Browse species preference and palatability of *Colobus guereza kikuyuensis* at the Denver Zoological Gardens

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ABSTRACT:

In the wild, folivorous primates have access to leaves of many different species. At the Denver Zoological Gardens (DZG), in Denver, Colorado, *Colobus guereza kikuyuensis* primates consume most vegetation presented to them, thus maintenance of vegetation in an animal exhibit is challenging. It is observed that insufficient browse often results in behavior concerns such as pacing or lack of mental stimulation. Therefore, regular addition of preferred and palatable browse may strengthen natural wild behavior and decrease habitual stereotypical captive behaviors. Palatability can play a role in the determination of browse preferences. Palatability can be defined as those plant characteristics stimulating an animal's selective response. The objective of this study was to examine the preferences of three adult (1 male, 2 female) *Colobus guereza kikuyuensis* monkeys for browse species commonly found at DZG. Five representative samples of browse were selected: *Salix irrorata; Carnus alba 'Bud's Yellow'; Salix discolor; Alnus tenuifolia;* and *Betula occidentalis.* Using paired preferences, plant selection by each primate was ranked and analyzed using SAS* statistical software, employing the Bradley-Terry categorical model. When comparing these species at DZG, *Alnus tenuifolia* was selected most often as the first or second choice among the three primates, whereas, *Salix irrorata* tended to be least popular. The primates as a group preferred low amounts of NDF, ADF, and ADL in the browse. However the most preferred plant, *Alnus tenuifolia*, did contain high amounts of NDF, ADF, and ADL, and in our opinion, this shows a preference for taste alone since the DZG diet is thought to meet all known nutrient needs of the Colobus. Knowledge of palatability and preference of browse species allows for informed decisions to be made providing a more palatable and preferred nutritive supplement for captive leaf monkeys, and may alleviate behavioral concerns. Thus, a preference study, demonstrating natural folivorous tendencies and preference for browse, is warranted, and may lead to future palatability studies of browse.

Keywords: Browse; Palatability; Preference; *Colobus guereza kikuyuensis*

INTRODUCTION:

* SAS System for Windows Version 6.12

Dietary formulation can have significant impacts on the behavior of animals. Proper management (Dwyer, 1964), inclusive of dietary analysis, can achieve behavioral enhancement. Awareness of an animal's preference for certain foods is equally important in achieving the goal of behavioral enhancement. Palatability can play a role in the determination of those dietary preferences. According to Heady (1964), palatability can be defined as "plant characteristics or conditions which stimulate a selective response by animals" and preference can be defined as "selection by the animal and is essentially behavioral." Heady continues to describe relative preference as a "proportional choice among two or more foods." It is known that mammalian herbivores do not randomly select their choice of food components. They may select their diet based on preference, which may focus on certain instinctual parameters, such as high energy or secondary defense compounds (Danell, 1994). While this study looks at the preference of *Colobus guereza kikuyuensis* (folivorous primates) by using relative preference for five browse species found at the Denver Zoological Gardens (DZG), it does not directly address palatability.

Colobus guereza, commonly referred to as black and white Colobus monkeys, are found in areas ranging from eastern Nigeria to Ethiopia and Tanzania (Nowak, 1991). They are part of the family Cercopithecidae which consists of 37 species in six genera, found in south and southeast Asia and equatorial Africa (Macdonald, 1989). *C. guereza* is black with a white beard and white mantle from the shoulders to the lower back and has a large white tuft on the end of the tail (Nowak, 1991). There is usually one male within a tight group of 3-15 animals.

Colobines are forestomach fermentors (Robbins, 1993) being unique in that the upper region is expanded from its lower acid region (Macdonald, 1989). This upper region has a neutral medium for fermentation of plants by anaerobic bacteria. The buffer fluid between the acidic and neutral regions of the stomach consists of salivary mucus. Colobus digest leaves more efficiently than any other primates due to this upper region of the stomach. Through evolution, the diet of Cercopithecines evolved from fruit consumption to a more broad diet containing fruits, flowers and leaves. This new diet allowed them to more readily survive on open Savannah and woodland habitats. The Colobus further progressed, relative to other Cercopithecines, to become almost solely folivorous (Macdonald, 1989).

MATERIALS AND METHODS:

Subjects

This study, at the Denver Zoological Gardens, examines adult *C. guereza* leaf monkey's preference for five browse plant species. One male (12 years old) and two female adult (between 11 and 12 years of age) primates, Christopher, Camille and Clare, were observed. These adults were a subset of a family of seven primates. Animal regulations at DZG require that the animals remain together at all times during the study. The four additional primates within the family were not included in the study since they were less than two years old. Sexual maturity for this species occurs between four and six years of

age (Nowak, 1991; Macdonald, 1989). An evident hierarchy existed within this group. Christopher, the only adult male, is the dominant adult. Camille and Clare are subordinate to Christopher, while Camille is dominant to Clare. The younger animals were permitted to sample the browse, however they did not interfere with the collection of data due to the existing dominance structure of the group. Therefore, the study was focused on preference rather than intake or bite counts, which would provide additional quantitative data.

Environment

Presentations of browse were conducted in the morning between 8 a.m. and 11 a.m. Data collection and a period of acclimation spanned from July 21 to August 4, 1999, with summer season temperatures of an average daily temperature of 20.5°C (69°F) with a range of 12.2-18.8°C (54-66°F) minimum to 21.6-33.3°C (71-92°F) maximum. From early morning through evening, the group of *C. guereza* remained outdoors in a semi-circular chain-link fence enclosure. Contained within the exhibit are suspended ropes, tall artificial and natural tree trunks, rope hammocks, a crescent-shaped concrete pond, and grass groundcover. Water is provided at all times to the animals via a manually operated water spigot. The lack of natural vegetation is immediately apparent, making supplementation of browse both desirable and mandatory. A small portion of the enclosure itself is made of thick glass to facilitate observation by zoo patrons. During data collection and observation, the experimenter presented food pairs from a portion of the fenced enclosure accessible only to zoo personnel. Therefore, zoo patrons did not interfere with data collection or plant presentation to the primates.

Plant Species

Five representative species of browse (Table 1) were selected from the available browse species grown at DZG: *Salix irrorata*; *Carnus alba 'Bud's Yellow'*; *Salix discolor*; *Alnus tenuifolia*; and *Betula occidentalis*. These plant species are commonly supplemented to DZG animals and are included in the Zoological Database being developed at Colorado State University (Kirschner, 1998). These plants were included because of availability and the previous exposure of the primates to these plants, as well as permission from the Horticulture Department of DZG. Rationale for the selections are as follows: *Salix irrorata* was chosen for its high ratio of leaves per branch, which may provide greater tactile stimulation and availability of leaves during the leaf picking process; *Betula occidentalis* was selected for its sticky, maple-like characteristics providing potentially positive palatability; *Carnus alba* was selected for its succulent and moist appearance; and both *Salix discolor* and *Alnus tenuifolia* were included as intermediates to the other succulent, abundant, and "sticky" plants. Furthermore, these characteristics are representative of the browse available at DZG.

Diet

Throughout this study, the primates remained on their normal diet (Table 2 and 3). This diet consisted mainly of vegetables and a high fiber Leafeater biscuit (Marion Zoological), as well as regulated treats, such as plums, peaches, tomatoes, and snow peas. The afternoon diet components are provided on a rotation schedule to increase diet

variety. Daily, for a period of four to five days prior to the study, the group was presented with samples of the five browse species, in the morning, to acclimate them to both the browse and the methods employed during the study.

Presentation of browse

Selected browse was presented to the primates and their choices were recorded. A simple method of paired preference was used to investigate food preference (Agresti, 1996; White, 1981). Each of five browse species was compared to the remaining four species for a total of ten comparisons (Table 4), presented over a period of three days. The comparisons were presented in random order. Three replications of these comparisons were conducted over a total observation period of nine days.

Each day of the study began with the collection of browse for that day. Comparisons required four bundles of five branches, two bundles per plant species. Ten inches of new growth of these plants was cut in the amount of ten branches per use of that specific plant. Those four bunches were attached to a rope with clips at either end. The plants were placed on the rope in alternating order using electrical ties. For example, a comparison of *Carnus alba* to *Salix irrorata* would be presented as: —5 branches of *Carnus alba*—5 branches of *Salix irrorata*—5 branches of *Carnus alba*—5 branches of *Salix irrorata*—. This mode of presentation allowed for each of the three adult *C. guereza* to have the opportunity to choose any of four bunches. The rope with the plants was attached to the exterior of the fence at approximately 18 inches from the ground. The choice of each animal was recorded. Once each of the observed adults had made their choice, the rope was removed and the next comparison was attached and presented. This procedure continued until the comparisons for that day were completed. Over nine days, three replications of ten comparisons were conducted.

Statistical Analysis

The Bradley-Terry categorical model for paired comparisons (Agresti, 1996) was used to analyze the preference data. Statistics were generated using SAS System for Windows, Version 6.12. General trends in preference ranking for each of the individual primates and the pooled data as a group were generated.

Leaf samples of the five plant species were collected for nutrient analysis. One portion of the plant samples were dried at 100°C for 48 hours for dry matter percent and at 50-60°C for 48 hours to subsequently be ground through a 1mm Wiley Mill screen. Fiber values for the leaves (being the portion of the branch eaten by the primates) were calculated for the five browse species collected, using ANKOM fiber analysis with detergent fibers. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) values were generated. Trends were generated from this data between preference and fiber content of the leaves.

RESULTS:

The data was analyzed using the Bradley-Terry Model for paired preferences. The plant choices were ranked from highest to lowest for each primate. Table 5 shows the ranking of each plant for each individual primate. The results demonstrate that Christopher, Camille, and Clare most often chose *Carnus alba*, *Alnus tenuifolia*, and *Salix discolor*, respectively. It is interesting to note that each animal based their highest-ranking choice on the selection left after the more dominant animals had chosen. Both Camille and Christopher preferred *Salix irrorata* the least where Clare chose *Salix irrorata* fourth, with *Betula occidentalis* as her last choice. We can look at whether the choice within a pair of comparisons was significant if the *p* value is less than $\alpha = 0.05$, showing that the number of times species *i* (defined as the first plant in the comparison) was chosen over *j (defined as the second plant in the comparison)* was significantly different. With Camille, the number of times *Carnus alba* was chosen over *Betula occidentalis* was significantly different whereas Christopher and Clare showed no preferences differed a significant number of times. This, however, does not invalidate the ranking of choices for each individual.

Results also showed *probabilities* that species *i* would be chosen over species *j* when paired together. The probabilities are listed in Table 6 for each of the three adult *Colobus*. The highest probabilities of choice occurred for Christopher, choosing *Carnus alba* over both *Salix irrorata* (94.2%) and *Betula occidentalis* (91.9%); for Camille, choosing *Alnus tenuifolia* over both *Salix irrorata* (97.8%) and *Salix discolor* (96.6%); and for Clare, choosing *Salix discolor* over both *Betula occidentalis* (74%) and *Salix irrorata* (70%). We did not expect Clare, in this case, to have a high probability of plant choice since she clearly showed the effect of the dominance hierarchy, her being the subordinate female. She was not able to make consistent choices due to the hierarchy.

One can also look at the goodness of fit of the Bradley Terry logit model to investigate the relationship between the species that "won" and the number of replications performed. By using the Chi Squared (χ^2) value with a degree of freedom of 6, we can see how well our model fits the data or a more positive relationship between species wins and number of replications. Results indicated that Christopher and Camille showed a significant relationship between the two variables with a χ^2 of 12.14 and 9.44, respectively. Therefore, lower the value of χ^2, the better the fit of the model. Again in this case, we did not expect the model to be as good of a fit for Clare (15.15) due to Christopher and Camille having first choice of the plants.

We can also look at whether a particular species of plant was chosen over another *a significant number of times*. Using the standard error of each comparison, a confidence interval can be generated. If the confidence interval contains the value of zero, then neither plant in the comparison was chosen over the other a significant *number* of times. Christopher and Camille significantly preferred *Carnus alba* when paired with *Betula occidentalis* and when *Salix irrorata* was paired with *Alnus tenuifolia*. Christopher choosing *Carnus alba* when paired to *Betula occidentalis* confirms his overall ranked preference for *Carnus alba*. However, when *Carnus alba* was paired with *Salix irrorata*, *Carnus alba* was not the preferred choice, though this did not invalidate his primary

preference for *Salix irrorata* over *Alnus tenuifolia*. Camille also chose her lowest ranked choice of *Salix irrorata* over her first ranked of *Alnus tenuifolia*. Clare did not make any significant choices, again perhaps due to subordinance factors.

The data was also pooled rather than looking at each animal as an individual. As a group, the preferred choice ranking remained closely similar to the general trends seen in each individual animal- first choice of *Alnus tenuifolia*, then *Salix discolor*, *Carnus alba*, *Betula occidentalis* and *Salix irrorata* as the lowest ranking preference. In this respect, *Alnus tenuifolia* remained in the top two choices and *Salix irrorata* remained in the last two choices. *Alnus tenuifolia* was also the only plant that was preferred a significant number of times to *Betula occidentalis* ($p=0.0177$, $\alpha=0.05$).

The NDF content of the leaves ranged from 10.97% to 3.57%, where *Salix discolor* and *Carnus alba* contained the most and least total amounts NDF, respectively. Acid detergent fibers ranged from 1.46% to 8.6% (*Carnus alba to Salix discolor*) and ADL ranged from 0.54% to 5.2% (*Salix discolor* to *Alnus tenuifolia)*. These data were ranked from lowest to highest values of NDF, ADF, and ADL and were matched to the ranked sum of the plant preference rankings of the primates as a group (Table 5). Rankings show that the primates appeared to prefer the lower values of NDF, ADF, and ADL. However, *Alnus tenuifolia* ranked the highest in NDF and ADF and second highest in ADL, while at the same time ranking as the first and second most preferred of the group as a whole. In this case, it appears that taste was preferred over amounts of NDF, ADF, and ADL content, as the study observed that the other plants were chosen by what appears to be a choice for nutrients.

DISCUSSION:

Palatability may not be considered quality of the food since it relies on the metabolic state and prior experience of the animals being studied. It is well known that animals will associate the exterior appearance and characteristics of a food with either its negative or positive metabolic consequences. Palatability is not easy to measure or to define since it depends on so many factors (Dwyer, 1964), such as, visual cues, taste, and texture, learning, position, and prior exposure to the food. Without visual and tactile stimuli, an animal cannot determine the food's metabolic potential (Forbes, 1995).

Forbes (1996) defines palatability as "the overall impression of the food given by all the animal's senses." Young (1948) describes three systems, which contribute to the acceptance of particular foods and thus their palatability (Heady, 1964). The first of these influences is related to the internal biology of the animal, such as energy release initiating nerve stimuli, body temperature, digestive tract movement, and general fatigue of the animal. The second is learned behavior from previous feeding experiences. Finally, the last system comprises the immediate environment, both physical and nutritional. In tying these systems together, Heady suggests that rather than looking at the typical definition by Webster Dictionary that palatability is pleasing to the taste and thus the mind, "selective response" may be a better descriptor of palatability than "taste response".

In light of discussing palatability, it is necessary to discuss dietary preference. In much research, palatability is measured by presenting two or more feeds to an animal so it can express its preference for one of the feeds (Pond, 1974). Due to using preference as a part of palatability, these two terms cannot be used interchangeably and must be defined in their own terms. The focus of this study is preference because many of the parameters for determining palatability are gained through the chemical components of the feed, as well as results from previous preference studies. This study did not have access to separation of the animal subjects to determine the actual intake or biomass consumed by the animal, some of the components necessary to determine palatability. Hence, this study should be viewed as a precursor to future palatability studies involving the preferences of captive Colobus primates.

The benefits of browse are not only nutritional but behavioral as well. Many zoos have seen that captive environments can cause "stereotypic movements, aberrant sexual behavior, excessive inactivity, or abnormal maternal care" (Hancocks, 1980). It has been documented that environmental enhancement is beneficial to the reduction of stereotypic behavior of captive felines (Lyons, 1997) and captive apes (Wilson, 1982). In addition to increasing the size of captive animal exhibits, the complexity of the environment must also be increased (Wilson, 1982; Hancocks, 1980). By providing a more natural diet in captivity, the primate can make use of the plants nutritional and physical, i.e. food search, throwing the branches, and searching the out-of-reach feature of their environment, characteristics (Baker, 1997). In the wild, leaf monkeys have access to trees, shrubs, and other browse species (Nowak, 1991) providing appropriate stimuli for normal behavior (Lyons, 1997). At DZG, *Colobus guereza kikuyuensis* primates consume most of the available vegetation presented to them, leaving the environment with inadequate vegetation for consumption and as part of their environment. Regular addition of preferred and palatable browse would strengthen natural wild behavior and decrease habitual stereotypical captive behaviors (Lyons, 1997), as well as improving the appearance of the exhibit as a whole. The position of the offered browse would enhance the exercise and activity level of these primates.

Results of this preference study show that the Colobus at DZG prefer browse lower in NDF, ADF, and ADL. We found that they selected browse containing low NDF, ADF, and ADL by demonstrating that the choices made were inversely related to the amount of fibers in the plant. Four of their 5 choices were ranked from most to least favorite and lowest to highest in fibers. This may effectively demonstrate their distinct preference for low fiber foods. However, the most preferred plant, *Alnus tenuifolia*, was found to contain high NDF, ADF, and ADL values. Overall, the primates preferred *Alnus tenuifolia*, which is validated by the fact that the DZG diet is thought to meet all known nutrient needs of the Colobus. In our opinion, the primates chose *Alnus tenuifolia* on taste alone.

CONCLUSION:

For some animal species, especially folivores, foliage from trees and shrubs is the main food source. Therefore, the nutritive value and palatability, or preference, is essential to the animal as well as the animal's caretaker (Dietz, 1971). However, Colobus are an exception to the rule of the Cercopithecidae primate family and are more folivorous than frugivorous as the family characteristics indicate. The zoo community is aware that Colobus are almost exclusively folivorous. This leads to the potential to feed more foliage to the Colobus in addition to the regular and available items currently in their diet. Understanding the kinds and amounts of food consumed in the natural environment is important towards understanding an animal's natural history, but simply comprising a list of dietary items shows almost nothing about the process of food selection. Many items that influence diet makeup and selection include digestibility, palatability, and availability of foodstuffs; thus studies of food dietary habits are varied in content (Gillingham, 1987). Knowledge of the palatability and preference of browse species allows for more informed decisions to be made providing a more preferred and palatable nutritive supplement for captive leaf eating primates. Thus, a preference study, demonstrating natural folivorous tendencies and preference for browse, is warranted.

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Table 1. Scientific and common names of plants chosen for the Colobus preference study at the Denver Zoological Gardens

Scientific Name	Common Name
Salix irrorata	Bluestem Willow
Carnus alba 'Bud's Yellow'	Bud's Yellow Dogwood
Salix discolor	French Pink Pussy Willow
Alnus tenuifolia	Thin Leaf Alder
Betula occidentalis	Native River Birch

Table 2. Daily diet, in grams as fed, offered to *Colobus guereza kikuyuensis* (single animal) at the Denver Zoological Gardens

AM		PM			
Daily	(grams)	Sun, Tues, Thurs, Sat	(grams)	Mon, Wed, Fri	(grams)
Yam	90	Broccoli	100	Cabbage	187
Potato	62	Cucumber	126	Green beans	36
Greens	90	Ear corn	75	Green peppers	50
Leafeater Biscuit [a]	48	Apples	32	Pear	25
		Orange	11	Orange	11
		Leafeater Biscuit [a]	94	Leafeater Biscuit [a]	94
		Greens	180	Greens	180

[a] Marion Zoological Leaf Eater Biscuit

Table 3. Diet analysis of daily diet offered to *Colobus guereza kikuyuensis* at the Denver Zoological Gardens

		Diet analysis	Requirement[b]
ME[a]	(Kcal/g)	1.545	-
Crude Protein	(%)	21.4	16.7
ADF	(%)	8.6	-
Crude Fat	(%)	4.2	-
Calcium	(%)	0.87	0.56
Phosphorus	(%)	0.56	0.44
Ascorbic Acid	(mg/kg)	1640	111
Vitamin E	(mg/kg)	179	55.6

[a] Metabolizable Energy; [b] Non-human primate, Old World, all stages, NRC, 1978

Table 4. Paired plant comparisons used for Colobus Preference Study at the Denver Zoological Gardens

Plant Species	Will be compared to:
A	B C D E
B	C D E
C	D E
D	E

Table 5. Ranking of browse species preference (three replications) for each of three adult *Colobus guereza kikuyuensis* primates at the Denver Zoological Gardens over three replications.

Plant Species	Christopher[a,d]	Camille[b,d]	Clare[c,d]	Ranked sum	Pooled[d]	% NDF[e,f]	% ADF[e,g]	% ADL[e,h]
A. tenuifolia	4	5	4	13	5	2	2	1
S. discolor	3	3	5	11	4	4	4	5
C. alba	5[*]	2	3	5	3	5	5	3
B. occidentalis	2[*]	4	1	5	2	3	3	4
S. irrorata	1	1	2	4	1	1	1	2

[a]Dominant male; [b]Dominant female; [c]Subordinate female; [d]The ranking of "5" is the most preferred and "1" is the least preferred; [e]The ranking of "5" is the lowest % value of fiber; [f]Neutral Detergent Fiber; [g]Acid Detergent Fiber; [h]Acid Detergent Lignin; [*] *C. alba* is the only plant that is significantly ($p=0.0242$, $\alpha=0.05$) chosen more often than B. occidentalis for Christopher; c Most preferred ranking plant

Table 6. Probabilities of individual primate choosing species *i* over species *j* when *i* and *j* are paired.

Species *i*	Species *j*	Christopher P*ij*	Camille P*ij*	Clare P*ij*
S. irrorata	*C. alba*	5.80%	39.40%	43.00%
S. irrorata	*S. discolor*	13.80%	12.50%	30.00%
S. irrorata	*A. tenuifolia*	10.60%	2.20%	39.00%
S. irrorata	*B. occidentalis*	41.10%	12.50%	55.00%
C. alba	*S. irrorata*	**94.20%**	60.60%	57.00%
C. alba	*S. discolor*	72.20%	18.00%	36.00%
C. alba	*A. tenuifolia*	65.80%	3.40%	45.00%
C. alba	*B. occidentalis*	91.90%	18.00%	61.00%
S. discolor	*S. irrorata*	86.20%	87.50%	70.00%
S. discolor	*C. alba*	27.80%	82.00%	64.00%
S. discolor	*A. tenuifolia*	42.50%	13.80%	59.00%
S. discolor	*B. occidentalis*	81.40%	50.00%	**74.00%**
A. tenuifolia	*S. irrorata*	89.40%	**97.80%**	61.00%
A. tenuifolia	*S. discolor*	34.20%	96.60%	55.00%
A. tenuifolia	*C. alba*	57.50%	86.20%	41.00%
A. tenuifolia	*B. occidentalis*	85.50%	86.20%	66.00%
B. occidentalis	*S. irrorata*	58.90%	87.50%	45.00%
B. occidentalis	*S. discolor*	8.10%	82.00%	39.00%
B. occidentalis	*A. tenuifolia*	18.60%	50.00%	26.00%
B. occidentalis	*C. alba*	14.50%	13.80%	34.00%