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Research Article

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

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Detrimental physiological effects due to stressors can contribute to the low captive success of primates. The objective of this research was to investigate the potential impact of diet composition on cortisol concentrations in feces and saliva in woolly ($n = 27$) and spider monkeys ($n = 61$). The research was conducted in three studies: the first investigated spider monkeys in the United States, the second investigated spider monkeys within Europe, and the third investigated woolly monkeys within Europe. Fecal cortisol in spider monkeys in US zoos varied ($P = .07$) from 30 to 66 ng/g. The zoo with the highest fecal cortisol also had the highest salivary cortisol ($P \leq .05$). For European zoos, fecal cortisol differed between zoos for both spider and woolly monkeys ($P \leq .05$). Spider monkeys had higher fecal cortisol than woolly monkeys ($P \leq .05$). Zoos with the highest dietary carbohydrates, sugars, glucose, and fruit had the highest cortisol. Cortisol was highest for zoos that did not meet crude protein requirements and fed the lowest percentage of complete feeds and crude fiber. Differences among zoos in housing and diets may increase animal stress. The lifespan and reproductive success of captive primates could improve if stressors are reduced and dietary nutrients optimized.

1. Introduction

Spider *(Ateles spp.)* and woolly *(Lagothrix ssp.)* monkeys are two of the largest New World primates with a weight range of 5.5 to 11 kg in the wild [1]. They live in South American rainforests in Brazil, Colombia, Peru, and Ecuador. Approximately 1000 spider monkeys are reportedly housed in captivity worldwide and only 85 captive woolly monkeys are reported worldwide [2]. Both spider and woolly monkeys are considered threatened species in the wild [3, 4]. Woolly monkeys are considered extremely difficult to breed and successfully maintain in captivity [5–8]. The natural diets of both spider and woolly monkeys are primarily frugivorous. They rely on more than 80% ripe fruits in their diet [9–12]. Zoological institutions typically feed the majority of woolly and spider monkeys diets as fruit. It is suspected, however, that human cultivated fruits differ significantly when compared to wild fruits [13, 14]. Wild fruits have higher contents of fibers, minerals, proteins, and vitamins as well as a lower content of total sugar [14, 15]. In addition, there is reportedly less sucrose and more fructose and glucose in wild fruits than cultivated fruits [14, 15]. 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 substantiality over time due to both item availability and composition and this seasonal variation is not typically reflected in human-cultivated fruits [14].

Elevated levels of dietary sugars and fats may increase sympathetic nervous system release of cortisol in mammals [16]. 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 [17–21]. These negative conditions are also associated with the poor life expectancy of woolly monkeys in captivity [5]. Various other management factors within captive primate populations such as housing space, competition for resources, age, and gender can also cause elevated levels [17, 22–24]. Studies have successfully evaluated cortisol concentrations in both feces [24–26] and saliva as a measure of stress in primates [27–30].

The objectives of the current research were to (1) investigate the fecal and salivary cortisol concentrations at multiple zoological institutions in woolly and spider monkeys, (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 due to their noted poor success in captivity, woolly monkeys are more responsive to diet as measured by cortisol than spider monkeys.

2. Materials and Methods

2.1. 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 three of the studies, the age, gender, animal exhibit dimensions, species and subspecies of monkey, and birth location (whether born in captivity or in 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 [1, 10].

2.2. Diet Collection and Analyses. For all studies, zoos maintained the same daily monkey diets for at least three days prior to data collection and animal keepers did not change the diets during the research period. At all institutions, except number 4 and number 5, diet consumption data were collected consecutively for three days (during which samples for cortisol analyses were also collected). For institutions number 4 and number 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. Evaporative losses were calculated for all remaining feed items. 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, USA) and Zoo Diet Analysis (ZDA; Allen and Baer Associates, Michigan State University (East Lansing, Mich, USA), and Zoological Society of San Diego (San Diego, Calif, USA)). 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; 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.

2.3. Animals and 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, Tex, USA ($n = 16$); Omaha's Henry Doorly Zoo, Omaha, Neb, USA ($n = 6$); Highwater Farms, Kipling, NC, USA ($n = 5$); Little Mans Zoo, Chadbourne, NC, USA ($n = 8$); Little Rock Zoo, Little Rock, Ark, USA ($n = 10$). 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 collections. 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 (*Lagothrix ssp.*) 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. 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.

2.4. 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 to the USA, two separate laboratories were used to analyze fecal samples for cortisol. Sample analyses procedure was duplicated between laboratories. After the samples were collected, they were immediately frozen and shipped overnight using dry ice to either North Carolina State University (Raleigh, NC, USA) or Wageningen University (Wageningen, The Netherlands). Samples 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 $2500 \times$ 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, Calif, USA). 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, Calif, USA) 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 μg/dL.

2.5. 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 [30], 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 $2500 \times$ 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, USA, until it was analyzed for cortisol. Samples were thawed and again centrifuged at $2500 \times$ 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 [27, 30]. 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. [27] 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.

2.6. 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 included zoo. The least square means procedure was used to calculate fecal and salivary cortisol means and SEM by zoo. Significances were noted at $P \leq .05$ and tendencies were considered at $.05 < P \leq .10$.

Studies 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 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.

Table 1: Monkey management and housing information from the zoological institutions housing spider and woolly monkeys.

Zoo number	N	Monkey 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.

Significances were noted at $P \leq .05$ and tendencies were considered at $.05 < P \leq .10$.

In addition, two zoological institutions in studies 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.

3. Results

3.1. Animal Housing and Management. Details on monkey management and housing information from the zoological institutions are given in Table 1 for all three studies, 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 ($35\,m^3$ versus $150\,m^3$) and only one of the zoos held more than one species of spider monkeys. In study 3, the space allotted per woolly monkey also varied greatly ($60\,m^3$–$250\,m^3$) and only Zoo 6 had woolly monkeys that were born in the wild.

3.2. 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, USA); Zoos 2 to 5 fed Mazuri New World Primate (PMI Nutrition International, St. Louis, Mo, USA); Zoo 5 also fed Mazuri Old World Primate (PMI Nutrition International, St. Louis, Mo, USA). 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.

3.3. Fecal and Salivary Cortisol Concentrations. For all three studies, fecal cortisol concentrations were highest within each study when total sugars and fruit percentages were highest and fiber was lowest (Tables 1 to 3). Fecal cortisol concentrations were also highest for all three studies in the zoos with the highest levels of carbohydrates. Finally, for fecal

Table 2: Spider monkey diet nutrient analyses, food group percentages, and fecal and salivary cortisol concentrations (±SEM) from five zoological institutions in the US.

Study 1	Zoo 1	Zoo 2	Zoo 3	Zoo 4	Zoo 5
Dietary nutrients, % (dry matter basis)					
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, % (as fed)					
Fruit	83	42	44	34	60
Vegetable	5	27	32	57	0
Nutritionally complete feed[‡]	10	18	22	9	20
Breads and grains	2	5	2	0	11
Miscellaneous	0	8	0	0	9
Cortisol analyses					
Fecal cortisol, ng/g*	66 ± 10.9	49 ± 40.6	30 ± 15.4	NA[€]	31 ± 18.2
Salivary cortisol, μg/dL[£]	17 ± 3.7[a]	NA	7 ± 3.2[b]	11 ± 5.9[a,b]	2 ± 3.2[b]

[‡]Zoo 1 fed Mazuri High Protein Primate (PMI Nutrition International, St. Louis, Mo, USA); Zoos 2 to 5 fed Mazuri New World Primate (PMI Nutrition International, St. Louis, Mo, USA); Zoo 5 also fed Mazuri Old World Primate (PMI Nutrition International, St. Louis, Mo, USA).
*Fecal cortisol is measured on a DM basis.
[£]Significant difference in salivary cortisol concentration among institutions ($P < .05$) is indicated by superscripts a, b.
[€]NA: information not available.

cortisol comparisons, the zoos with the lowest percentage of nutritionally complete primate diet had the highest cortisol concentrations within all three studies.

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, $P = .21$) 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; $P = .07$) and 5 (31 ± 18.2 ng/g; $P = .12$).

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 ($P = .05$).

Studies 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) ($P \leq .003$). In addition, spider monkey fecal cortisol concentration means were higher (171 ± 15.4) than woolly monkey means (62 ± 12.6) ($P \leq .0001$).

4. 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 [12, 31]. 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 nonhuman 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

Table 3: Spider and woolly monkeys diet nutrient analyses, food group percentages, and fecal cortisol concentrations (±SEM) from three zoological institutions in Europe.

	Zoo 6	Zoo 7	Zoo 6	Zoo 7	Zoo 8
	Study 2 spider monkeys		Study 3 woolly monkeys		
Diet nutrients, % (dry matter basis)					
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, % (as fed)					
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 ± 40.6^{a}	227 ± 40.6^{b}	87 ± 26.8^{x}	122 ± 20.3^{x}	269 ± 20.8^{y}

‡The nutritionally complete feed utilized by Zoos 6 and 7 was Leaf Eater Primate (Mazuri Zoo Foods, Witham, Essex, UK).
*Fecal cortisol is measured on a DM basis.
£Significant difference in fecal cortisol concentration between spider monkey institutions ($P < .05$) are indicated by superscripts a, b and among woolly monkey institutions by superscripts x, y.

[13, 14]. 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 [32].

The large variation in diet food category percentages and nutrients between and within zoos from all three studies is a potential for concern. The high levels of fruit and low levels of nutritionally complete foods are not in agreement with the National Research Council (NRC) nonhuman primate nutrient requirements [33]. 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 monkeys consuming up to 68% of their diet from vegetables. In addition, 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 [14]. 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 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 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 [33]. The highest concentrations of fecal and salivary cortisol were observed in zoos that did not meet the crude protein requirement. In addition, in study 1, the highest concentrations of salivary cortisol were measured in the two zoos that did not meet the crude protein requirement.

Seematter et al. [16] 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 2 diabetes, ulceration in the gastrointestinal tract, decreased reproduction, osteoporosis, and immunosuppression [17, 19–21]. 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 [24–26, 34] and saliva to determine stress levels [27–30]. New World primates and Old World primates reportedly differ in their circulating cortisol levels as well the metabolism of cortisol [35]. New World primates typically have a 10-fold higher concentration than the Old World primates [36]. Spider and woolly monkeys are both considered New World primates. The spider monkey salivary cortisol range reported within study 1 (2–17 μg/dL) was lower than the New World monkey values previously reported for squirrel monkeys *(Saimiri sciureus)* ($28 \pm 2.3\,\mu$g/dL) but was approximately 10-fold higher than the Old World monkey range (0.3–1.8 μg/dL) reported for rhesus monkeys *(Macaca mulatta)* [29, 30]. Thus, circulating levels differ among monkey species and it is important to establish normal concentrations for all species.

Since spider and woolly monkeys are both New World primates and extremely closely related [7, 8], differences in fecal cortisol concentrations between the two were not expected. In addition, the higher fecal cortisol concentration for spider monkeys was also unexpected since these species tend to be more successful in captivity with regard to maintenance and reproduction when compared to woolly monkeys.

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 [37]. 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 allow more samples to be collected without disturbing behavior [24, 37].

It has been reported that physical stress, insufficient living space, and obesity are all factors that can cause hypertension and increase cortisol concentrations [16, 38, 39]. 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 was 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 [40]. 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 [35]. Due to these possible influencing variables and the fact that study 1 cortisol concentrations were analyzed in an American laboratory while studies 2 and 3 were analyzed in European laboratories, we did not compare the fecal analyses between and among all zoos within both countries holding spider monkeys (study 1 versus study 2). In addition, the subspecies of spider monkeys varied slightly among the two studies and this could have provided cortisol variations. It is important to note, however, that all zoos and all studies 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 [41], 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.

5. 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 compare the cortisol levels of both spider and woolly monkeys. It can be hypothesized that 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 may 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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