as these two and a fourth had the same father. Due to the small number of animals in captivity, inbreeding and low genetic diversity is common among all populations. All of the animals were housed similarly, having access to both inside and outside areas. Four of the animals were housed together and the other two were housed together in a separate yet similar enclosure. Body weight observations during this study were made by the primary woolly monkey caretaker who has more than twenty years experience working with this species in both captivity and the wild.

Diet evaluation: A three-day diet disappearance study was conducted for these monkeys at The Louisville Zoo, Kentucky, USA. This disappearance study is a measure of the amounts of dietary items provided for 72-hours prior to each animal being sedated for blood collections minus the food items they did not consume. The animal keepers and researchers measured the amount of each food item fed to the woolly monkeys for both their morning and evening meals and weighed back the portions of the food that the monkeys did not consume after each meal. These four animals were fed in separated pairs of two. The portions of the favored diet items were hand-fed. The items that were not hand-fed were assumed to be consumed equally by the primates due to animal keeper and researcher observations. The consumed diet was determined for each individual woolly monkey. Nutrient composition of the consumed diets was calculated using the ZDA (Zoo Diet Analysis; Allen and Baer Associates, Michigan State University and Zoological Society of San Diego) computer software program. The nutrients analyzed include: carbohydrates, fiber, total sugars, protein, fat, vitamin A, vitamin D, vitamin E, vitamin B₁, vitamin B₂, niacin, vitamin B₆, vitamin B₁₂, pantothenic acid, vitamin C, calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K),

sodium (Na), iron (Fe), selenium (Se), zinc (Zn), iodine (I), copper (Cu) and manganese (Mn).

Blood sampling and analysis: After diet disappearance was measured for 72 hours, the animals were fasted overnight as appropriate for sedation. The research protocol was performed under the approval of the Louisville Zoo Animal Research Committee. During the annual exam for each woolly monkey, the animals were sedated by a blow dart using ketamine hydrochloride intra muscular injection (22-29 mg/kg body weight). After blood pressures were measured, monkey anesthesia was maintained using isoflurane (1-4 %). Blood was collected from the femoral vein using a 19 gauge butterfly catheter into a vacuum tube and serum was subsequently analyzed for fructosamine, glucose, glycated (glycosylated) hemoglobin, high density lipoprotein (HDL) - cholesterol, insulin, low density lipoprotein (LDL) - cholesterol, total cholesterol and triacylglycerides. If required for analyses, the samples were centrifuged at the Louisville Zoo Veterinary laboratory using the Ultra-8 centrifuge at 1000 x g.

Fructosamine was analyzed by Antech Diagnostics (Alsip, Illinois, USA) by a Roche Hitachi 911 using Roche calibration reagents standardized via glycated poly-L-lysine with 300 μ L of serum (Cefalu et al. 1991). Glucose was analyzed by Antech Diagnostics using a Hitachi 747 analyzer Roche Hitachi 747 Analyzer (Roche Diagnostics Corp., Indianapolis, IN, USA) with 200 μ L of serum. In addition, 150 μ L serum was collected, centrifuged and immediately frozen for insulin analysis. Antech Diagnostics conducted the analyses by radio immunoassay (RIA) with a Coat-A-Count[®] Insulin Diagnostic Product Corporation (DPC) kit which uses an anti-human polyclonal insulin antibody attached to the wall of a polypropylene tube. (Los Angeles, CA, USA).

To measure glycated hemoglobin one ml of whole blood was collected in a tube

containing ethylenediaminetetraacetic acid (EDTA), chilled overnight and immediately shipped to be analyzed by Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, Louisiana, USA using the Helena Labs (Beaumont, Texas, USA) GLYCO-Tek affinity column kit (#5351). These GLYCO-Tek Affinity columns detect all glycosylated hemoglobins, not just HbA1.

The lipid profile (total cholesterol, triacylglycerides, HDL-Chol and LDL-Chol) was analyzed by The Simian Diagnostic Laboratory (Rockville, MD, USA) using the Alfa Wassermann Vet Ace analyzer (Woerden, The Netherlands). The LDL-cholesterol method was a calculation based on a modification of the Friedewald formula using total cholesterol minus HDL-cholesterol and triacylglycerides divided by 6.25 (representing very low density lipoprotein (VLDL)) (Friedewald et al., 1972). This calculation is valid for triacylglyceride concentrations up to 4.56 mmol/L (Friedewald et al. 1972).

Urine: Urine was collected by either catheterization or cystocentesis from each of the woolly monkeys while they were sedated for their yearly physicals and Multistix Pro Reagent Strips (Bayer AG, Leverkusen, Germany) were used to determine glucose concentration.

Blood pressure measurement: Both direct and indirect blood pressure measurements during sedation were averaged for each woolly monkey over the three years prior to this research and current monkey body weights were recorded. Direct blood pressure averages were a result of the pressure as measured intra-arterial during sedation with ketamine as previously described while indirect measures were recorded via the brachial artery using an arm cuff. Over the three year period, indirect measures were recorded on some occasions with ketamine sedation and on some occasions without.

RESULTS

Diet: The woolly monkeys in this research appeared to have insufficient intakes of Ca, P, Se, vitamin A and vitamin D when compared to the new world primate nutrient guidelines (Table 1) (NRC 2003). A few other possible nutrient deficiencies were noted with respect to the vitamins B₁ and B₂ and the minerals Zn and Cu (Table 1). However, the possible nutrient deficiencies for these four nutrients were typically for only one day of the trial and are likely not a concern if the diet is calculated over a weekly basis.

Each of the diet items and the percentages consumed by the woolly monkeys during the three-day trial are presented (Table 2). The individual food choices and items offered varied greatly over the course of the three-day measurement period. The nutritionally complete non-human primate diet was not consumed in large quantities and likely contributed to the possible deficiencies previously mentioned.

Blood and Urine: With the possible exception of low HDL-cholesterol values, the blood parameters measured in this study (Table 3) did not differ from the normal average non-diabetic values for humans and for new world monkeys or old world monkeys. In addition to the blood parameters, which appeared normal, the urine from each woolly monkey did not contain glucose.

Blood pressure and weight: The direct and indirect systolic and diastolic blood pressure measurement averages during animal sedation for the preceding three years for each monkey was calculated (Table 4). Five of the six monkeys had both systolic and diastolic blood pressure values higher than the average normal for humans and those reported as normal for their close relative the spider monkey (Miller et al. 1995; NIH, 2006; Srinivasan et al. 1980). The three monkeys that had indirect blood pressure measurements taken without ketamine

sedation are also presented. The woolly monkey ages and weights are also reported in Table 4. The woolly monkeys in this research had similar weights as their free-ranging counterparts (Nowak 1999). Three of the six monkeys were close to the upper end of this range and thought to be slightly overweight although not reported as obese by their primary caretakers. The monkeys in this research are not considered geriatric. The average ages for captive woolly monkeys at death between 1990 and 2005 are 7.6 yr and 10.5 yr (male and female, respectively) (Timmer 2006). The males in the current study (Table 4) were all older than these averages. However, numerous captive woolly monkeys have lived and successfully bred after age 30. Therefore we did not consider any monkeys in this research geriatric.

Numerous medications were given to the woolly monkeys during the course of this research. These medication uses and dosages are described (Table 5).

Table 1. Three day ranges in nutrient composition of consumed diets for each of the six woolly monkeys.* †‡

	#1's	#2's	#3's	#4's	#5's	#6's	Requirement†
	Consumed Diet	Consumed Diet	Consumed Diet	Consumed Diet	Consumed Diet	Consumed Diet	
Nutrient, units							
Carbohydrate, %	51-59	43-56	40-57	42-59	51-59	44-60	NA
Fiber, %	8.0-9.5	8.8-11.0	8.6-10.8	9.6-10.9	9.5-11.3	8.8-10.9	NA
Total Sugars, %	26-40	27-41	22-41	26-40	28-35	28-41	NA
Protein, %	16-21	16-23	18-21	17-24	17-19	15-23	15-22
Fat, %	5.0-8.5	3.7-9.5	6.0-15.2	3.9-7.4	3.5-4.0	3.6-9.3	NA
Vit. A, IU/g	3.8-4.8	2.6-6.8	3.5-10.8	3.7-8.3	3.7-5.2	2.6-7.7	8.0
Vit. D, IU/g	1.4-2.0	1.8-2.1	0.7-2.8	0.7-3.1	2.1-2.2	2.0-2.3	2.5
Vit. E, mg/kg	190-239	218-269	123-229	119-270	220-267	222-266	100
Vit. B ₁ , mg/kg	8.9-10.1	9.9-10.2	5.5-11.7	6.6-10.8	10.0-11.4	9.9-10.6	3.0
Vit. B ₂ , mg/kg	9.3-10.9	10.1-13.1	5.1-12.8	6.7-13.3	10.8-12.2	11.2-13.0	4.0
Niacin, mg/kg	82-92	88-93	41-120	57-93	90-100	88-96	25
Vit. B ₆ , mg/kg	6.5-9.0	8.3-9.4	2.6-8.3	2.7-9.4	9.1-10.0	9.4-9.6	4.0
Vit. B ₁₂ , mg/kg	0.02-0.03	0.03	0.01-0.03	0.01-0.04	0.03	0.03	0.03
Pant. Acid, mg/kg	14-23	43-54	14-44	17-54	47-53	49-53	12
Vit. C, mg/kg	1306-1525	1611-1850	963-1881	1076-1793	1749-1774	1584-1781	200

Ca, %	<i>0.60-0.70</i>	<i>0.54-0.82</i>	<i>0.62-0.71</i>	<i>0.63-0.93</i>	<i>0.65-0.79</i>	<i>0.53-0.80</i>	0.80
P, %	<i>0.50-0.60</i>	<i>0.44-0.61</i>	<i>0.45-0.56</i>	<i>0.49-0.62</i>	<i>0.48-0.55</i>	<i>0.44-0.60</i>	0.60
Mg, %	0.16-0.18	0.16-0.19	0.16-0.17	0.15-0.20	0.16-0.19	0.18-0.19	0.08
K, %	1.3-1.6	1.6-1.7	1.4-1.8	1.5-1.6	1.5-1.7	1.6-1.7	0.40
Na, %	0.26-0.30	0.28-0.35	0.27-0.29	0.27-0.35	0.26-0.30	0.26-0.34	0.20
Fe, mg/kg	130-148	141-168	<i>89-153</i>	103-169	143-166	148-166	100
Se, mg/kg	<i>0.18-0.26</i>	<i>0.20-0.26</i>	<i>0.20-0.23</i>	<i>0.20-0.27</i>	<i>0.20-0.26</i>	<i>0.19-0.27</i>	0.30
Zn, mg/kg	<i>91-104</i>	<i>95-117</i>	<i>62-101</i>	<i>62-118</i>	<i>99-114</i>	<i>95-116</i>	100
I, mg/kg	0.67-0.91	0.83-0.84	0.39-0.83	0.39-0.99	0.87-.99	0.84-0.99	0.35
Cu, mg/kg	20-24	22-26	<i>17-22</i>	<i>16-26</i>	23-26	22-25	20
Mn, mg/kg	34-40	30-45	38-48	38-46	37-45	29-44	20

*The woolly monkeys are numbered from youngest to oldest with the only female being the youngest animal.

†Requirement is based on the new world primate estimated requirements as published by the NRC, 2003.

‡Deficiencies are noted in italics within the table

NA: Information is not currently available

Table 2. Daily diet items consumed and their contribution ranges to the total diet for the six woolly monkeys.

Diet Item	% of Diet
Apple	3.2 - 9.5
Applesauce	0 - 5.2
Banana	0 - 4.2
Blueberries	0 - 4.5
Cantaloupe	0 -17.5
Celery	11.6 - 31.6
Children's vitamin *	0.06 - 0.01
Cooked corn	0 - 12.5
Cooked eggplant	0 -8.8
Cooked onion	0 -16.3
Cucumber	0 -14.7
Flax seed oil	0 - 0.1
Grape Tomato	0 - 5.1
Grapes or Raisins	0 - 2.3
Graviola (<i>Annona muricata</i>)	0 - 0.5
Green peppers	0 - 8.4
Hard boiled egg	0 - 6.5
Juice cocktail (10% juice)	0 - 1.7
Kale	0 - 10.5
Kiwi, Mango or Papaya	0 - 22.0
Mealworm larvae	0 - 1.2
Non-human primate diet [‡]	4.3 - 10.5
Oatmeal	0 - 3.3
Oranges	0 - 5.6
Pears	0 - 5.4
Pediasure [€]	0 - 3.5
Peanut butter	0 - 1.6
Raisins	0 - 2.3
Romaine	0 - 20.7
Spinach	0 - 12.5
White bread	0 - 3.0
Yogurt [£]	0 - 3.5

*Zippy Zoo Children's vitamin and mineral supplement, The Kroger Co., Cincinnati, OH, USA.

[‡]Marion Leafeater Food, Marion Zoological, Inc. 2003 E. Center Circle. Plymouth, MN, USA.

[€]Pediasure®, (pediatric complete nutrition beverage), Abbott Laboratories, Abbott Park, IL, U.S.A.

[£]Dannon Creamy Fruit Yogurt, The Dannon Company, Inc. White Plain, NY, USA.

Table 3. Serum measurements of woolly monkeys at The Louisville Zoo for fasted diabetic parameters with published reference values for various other primate species* .

Woolly Monkey	Gender	Age yr	Fructosamine $\mu\text{mol/L}$	Glucose mmol/L	Glycated Hb %	Insulin $\mu\text{U/mL}$	Triacyl. mmol/L	Cholest. mmol/L	HDL-Chol mmol/L	LDL-Ch mmol/L
1	Female	3	203	4.8	4.1	7.0	0.38	3.0	0.8	2.2
2	Male	8	220	4.7	3.5	13.0	0.46	2.5	0.4	1.8
3	Male	11	193	3.8	3.8	8.6	0.42	5.1	1.6	3.2
4	Male	14	139	3.5	4.5	8.0	0.71	3.3	0.4	2.6
5	Male	15	219	2.2	4.7	6.2	0.58	3.3	0.4	2.7
6	Male	16	242	4.4	3.6	6.6	0.60	4.7	1.0	3.4
Average			203	3.9	4.0	8.2	0.52	3.6	0.8	2.7
Published Normal Reference Values										
New World Monkeys			NA	4.44 ^(1, 2)	4.9 \pm 0.65 ⁽³⁾	< 5 ⁽⁴⁾	0.6-1.6 ^(5,6)	2.9-5.5 ^(5,6,7)	1.2 ⁽⁵⁾	4.0 ⁽⁵⁾
<i>Old World Monkeys</i>			97 – 226 ⁽⁷⁾	3.2-4.8 ^(8, 9)	3.8 ⁽¹⁰⁾	21-72 ^(9,11)	0.2-0.9 ^(5,8)	2.0-5.1 ^(5,7,8,12)	1.1-1.7 ⁽⁵⁾	0.7-2.7 ⁽⁵⁾
<i>Human</i>			< 285 ^(13, 14)	\leq 5.6 ⁽¹⁵⁻¹⁷⁾	4-6 ^(13, 16, 17)	5–20 ⁽¹⁷⁾	< 1.7 ⁽¹⁷⁾	< 5.1 ⁽¹⁷⁾	\geq 1.0 ⁽¹⁷⁾	< 4.1 ⁽¹⁷⁾

*Numbers in superscript italics refer to the published references as follows: 1=Brady, 2000; 2=Simian, 2006; 3=Ausman and Gallina, 1978; 4=Lloyd et al., 1995; 5=Crissey et al., 1999; 6=Halperin et al., 1968; 7=Cefalu et al., 1993; 8=Kim et al., 2005; 9= Mythili et al., 2005; 10=Edwards et al., 2004; 11=Kemnitz et al., 2002; 12=Andrade et al., 2004; 13=Guthrie and Guthrie, 2006; 14=Merck, 1996; 15=ECDDM, 2003; 16=Lagua and Claudio, 2004; 17=Medline Plus, 2006.

NA = Data not available

Table 4. Age, gender, and body weight of woolly monkeys for the day of diabetic parameter blood measurements and averages \pm SEM for both the indirect and direct blood pressure (BP) measurements for the monkeys over the three years preceding the study^{*,†}.

Woolly Monkey #	Age (yr)	Gender	Weight (kg)	Indirect Systolic (mm Hg)	Indirect Diastolic (mm Hg)	Direct Systolic (mm Hg)	Direct Diastolic (mm Hg)
1	3	Female	5.3	140 \pm 22.4 (n=4)	98 \pm 14.9 (n=4)	160 (n=1)	101 (n=1)
2	8	Male	10.5	156 \pm 6.7 (n=22)	86 \pm 4.4 (n=22)	164 \pm 11.1 (n=6)	108 \pm 5.4 (n=6)
3	11	Male	10.6	172 \pm 25.8 (n=4)	100 \pm 18.4 (n=4)	198 \pm 26.3 (n=5)	119 \pm 16.3 (n=5)
4	14	Male	9.1	158 \pm 14.0 (n=7)	99 \pm 9.9 (n=7)	177 \pm 14.3 (n=10)	117 \pm 9.7 (n=10)
5	15	Male	10.5	172 \pm 13.1 (n=10)	101 \pm 10.0 (n=10)	170 \pm 17.4 (n=9)	98 \pm 11.2 (n=9)
6	16	Male	8.5	136 \pm 15.6 (n=6)	66 \pm 12.5 (n=6)	116 \pm 29.5 (n=3)	75 \pm 17.7 (n=3)
Average BP of the six monkeys				156	92	164	103
Average BP with sedation ^{‡,€}				165	102	NA	NA
Average BP without sedation				154	103	NA	NA
<i>Published average woolly weight</i>			5.5 – 10.8 ^[1]				
<i>Normal human BP</i>				< 120 – 140 ^[2,3]	< 80-90 ^[2,3]	NA	NA
<i>Reported spider monkey BP</i>				142 \pm 2.4 ^[4]	96 \pm 2.2 ^[4]	NA	NA

^{*}The number in parentheses is the number of times the monkey's blood pressure was measured during the preceding three years.

[†]Numbers in superscript italics refer to the published references as follows: 1=Nowak, 1999; 2=Mooney and Lee, 1999; 3=NRC, 2003; 4=Srinivasan et al., 1980.

[‡]NA = Not available

[€]Only three of the six monkeys had blood pressure measurements taken without sedation.

Table 5. Woolly monkey medication dosages during the current research trial^{*,†}.

Drug	Diuretic	Vasodilator	Ca channel	ACE inhibitor	Herbal nutritional	Clot	Alpha & Beta	Cardiac		
Action			blocker		supplements	prevention	receptor blocker	glycoside		
Drug Used	Furosemide	Minoxidil	Amlodipine	Enalapril	Captopril	Graviola	AHS	Aspirin	Labetalol	Digoxin
Monkey #	mg/day	mg/day	mg/day	mg/day	mg/day	g/day	mg/day	mg/day	mg/day	mg/day
1	10		0.63		25			10		
2	20	10	1.25		50		2400	20	50	
3	10	5	1.25		50			20	50	
4	20		1.25		50			20		0.1
5	20	10	1.25		50	2	2400	20	40	
6	20			2.5				20		

*In addition to the above medications, woolly monkey # 4 was given 25 mg/day of the beta blocker Atenolol and woolly monkey #1 was given 30 mg/day of the heart muscle supplement Coenzyme Q10.

†Manufacturer information for the medications are as follows: Furosemide: Furosemide Oral Solution 10 mg/ml, Morton Grove Pharmaceuticals, Inc., Morton Grove, IL 60053, USA; Atenolol: Tenormin, AstraZeneca Pharmaceuticals LP, Wilmington, DE 19850, USA; Minoxidil: Minoxidil tablet, USP, 2.5 mg, Mutual Pharmaceutical Co., Philadelphia, PA 19124, USA; Amlodipine besylate: Norvasc^R 2.5 MG tablet, Pfizer Labs, Division of Pfizer Inc., NY, NY 10017, USA; Enalapril: Enacard, Merial LTD., 3239 Satellite Blvd., Duluth, GA 30096, USA; Captopril: Captopril tablets, Bristol-Myers Squibb, 345 Park Avenue, New York, New York, USA 10154-0037, USA; Graviola: Annona muricata powder, Raintree Nutrition, Inc., Austin, TX 78758, USA; AHS: Amazon Heart Support herbal supplement, Raintree Nutrition, Inc., Austin, TX 78758, USA; Aspirin: Children’s Chewable Aspirin 81 mg, Qualitest Pharmaceuticals, Inc., Huntsville, AL 35811, USA; Coenzyme Q10: Vetri-Science Laboratories of Vermont, A Division of Food Science Corp., 20 New England Drive, Essex Junction, VT 05453, USA; Labetalol: Labetalol Hydrochloride tablets, USP 100 mg Eon Labs, Inc. Laurelton, NY 11413, USA; Digoxin: Cardoxin, Evsco Pharmaceuticals, Affiliate of IGI, Inc., Buena, NJ 08310, USA

DISCUSSION

All of the calculated woolly monkey diets were deficient in vitamin A supply. Four of the six woolly monkeys had diets that did not meet the recommended vitamin A concentration for all three trial days. Vitamin A deficiencies are of particular concern for growing animals and the reproductive health of females (Underwood and Arthur 1996). Therefore, it is critical to ensure proper amounts of this nutrient to the population, in particular to the juvenile female. While each of the six woolly monkeys had days that their diets were deficient in vitamin D, each animal also had days that the diet met the recommended requirement. Human vitamin D requirements can be met or exceeded by exposure to sunlight, therefore, we are not concerned because the animals are housed outdoors when appropriate. It is interesting to note, however; that vitamin D supplementation has been linked to a decrease in the prevalence of hypertension and Type I diabetes (Holick 2004). Diets of all animals were also deficient in Selenium. This is of particular concern because the monkeys live in a region of the United States that is not considered to have rich deposits of Se in the soil. Therefore they likely do not obtain significant amounts of Se from the plants in their enclosures (Kubota et al. 1967). Se is thought to have insulin like properties. Se deficiencies have been linked to higher prevalences of heart disease and both increased prevalence of diabetes and severity of diabetic lesions (Douillet et al. 1999). The slightly low levels of Ca and P could also be a concern due to the fact that some researchers associate diabetes as a risk factor for osteopenia and osteoporosis in humans and therefore low Ca and P levels and CA:P ratios could possibly lead to increased monkeys injuries (Bechtold et al. 2006; Leidig-Bruckner and Ziegler 2001). In general, the woolly monkeys in this trial did not consume the same amount each day nor did they consume enough of their nutritionally complete food (the non-human primate diet). They consumed between 4.3 and 10.5% of their diet as this complete item and it is recommended to alter the offered diet such that the monkeys are encouraged to consume at least 15 to 20% of

their daily diet as this item instead of less nutritionally dense items. A large percentage of the daily needed vitamins and minerals were provided by the offered children's dietary supplement and therefore this supplement is a critical part of the current diet. The diet consumed and the potential diet deficiencies noted at Louisville Zoo are reflective of many zoological institutions holding the woolly monkey and therefore the diet concerns reflect the entire captive population (Timmer 2006).

Diabetes mellitus can be diagnosed in humans by measuring a fasting plasma glucose concentration of ≥ 7 mmol/L on two subsequent occasions and increased risk of developing diabetes is evident by fasted glucose of 5.6 to 6.9 mmol/L (ECDDM 1999). This disease can occur at any age although it is more common with advanced age and in conditions of obesity. Moreover, human patients with Type II diabetes commonly have both elevated blood pressure and circulating serum lipids (Lagua and Claudio 2004).

Five of the six woolly monkeys have elevated indirect systolic blood pressure values as compared to published human measures and four of the six are elevated compared to reported spider monkey measures. In addition, four of the six are also elevated when compared to published indirect human and spider monkey measures for diastolic blood pressure. It is interesting to note, however, that the average direct measures of systolic (164 mm Hg) and diastolic (103 mm HG) blood pressures of these six animals are below those direct measures as reported by previous researchers documenting hypertension in woolly monkeys (194 mm Hg for systolic and 136 mm Hg for diastolic) (Giddens et al. 1987). Therefore, the medications prescribed for these animals may have some lessening effect for hypertension within the species. The majority of the current and previous measures were all gathered using ketamine for sedation. Three of the six monkeys in this trial did have some indirect measures taken without ketamine. The average indirect systolic blood pressure for these three monkeys is higher (165 mm Hg) when under sedation than when they were without (154 mm Hg).

While this is potentially interesting, it should be noted that the majority of available literature on non-human primate measurements do report using some type of sedation.

Three of these five were classified as slightly overweight relative to their body frame at the time of the trial although not obese. Their weights did not exceed the wild woolly monkey weight range (Nowak 1999). Although only six animals were studied, it does not seem that elevated age or weight are factors with regard to elevated blood pressure due to the fact that the thinnest and youngest animals did not have the lowest pressure results.

Glucose metabolic problems and unique transient times for glucose and insulin have been diagnosed in many species of non-human monkeys including squirrel monkeys (*Saimiri sp.*), which are also new world primates (Abee 1985; Ausman and Gallina 1978). Squirrel monkeys, in contrast to current theory regarding woolly monkeys, are reportedly predisposed to hypoglycemia (Brady et al. 1990; Brady et al. 1991; Brady 2000). Their average normal adult blood glucose concentration is 4.4 ± 1.55 mmol/L (Loeb and Quimby 1989). The woolly monkey concentrations of serum glucose averaged 3.9 mmol/L which is well within the normal range of reported primate values (3.2 to 5.6 mmol/L) and similar to the squirrel monkey values (Brady 2000; ECDDM 1999; Kim et al. 2005; Laguna and Claudio 2004; Medline Plus 2006; Mythili et al. 2005; Simian 2006). Therefore, it does not seem that serum glucose disturbances were a problem in the current research animals. This conclusion is confirmed by the absence of glucose in the urine of all six woolly monkeys.

The fructosamine test is conducted to determine the number of glucose molecules linked to protein molecules in the blood and provides information on an individual's blood glucose status for approximately the two to three previous weeks (Laguna and Claudio 2004). This is an advantage compared to checking blood glucose concentrations, the later ones are a more valuable immediate measure of the body's daily glucose status. It appears that our fructosamine values for the woolly monkeys (203 μ mol/L) are within the normal ranges

reported in humans (97-285 $\mu\text{mol/L}$) (Cefalu et al. 1993; Guthrie and Guthrie 2003; Merck 1996). It should be noted that Cefalu et al. (1993) reported diabetic cynomolgus monkeys (*Macaca fascicularis*) had fructosamine values of approximately 226 $\mu\text{mol/L}$. Non-diabetic cynomolgus monkeys had an average value of 97 $\mu\text{mol/L}$ (Cefalu et al. 1993). Therefore, the current fructosamine values may be indicative of species differences or potentially warrant further research comparisons across new and old world monkey species. This may be particularly warranted being that there are no well-documented reference values for serum fructosamine concentrations in new world primates. In addition, comparison for fructosamine among different primate species may not be valid because historically different analytical methods were used by researchers and there are no valid reference ranges for most species (Cefalu et al. 1991).

Glycated hemoglobin is a compound formed in an animal's red blood cell by the irreversible reaction of hemoglobin A with glucose. This measurement provides an estimate of the average blood glucose concentrations over the past two to three months or for the life span of the species red blood cells (Dutton et al. 2003; Laguna and Claudio 2004). Therefore, this is a reliable indicator of long-term diabetes mellitus status and is more commonly used than the fructosamine test (Medline Plus 2006). The animals in this study had normal glycated hemoglobin values (4.0 %) as compared to normal values for new world monkeys, old world monkeys and humans (3.8 to 6.0 %) (Ausman and Gallina 1978; Edwards et al. 2004; Guthrie and Guthrie 2003; Laguna and Claudio 2004; Medline Plus 2006). Diabetic cynomolgous monkeys (old world monkeys) have an average glycated hemoglobin value of 8.2 % (Edwards et al. 2004). Care should be taken when comparing glycated hemoglobin values across species or among laboratories due to differences between the rate of red blood cell turnover among species. Also, some laboratories measure only HbA1 instead of all Hb linked glucose (Cefalu et al. 1993).

Insulin concentrations in woolly monkeys in the current study were all within the normal range for humans (Medline Plus 2006). The values in literature for old world primates have a broad range (21 to 72 $\mu\text{U}/\text{ml}$) and our woolly monkey insulin average of 8.2 $\mu\text{U}/\text{ml}$ is also below it (Lloyd et al. 1995; Mythili et al. 2005). Kemnitz et al. (2002) reported values at the lower end of the reference range (21 $\mu\text{U}/\text{ml}$) for free-ranging baboons (*Papio spp.*). They showed that when animals had access to preferred diet items and exercise was limited that their insulin values increased. It should be noted that although woolly monkey number 2 did not have values outside of the human and old world monkey range, his values were considerably higher than the other monkeys in this study.

Valid insulin reference ranges for new world monkeys were scarce although it was reported that two non-pregnant female saki monkeys (*Pithecia pithecia*) had fasting plasma insulin values less than 5 $\mu\text{U}/\text{ml}$ (Lloyd et al. 1995). Although all of the woolly monkeys insulin values in the current research (average of 8.2 $\mu\text{U}/\text{ml}$) are higher than the reported Saki measurements we do not feel that this is a concern especially because it appears that the insulin values in woolly monkeys were normal compared to the available human and old world monkey literature. For correct validation, however, valid reference ranges for plasma insulin values from new world monkeys are needed.

It has been postulated that abnormal metabolism of lipoproteins may be secondary to diabetes and, therefore, that diabetes may be a contributing factor to atherosclerosis (Wagner et al. 1996). Triacylglycerides, total cholesterol and LDL-cholesterol concentrations for the woolly monkeys were within the normal ranges for new and old world monkeys and for humans (Andrade et al. 2004; Cefalu et al. 1993; Crissey et al. 1999; Halperin et al. 1968; Kim et al. 2005; Medline Plus 2006). However, the HDL-cholesterol values appeared to be low (0.8 mmol/L) compared to the reference ranges for primates (Medline Plus 2006; Crissey et al. 1999). Concentrations of HDL are inversely related to the prevalence of cardiovascular

disease (Lagua and Claudio 2004). This low value, however, can also be a result of both the daily diet and the medications prescribed for these animals. The diet was adequate with respect to lipid content although specific sources of fats were not analyzed.

The woolly monkeys in this study were given ketamine hydrochloride in order to anesthetize them. Ketamine significantly decreased glucose in both sexes of cynomolgus monkeys and decreased triacylglycerides and albumin to globulin ratios, while increasing total cholesterol in cynomolgus males (Kim et al. 2005). Therefore, it is feasible that this drug may alter results for other non-human primates as well. The monkeys in the current research also were given numerous veterinary recommended medications due to previous hypertension diagnoses and related conditions (heart enlargement, etc.). Some of them have documented effects on the diabetic parameters measured in this study (Drugs.com 2006). Furesomide is known to increase fasting plasma glucose, Enalapril is known to increase the capacity of insulin to control blood sugars, Captopril is thought to decrease fasting blood glucose measures, and Labetalol is reported to increase the risk of high blood glucose (Drugs.com 2006). Recent research with aspirin is less clear. Some researchers have indicated that it may impair insulin-mediated glucose utilization and reduce insulin clearance while others consider that it improves glucose metabolism (Bratusch-Marrain et al. 1985; Hundal et al. 2002). The natural supplements graviola and Amazon heart support are both reported to decrease blood glucose (AHS 2006; Graviola 2006). Coenzyme Q10 is reported to decrease blood pressure, blood insulin, blood glucose, and triacylglycerides (Singh et al. 1999). It is also important to note that some evidence suggests that antihypertensive medications or ACE inhibitors can be a causative factor in the development of diabetes and related cardiovascular disease (Bakris and Sowers 2004). Therefore, while it is feasible that the drugs may alter results it should be understood that the drugs mentioned are common in human medicine and may skew the standard values used for humans and other captive non-human primates.

Results of this study indicate that diabetes mellitus does not appear to be a problem within this sample of woolly monkeys and, therefore, that the monkeys do not have diabetic related diet abnormalities. The current study cannot make any general health observations regarding gestational diabetes and it should be noted that the prescribed medications could have impacted data interpretation. Further research is needed to ascertain the true nature of the problems faced by the woolly monkey and the preventative role nutrition may play.

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Chapter 6:

Diet Effects on Fecal and Salivary Cortisol Concentrations in Woolly (*Lagothrix lagotricha ssp.*) and Spider Monkeys (*Ateles spp.*)

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ABSTRACT

Detrimental physiological effects due to stressors such as unnatural diet can contribute to the low captive success of endangered primates. The objective of this study was to investigate the impact of diet composition in multiple zoos on cortisol concentrations in feces and saliva in woolly (n=27) and spider monkeys (n=61). Fecal cortisol concentrations in spider monkeys in US zoos varied ($P = 0.07$) from 30 to 66 ng/g. The zoo with the highest fecal cortisol also had the highest salivary cortisol ($P \leq 0.05$). For European zoos, fecal cortisol concentrations differed between zoos for both spider and woolly monkeys ($P \leq 0.05$). Spider monkeys had higher fecal cortisol than woolly monkeys ($P \leq 0.05$). Zoos with the highest dietary total carbohydrates, total sugars, glucose and amount of fruit had the highest cortisol concentrations. Cortisol was highest for zoos that did not meet crude protein requirements and fed the lowest percentage of complete primate feeds and crude fiber. Large differences exist among zoos in housing and diets of spider and woolly monkeys, which may increase their susceptibility to stress. The lifespan and reproductive success of captive primates will improve if stressors can be reduced and dietary nutrients optimized.

Keywords: cortisol, woolly monkey, spider monkey, stress, diet

INTRODUCTION

Spider (*Ateles spp.*) and woolly (*Lagothrix spp.*) monkeys are two of the largest New World primates with a weight range of 5.5 to 11 kg in the wild (Nowak, 1999). They live in South American rainforests in Brazil, Colombia, Peru, and Ecuador. Approximately 1,000 spider monkeys are reportedly housed in captivity worldwide and only 85 captive woolly monkeys are reported worldwide (Ange-van Heugten et al., in review A; ISIS, 2007). Both spider and woolly monkeys are considered threatened species in the wild (CITES, 2007; IUCN, 2004). Woolly monkeys are considered extremely difficult to breed and successfully maintain in captivity (Ange-van Heugten et al., 2008; Dunlap et al., 1985; Ford and Davis, 1992; Strier, 1992). The natural diets of both spider and woolly monkeys are primarily frugivorous. They rely on more than 80% ripe fruits in their diet (Defler and Defler, 1996; Mooney and Lee, 1999; Peres, 1994; Stevenson et al., 2000). Zoological institutions typically feed the majority of woolly and spider monkey diets as fruit. It is suspected, however, that human cultivated fruits differ significantly when compared to wild fruits (Crissey and Pribyl, 1997; Milton, 1999). Wild fruits have higher contents of fiber, minerals, protein and vitamins and a lower content of total sugar (Baker, 1998; Milton, 1999). There is reportedly less sucrose and more fructose and glucose in wild fruits than cultivated fruits (Baker and Baker, 1998; Milton, 1999). Although zoological institutions attempt to replicate dietary items consumed in the wild, the actual dietary nutrients fed to the monkeys in captivity may be very different from that. This is especially true with spider and woolly monkeys which do not have their free-ranging dietary items analyzed for nutrient content within available published literature. The seasonality of wild fruits cause the nutrient content of diets for free-ranging monkeys to differ substantially over time due to both item availability and composition and this seasonal variation is not typically reflected in human cultivated fruits (Milton, 1999).

Elevated levels of dietary sugars and fats may increase sympathetic nervous system release of cortisol in mammals (Seematter et al., 2005). Increased concentrations of cortisol for chronic or long-term periods of time have been associated with negative health conditions such as hypertension, immune system suppression, insulin resistant diabetes, and poor reproductive success (Abbot et al., 2003; McEwen and Seeman, 1999; Mostl and Palme., 2002; Pride, 2005; Sapolsky and Share, 1994). These negative conditions are also associated with the poor life expectancy of woolly monkeys in captivity (Ange-van Heugten et al., 2008). Various other management factors within captive primate populations such as housing space, competition for resources, age, and gender can also cause elevated levels (Abbott et al., 2003; Boinski et al., 1999; Cavigelli, 1999; Whitten et al., 1998a). Studies have successfully evaluated cortisol concentrations in both feces (Bahr et al., 2000, Stavisky et al., 2001, Whitten et al., 1998a) and saliva as a measure of stress in primates (Cross et al., 2004, Kuhar et al., 2005, Lutz et al., 2000, Tiefenbacher et al., 2003).

The objectives of the current research were to: 1) investigate the impact of diet composition, particularly simple sugars, at multiple zoological institutions on fecal and salivary cortisol concentrations in woolly and spider monkeys; and 2) compare cortisol measurements between spider and woolly monkeys and how they relate to diet composition and zoo management. It was hypothesized that diets high in sugar are associated with high fecal and salivary cortisol levels and that woolly monkeys are more responsive to diet as measured by cortisol than spider monkeys.

MATERIALS AND METHODS

Animal housing and management

Due to the scarcity of captive woolly monkeys and the challenges of transporting biological samples from endangered primates, the current research was completed in three

separate studies. In all studies, the age, gender, animal exhibit dimensions, species and subspecies of monkey, and birth location (whether born in captivity or the wild) were noted for every monkey. Age was organized into three groups (Group 1 was 0 to 6 years; Group 2 was 7 to 20 years; Group 3 was 21+ years). In ascending order, these groups are broadly considered youth, adult, and geriatric. Woolly monkey lifespans are assumed shorter than those for spider monkeys and thus these age ranges are approximate (Ange-van Heugten et al., in review A; Mooney and Lee, 1999; Nowak, 1999).

Diet collection and analyses

For all studies, zoos maintained the same daily monkey diets for at least three days prior to data collection and they did not change the diets during the research period. At all institutions, except # 4 and # 5, diet consumption data were collected consecutively for three days (during which samples for cortisol analyses were also collected). For institutions #4 and #5, diet consumption data were collected for only one day and samples for cortisol analyses were also collected for one day. The diet disappearance study consisted of a measure of the exact amounts of dietary items provided for 24 or 72 hours minus the dietary items they did not consume. The daily consumption data were then entered into diet analysis software to determine percentages of nutrients in the daily diet. Two separate diet analyses programs were used: Zootrition (St. Louis Zoo, St. Louis, MO 63146) and Zoo Diet Analysis (ZDA; Allen and Baer Associates, Michigan State University and Zoological Society of San Diego). Depending on the foods used in the monkey diets, these software programs use both nutrient percentages listed in table values as well as laboratory measured values to calculate complete diet nutrient composition. The Zootrition software program was used to calculate crude fat, crude fiber, crude protein, fructose, glucose, sucrose, and total carbohydrates, and ZDA was used to calculate total sugars (the sum of disaccharides and monosaccharides). Diet items

were grouped into food categories, consisting of: breads and grains, fruits, nutritionally complete primate diet, vegetables, and miscellaneous items.

Animals & Zoological Facilities

Study 1: Five zoological institutions in the United States that housed spider monkeys contributed data to this research study: Gladys Porter Zoo, Brownsville, TX (n = 16); Henry Doorly Zoo, Omaha, NE (n = 6); Highwater Farms, Kipling, NC (n = 5); Little Mans Zoo, Chadbourne, NC (n = 8); and Little Rock Zoo, Little Rock, AR (n = 10). Zoo identity was blinded by giving them a random number. The zoos are hereafter referred to as Zoos 1 to 5 in random order. Four zoos contributed data for the fecal collection and four zoos also contributed to the saliva collection. Three zoos contributed to both saliva and fecal collection. Three species of spider monkey were used in this research project (*Ateles chamek*, *Ateles fusciceps*, and *Ateles geoffroyi*). All animals had access to both indoor and outdoor exhibits during the study period. Samples were collected in the fall of 2005.

Study 2: Two European zoos housing spider monkeys contributed data to this research study: Apenheul Primate Park, Apeldoorn, The Netherlands (n = 9) and Twycross Zoo, Birmingham, England, UK (n = 10). To preserve zoo identity the zoos are hereafter referred to as Zoos 6 and 7. Three species of spider monkey were used in this research project (*Ateles belzebuth*, *Ateles fusciceps*, and *Ateles geoffroyi*). All animals had access to both indoor and outdoor exhibits during the study period. Samples were collected during the summer of 2006.

Study 3: Three zoological facilities housing woolly monkeys contributed data to this research study: Apenheul Primate Park, Apeldoorn, The Netherlands (n = 7), The Monkey Sanctuary, Looe, UK (n = 10) and Twycross Zoo, Birmingham, England, UK (n = 10). Zoos are hereafter referred to as 6, 7 and 8 in random order. Of the two naturally occurring woolly monkey species, only *Lagothrix lagotricha ssp.* are kept in captivity and thus represented in

this research (Nowak, 1999; CITES, 2007). All animals had access to both indoor and outdoor exhibits during the study period. Samples were collected during the summer of 2006.

It should be noted that the two zoos in study 2 housing spider monkeys were also two of the three zoos within study 3 with woolly monkeys (Zoos 6 and 7). Samples from both monkey types were collected at the same time.

Fecal sampling and analyses

In all studies, fecal samples were only collected if they were fresh (as quickly after voiding as possible) and not contaminated with urine. Researchers and monkey keepers routinely watched the monkeys and collected feces from defecations they witnessed. The animal enclosures were cleaned at least twice daily to ascertain freshness of the samples. The monkeys were housed in either pairs or groups and, therefore, it was not possible to isolate fecal samples from all monkeys or confirm that each monkey contributed a sample. Due to the complications of transporting fecal samples from three European zoos into the USA, two separate laboratories were used to analyze fecal samples for cortisol. For Study 1, after samples were collected they were immediately frozen and shipped overnight using dry ice to North Carolina State University, Raleigh, NC. They were immediately stored at -20°C until ready for assay. For analysis, 0.5 g of dried feces was mixed with 4.5 ml of 90% methanol in deionized water by shaking for 40 minutes. The mixture was then centrifuged at 2500 x g for 15 minutes at 4°C. The supernatant was transferred to another tube and then evaporated to dryness under nitrogen gas (99.9% purity) and then reconstituted in 0.15 ml of cortisol zero calibrator (25COZ, Siemens Medical Diagnostics, Los Angeles, CA). Spiked samples with 2.5, 5.0, 10.0 or 20.0 ng of cortisol added to them were tested for recovery, which averaged 87%. Serial dilutions of pooled fecal extracts were done and exhibited parallelism with the standard curve. Cortisol concentrations were determined using the Coat-A-Count cortisol kit

(Siemens Medical Diagnostics, Los Angeles, CA) according to the instructions provided by the manufacturer. Fifty μl of the reconstituted sample was used and samples were assayed in duplicate. Inter and intra-assay coefficients of variation were 10.4% and 5.6% respectively. Sensitivity of the assay was 0.2 $\mu\text{g}/\text{dl}$. In studies 2 and 3, procedures for fecal cortisol analyzes at Wageningen University, Wageningen, The Netherlands were identical to those published previously (Ange-van Heugten, in review B).

Saliva Sampling and Analysis

Study 1: Spider monkeys from the four zoos willing to collaborate with the salivary portion of this research study collected samples for three consecutive days. Salivary collections were attempted in the morning before monkey feeding time and in the afternoon before the last daily feeding time. If possible, additional samples were occasionally taken during the day. During the collection period, the monkeys remained in their cages and saliva collection was completely voluntarily. Not every monkey contributed salivary samples. As described previously (Tiefenbacher et al., 2003), saliva was collected by letting monkeys chew on one inch sections of cotton dental rope (Richmond Dental, Charlotte, NC, USA) held by metal clamps. The monkey had to chew on the rope for a minimum of one minute for the sample to be considered suitable for analyses. If more than one monkey contributed to a sample, it was discarded.

Immediately after collection, the saturated dental ropes were placed in Salivette tubes (Sarstedt, Nuernbrecht, Germany) and centrifuged for 15 minutes at 2500 x g at 4°C to remove the saliva. The extracted saliva was then frozen at -20°C and shipped overnight to North Carolina State University, Raleigh, NC until it was analyzed for cortisol. Samples were thawed and again centrifuged at 2500 x g for 15 minutes at 4°C. Fifty μl samples were assayed using the Coat-A-Count kit according to the manufacturer's instructions with the

following exception. Another standard point (0.5 ng/ml) was created by diluting a portion of the provided 10 ng/ml standard. The inter and intra-assay coefficients of variation were 8.7 and 6.1% respectively.

From preliminary studies and previous published literature it was noted that monkeys were more willing to offer salivary samples if a small food incentive was added to the dental rope (Cross et al., 2004; Tiefenbacher et al., 2003). Thus, the dental rope was lightly dipped into grape jelly or touched to a slice of banana prior to sample collection. This was completed by the same researcher to minimize variation. To account for dilution effects of jelly or banana, a conversion factor was established using 24 human volunteers. Each volunteer chewed on three pieces of dental rope in random order for one minute. The three pieces included one without food additive, one with banana and one with grape jelly. Samples with grape jelly had salivary cortisol concentrations that were 17% lower than samples without food additive and samples with banana added had salivary cortisol concentrations that were 51% lower than those without additive. Cross et al. (2004) had previously developed correction factors for banana added to dental rope using marmosets (*Callithrix jacchus*) in a similar manner. To correct for food adhesion, cortisol concentrations obtained using dental rope dipped in jelly or banana were multiplied by 1.17 and 1.51, respectively. Of the 66 samples analyzed, 61 were collected using jelly and 5 were collected using banana.

Statistical Analyses

Study 1: Multiple samples were collected for some animals and data were averaged by animal identification (ID) number such that each monkey contributed to the data only once to avoid skewing of the data. Statistical analyses were conducted using General Linear Models procedures of SAS (Cary, NC, USA). The model only included zoo. The Least Square Means

procedure was used to calculate fecal and salivary cortisol means and SEM by zoo.

Significances were noted at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

Study 2 and 3: Animal ID could not be preserved for samples collected in these studies, therefore, each sample was considered a unique observation in the data analysis. Statistical analyses were conducted using General Linear Models procedures of SAS (Cary, NC, USA). The model only included zoo. The Least Square Means procedure was used to calculate fecal cortisol means and SEM by zoo for the spider monkeys in study 2 and the woolly monkeys in study 3. Significances were noted at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

Two zoological institutions in study 2 and 3 both held spider and woolly monkeys and sample collections were conducted at the same time for both species. Data from these institutions were analyzed using the General Linear Models procedures of SAS using zoo, monkey species, and the zoo by monkey species interaction in the model. Least square means were calculated by zoo and monkey species to compare fecal cortisol concentrations between species within zoos.

RESULTS

Animal housing and management

Details on monkey management and housing information from the zoological institutions is given in Table 1, including number of monkeys studied at each zoo, monkey species, number of species per zoo, average space per monkey, percentage born in captivity, male to female ratio, number of monkeys per age group, and laboratory used for cortisol analyses. The five zoos that participated in study 1 held 45 spider monkeys while the two zoos that participated in study 2 held 19 spider monkeys. Study 3 had three contributing zoos holding a total of 27 woolly monkeys. The same zoo in study 1 (Zoo 1) was the only institution that

housed more than one spider monkey species as well as housing monkeys that had been born in the wild. In study 2, the space allotted per monkey varied greatly between the two zoos (35m^3 versus 150m^3) and only one of the zoos held more than one species of spider monkey. In study 3, the space allotted per woolly monkey also varied greatly (60m^3 - 250m^3) and only Zoo 6 had woolly monkeys that were born in the wild.

Diet Composition and Nutrient Content

Food groups used in the monkey diets and nutrient percentages varied substantially among zoological institutions for American zoos housing spider monkeys within study 1 (Table 2) and European zoos housing spider and woolly monkeys within studies 2 and 3 (Table 3). Most notably, total sugars ranged from 17 to 42 % of the diet among American zoos housing spider monkeys and from 20 to 41 % among the zoos housing European spider and woolly monkeys. There was large variation between zoos in the amounts of fruits, vegetables, nutritionally complete primate feeds, breads and grains, and treats. The fruit category ranged from 34 to 83 % within study 1, 30 to 49 % in study 2, and 19 to 44 % in study 3. Similarly, the percentage of vegetables fed ranged from 0 to 68 % when considering all three studies, and the monkey complete feed ranged from 0 to 22 % for the three studies. The nutritionally complete primate feeds utilized by each zoo also varied substantially. Zoo 1 fed Mazuri High Protein Primate (PMI Nutrition International, St. Louis, MO 63166); zoos 2 to 5 fed Mazuri New World Primate (PMI Nutrition International, St. Louis, MO 63166) and zoo 5 also fed Mazuri Old World Primate (PMI Nutrition International, St. Louis, MO 63166). Zoos 6 and 7 fed Leaf Eater Primate (Mazuri Zoo Foods, Witham, Essex, UK) and Zoo 8 did not feed a nutritionally complete primate diet at all. All US zoos in study 1 fed the animals twice daily while European zoo 6 fed twice daily and zoos 7 and 8, fed three meals per day.

Table I. Monkey management and housing information from the zoological institutions housing spider and woolly monkeys

Zoo #	Monkey n	Species Type	Species Housed	Average Space (m ³)*	Percentage Captive Born	Gender Ratio Male:Female	Young Age Group	Adult Age Group	Geriatric Age Group	Lab Used
Study 1										
1	16	Spider	2	45	34	5:11	2	8	6	1
2	6	Spider	1	34	100	2:4	3	2	1	1
3	10	Spider	1	56	100	2:8	0	6	4	1
4	5	Spider	1	26	100	2:3	1	3	1	1
5	8	Spider	1	39	100	6:2	5	3	0	1
Study 2										
6	9	Spider	1	35	70	2:7	2	2	5	2
7	10	Spider	3	150	70 [†]	6:4	1	6	3	2
Study 3										
6	7	Woolly	NA	60	71	2:5	3	4	0	2
7	10	Woolly	NA	250	100	8:2	3	2	5	2
8	10	Woolly	NA	125	100	7:3	1	9	0	2

*Average space per individual monkey.

[†] Zoo 7 had 7 spider monkeys born in captivity, 1 born wild and 2 with unknown birth locations.

TABLE II. Spider monkey diet nutrient analyses^{*}, food group percentages and fecal and salivary cortisol concentrations (\pm SEM) from five zoological institutions in the US.

	<i>Zoo 1</i>	<i>Zoo 2</i>	<i>Zoo 3</i>	<i>Zoo 4</i>	<i>Zoo 5</i>
Dietary Nutrients, %					
Protein	14	17	21	11	17
Fat	7	9	4	4	5
Fiber	3.8	4.1	6.5	4.0	4.0
Carbohydrates	71	64	67	78	68
Total Sugar	42	17	18	25	20
Sucrose	5.3	4.0	1.0	6.0	9.1
Fructose	13.4	6.0	2.4	6.2	5.0
Glucose	6.4	4.1	1.0	4.1	4.0
Diet Food Group, %					
Fruit	83	44	44	34	60
Vegetable	5	29	32	57	0
Nutritionally complete feed [‡]	11	19	22	9	20
Breads and Grains	2	5	2	0	11
Miscellaneous	0	8	0	0	9
Cortisol Analyses					
Fecal Cortisol, ng/g [£]	66 \pm 10.9	49 \pm 40.6	30 \pm 15.4	NA [€]	31 \pm 18.2
Salivary Cortisol, ug/dl	17 \pm 3.7 ^a	NA	7 \pm 3.2 ^b	11 \pm 5.9 ^{a,b}	2 \pm 3.2 ^b

^{*} Nutrient analyses are expressed on a DM basis per day

[‡] Zoo 1 fed Mazuri High Protein Primate (PMI Nutrition International, St. Louis, MO 63166); zoos 2 to 5 fed Mazuri New World Primate (PMI Nutrition International, St. Louis, MO 63166) and zoo 5 also fed Mazuri Old World Primate ((PMI Nutrition International, St. Louis, MO 63166).

[€] NA = Information not available

[£] Significant difference in salivary cortisol concentration among institutions ($P < 0.05$) are indicated by superscript a,b.

TABLE III. Spider and woolly monkey diet nutrient analyses^{*}, food group percentages and fecal cortisol concentrations (\pm SEM) from three zoological institutions in Europe.

	<i>Zoo 6</i>	<i>Zoo 7</i>	<i>Zoo 6</i>	<i>Zoo 7</i>	<i>Zoo 8</i>
	<i>Study 2</i> <i>Spider Monkeys</i>		<i>Study 3</i> <i>Woolly Monkeys</i>		
Diet Nutrients, %					
Protein	18	12	22	15	12
Fat	19	8	12	12	7
Fiber	8.5	4.1	9.0	8.3	5.6
Carbohydrates	54	75	55	62	75
Total Sugar	20	35	22	28	41
Sucrose	4.8	3.7	3.2	3.7	4.9
Fructose	10.7	9.9	6.7	8.0	13.8
Glucose	6.5	8.1	3.5	4.6	6.9
Diet Food Group, %					
Fruit	30	49	19	30	44
Vegetable	60	42	68	60	48
Nutritionally complete feed [‡]	6	4	5	4	0
Breads and Grains	2	5	0	2	6
Miscellaneous	2	0	8	4	2
Cortisol Analyses					
Fecal Cortisol, ng/g [£]	115 \pm 40.6 ^a	227 \pm 40.6 ^b	87 \pm 26.8 ^x	122 \pm 20.3 ^x	269 \pm 20.8 ^y

^{*} Nutrient analyses are expressed on a DM basis per day.

[‡] The nutritionally complete feed utilized by zoos 6 and 7 was Leaf Eater Primate (Mazuri Zoo Foods, Witham, Essex, UK)

[€] NA = Information not available

[£] Significant difference in fecal cortisol concentration between spider monkey institutions ($P < 0.05$) are indicated by superscript a,b and among woolly monkey institutions by superscript x,y

Fecal and Salivary Cortisol Concentrations

For all three studies, fecal cortisol concentrations were highest when total sugars and fruit percentages were highest and fiber was lowest (Tables 1 to 3). Fecal cortisol concentrations were also highest for all the zoos with the highest levels of carbohydrates. Finally, for fecal cortisol comparisons, the zoos with the lowest percentage of nutritionally complete primate diet had the highest cortisol concentrations.

Study 1: Fecal samples were collected from four zoological institutions. A total of 113 fecal samples were taken. There was no overall difference in fecal (main zoo effect, $P=0.21$) cortisol concentrations among the zoological institutions. However, Zoo 1 cortisol concentration (66 ± 10.9 ng/g) was twice the concentrations of Zoos 3 (30 ± 15.1 ng/g; $P = 0.07$) and 5 (31 ± 18.2 ng/g; $P = 0.12$).

Saliva samples were taken in four institutions, holding together 37 spider monkeys. A total of 66 samples were taken and analyzed. Salivary cortisol concentrations differed among zoological institutions with Zoo 1 concentrations being higher than Zoo 3 and Zoo 5 ($P=0.05$).

Study 2 and 3: There were 39 fecal samples analyzed for the European spider monkeys in study 2 and 120 fecal samples for the woolly monkeys in study 3. The spider monkeys in study 2 differed with fecal cortisol concentrations for Zoo 7 being higher than Zoo 6 (Table 3). The woolly monkeys in study 3 also differed with fecal cortisol concentrations for Zoo 8 being higher than both Zoos 6 and 7 (Table 3).

When both of the zoos that housed spider and woolly monkeys were compared by zoo, overall fecal cortisol concentrations were higher at Zoo 7 (142 ± 12.1) than at Zoo 6 (91 ± 15.8) ($P \leq 0.003$). Spider monkey fecal cortisol concentration means were higher (171 ± 15.4) than woolly monkey means (62 ± 12.6) ($P \leq 0.0001$).

DISCUSSION

Although termed frugivorous, spider and woolly monkeys typically do not live on fruit alone. In the wild they procure various animal and plant sources to acquire additional nutrients throughout different seasons (Stevenson et al., 2000; Stevenson, 2004). In captivity, the fruit diets are supplemented by nutrients within breads and grains, nutritionally complete primate diets, vegetables, and other miscellaneous items. Most of the food items consumed by non-human primates in the wild are not available for captive primates commercially. Products grown for human consumption, which are fed routinely to captive primates, do not typically have the same nutrient profile as similar items consumed in the wild. This is because products such as fruits and vegetables available to captive primates are traditionally higher in water and sugars, lower in fiber and more digestible than the natural diet of the animal (Crissey and Pribyl, 1997; Milton, 1999). Analyses of the composition of wild primate foods for comparison, however, are scarce. Often captive animal diets are formulated by trying to equate wild food groups to what can be fed in captivity. Wild monkey food selection criteria are not based on food groups. They choose different plant parts based on resource availability and nutrient content (Silver et al., 2000).

The large range in diet food category percentages between and within zoos from all three studies is interesting. Some zoos had their monkeys consuming as little as 19% fruit while other zoo's monkeys consumed as high as 83%. Similarly, some zoos fed no vegetables while others had their monkey's consuming up to 68% of their diet from vegetables. Some zoos did not feed any bread or grain items or any primate nutritionally complete foods. Within study 1, the zoo that fed the highest percentage of the daily diet as fruit had the highest fecal and salivary cortisol concentrations in the studied animals. Monkeys in both study 2 and study 3 also had the highest fecal cortisol concentrations within the zoo that had the highest fruit content. The fecal cortisol concentrations were also the highest for each of the three studies

within the zoos with the greatest fruit percentage, the highest carbohydrates, highest total sugar, highest glucose, and lowest total fiber. There was not a clear relationship with high sucrose levels having higher cortisol levels although previous research has indicated that wild fruits consumed by primates have decreased sucrose content compared to cultivated fruits (Milton, 1999). Interestingly, the highest fecal cortisol concentrations were also found at the zoos that fed the least amount of nutritionally complete primate feed. There did not appear to be a clear relationship for cortisol concentrations for any of the studies with respect to protein, fat, or breads and grains percentages within the zoo diets.

The current research shows that within each of the three current studies, the zoos with the highest concentrations of nutritionally complete feeds actually had the lowest cortisol concentrations. In conjunction, low levels of protein also were associated with increased fecal and salivary cortisol concentrations. Being that the majority of the fruits and vegetable consumed by the monkeys are low in protein these animals typically acquire a large percentage of their protein from the nutritionally complete feeds. It has been estimated that new world primates require 15% of their diet as crude protein (NRC, 2003). The highest concentrations of fecal and salivary cortisol were observed in zoos that did not meet the crude protein requirement. In study 1, the highest concentrations of salivary cortisol were measured in the two zoos that did not meet the crude protein requirement.

These results are consistent with the results of Seematter et al. (2005), who showed that increased cortisol concentrations may lead to visceral fat deposition, with adverse metabolic consequences such as decreasing insulin sensitivity. It has long been recognized that chronic activation of the hypothalamic pituitary adrenal axis resulting in increased cortisol levels can have deleterious physiological effects. These effects include the induction or worsening of hypertension, Type II diabetes, ulceration in the gastrointestinal tract, decreased reproduction, osteoporosis, and immunosuppression (Abbot et al, 2003; Pride, 2005; Möstl and Palme,

2002 Sapolsky and Share, 1994). It appears that the zoo diets among all three studies may be a contributing factor to the elevation of cortisol levels and potential health concerns. Future diet formulations for these species should attempt to reflect nutrient needs instead of trying to copy food group percentages.

Several studies have previously measured primate cortisol concentration in feces (Bahr et al., 2000; Stavisky et al., 1994; Stavisky et al., 2001; Whitten et al., 1998a) and saliva to determine stress levels (Cross et al., 2004; Kuhar et al., 2005; Lutz et al., 2000; Tiefenbacher et al., 2003). New World primates and Old World primates reportedly differ in their circulating cortisol levels as well the metabolism of cortisol (Hernandes-Jauregui et al., 2005). New World primates typically having a 10-fold higher concentration than the Old World primates (Boyce et al. 1995). Spider and woolly monkeys are both considered New World primates. The spider monkey salivary cortisol range reported within study 1 (2 – 17 ug/dl) was lower than the new world monkey values previously reported for squirrel monkeys (*Saimiri sciureus*) ($28 \pm 2.3 \mu\text{g/dl}$) but was approximately 10 fold high than the old world monkey range (0.3 – 1.8 ug/dl) reported for rhesus monkeys (*Macaca mulatta*) (Lutz et al., 2000; Tiefenbacher et al., 2003). Thus, the spider monkey salivary results from the current work appear valid.

Spider and woolly monkeys are both New World primates and extremely closely related and therefore differences in fecal cortisol concentrations between the two were not expected (Ford and Davis, 1992; Strier, 1992). In addition, the higher fecal cortisol concentration for spider monkeys was also unexpected because these species tend to be more successful in captivity with regard to maintenance and reproduction when compared to woolly monkeys (Ange-van Heugten, in review A).

Salivary cortisol concentrations were only collected for study 1. However, it is noteworthy that the highest fecal and salivary cortisol measures within spider monkeys in this

study came from the same zoo. Saliva samples were more difficult to obtain compared to the fecal samples and the stress caused by the sampling could potentially skew subsequent results. While some animals were excited to contribute saliva samples, others were frightened by the procedure and some dominant animals would not allow the subordinate ones near the collection ropes. Thus, collection of salivary samples was discontinued in studies 2 and 3. In general, fecal cortisol represents secretion and metabolism over a number of hours which can be different than measures of stress from the single moment in time estimate provided by salivary cortisol (Whitten et al., 1998b). Although fecal cortisol is not as sensitive to the intensity of an acute event as serum and salivary cortisol concentrations, they have the advantage of being easier to collect and allowing more samples to be collected without disturbing behavior (Whitten et al., 1998a and Whitten et al., 1998b).

It has been reported that physical stress, insufficient living space, and obesity are all factors that can cause hypertension and increase cortisol concentrations (Müller et al. 1989; Kirshbaum et al., 1999; Seematter et al., 2005). Housing and management differed between zoos and could have impacted cortisol concentrations measured within the current research. It is interesting that for study 1, the only zoo to house spider monkeys born in the wild as well as more than one species of spider monkey had the highest fecal and salivary cortisol results. Decreased amount of space per individual monkey did not appear to increase cortisol being that some monkeys with the most space had the highest cortisol measures while conversely some of the monkeys with the least space had the lowest cortisol measures. There were not enough representatives from all enclosure size categories to statistically analyze the effect of space on the cortisol data. Similarly, the time of day that the cortisol samples were taken were not analyzed statistically due to the reasons previously described. However, previous work has shown that spider monkey fecal samples do not appear to change with respect to the time of day the sample is taken (Ange-van Heugten, in review B). Similarly, the possible effects of

gender and age were not able to be examined within this study. These factors can possibly also influence cortisol concentrations and cortisol metabolism (Hernandes-Jauregui et al., 2005). However, all zoos did hold monkeys from both genders and most age groups. Future studies further evaluating the effects of animal housing and management are recommended before making concrete conclusion about the zoo diet composition being the only cause of increased cortisol. However, as previously suggested (Vermeer, 1994), diet alterations such as changing the monkey daily feedings so that the monkeys do not have large quantities of sugar (or glucose) available at any one point in the day or drastically reducing the total sugars available to the woolly monkeys could potentially decrease the captive health problems affecting this species.

CONCLUSION

This work demonstrates that large differences exist between zoos with respect to housing facilities and diets of spider and woolly monkeys. Measuring cortisol concentrations seems to be a reliable method to determine the cortisol levels of both spider and woolly monkeys. High amounts of carbohydrates, total sugars, glucose and fruits and low amounts of nutritionally complete diets may cause spider and woolly monkeys to be more susceptible to stress which can in turn cause metabolic, reproduction, and cardiovascular problems. The lifespan and breeding success of captive woolly and spider monkeys will improve if the stressors and negative effects of nutrition on the health status can be reduced and dietary nutrients can be optimized.

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Chapter 7:

Effect of Dietary Inulin-Type Fructans on Fecal Dry Matter and Fecal Cortisol in Spider and Woolly Monkeys

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ABSTRACT

Dietary inulin-type fructans (ITF) are fermented in the gastro intestinal tract (GIT) and may alter GIT microfloral activity and immune function. These ITF effects may be pertinent to the survival of captive woolly monkeys (*Lagothrix ssp.*) specifically by their potential to alleviate diabetes, hypertension, and observed stress while increasing reproductive and immune functions. It is hypothesized that if ITF's ease health problems that fecal cortisol concentrations could be an assessment tool to track positive change. The current research studied the effect of ITF added to the daily diet for spider (*Ateles spp.*; n = 9) and woolly monkeys (n = 7). A three day diet disappearance study was conducted and indicated that, in general, the diets met nutrient recommendations. However, vitamin A concentrations, which included β -carotene, were 10 to 30 times above recommendations. Monkeys were given ITF supplements (containing 90 to 94% inulin), individually, at 5 g per day for four weeks. Fecal samples were collected prior to supplementation, after two weeks and after four weeks. Overall, spider monkeys had lower fecal DM content ($P < 0.05$) than woolly monkeys (19% *versus* 23%), regardless of ITF supplementation. Fecal cortisol concentrations were not affected by the addition of ITF, however, fecal cortisol was numerically decreased after the 4 week ITF supplementation, primarily in spider monkeys (110 *versus* 65 ng/g dry feces). These results suggest that ITF supplementation may have positive effects on captive primate welfare. However, additional research with larger populations of monkeys using higher doses of ITF is recommended.

Key Words: cortisol, inulin-type fructan, stress, woolly monkey, spider monkey

INTRODUCTION

Prolonged or chronic activation of the mammalian stress response can have detrimental physiological effects on human and animal well-being (McEwen and Seeman 1999). Some of these effects can seriously affect woolly monkeys (*Lagothrix lagotricha* sp.) such as the induction or worsening of hypertension and insulin-resistant diabetes. The consequences of these health impairments can include increased infant mortality, gastrointestinal tract ailments, lack of ovulation, impotence, and decreased immune response (Abbot et al. 2003; Pride 2005; Möstl et al. 2002). The woolly monkey is facing endangerment in the wild and zoological institutions have very limited success housing and breeding this species (Ange-van Heugten 2008; Ange-van Heugten in review). Therefore, it is vital to determine whether prolonged stress responses contribute to poor survival and reproduction of these animals during captivity. Measuring the level of cortisol in feces can be a valid method to represent stress responses in primates (Bahr et al. 2000; Stavisky et al. 2001; Whitten et al. 1998a; Whitten et al. 1998b). It can be conducted without interfering with animal behavior and it does not cause additional stress to the animal.

Inulin type fructans (ITF) are fructo-oligo polysaccharides that are naturally found in many plant species and are readily consumed by humans and animals (Roberfroid 1999; Toma and Pokrotnieks 2006). ITF are linearly attached fructose molecules by β -2 \rightarrow 1 fructosyl - fructose linkages and are classified as belong to non-digestible carbohydrates or soluble fibers. They are rapidly fermented in the intestinal tract for most monogastric mammals (Roberfroid 2005a) and short chain fatty acids are produced. A product is considered an ITF if the degree of polymerization (DP) is between 2 and 60+ monomer units (Roberfroid 2005a). ITF have been added to diets for humans, domesticated livestock, companion animals, and recently also to exotic animals (Verdonk et al. 2005). Research results regarding ITF effects are not consistent. For example, Houdijk (1998) found that if

production and health conditions were challenging for piglets that added dietary fructo-oligosaccharides had some positive effects on growth. However, these positive effects were absent when housing conditions were very good (Houdijk 1998). Prebiotic ITF, however, have also been reported to have positive effects on the gastro-intestinal tract microflora and host health. These positive effects include an increase in calcium absorption and bone mineralization and are associated with the reduction of atherosclerotic cardiovascular disease and insulin resistance by improving lipid profiles (Roberfroid 1999; Roberfroid 2005a; Verdonk et al. 2005). Non-starch polysaccharide work with sows has shown a positive effect on reproduction *via* increased live births (van der Peet-Schwering et al. 2003). Positive ITF effects vary depending on the particular product used, the dosage used, and duration of supplementation.

Woolly monkey health problems associated with hypertension and diabetic type disorders can seriously impact their ability to survive in captivity while their close relatives the spider monkey (*Ateles sp.*) have fewer problems (Ange-van Heugten et al. 2008; Ange-van Heugten et al. in review). ITF have been previously shown to induce relaxing effects and increased general activity in rats (Messaoudi et al. 2005). Therefore it was hypothesized that similar effects may take place in other species and that adding ITF to the diet may benefit the monkeys by reducing cortisol levels.

The current research objectives were to: 1). document fecal cortisol levels in woolly monkeys and spider monkeys for comparison and to establish normal levels; and 2) add ITF to the daily diets of both woolly and spider monkey populations housed within the same zoological institution and determine effects on fecal consistency and fecal cortisol.

ANIMALS AND METHODS

Animals

The woolly monkey (*Lagothrix lagotricha sp.*) (n=7; 2:5 male to female ratio) and spider monkey (*Ateles fusciceps robustus*) (n=9; 2:7 male to female ratio) populations at Apenheul Primate Park, The Netherlands, were used in this study. The woolly monkey age range was 1 to 15 with an average age of 7.0 years while the spider monkey age range was 1 to 35 years with an average age of 17.9 years. At Apenheul, average adult woolly monkey weights for males and females are 8 kg and 5.5 kg, respectively. Adult spider monkeys at this institution have a higher average body weight of 12 kg for males and 10 kg for females. The woolly monkeys were all housed together and fed the same diet (Table 1). Similarly, the spider monkeys were also housed together and fed the same diet (Table 1). All animals had access to indoor and outdoor exhibits. This research was conducted during July and August, 2006.

Diet Collection

Immediately prior to the initiation of this research project, a three day diet disappearance study was conducted to determine consumption data and compare it to estimated nutrient requirements (NRC 2003). This disappearance study was a measure of the amounts of exact dietary items provided for 72 hours prior to each monkey group starting the ITF supplementation trial minus the dietary items they did not consume. The animal keepers and researchers measured the amount of each food item fed to the monkey groups for both their morning and evening meals and weighed back the portions of the food that the monkeys had not consumed after each meal. Dietary nutrients were calculated using Zootrition software (St. Louis, MO, USA) and comparative estimated requirements were taken from the National Research Council (NRC) non-human primate publication (NRC, 2003). For calculation

purposes, the items that were group fed were assumed to be consumed equally by the monkeys according to animal keeper and researcher observations.

Fecal Collection and ITF Addition

Fecal samples were only collected if they were fresh (as quickly after voiding as possible). The monkey keepers routinely watched the monkeys and collected feces from defecations they witnessed. The animal enclosures were cleaned at least twice daily to ascertain freshness of the samples. Due to the monkeys being housed in groups, it was not possible to isolate fecal samples from all monkeys or confirm that each monkey contributed a sample. The same animal keepers managed both the spider and woolly monkey populations. Therefore, diet preparation and sample collection for the two species was identical. The first fecal collection period was during the three days immediately prior to ITF supplementation. This also was the diet collection period previously discussed. A dietary ITF product (Beneo™ Synergy 1, Orafit Group, Tienen, Belgium) was then added to the daily diet for the two groups of animals. This product is an enriched chicory inulin powder containing a selected mixture of oligofructose and long inulin-type fructan chains. It contains 90 to 94 % inulin with a remainder of glucose + fructose + sucrose. The ITF product was added at the rate of 5 g per day per monkey. Each animal received this additive as it was individually hand fed once daily *via* an approximately 3 cm moistened bolus. This bolus consisted of the pelletized nutritionally complete monkey food portion of their diet plus the powdered ITF. All the monkeys were adjusted to the ITF by providing 2 g per day during the first week of the trial. After the one week adjustment period, the ITF amount was increased to 5 g per day per animal. Each group of monkeys had the 5 g ITF added for two weeks and then fecal samples were again collected for three days. The ITF supplementation was continued and after two additional weeks with consumption of the ITF the fecal samples were collected for

a third time for three days. The addition of ITF was the only change, diet and housing conditions did not change for the duration of the trial.

Cortisol Analyses

After collection, the feces were placed in appropriate containers, immediately labeled and frozen at -20°C until further analyses were conducted. Samples were freeze dried using a Tri-Philizer MP (FTS Systems, Stone Ridge, New York, USA). The freeze dried feces was homogenized (Moulinex Type 505, Brevete, France) and 200 mg of each sample was then analyzed. Cortisol was measured in each fecal sample in duplicate in the following manner. Fecal samples and 2500 cpm cortisol tracer for individual recovery were added together with 1 ml deionized water and mixed (S/P Multi-Tube Vortexer, Baxter Scientific Products, Boom B.V., Meppel, The Netherlands) for 60 minutes at room temperature. Four ml of methanol (Merck 6009, VWR International B.V., Amsterdam, The Netherlands) was added to the samples followed by 40 minutes of mixing and extraction. Samples were then centrifuged (Centra-8R Centrifuge, IEC, Boom B.V., Meppel, The Netherlands) at 1300 x g for 20 minutes at 4 °C. Supernatant (1.5 ml) was collected and dried under nitrogen (>99.9% purity, Praxair, Vlaardingen, The Netherlands) at 60°C using a sample concentrator and Techne Dri-Block DB-3 (Den Hartog Scientific B.V., Terschuur, The Netherlands), re-dissolved in 0.15 ml of Cortisol 0 Calibrator (25COZ Siemens, Nuclilab, Ede, The Netherlands) and mixed for 60 minutes. Recovery was measured using a Wallac Wizard 1470 Automatic Gamma Counter (Perkin Elmer, Groningen, The Netherlands); 25 µl of each sample was assayed in duplicate using the Coat-A-Count Cortisol kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA, U.S.A.). The intra-assay and inter-assay variation were 5.5% and 11.2%, respectively.

Statistical Analyses

Statistical analyses were conducted using General Linear Models procedures of SAS (Cary, NC, USA) to determine effects of monkey type, time period of supplementation, and time of day of fecal collection according to the following model:

$$Y_{ijk} = \mu + M_i + P_j + (M_i \times P_j) + T_k + e_{ijk}$$

where, Y_{ijk} = dependent variable, μ = overall mean, M_i = main effect of monkey type, P_j = main effect of time period, $M_i \times P_j$ = the interactive effect of monkey and time period, T_k = main effect of time of collection, and e_{ijk} = residual error. Monkey type included spider and woolly monkeys and time period included ITF supplementation of 0, 2, and 4 weeks. Time of day was whether the fecal sample was collected before noon (AM) or after noon (PM).

Least squares means and SEM were calculated for monkey type, period length and monkey by period interactions. Significances were noted at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

RESULTS

The diet fed to both spider and woolly monkey populations met or exceeded the nutrient recommendations for non-human primates (NRC 2003) for nearly all nutrients (Table I). The diet items fed to both species were very similar with regard to percentages of vegetables, fruits, and other feed items provided (Table II). The nutritionally complete non-human primate diet used was identical between species (Table II). However, vitamin D and choline were both slightly lower than recommendations for both monkey species for each of the three days evaluated. Diets for spider monkeys with regard to calcium, phosphorus, selenium, vitamin B₁₂ and zinc for all three days were also slightly lower than recommendations. Biotin and chloride were variable but were adequate if calculated on a three-day basis.

Table I. Daily diet items consumed and their contribution ranges to the total diet for spider and woolly monkeys*

Nutrient, units	Spider Monkey Diet	Woolly Monkey Diet	Requirement [†]
Crude Protein, %	13-20	20-23	15-22
Crude Fat, %	7-20	8-20	na [‡]
Crude Fiber, %	5-7	5-7	na [§]
Vitamin A, IU/g [¥]	236-271	89-285	8
Vitamin D, IU/g [£]	1.0-1.5	1.0-1.6	2.5
Vitamin E, mg/kg [Ⓛ]	114-122	102-218	100
Thiamin, mg/kg	7-8	8-18	3
Riboflavin, mg/kg	7-10	9-20	4
Niacin, mg/kg	62-70	57-114	25
Pyridoxine, mg/kg	9-10	9-19	4
Biotin, mg/kg	0.1-0.2	0.1-0.2	0.2
Folacin, mg/kg	6.6-7.5	6.7-8.6	4.0
Vitamin B12, mg/kg	0.01-0.02	0.02-20.4	0.03
Pantothenic acid, mg/kg	43-53	42-65	12
Choline, mg/kg	434-567	471-623	750
Vitamin C, mg/kg	1109-1689	1366-2397	200
Calcium, %	0.5-0.7	0.7-0.8	0.8
Phosphorus, %	0.4-0.5	0.5-0.6	0.6
Magnesium, %	0.16-0.21	0.21-0.22	0.08
Potassium, %	1.8-2.0	1.8-1.9	0.4
Sodium, %	0.2-0.3	0.2-0.4	0.2
Chloride, %	0.1-0.2	0.1-0.2	0.2
Iron, mg/kg	178-216	191-238	100
Selenium, mg/kg	0.1-0.2	0.2-0.3	0.3
Zinc, mg/kg	62-75	75-320	100
Iodine, mg/kg	0.33-0.42	0.35-0.39	0.35
Copper, mg/kg	14-17	15-20	20
Manganese, mg/kg	51-62	56-61	20

* Diet nutrients are presented on a dry matter basis.

† Estimated nutrient requirements based on NRC, 2003.

‡ It is recommended that primates receive 0.5 % essential n-3 fatty acids and 2 % essential n-6 fatty acids.

§ It is recommended that primates receive 10-30 % of their diet as neutral detergent fiber and 5-15 % of their diet as acid detergent fiber.

¥ Vitamin A in the analyzed diets was retinol, carotenoids or both.

£ D₃ percentage only.

ϕ Vitamin E in the analyzed diets was either α -tocopherol or total tocopherol, depending on the source of the data.

Table II. Daily diet items consumed and their contribution ranges to the total diet for the spider and woolly monkey groups.

Diet Item	Spider Monkey % of Diet	Woolly Monkey % of Diet
Apple	13.4 - 21.3	13.7 - 19.1
Avocado	0 - 12.4	0
Beets	0	0 - 8.8
Belgian endive	17.0 - 18.2	14.9 - 15.8
Blueberries	0 - 9.5	0
Broccoli	0	0 - 6.9
Celery	0 - 5.7	0
Cheese	0	0 - 4.2
Carrots	7.7 - 10.9	0 - 10.3
Chinese cabbage	0	15.0 - 22.2
Cucumber	0	0 - 6.6
Endive	16.5 - 17.2	18.3 - 22.8
Green beans	0 - 5.3	0
Green peppers	5.2 - 11.3	3.6 - 6.8
Hard boiled egg	0 - 3.0	0 - 3.1
Melon	0 - 5.2	0
Non-human primate diet*	5.1 - 6.1	5.2 - 5.8
Papaya	0	5.7-6.4
Pears	0	0 - 8.2
Reed (collected onsite)	0	0 - 3.1
Radish	0 - 3.7	0
Scallions	0	0 - 4.2
Sunflowers seeds	0	1.4 - 1.8
Turnip greens	0 - 15	0 - 8.1
Walnuts	0 - 5.6	0 - 2.0
White radish	0	0 - 5.3
Zucchini	0	0 - 6.2

*The non human primate diet was Leaf Eater Primate (Mazuri Zoo Foods, Witham, Essex, UK)

In contrast, diets for both species had very high levels of vitamin A (89 to 285 IU/g) compared to the recommendation of 8 IU/g (NRC 2003). These levels are somewhat inflated due to the fact that β -carotene contributions are listed as vitamin A values in the Zootrition computer database. Of less concern is the surplus in niacin and vitamin C. They are relatively abundant in the diet of both species compared to estimated recommendations (57 to 114 mg/kg *versus* 25 mg/kg for niacin and 1109 to 2397 mg/kg *versus* 200 mg/kg for vitamin C).

A total of 87 fecal samples were collected and analyzed. Overall, spider monkeys had lower fecal DM content ($P < 0.05$) than woolly monkeys (19 % *versus* 23 %; Table III).

Table III. Dry matter percentage and fecal cortisol results for spider and woolly monkeys with inulin-type fructan supplementation *

	Period (wk)			
	0	2	4	Overall
Dry matter, %				
Spider monkeys	16 \pm 2.3 (n=15) [†]	18 \pm 2.6 (n=12)	23 \pm 2.8 (n=9)	19 \pm 1.6 (n=36) [‡]
Woolly monkeys	24 \pm 1.8 (n=21)	22 \pm 2.7 (n=10)	23 \pm 1.9 (n=20)	23 \pm 1.3 (n=51) [‡]
Monkey overall	20 \pm 1.5 (n=36)	20 \pm 1.9 (n=22)	23 \pm 1.7 (n=29)	
Fecal cortisol, ng/g [‡]				
Spider monkeys	110 \pm 24.0 (n=15)	103 \pm 26.3 (n=12)	65 \pm 28.5 (n=9)	93 \pm 16.3(n=36)
Woolly monkeys	65 \pm 19.0 (n=21)	88 \pm 28.0 (n=10)	80 \pm 19.0 (n=20)	78 \pm 13.3 (n=51)
Monkey overall	88 \pm 15.8 (n=36)	95 \pm 19.8 (n=22)	73 \pm 17.3 (n=29)	

* Mean values in the same column marked via * were significantly different ($P=0.05$).

[†] n = number of fecal samples analyzed in each category

[‡] Cortisol represented as concentration present in dry feces.

The addition of ITF did not alter the DM of feces when analyzed by time period (0, 2, and 4 week supplementation) or the species by time period interaction (Table III). Fecal cortisol concentrations were not affected by the addition of ITF for either spider or woolly monkeys. There was also no time period effect (0, 2, or 4 weeks of ITF supplementation) noted (Table III). No interactive effects of species by time period on cortisol concentrations were observed (Table III). While not significant, cortisol tended to be decreased during the 4 week ITF supplementation (most notably in spider monkeys). Time of day that samples were collected was also analyzed and there was no difference between DM % (20.8 ± 1.08 versus 21.2 ± 1.87) or fecal cortisol (90 ± 11.0 versus 80 ± 19.3 , ng/g dry feces) measures when AM was compared to PM, respectively.

DISCUSSION

Only a few nutrients deviated from the estimated requirements for spider and woolly monkeys. Overall, the diets had sufficient amounts of all nutrients. If the diets were evaluated over a weekly period instead of the three day trial in the current research the occasional daily deficiencies calculated would likely disappear. This is due to the scheduled rotations in daily vegetable, fruit and other dietary items at the primate facility. The primary concern regarding diet was the high levels of vitamin A. However, the high levels reported include β -carotene from the Zootrition diet analysis software. Thus vitamin A values may appear somewhat artificially inflated. This nutrient can be stored by the body and can have both toxic and sub toxic effects in primates when given in high concentrations (NRC 2003; Penniston and Tanumihardjo 2001). For example, rhesus monkeys which received a daily diet with 40 IU/g vitamin A showed toxic effects on the liver (Penniston and Tanumihardjo 2001). The levels noted for both spider and woolly monkeys were 10 to 30 times the recommendation (89 to 285 IU/g versus the recommendation of 8 IU/g) and are potentially problematic. Toxic and

sub toxic effects of vitamin A can be associated with reproduction disorders (NRC 2003) which are commonly seen in woolly monkeys.

The high levels of vitamin C and niacin are not a concern because they are water soluble and thus excreted from the body relatively easily (NRC 2003). New world monkeys are suspected to have higher vitamin D requirements than other primates (NRC 2003) and, therefore, it may be a concern that vitamin D as well as Ca and P were low in the diets. However, human vitamin D requirements can be met or exceeded by exposure to sunlight, therefore, the current diet is likely sufficient because all the animals had continuous access to outdoors (NRC 2003). It is interesting to note, however, that vitamin D supplementation has been linked to a decrease in the prevalence of hypertension and Type II diabetes (Holick 2004) both of which woolly monkeys may be predisposed to.

The monkey diets and the calculated nutrient consumptions did not differ between the two primate species. Thus, the ITF addition may be responsible for the changes over time noted in this research. It should be noted that ITF and diet nutrient effects may change with increasing animal age and the fact that the woolly monkey population average age was less than half of that of the spider monkey average age could have affected species comparisons (Roberfroid 2005a; Verdonk 2005).

The ITF used at the daily dosage of 5 g per day was well tolerated by both monkey species. In agreement with published data (Roberfroid 2005b), ITF did not significantly alter the DM content of feces. ITF are typically considered positive supplements for the treatment of constipation as they increase fecal water content (Den Hond et al. 2000). In contrast, the numerically higher DM in week 4 in the present study could potentially be because ITF are known to normalize stool frequency, stimulate bowel movements, and potentially eliminate deleterious bacteria that may cause diarrhea or inflammatory bowel disease (Roberfroid 2005c). Feces from spider monkeys had lower overall DM content than woolly monkeys,

primarily due to samples collected at time 0 for spider monkeys being much lower than those collected at time 0 for woolly monkeys (16% *versus* 24%). This difference disappeared after four weeks of ITF supplementation. The reason for this observation is not clear. It may be related to positive gastro-intestinal tract functions due to the addition of ITF because cortisol content decreases with time. Nijboer et al (2006) has shown normal fecal DM in François langurs (*Trachypithecus francoisi*) to be approximately 23 to 27% and human fecal DM has been reported to be approximately 21% (Moore and Holdman 1974). However more research with free-ranging monkeys consuming their natural diets is needed to ascertain the normal fecal consistencies for spider and woolly monkey species.

Fecal cortisol is proven to be a valid method to represent long term measures of stress in primates (Bahr et al. 2000; Carlsson et al. 2007; Stavisky et al. 2001; Whitten et al. 1998a). The current data report fecal cortisol levels from two primate species housed in captivity. In the published literature, no comparative data for these species were found. Zeigler (2001) has conducted limited fecal cortisol research with woolly monkeys but cited methodology for hormone analyses were not available for comparison. The method in the current research included freeze drying of feces after the samples were analyzed. Thus, these results refer to fecal dry matter and variation in cortisol concentrations due to fecal moisture content is avoided. This is very important when comparing cortisol measures among animals or species. Fecal measures are not very sensitive to an acute event, especially those just prior to voiding. They are considered more sensitive to cumulative or chronic stress. Thus, they can be considered more appropriate for studies of stressors affecting the well-being of captive animals (Whitten et al. 1998b). Fecal samples are easier to collect and allow sampling more often without disturbing ongoing behavior of animals or increasing stressors by human intervention. This will allow assessments of change in animal well-being before detrimental markers such as weight loss, infertility, and poor health become apparent (Sapolsky et al.

1994; Whitten et al. 1998a). Pertinent to the comparisons within the present study, both woolly and spider monkeys are frugivorous primates and have rapid gastro-intestinal retention times compared to more folivorous old world primates. Previous research with wild monkeys reported woolly monkey gastro-intestinal transit times ranging between 2 to 14 hours (Yumoto et al. 1999) while spider monkey transit times have been reported as 4.4 ± 1.53 hours (Milton 1981). The 14 hour woolly monkey transit time was thought to be an anomaly. It is therefore unlikely that transit time of the ITF would affect the outcome of the dry matter or fecal cortisol results in the current research.

While previous research has shown a diurnal pattern with fecal cortisol being lower in the afternoon (Sousa and Ziegler 1998), this was not evident in our research. In addition, feces were routinely collected once in the AM and once in the PM to address possible diurnal issues with the hormone analyses. To further address the possible diurnal issue, it has been previously reported that the diurnal pattern of cortisol secretion is lessened in fecal extracts, making it possible to collect samples at any time of day (Möstl et al. 2002; Pride 2005).

High amounts of dietary fat and carbohydrates in humans lead to increased cortisol release *via* the sympathetic nervous system (Seematter et al. 2005). Furthermore, increased cortisol concentrations may lead to visceral fat deposition (Epel et al. 2000) with adverse metabolic consequences, such as a decrease in insulin sensitivity. It has long been recognized that prolonged activation of the stress response or prolonged cortisol elevation can have deleterious physiological effects. These include those pertinent to woolly monkeys, such as the induction or worsening of hypertension, insulin-resistant diabetes, gastrointestinal tract abnormalities, ovulation abnormalities, impotence and immune system depression (Abbot et al. 2003; Pride 2005; McEwen and Seeman 1999; Möstl et al. 2002). It has been shown by Messaoudi et al. (2005) that adding ITF supplements can potentially relax rats because they noted less startle responses and less fear in animals when exposed to novel items. ITF have

been reported to potentially help control blood glucose levels and to reduce serum cholesterol and triacylglycerides (Beylot 2005; Burcelin 2005). This can lead to decreasing related diabetic conditions as well as lower the prevalence of obesity (Beylot 2005; Burcelin 2005). On the basis of the cortisol results in the current trial it can be suggested that ITF supplements should be further examined as a possible way to decrease fecal cortisol. This could be a potential indicator of positive changes in animal welfare. Spider monkeys are noted to be more successful breeders in captivity than woolly monkeys and thus, were expected to have lower cortisol concentrations (Ange-van Heugten et al. in review). The high average age of the spider monkeys compared to the lower average age of the woolly monkeys and the resulting increased potential for disease with age could explain this interesting trend. It is also possible that woolly monkeys with the highest cortisol levels die younger and thus are already removed from the sampled population. The spider monkeys in this study had higher body weights compared to the woolly monkeys. Therefore, the spider monkey daily dosage of ITF was a lower on a metabolic body weight basis than it was for the woolly monkeys. It should be noted that free-ranging woolly monkeys are reported to be lighter than spider monkeys (Nowak 1999). Therefore, it is possible that the spider monkeys in this research are more overweight than the woolly monkeys and more prone to disease. Thus, ITF supplementation was more useful for them.

The current trial lasted for four weeks and previous research has demonstrated that such a time period should be enough for ITF to generate gastrointestinal health benefits (Verdonk et al. 2005). Thus, potentially the supplementation dosage in the current trial should have been higher. Although some researchers argue that ITF dosage is not a major factor in eliciting results (Roberfroid 2007), a higher dose could have elucidated more clear information. The current moderately low dosage was selected due to the novelty of this type of research with endangered animals. However, the type of ITF, the dosage and the duration can be very

species specific. Although spider and woolly monkeys are closely related, they may respond differently to ITF supplementation (Ford and Davis 1992; Roberfroid 2005a; Strier 1992; Verdonk et al. 2005). Thus, the most appropriate dose of ITF for the prevention or treatment of disease in these monkey species needs to be further investigated. The ITF was added to the diets of these monkeys one time per day, which may have impacted the outcome of this study. This is especially true considering the fairly rapid GIT transit times of these species. Finally, while it is not possible to separate the animals in either the spider or the woolly monkey groups by age or by gender at Apenheul Primate Park, research with controlled laboratory monkeys could provide more individual age and gender specific information.

CONCLUSION

The current research demonstrated that collecting fecal samples to measure cortisol measures, and therefore stress, is an acceptable practice for captive zoological primates. The fecal cortisol measures established for spider and woolly monkeys are important standards for future individual and species comparisons. The DM % and fecal cortisol concentration from this research demonstrate that ITF supplementation may have positive effects on captive primate welfare. While more research into the potential positive effects of ITF supplements on the health of new world monkeys is needed, the data are encouraging. In addition, Vitamin A and β -carotene levels should be examined in both woolly monkey diets and nutritional software programs in order to better examine requirements and possible toxicities. Considering the detrimental health concerns facing the woolly monkey in captivity it is vital to continue nutritional research for this species in an attempt to find dietary factors such as prebiotics, probiotics and synbiotics to improve their health.

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Chapter 8:

General Discussion

INTRODUCTION

Woolly monkeys (*Lagothrix spp.*) are unique non-human primates. Their management in captivity causes numerous concerns. This primate species has declined dramatically in numbers in captivity during the 20th century. Over 16,000 woolly monkeys were imported into the United States beginning in the early 1960's (Franceshini et al., 1997), but less than 10 remained by the turn of the century (Ange-van Heugten et al., in review; ISIS, 2007). Although the potential nutritional problems associated with rearing and housing woolly monkeys have been suspected for many years, very little research has been conducted to determine the causes of these nutritional problems or how to treat them. Some of the primary woolly monkey concerns include low fertility and reduced body mass during pregnancy. The latter is often associated with abortion without obvious reasons. In addition, offspring frequently die at young ages (Müller et al., 1989). Thus, animals probably do not consume sufficient quantities of nutrients to maintain or increase body weight during pregnancy or have viable young (Mooney and Lee, 1999). Other complications that frequently arise in woolly monkeys are similar to those of diabetes mellitus and hypertension in humans and are of possible nutritional origin. These complications include congestive heart failure and renal failure (Giddens et al., 1987; Miller et al., 1995). The primary goal for the research presented within this dissertation was to determine if the woolly monkey health problems mentioned above are in fact nutrition-related. If so, the secondary goal was to then research possible ways to alleviate these concerns.

CHALLENGES RELATED TO CONDUCTING RESEARCH WITH EXOTIC ANIMALS

Research with exotic animal species, particularly those which are endangered such as the woolly monkey, present many unique challenges. Only a few zoological institutions house

woolly monkeys and, therefore, the research reported here involves zoos in several countries. Most institutions were ultimately willing to contribute to the research, while a few were very hesitant. Primary reasons for the hesitation or not participating in the research included the opinion that this species was not a priority area and a concern about the public's perception. Research into problem species have previously caused the public to misinterpret the zoos status of animal welfare, not understanding that the zoo is not to blame, but instead to be commended for attempting to assist the animal survival. Most zoological institutions have experienced very limited success in breeding woolly monkeys in captivity and have adopted a strategy of maintaining their remaining population, rather than continuing breeding programs. Understandably, zoo conservation efforts have to focus on those species that are most likely to survive and thrive in captivity. Due to budget constrains and welfare concerns woolly monkeys are not considered a feasible species for captivity and, therefore, are not bred by most holding institutions.

Many zoological workers in charge of woolly monkeys have cared for these animals for many years and have formed emotional bonds with them. It is critically important for research programs to be successful to actively involve animal keepers in the development and implementation of research protocols. In particular, animal procedures need to be clearly outlined and justified as not being harmful to the animal and essential to ensure the survival and well-being of the animal. In our projects, the mutual understanding of the objectives of the research and the exact protocol to obtain the data that was established proved invaluable.

Woolly monkeys are endangered and are perceived to be very stress sensitive in nature, suffering from high captive mortality rates. Therefore, most zoological institutions do not allow biological samples to be collected from their monkeys. Opportunistic sampling provides an important avenue to collect information for studies. For example, we collected serum, urine and hair samples during routine bi-yearly physical exams for woolly monkeys

from one of the few institutions that perform physicals on this species. Collection of non-invasive samples that do not cause stress or interference with normal animal behavior, such as fecal sampling, can be justified.

Difficulties in transporting biological samples, especially from non-human primate species, across country borders is another considerable challenge when conducting research at multiple institutions in several countries. The current research involved samples from three different countries and, to avoid legal complications and potential loss of these samples, two different laboratories were used for sample analysis. This provides another potential concern related to confounding of results due to differences between laboratories. To minimize the impact of inter-laboratory variation, standardizing laboratory methods as well as the proper use of identical analytical standards can provide an acceptable solution.

UNDERSTANDING THE HISTORY OF THE PROBLEM

In order to better understand the unique health problems associated with woolly monkeys, it was imperative to first conduct a concise literature review of the research previously conducted with this species. After compilation, it was obvious that woolly monkeys perform poorly in captivity. It was equally clear that many of the health complications noted in captivity could have an underlying nutritional cause. In this respect, reported cases of diseases due to immunosuppression, hypertension, liver disease, reproductive problems, and diabetic type symptoms (Ange-van Heugten et al., in review; Giddens et al., 1987; Miller et al., 1995) are of considerable interest. Thus these health problems needed to be studied with comparisons to the nutrient contents of the diets and serum blood parameters within woolly monkeys in captivity. These newly identified primary study areas were vital to gain insight into the primary medical conditions that threaten woolly monkeys in captivity. The survival of woolly monkeys in the wild is vital not only due to the potential loss of one species, but

mandatory for overall rainforest health and diversity *via* seed dispersal, as well as this monkey being an important part of the rainforest food chain (Defler, 1995).

In order to ascertain their true current captive status, a 16 year historical analysis was undertaken to determine whether woolly monkeys, in fact, thrive less in captivity than their close relative the spider monkey (*Ateles spp.*). These retrospective data were alarming. It is known that woolly monkeys are endangered in the wild and it now appears certain that zoological institutions have limited success maintaining captive populations (Ange-van Heugten et al., in review; CITES, 2007; IUCN, 2007; Nowak, 1999). In the past 16 years, the total woolly monkey population decreased by 11% and the birth to death ratio was below one (0.65) and much less than the spider monkey (1.26). Thus, one woolly monkey death was reported for every 0.65 births and this was even more pronounced in the female population (0.47 birth to death ratio *versus* 0.74 in males). In addition, only one zoological facility managed to breed several generations of the woolly monkey successfully. This colony of animals was genetically responsible for the majority of the reproductive events analyzed at all facilities. Thus, lack of diversity in woolly monkey genetics is a concern.

SERUM NUTRITIONAL PROFILES

It was confirmed that woolly monkeys have lower and less productive lifespans in captivity than in the wild. Thus their historical nutritional serum chemistry profiles were examined and compared to their closest relatives (spider and howler monkeys (*Aloutta spp.*)). The objective was to try and isolate potential abnormalities in the woolly monkey population to help with future health diagnostics. These nutritional profiles were taken from historical veterinary records collected over the previous 12 years from two institutions known for housing large woolly monkey populations and keeping species studbook records. Interestingly, in comparison to howler and spider monkeys, all measured woolly monkey

serum concentrations appeared within normal baseline values with the possible exceptions of alanine transferase (ALT) and gamma-glutamyl-transferase (GGT) being elevated while creatinine and phosphorus were lower. The potential abnormalities for ALT, GGT and creatinine could be indicative of the cardiovascular and liver disease noted within captive woolly monkey populations. Glucose was slightly higher in woolly monkeys as compared to humans and spider monkeys. Statistical differences in glucose were noted between age groups, sexes, and institutions, but, these differences may have been related to variables not controlled in this study such as age. The higher serum glucose concentrations found in woolly monkeys may be indicative of Type II diabetes, which is a problem often mentioned in logbooks kept in zoos on woolly monkeys. Thus, our next research efforts were focused on the association of diabetes and hypertension as the primary health concern for this animal species.

DIABETES DETERMINANTS

During routine physical examinations of six woolly monkeys, blood and urine samples were collected to test for possible indicators of diabetes. The monkeys in this study were all diagnosed and being treated for various health problems associated with hypertension and possible diabetes. All six monkeys had blood pressure historical records that had been collected for three years prior to our research and overall they were elevated compared to human and primate reference ranges, despite daily medications for high blood pressure. Serum samples were collected and analyzed for common markers used to diagnose diabetes in humans, including fructosamine, glucose, glycated hemoglobin, insulin, triacylglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol, and urine was analyzed for glucose concentrations. Overall, the serum and urine diabetes determinants appeared within normal reference ranges when compared to humans and similar species. Diet consumption data were

also collected for three days prior to physical examinations. Potential dietary deficiencies were noted for vitamin A, vitamin D, calcium, phosphorus, and selenium (NRC, 2003). However, when compared to the available primate reference ranges, the diet composition did not raise concerns as a causative factor associated with diabetes mellitus, glucose metabolism problems, or the diagnosed hypertension. However, we were not able to make any general health observations regarding gestational diabetes, and it was noted that monkey prescription medications could have impacted the data. From this study, it appears that diabetes may generally not be a concern in woolly monkeys.

CORTISOL STRESS DETERMINANTS

In our next studies, we focused on the impact of management and nutrition on stress responses in woolly monkeys compared to spider monkeys. It is well-known that cortisol can be elevated by high fat and high sugar diets (Seematter et al., 2005) and we hypothesized that stress and hypertension could be related to diet composition. Fecal and salivary cortisol concentrations were analyzed from spider monkeys and fecal concentrations were analyzed from woolly monkeys. As expected, cortisol concentrations in both fecal and salivary samples were highest in zoological institutions that fed the highest levels of total carbohydrates, calculated total sugars, and glucose as well as the most fruit content. The highest cortisol concentrations were also noted from the zoos that had the lowest percentages of crude fibers and nutritionally complete primate foods. Spider monkey cortisol concentrations were higher than those for woolly monkeys, which was unexpected, considering that spider monkeys are maintained in captivity with much more success than woolly monkeys. Three conclusions could be derived from this research: 1) diets for both woolly and spider monkeys contain improper amounts of sugars and fruits and should be re-evaluated; 2) captive spider monkey are able to physiologically handle stress better than

woolly monkeys but could still be experiencing underlying negative health effects; 3) stress is not the major underlying cause of the problems noted in woolly monkeys because they had lower cortisol concentrations than their more successful close relative the spider monkey.

INULIN-TYPE FRUCTANS

High levels of dietary total carbohydrates, total calculated sugars and glucose may increase cortisol concentrations in spider monkey and woolly monkey feces. We hypothesized that dietary inulin-type fructans (ITF) may indirectly reduce stress responses in both woolly and spider monkeys. ITF's are fermented in the gastro-intestinal tract (GIT) and, as a result, physical and chemical conditions of the chyme are changed. These changes include altering GIT microflora activity and changed gut immune function (Roberfroid, 1999; Roberfroid, 2005; Verdonk et al., 2003). These ITF effects may be pertinent to the survival of captive woolly monkeys (*Lagothrix ssp.*) specifically by their potential to alleviate diabetes, hypertension, and alleviate indicators of stress while increasing reproductive and immune functions.

Numerical trends for increased fecal dry matter percentage and decreased fecal cortisol concentrations in spider monkeys with ITF supplementation demonstrate that ITF may have positive affects on captive primate well-being. It is interesting to note that spider monkeys showed higher fecal cortisol levels than woolly monkeys and that now the ITF supplementation tended to decrease fecal cortisol more in the spider monkeys than in the woolly monkeys. Perhaps it could be argued that ITF supplementation worked better where it was needed most. While more research into the potential positive effects of ITF supplements on the health of new world monkeys is needed, the data are encouraging. In addition, being that vitamin A levels were extremely elevated in the monkey diets, they should be examined

in both captive woolly and spider monkey diets in conjunction with β -carotene concentrations to better examine requirements and possible toxicities. Considering the detrimental health concerns for the woolly monkey in captivity, it is vital to continue nutritional research for this species in an attempt to find dietary factors, such as prebiotics, probiotics and synbiotics, to improve their health.

FINAL DISCUSSION AND CONCLUSIONS

Many zoo conservationists perceive the woolly monkey as a species that is too difficult to maintain in captivity. The exact causes of the problems in captive woolly monkey populations have, however, not been isolated. However, our research suggests that diabetes is not the primary problem affecting these monkeys; although, their captive diet is potentially too high in total sugars. It is apparent that complete analyses of diets and serum nutrition parameters from wild populations are needed. For example, research with macaques (*Macaca spp.*) have shown that free-ranging animals have much lower total cholesterol and circulating lipoproteins when compared to their more prone to cardiovascular disease captive counterparts (Takenaka et al., 2000). Woolly and spider monkey data from wild animals for comparison with the captive diet and serum chemistry results presented within this dissertation could ensure that captive diets are as similar as possible to those of free-ranging animals. Free-ranging animals may experience nutrient inadequacies; for some periods however, this has not affected them very much during their breeding period because their population has been sustained in the wild unlike their captive relatives. Our research further indicates that supplementation with prebiotics may have beneficial effects on exotic animal health. Thus, research with dietary supplements should be continued. Our research has also provided critical comparative data from spider monkeys that offer insight into the woolly monkey, but also provide vital nutritional information to help maintain these species in

captivity as well. Captive woolly monkeys are in severe jeopardy and immediate action is mandatory to successfully maintain captive populations.

REMAINING QUESTIONS

Regardless of the insights we have made into the nutritional issues facing the woolly monkeys, we have unearthed new questions and areas for needed research. The most critical area of nutritional research needed for the woolly monkey is information about the diets of wild animals to obtain information for comparative analyses. This presents large challenges due to country politics, expense, seasonal challenges dealing with the South American wet season and sample handling and analyses. It is also critical to better understand the genetic make-up of the captive population. This is due to the potential for inbreeding within a very small captive population and the fact that it appears that some woolly monkey family lines are able to breed while others are not. Genetic analyses compared with nutrition and reproduction histories could provide important information for this species. Thus, answering the following questions could lead to a successful captive woolly monkey zoological program: 1) Do monkeys within zoos with the highest fecal cortisol concentrations have lower life expectancies and lower reproductive rates?, 2) Do diets of wild woolly monkeys have different total carbohydrate, total sugars, crude fiber, crude protein and glucose concentrations than diets of those in captivity?, and 3) Are woolly monkeys in zoos with lower fecal cortisol concentrations genetically distinct from those with higher cortisol concentrations?

Preserving exotic species in captivity and the wild are imperative to ensuring the ecological diversity upon which we are accustomed. With each species that goes extinct, future species (plant and animal) are in increased jeopardy. Preservation of many of these species, such as the woolly monkey, cannot happen without the joint scientific efforts of

researchers within the nutrition community. While exotic animal research is challenging, developing trusting relationship with animal care givers and formulating non-invasive research techniques can gleam diagnostic and preventative answers for factors that cause negative animal well-being.

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Summary

INTRODUCTION

Spider (*Ateles spp.*) and woolly (*Lagothrix spp.*) monkeys are two of the largest New World primates with a wild weight range of 5.5 to 11 kg. Approximately 1,000 spider monkeys are reportedly housed in captivity worldwide, while only 85 captive woolly monkeys are reported worldwide. Both spider and woolly monkeys are considered threatened species in the wild and woolly monkeys are considered extremely difficult to breed and successfully maintain in captivity. Captive breeding does not result in a sufficient number of woolly monkeys. Attaining a long normal lifespan (over 15 years) for these monkeys in most captive locations throughout the world has had very limited success. The majority of the health complications in woolly monkeys are probably nutrition related, based on the fact that the nature of their problems are similar to diabetes mellitus and hypertension in other primates. As with humans, these woolly monkey health conditions are often more pronounced during pregnancy and may result in very large, difficult to deliver, infants.

The current research had two primary objectives. The first one was to determine whether a nutritional cause for the primary disorders afflicting captive woolly monkeys could be isolated *via* historical research. The second primary objective was to investigate the effects that carbohydrates (particularly sugars) and lipids have on woolly monkey serum nutritional profiles, serum diabetic determinants, and stress hormone (cortisol) concentrations.

HISTORICAL DATA ANALYSIS

Written survey reports, International Species Information System historical record analysis, and woolly monkey specific zoological studbook record analysis were completed to gather data on the survivability of the captive woolly monkey from 1990 to 2005. Data on spider monkeys, one of the closest living relatives of the woolly monkey, also were collected from five representative institutions for comparative analysis. In the past 16 years, the total

woolly monkey population has decreased by 11%. The number of institutions holding this species has decreased and the birth to death ratio is well below 1 (0.65) compared to the spider monkey (1.26). This is even more pronounced in the female population (0.47 birth to death ratio *versus* 0.74 for males). The primary two known causes of death in woolly monkeys included infant and maternal failures to thrive (53%) and cardiovascular events (15%). These primary causes all need further examination with particular interest in the nutritional aspects potentially involved.

Only one institution has managed to breed several generations of the woolly monkey. Many of these successful offspring have been transferred to other facilities where they are typically one of the few successful monkeys reproductively. Thus, lack of genetic diversity in captive woolly monkeys may have a negative influence on their reproductive success. It is, therefore, possible that genetic abnormalities in nutrient metabolism or overall unnatural diet nutrient composition is responsible for the poor captive success noted in woolly monkeys. The following important possible research possibilities are proposed: 1) do woolly monkeys have unique nutritional or housing specifications when compared to their close relatives; and 2) do woolly monkeys acquire metabolic conditions more easily than their close relatives and therefore their diets need to be more closely monitored.

SERUM CHEMISTRY

Serum analyses from 30 woolly monkeys housed at two institutions (Apenheul, The Netherlands and The Louisville Zoo, USA) over 12 years were collected. Primary objectives are: 1) to determine baseline serum concentrations for captive woolly monkeys; 2) compare means by gender, age groups and zoological institution; 3) compare overall serum concentrations to the closest relatives of the woolly monkey; and 4) isolate abnormalities that may affect captive woolly monkey longevity. Serum chemistry means were similar to

previously reported concentrations for howler (*Aloutta sp.*) and spider monkeys (*Ateles sp.*) with the possible exception of alanine aminotransferase (ALT) and gamma-glutamyl-transferase (GGT) concentrations were higher, while creatinine and phosphorus concentrations were lower. Serum glucose (6.7 mmol/L) was above the baseline range when compared to both humans and spider monkeys. Alkaline phosphatase, ALT, and sodium (Na) were higher in females and magnesium (Mg) was higher in males ($P < 0.05$). Alkaline phosphatase, Mg, and phosphorus were highest ($P < 0.05$) and calcium, Na, and ferritin tended to be highest ($P < 0.10$) in the oldest animals. Albumin, alkaline phosphatase, chloride, Na and total bilirubin were higher for Zoo A while GGT, glucose and lactate dehydrogenase were lower ($P < 0.05$). Although potential abnormalities were noted, future research studies are needed to determine serum concentrations of free-ranging woolly monkeys to elucidate parameters that contain aberrant concentrations and decrease health status.

DIABETES

Six woolly monkeys with known hypertension problems were used to determine if Type II diabetes and daily diet composition were underlying links to health problems for the captive population of this species. Fasting concentrations of glucose (real-time indicator of insulin function), insulin (determinant of insulin insufficiencies), fructosamine (indicator of blood glucose control over the past two or three weeks), and glycated hemoglobin (indicator of long term blood glucose status) were determined. Circulating lipids (high density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), total cholesterol, & triacylglycerides) were evaluated as indicators for potential risk of heart and vascular disease. Urine was collected and analyzed for glucose concentrations. Diet disappearance was determined for three days prior to blood collection and nutrient content

was calculated. Serum analyses were within normal ranges (fructosamine (139 to 242 $\mu\text{mol/L}$), glucose (2.22 to 4.78 mmol/L), glycated hemoglobin (3.52 to 4.73 %), insulin (6.2 to 13.0 $\mu\text{U/ml}$), triacylglycerides (0.38 to 3.4 mmol/L), total cholesterol (2.5 to 5.1 mmol/L), HDL-cholesterol (0.4 to 1.6 mmol/L), and LDL-cholesterol (1.8 to 3.4 mmol/L)) when compared to New World monkeys, Old World monkeys and humans. Urine glucose concentrations were below the detection limit. Diets were not limiting in starch and total sugars and were similar in nonstarch polysaccharides. Potential dietary deficiencies were noted for vitamin A, vitamin D, calcium, phosphorus, and selenium. Results of this study indicate that diabetes mellitus does not appear to be a problem within this sample of woolly monkeys. The current study cannot make any general health observations regarding gestational diabetes and it should be noted that prescribed medications could have impacted data interpretation.

FECAL AND SALIVARY CORTISOL

Potential stressors, such as an unnatural diet, can reduce the success of raising endangered primates because chronic activation of the stress response can have detrimental physiological effects on animal well-being. Thus, the objectives of this experiment were to investigate the impact of diet composition, particularly simple sugars, at multiple zoological institutions on fecal and salivary cortisol concentrations in woolly and spider monkeys. Fecal (272) and salivary (66) samples from woolly ($n=27$) and spider monkeys ($n=61$) were collected from four US and three European zoos and analyzed for cortisol concentrations. Within US zoos, the highest fecal cortisol concentration ($66 \pm 10.9 \text{ ng/g}$) in one zoo was twice as high as the two lowest zoos (30 and 31 ng/g ; $P = 0.07$ and 0.12 , respectively). The same zoo with the highest fecal cortisol also had the highest salivary cortisol ($P \leq 0.05$). For European zoos, fecal cortisol concentrations differed between zoos for both spider and woolly monkeys ($P \leq$

0.05) and spider monkeys fecal cortisol concentrations were higher than those from woolly monkeys within the same zoos ($P \leq 0.05$). From each study, the zoos with the highest dietary carbohydrates, total sugars, glucose and fruit content had the highest cortisol. In addition, cortisol concentrations were highest for the zoos that did not meet crude protein requirements and fed the lowest percentage of nutritionally complete primate feeds and crude fiber. This study demonstrates large differences exist between zoos with respect to housing and diets of spider and woolly monkeys. Differences between diets may cause spider and woolly monkeys to be more susceptible to stress which can lead to metabolic, reproduction, and cardiovascular problems. The lifespan and reproductive success of captive primates will improve if stressors and negative effects of nutrition on the health status can be reduced and dietary nutrients can be optimized.

INULIN-TYPE FRUCTANS

It was hypothesized that ITF may alleviate diabetes, hypertension, and stress in woolly monkeys, while increasing reproductive and immune functions. It was then further postulated that fecal cortisol concentrations could be an assessment tool to track these positive changes. The objectives of this study were to: 1) compare fecal cortisol levels in woolly and spider monkeys and to establish normal levels; and 2) evaluate the effect of ITF supplementation in the daily diets of both woolly and spider monkey on fecal dry matter and fecal cortisol. A three day diet disappearance study was conducted and indicated that, in general, the diets met nutrient recommendations. However, vitamin A concentrations (including β -carotene), were 10 to 30 times above recommendations. Subsequently, ITF (containing 90 to 94% inulin) was added to the daily diet for spider (*Ateles sp.*; $n = 9$) and woolly monkeys ($n = 7$), individually, at 5 g per day for four weeks. Fecal samples were collected prior to supplementation, after two weeks and after four weeks. Overall, spider monkeys had lower fecal DM content ($P <$

0.05) than woolly monkeys (19% *versus* 23%), regardless of ITF supplementation. Fecal cortisol concentrations were not affected by ITF, however, fecal cortisol was numerically decreased after the 4 week ITF supplementation, primarily in spider monkeys (110 *versus* 65 ng/g dry feces). These results suggest that ITF supplementation may have positive effects on captive primate well-being. However, additional research with larger populations of monkeys using higher doses of ITF is recommended.

CONCLUSION

Zoos and concerned monkey keepers have tried various methods to reduce stressors and health problems within captive woolly monkey populations. These methods include medications, removing the woolly monkeys from public exhibits, altering feeding schedules to deliver sugar loads gradually, altering diet items to perceived natural foods and stopping breeding. However, cortisol measures have not been taken before and after the removal of these potential stressors and, therefore, little is known about the success of such techniques. It is obvious from the research within this dissertation that woolly monkeys are a threatened species in captivity. From this research, it also is obvious that unnatural diets may contribute to the poor success of this species. With free-ranging data on woolly monkey natural diet, natural fecal cortisol concentrations, serum nutritional analyses and genetic lineage, it is probable that the exact underlying problem affecting captive woolly monkeys can be determined and altered.

Samenvatting

INLEIDING

Spinapen (*Ateles spp.*) en wolapen (*Lagothrix ssp.*) zijn twee van de grootste Nieuwe Wereld primaten. Zij hebben in het wild een gewicht van 5,5 tot 11 kg. Naar verluidt zijn er ongeveer 1.000 spinapen wereldwijd in gevangenschap, terwijl er slechts 85 wolapen wereldwijd in gevangenschap worden gehouden. Zowel spinapen als wolapen worden beschouwd als bedreigde diersoort. Speciaal de wolapen planten zich bijzonder moeilijk voort in gevangenschap en zijn ook anderszins heel moeilijk succesvol te houden in gevangenschap. Het fokken van wolapen in dierentuinen levert niet voldoende wolapen op om de populatie in stand te houden. Bovendien bereiken deze dieren vaak niet de normale levensduur van boven de 15 jaren in dierentuinen vanwege gezondheidsproblemen. De meerderheid van de gezondheidscomplicaties in wolapen is waarschijnlijk gerelateerd aan voeding. Dit wordt afgeleid uit het feit dat hun problemen vergelijkbaar zijn met diabetes mellitus en hypertensie bij andere primaten. Net als bij mensen zijn deze gezondheidsproblemen vaak meer uitgesproken tijdens zwangerschap en na de geboorte.

Het huidige onderzoek had twee hoofddoelen. Het eerste doel was om vast te stellen of voeding als een van de voornaamste oorzaken voor de gezondheidscomplicaties in wolapen via onderzoek uit (historische) gegevens van dierentuinen afgeleid kon worden. Het tweede doel was om de invloed van koolhydraten (vooral suikers) en vet in de voeding op het serumprofiel van een aantal indicatoren voor diabetis en op cortisolconcentratie (als maat voor stress) te onderzoeken.

ANALYSE VAN HISTORISCHE GEGEVENS

Data werden verzameld via schriftelijke enquête en via data analyse uit gegevens van het International Species Information System. Ook werden zootechnische gegevens uit het fokboek van wolapen bestudeerd om de levensduur en sterfte van wolapen in gevangenschap

over de periode van 1990 tot 2005 te bepalen. Data voor spinapen, een van de meest nauwe verwanten van de wolaap, werden van vijf representatieve instellingen voor een vergelijkende analyse verzameld.

In de afgelopen 16 jaren is de totale populatie van wolapen met 11% afgenomen. Het aantal instellingen die deze primaten huisvest is ook afgenomen. De verhouding in geboorte tot sterfte is minder dan 1 (0,65). In vergelijking daarmee is deze verhouding bij spinapen veel hoger (1,26). De lage verhouding is meer uitgesproken bij de vrouwelijke wolapen (0,47) dan bij de mannelijke wolapen (0,74). De voornaamste twee sterfteoorzaken in wolapen zijn een lage levensvatbaarheid bij babies en complicaties gedurende zwangerschap (53% van de gevallen) en ook cardiovasculaire complicaties (15%). Verder onderzoek is nodig, in het bijzonder naar mogelijke voedingsaspecten die ten grondslag liggen aan deze doodsoorzaken.

Slechts een enkele instelling is succesvol geweest om een aantal generaties van de wolaap te fokken. Veel van de nakomelingen die in deze instelling ter wereld kwamen zijn naar andere faciliteiten overgebracht. En zij behoren daar tot de weinige apen die reproductief succesvol zijn. Dit betekent ook dat er wellicht een afname is in genetische variatie bij wolapen in gevangenschap en dit kan waarschijnlijk ook een negatieve invloed hebben op hun reproductief succes. Het is daarom niet uitgesloten dat genetische afwijkingen in metabolisme of geen goede voeding (in vergelijking met voeding in de natuur) verantwoordelijk is voor problemen bij het houden van wolapen in dierentuinen.

De volgende onderzoeksvragen zijn van belang:

- 1) zijn wolapen uniek in hun voeding en/of huisvesting vergeleken met andere, verwante, primaten.

- 2) zijn wolapen meer gevoelig voor metabolische problemen vergeleken met verwante primaten en is het daarom nodig om meer op hun voeding te letten.

SERUM PROFIELEN

Data van serum analyses van 30 wolapen gehuisvestigd in twee instellingen (Apenheul in Nederland en De Louisville Dierentuin in de VS) zijn over 12 jaren verzameld. De voornaamste doelen waren: 1) een basislijn voor serum concentraties voor wolapen in gevangenschap vast te stellen; 2) serum concentraties bij de twee geslachten, bij verschillende leeftijdsgroepen en bij verschillende instellingen te bepalen; 3) serum concentraties te vergelijken met de meest verwante primaten van de wolaap; en 4) afwijkingen bepalen die in relatie kunnen staan tot de korte levensduur van wolapen in gevangenschap.

Een aantal gehalten van chemische parameters in het serum van wolapen waren vergelijkbaar met concentraties die gerapporteerd zijn voor brulapen (*Alouatta* sp.) en spinapen (*Ateles* sp.) met uitzondering van alanine aminotransferase (ALT) en gamma-glutamyl-transferase (GGT) concentraties. De laatste twee waren hoger voor wolapen terwijl creatinine en fosfor concentraties lager waren dan voor brulapen en spinapen. Het serum glucose gehalte (6,7 mmol/L) was hoger dan de basislijn waarden in mensen en spinapen. Alkalisch fosfatase, ALT en natrium (Na) was het hoogste in vrouwelijke apen en magnesium (Mg) was hoger in mannelijke apen ($P < 0,05$). Bij de oudere dieren waren de gehalten aan alkalische fosfatase, Mg en fosfor het hoogste ($P < 0,05$) en calcium, Na en ferritin waarden tenderden naar hoger ($P < 0,10$). Gehaltes aan albumine, alkalisch fosfatase, chloride, Na en total bilirubin waren het hoogst bij de dieren gehouden in dierentuin A, terwijl GGT, glucose en lactate dehydrogenase gehalten in deze dierentuin lager waren ($P < 0,05$). Om goed te kunnen vergelijken is veel meer onderzoek nodig om serum

concentraties vast te stellen bij in het wild levende wolapen. Dit kan dan inzicht geven in parameters waarvoor bij in gevangenschap levende dieren abnormale concentraties gevonden worden alsmede hun invloed op gezondheidsstatus

DIABETIS

Zes wolapen met hypertensie werden gebruikt om vast te stellen of type II diabetes en dagelijkse dieet compositie ten grondslag kunnen liggen aan de gezondheidsproblemen bij deze diersoort in gevangenschap. Concentraties in nuchtere staat voor glucose (directe indicator van insuline functie), insuline (indicator van insuline deficit), fructosamine (indicator van bloed glucose controle gedurende de afgelopen twee tot drie weken) en glycated hemoglobine (indicator van lange termijn bloed glucose status) werden vastgesteld. Serum lipiden (high density lipoproteïne cholesterol (HDL-Chol), low density lipoproteïne cholesterol (LDL-Chol), totaal cholesterol, & triacylglycerides) werden geëvalueerd als indicators voor het risico voor hart- en vasculaire ziekte. Urine werd verzameld en geanalyseerd op glucose concentraties. Voeropname bij de dieren werd bepaald gedurende een periode van drie dagen voor de bloedafname. Uit deze gegevens en de nutriënten inhoud werd berekend hoeveel nutriënten de dieren opnamen. Resultaten van serum analyse lieten waarden zien binnen het normale bereik (fructosamine: 139 tot 242; glucose: 2,22 tot 4,78 mmol/L; glycated hemoglobine: 3,52 tot 4,73%; insuline: 6,2 tot 13,0; triacylglycerides: 0,38 tot 3,4 mmol/L; total cholesterol: 2,5 tot 5,1 mmol/L; HDL-Chol: 0,4 tot 1,6 mmol/L; en LDL-Chol 1,8 tot 3,4 mmol/L) wanneer ze vergeleken worden met Nieuwe en Oude Wereld apen en met mensen. Glucose concentraties in urine waren niet meetbaar. Voersamenstelling was niet limiterend in zetmeel en total suikers. Ook de gehalten aan verteerbare overige organische stof was niet afwijkend. Potentiële tekorten in het voer werden gevonden voor vitamine A, vitamine D, calcium, fosfor en seleen. Resultaten van deze studie duiden ook aan

dat diabetes mellitus geen probleem schijnt te zijn binnen deze specifieke populatie van wolapen. In de huidige studie werden geen algemene gezondheidswaarnemingen gedaan met betrekking to zwangerschapsdiabetes. Het kan echter ook zijn dat de voorgeschreven medicaties zoals die in deze instelling werd toegepast de interpretatie van gegevens heeft beïnvloed.

CORTISOL IN FECES EN SPEEKSEL

Potentiële stressors, samenhangend met een onnatuurlijk dieet, kunnen het houden van bedreigde primaten bedreigen omdat chronische activering van de stress response een nadelig effect heeft op het welzijn van een dier. Dus het doel van dit experiment was om de effecten van dieet compositie, in het bijzonder eenvoudige suikers, bij verschillende zoölogische instellingen op fecaal- en speekselcortisol concentraties in wolapen en spinapen te onderzoeken. Fecaal (272) en speeksel (66) monsters van wolapen (n=27) en spinapen (n=61) werden verzameld in vier dierentuinen in de VS en in drie in Europa. Monsters werden geanalyseerd voor cortisol concentraties. Bij een instelling in de VS werd een hoge fecale cortisol concentratie ($66 \pm 10,9$ ng/g) in de dieren gemeten. Deze was in deze dierentuin tweemaal zo hoog als in de andere twee, die hadden concentraties van respectievelijk 30 en 31 ng/g; ($P = 0,07$ en $0,12$, respectievelijk). De dierentuin met de hoogste fecaal cortisol gehalte liet ook de hoogste speeksel cortisol gehalte zien ($P = 0,05$). In Europese dierentuinen verschilden de fecale cortisol concentraties tussen dierentuinen voor zowel spinapen als wolapen ($P < 0,05$). Wanneer vergeleken werd binnen dezelfde dierentuin was de fecale cortisol concentratie bij spinapen hoger dan bij wolapen ($P < 0,05$). De dierentuinen die de hoogste concentratie koolhydraten, totale suikers, glucose en fruit in het voer gaven hadden ook de dieren met een hoog cortisol niveau. Bovendien waren de cortisol concentraties het hoogste bij dieren in dierentuinen die een rantsoen gaven dat niet voldeed aan de ruwe eiwit

behoefte. Deze dieren werden een rantsoen gegeven dat minder van een volledige prismaat voer bevatte en ook een mindere vezel fractie in het voer had. Deze studie laat zien dat er grote verschillen bestaan tussen dierentuinen met betrekking tot de huisvesting en voer samenstelling van spinapen en wolapen. Een niet optimale voersamenstelling kan er bij beide soorten apen (spinapen en wolapen) toe leiden dat ze gevoeliger worden voor stress. Dat kan leiden tot metabole, reproductieve, en cardiovasculaire problemen. De levensduur en het reproductiesucces van primaten in gevangenschap kan wellicht verbeteren als de voedingssamenstelling meer optimaal kan worden gemaakt.

INULIN-TYPE FRUCTANS (ITF)

In de literatuur wordt wel voorgesteld dat ITF in het rantsoen het optreden van diabetes, hypertensie en stress bij wolapen zou kunnen verminderen, terwijl reproductie en immuun functie verbeterd zou kunnen worden. In literatuur studies wordt verder gepostuleerd dat als door ITF in de voeding de fecale cortisol concentraties verlaagd worden dit een positieve invloed op het dier heeft. Het doel van deze studie was als volgt: 1) een vergelijking te maken in fecale cortisol concentraties in wolapen en spinapen en normale waarden vast te stellen; en 2) het effect van ITF supplementatie in het dagelijkse voer na te gaan op zowel wolapen als spinapen met betrekking tot droge stof in feces en in fecaal cortisolgehalte.

Gedurende drie dagen werd consumptie van voer en nutriënten vastgesteld. Resultaten lieten zien dat in het algemeen de voedselsamenstelling voldeed aan de aanbevelingen voor nutriëntenopname. Echter, vitamine A hoeveelheid (inclusief β -caroteen) was 10 tot 30 keer zo hoog dan de aanbevolen hoeveelheid. Het ITF (bevatte 90 tot 94% inuline) werd vervolgens toegevoegd aan het dagelijkse dieet voor spinapen (*Ateles sp.*; n = 9) en wolapen (n = 7) en werd individueel verstrekt als 5 g per dag gedurende vier weken. Fecale monsters werden drie keer verzameld: 1) voor supplementatie 2) na twee weken en 3) na vier weken.

In het algemeen hadden spinapen een lager fecale droge stof gehalte ($P < 0,05$) dan wolapen (19% versus 23%). Dit was onafhankelijk van ITF supplementatie. Fecale cortisol concentratie werd niet beïnvloed door ITF, alhoewel fecal cortisol numeriek lager was na 4 weken ITF supplementatie, voornamelijk in spinapen (110 versus 65 ng/g droge stof). Deze resultaten suggereren dat ITF supplementatie een positieve invloed op het welzijn van primaten in gevangenschap kan hebben. Verder onderzoek is echter nodig met grotere populaties van apen en met verschillende doses van ITF.

CONCLUSIES

Dierentuinen en diervverzorgers hebben verschillende methoden geprobeerd om stressors en gezondheidsproblemen binnen wolapen populaties te verminderen. Deze methoden omvatten medicatie en het verwijderen van wolapen zodat ze niet meer voor het publiek te zien zijn.

Ook voer schema's veranderen om suikers in het voer meer regelmatig en geleidelijk te verstrekken wordt toegepast. Verder probeert men meer natuurlijke voedingsmiddelen te verstrekken. Ook worden fokprogramma's gestaakt om complicaties tijdens zwangerschap te voorkomen. Echter, cortisol metingen zijn nooit eerder gedaan voor en na het verwijderen van potentiële stressors. Het succes van deze technieken is daarom niet bekend. Het is duidelijk uit het onderzoek in deze dissertatie dat wolapen in gevangenschap een bedreigde diersoort is. Uit dit onderzoek is verder duidelijk dat onnatuurlijke dieeten kunnen leiden tot het verminderen van aantallen van deze soort in gevangenschap. Data met betrekking tot het dieet van wolapen in het wild als ook fecale cortisol concentraties en serum concentraties bij in het wild levende wolapen, zouden kunnen leiden tot het vaststellen van het onderliggende probleem bij wolapen. Daaruit ontstaan misschien mogelijke oplossingen.

Curriculum Vitae, Publications of the Author, and Notes

CURRICULUM VITAE

Kimberly Ange-van Heugten was born on August 8, 1974 in Washington, North Carolina, USA. She graduated from Pungo Christian Academy in 1992 and then started her studies at North Carolina State University (NCSU) in the same year. She received her BS degree with honors in animal science with a minor in nutrition in 1996 and her MS degree in nutrition with a minor in animal physiology in 1999. Her MS work was conducted under the supervision of Dr. Joan Eisemann and evaluated water disappearance and the effects of buffered water on proximal stomach pH in swine. Subsequently, she worked for two years as an associate animal nutritionist at The Bookfield Zoo near Chicago, IL, USA. She then was appointed as animal science lecturer at NCSU, where she has been since 2001. She develops and teaches nutrition courses, companion animal science courses, and introductory agricultural science courses. She also is involved in novel research, and conducts nutrition consulting for zoological facilities. She began her PhD as an external student at Wageningen University in late 2003.

LIST OF PUBLICATIONS AND AWARDS**Peer Reviewed Publications:**

Ange-van Heugten, K.D.; van Heugten, E.; Timmer, S.; Bosch, G.; Elias, A.; Whisnant, S.;

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Sep. 16, 2003: Pets need special care during hurricanes, too. North Carolina State University College of Agriculture and Life Sciences news release.

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Dec. 5, 2002: Pets need special care when the power's off. North Carolina State University College of Agriculture and Life Sciences news releases.

<http://www.cals.ncsu.edu/agcomm/writing/newsrsls/archive/2002/12-06-02a.htm>

Television Appearances

Dec. 22, 2002 & Dec. 28, 2003: Guest appearance for Fox 50's 30 minute Sunday morning show "Tarheel Talk" at 6 and 8 am. The interview was to inform pet owners about potential holiday hazards.

Awards

Received the 2007 Gertrude Cox 2nd place award for Innovative Excellence in Teaching and Learning with Technology.

Received a 2003 Pride of The Wolfpack Award, North Carolina State University.

Received the 1997 CALS ANS department Graduate Student Teaching Award.

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