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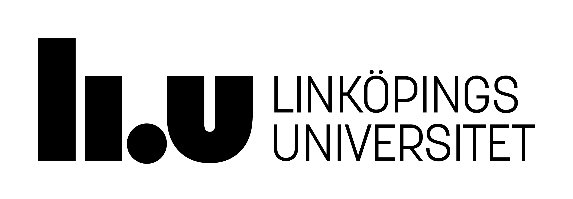
Food preferences and nutrient composition in zoo-housed ring-tailed lemurs *(Lemur catta)*

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*Lemur catta*, food preferences, nutrient composition

**Sammanfattning**

Abstract

The purpose of this study was to assess the occurrence of food preferences in zoo-housed ring-tailed lemurs (*Lemur catta*) and to analyse whether these preferences correlate with nutrient composition. By using a two-alternative choice test four ring-tailed lemurs were repeatedly presented with all possible binary combinations of 12 food items which were all familiar to the lemurs. The following rank order of preference was found: Apple > Sweetpotato > Melon > Beetroot > Carrot > Egg > Eggplant > Pumpkin > Cucumber > Tomato > Cabbage > Meal worm. Correlational analyses revealed that this preference ranking showed a significant positive correlation with the total content of carbohydrates and the content of sucrose of the food items. The preferences did not correlate with the content of protein, lipids, water or any other macro- or micronutrient such as vitamins or minerals. These results suggest that *Lemur catta* are not opportunistic feeders with regard to energy gain but rather seek to meet their requirements of metabolic energy by preferring foods that are high in soluble carbohydrates. The findings of this study might be useful when composing diets for zoo-housed ring-tailed lemurs and knowledge about their nutritional choices are important to provide a good health and welfare status. The lemurs’ preference for carbohydrate-rich foods is in line with findings in pigtailed macaques and white-handed gibbons, but differs from findings in squirrel monkeys and spider monkeys which both are opportunistic feeders with regard to meeting their energy requirements.

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# Abstract

The purpose of this study was to assess the occurrence of food preferences in zoo-housed ring-tailed lemurs (*Lemur catta*) and to analyse whether these preferences correlate with nutrient composition. By using a two-alternative choice test four ring-tailed lemurs were repeatedly presented with all possible binary combinations of 12 food items which were all familiar to the lemurs. The following rank order of preference was found: Apple > Sweetpotato > Melon > Beetroot > Carrot > Egg > Eggplant > Pumpkin > Cucumber > Tomato > Cabbage > Meal worm. Correlational analyses revealed that this preference ranking showed a significant positive correlation with the total content of carbohydrates and the content of sucrose of the food items. The preferences did not correlate with the content of protein, lipids, water or any other macro- or micronutrient such as vitamins or minerals. These results suggest that *Lemur catta* are not opportunistic feeders with regard to energy gain but rather seek to meet their requirements of metabolic energy by preferring foods that are high in soluble carbohydrates. The findings of this study might be useful when composing diets for zoo-housed ring-tailed lemurs and knowledge about their nutritional choices are important to provide a good health and welfare status. The lemurs’ preference for carbohydrate-rich foods is in line with findings in pigtailed macaques and white-handed gibbons, but differs from findings in squirrel monkeys and spider monkeys which both are opportunistic feeders with regard to meeting their energy requirements.

Keywords:

*Lemur catta,* food preferences, nutrient composition

# Introduction

Primates are known to feed on a diverse array of both plant items and animal material to meet their nutritional needs (Coiner-Collier, 2016). However, most primate species are highly selective in their food choices in order to optimize their nutritional intake (Chivers, 1998). The optimal foraging theory predicts that natural selection will favor those animals that maximize their net intake of energy or other critical nutrients (Stephens & Krebs, 1986).

Food choices of primates can be based on different factors, and in the case of plants this includes the nutritional content of the plant part in question and/or the concentrations of plant secondary compounds (Freeland & Janzen, 1974). Another important factor that affects food choices is the relative spatial and temporal availability of the food items (Stevenson, 2003). According to Yamashita (2008) animals make decisions concerning the costs and benefits of procuring, processing and digesting potential food items. A number of studies reported previously that food choices of primates correlate negatively with the content of plant secondary compounds that can be toxic or inhibit the digestion of nutrients (Glander 1982; Wrangham *et al*., 1998). On the other hand, only few studies on non-human primates so far assessed positive correlations between food selection and the content of specific nutrients that should be beneficial to the animal (Laska *et al*., 2000, Laska, 2001, Jildmalm *et al*., 2008).

One approach to obtain information as to which nutrients might affect primate food selection positively is to present animals with food items that are known to contain only small and thus presumably negligible amounts of plant secondary compounds and to assess whether their choice behavior correlates positively with any nutrient. Using cultivated fruits and vegetables complies with this idea as they are both low in plant secondary compounds and well characterized in their nutrient contents (Souci *et al*., 1989; Food Standards Agency, 2002).

Previously, food choices in relation to nutrient composition have been investigated in both primates and non-primates. Laska (2001) and Jildmalm *et al*. (2008) reported that captive pigtail macaques *(Macaca nemstrina)* and white-handed gibbons *(Hylobates lar)* are selective feeders with regard to maximizing net gain energy by preferring foods that are high in carbohydrates. Neither of these species preferred foods with energy from proteins, lipids or from the total content of energy. In contrast, Squirrel monkeys *(Saimiri sciureus)*, Spider monkeys *(Ateles geoffroyi)* and Pacas *(Agouti paca)* were found to display food preferences which significantly correlated with the total energy content, irrespective of the source of energy (Laska, 2001; Laska *et al*., 2000). Thus, there are different strategies with regard to food choices that an animal can adopt to meet its energy requirements.

Free ranging ring-tailed lemurs have been reported to consume a wide variety of foods. According to La Fleur & Sauther (2015) *Lemur catta* are opportunistic frugivores/folivores and their diet is highly adapted to seasonal changes. Wild ring-tailed lemurs have also been reported to include some flowers, dead wood, sap and bark into their diet (Dierenfeld & McCann, 1999). O’Mara (2012) also reported that they catch flying insects, however the capture requires skills that are not attained until adulthood. *Lemur catta* has been observed to spend up to 15.7% of their feeding time foraging on insects such as cicadas and caterpillars (Soma, 2006). Therefore, it should also be interesting to assess whether the lemurs’ food preferences correlate with the content of protein which are the predominant source of energy in animal matter, such as insects.

It has been observed that ring-tailed lemurs consume soil, this behaviour is thought to help meet their sodium requirements (Ganzhorn, 1987). During the present study, it was therefore interesting not only to assess whether the macronutrients of the food items may correlate positively with the lemurs’ food preferences, but also micronutrients such as minerals. To meet their need for water, *Lemur catta* drink from open sources such as streams or springs on a daily basis (Wilson & Hanlon, 2010). This makes it also interesting to assess whether water content might correlate with the lemurs’ food preferences.

The young leaves and fruits from *Tamarindus indica* (tamarind tree) are an important part of the ring-tailed lemurs’ diet in the wild (Mowry *et al*., 1996). According to Yamashita (2008) ring-tailed lemurs are high-level consumers of sugar throughout the year while another species of lemur, the Verreaux’s sifaka (*Propithecus verreauxi*), are selecting food with high levels of protein. *Lemur catta* has also been reported to be highly sensitive to the bitter taste of plant secondary metabolites which is thought to be an evolutionary adaptation to avoid ingestion of potentially noxious substances (Simmen *et al*., 2006). Little is known so far about which nutrients of its diet affect the food choices of the ring-tailed lemur in a positive manner. It was therefore the aim of the present study to determine food preferences in a group of zoo-housed ring-tailed lemurs for a variety of cultivated fruits and vegetables as well as foods of animal origin which differ markedly in their contents of macronutrients and/or micronutrients. I also assessed whether these food preferences correlate with the abundance of macro- or micronutrients. My hypothesis was that the lemurs will show a preference for specific food options based on nutrient composition.

# Materials and Methods

## **3.1 Animals**

This study was carried out using four Ring-tailed lemurs (*Lemur catta*) at Furuvik Zoo, Gävle, Sweden. Two of the individuals were adult females of thirteen (Ester) and seventeen (Bi) years of age. The other two were infants siblings, a one-year old female (Lily) and a male of three months (Vide) at the start of the study. All lemurs except the oldest female were born at Furuvik. The lemurs were housed in an indoor enclosure of 200 m3, with access to a 5,000 m2 outdoor enclosure. A small corridor connected the indoor and outdoor enclosure. The lemurs were fed a variety of vegetables and two kinds of commercial primate pellets (Zoo primate high fiber pellets and leaf eating primates pellets, from Granovit, Kaiseraugust, Switzerland) two times per day. Fresh leaves, grass and other plant matter were available from the natural vegetation outdoors. Water was always available from a drinking bowl. Due to problems with overweight the group of lemurs in this study had been switched from a diet which was based on a high proportion of fruits to a diet which now is based on vegetables and includes only small amounts of fruit as rewards when training sessions occur. The switch of diet occurred 2 years before the start of this study.

## **3.2 Procedures**

Food preferences were assessed using a two-alternative choice test. Tests were performed in the morning between 07:30-08:30 before the lemurs were given breakfast. Later during the day the lemurs were given two more meals. This time of day was chosen to maximize the chances that the lemurs had some appetite and were motivated to participate in the tests. During this study each lemur was presented with all binary combinations of twelve food items regularly fed to them. The food items used were cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), carrot (*Daucus carota*, subsp. *sativus*), aubergine (*Solanum melongea*), beetroot (*Beta vulgaris*), sweet potato (*Ipomoea batatas*), butternut pumpkin (*Cucurbita moschata*), napa cabbage (*Brassica rapa* subsp *pekinesis*), apple (*Malus pumila*), honey melon (*Cucumis melo*), meal worm (Larva of *Tenebrio molitor*) and hardboiled egg (egg from *Gallus gallus domesticus*). The reason for choosing these food items was that they are all part of the lemurs’ regular diet at Furuvik and that the nutrient composition of these foods are known allowing me to assess possible correlations between food preferences and nutrient composition (Food Standards Agency, 2002). All foods were cut into pieces of approximately 1 x 1 x 1 cm when presented to the lemurs. On average each lemur was presented with eight combinations per session. Recordings were made with regard to which item of a given pair of foods the lemurs picked as the first one. All 66 combinations of the twelve foods were presented for a total of 10 times, meaning that each lemur made 660 choices.

Two methods of presenting the food items to the lemurs were used. Two of the lemurs learned to voluntarily enter the small corridor singly where they were presented with two food items placed on a wooden shelf on the outside of the mesh (Fig.1). Since the width of the mesh was large enough to fit the lemurs’ hands they could pick up the food items through the mesh. With the other two lemurs, I entered the indoor enclosure with a metal tray (10x16 cm) presenting the food items to the lemurs (Fig 2). All individuals adapted rather quickly to the procedure and did not disturb each other. Caution was taken not to present a food item that was in the previous pair to prevent any bias. Sessions ended when the lemurs did not show any more interest in participating in the tests or after a maximum of 8 pairs.

En bild som visar inomhus, mat, sitter, katt

Automatiskt genererad beskrivning En bild som visar djur, däggdjur, snö, sitter

Automatiskt genererad beskrivning En bild som visar stängsel, sitter, inomhus, djur

Automatiskt genererad beskrivning

Figure 1. The test set-up where the lemur is making a choice between two food items presented in a wooden tray outside of the mesh.

En bild som visar däggdjur, djur, katt, hund

Automatiskt genererad beskrivning

Figure 2. The test set-up where the lemur is making a choice between two food items presented on a tray inside the enclosure.

## **3.3 Data analysis**

All statistical tests were carried out using the webpage Social science statistics.

A total of 2640 choices (66 binary combinations x 10 presentations per animal x 4 animals) were recorded.

For the analysis of food preferences, each food item that was consumed first by the lemurs when presented with a given pair of food items was given 1 point and the alternative 0. If the lemur did not make a choice between two items presented within 10 seconds, both items were assigned 0.5 points. When the data collection was finished the points for all the food items were summarized.

To establish food preference rankings two different criteria were used:

Criterion 1 (individual level)

The sum of the choices for each of the 12 food items was built for each individual lemur. The theoretical maximum score for any type of food with this criterion was 110 (11 combinations x 10 presentations x 1 animal).

Criterion 2 (group level)

The choices from criterion 1 were summed up across all four lemurs. Here, the theoretical maximum score for any type of food with this criterion was 440 (11 combinations x 10 presentations x 4 animals).

To test whether all four lemurs displayed similar food preference rankings, the Spearman rank order correlation test was used. All six possible combinations between the four individuals were tested. To test whether there would be a difference between the two youngsters (Vide and Lily) and the adults (Bi and Ester) I performed a Spearman rank correlation test between the two age groups. The binomial test using the sum total of choices for each member of a given pair of food items was used to assess significant food preferences at the individual level (criterion 1) and the group level (criterion 2). Correlations between the food preference rankings and the nutritional values of the food items were calculated using the Spearman rank-order correlation coefficient.

# Results

## **4.1 Food preferences**

Table 1 summarizes the choices from all four lemurs for each member of a given pair of food items. With 46 out of the 66 binary combinations the lemurs displayed a significant preference for one of the two options. Apple was significantly preferred over all other food items. Sweet potato, melon, beetroot, carrot and egg were also significantly preferred over several other items. Eggplant, pumpkin, cabbage, cucumber, tomato and meal worm were never preferred over any other food item.

Table 1: The choice behaviour of the lemurs (N=4). The first value applies to the food item on the left and the second value to the food item on the top. Indicates a significant preference for the food item on the left. n.s indicates a lack of a significant preference.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Apple | Sweetpotato | Melon | Beetroot | Carrot | Egg | Eggplant | Pumpkin | Cabbage | Cucumber | Tomato | Mealworm |
| Apple | X |  |  |  |  |  |  |  |  |  |  |  |
| Sweetpotato | 11:29 | X |  | n.s |  |  |  |  |  |  |  |  |
| Melon | 3:37 | 14:36 | X | n.s |  |  |  |  |  |  |  |  |
| Beetroot | 9:31 | 15:25 | 15:25 | X | n.s |  |  |  |  |  |  | n.s |
| Carrot | 2:38 | 6:34 | 6:34 | 12.5:27.5 | X | n.s |  |  |  |  |  |  |
| Egg | 2:38 | 6:34 | 7:33 | 11:29 | 16:24 | X |  |  |  |  |  |  |
| Eggplant | 1:39 | 0:40 | 0.5:39.5 | 2.5:37.5 | 2.5:37.5 | 7.5:32.5 | X | n.s | n.s | n.s | n.s | n.s |
| Pumpkin | 0:40 | 0:40 | 0.5:39.5 | 3.5:36.5 | 2.5:37.5 | 9:31 | 21:19 | X | n.s | n.s | n.s | n.s |
| Cabbage | 0:40 | 1:39 | 0:40 | 4:36 | 3:37 | 8.5:31.5 | 17:23 | 19:21 | X | n.s | n.s | n.s |
| Cucumber | 0:40 | 0:40 | 1:39 | 4:36 | 2:38 | 6:34 | 17:23 | 20:20 | 21.5:18.5 | X | n.s | n.s |
| Tomato | 0:40 | 0:40 | 0:40 | 5:35 | 1:39 | 8.5:31.5 | 16:24 | 18:22 | 21.5:18.5 | 19.5:20.5 | X | n.s |
| Meal worm | 0:40 | 0:40 | 0:40 | 3:37 | 0:40 | 6:34 | 16.5:23.5 | 17:23 | 20:20 | 20.5:19.5 | 18.5:21.5 | X |

## **4.2 Food preference rankings**

Table 2 summarizes the choices made by the lemurs according to criterion 1 and 2. Apple was clearly the most attractive among the 12 food items, followed by sweet potato and melon. In contrast, meal worm, cabbage, tomato and cucumber were clearly the least attractive food items.

All four lemurs displayed similar rankings of preference for the 12 food items as their rankings all significantly correlated with each other (Spearman rs ≥ 0.62, P<0.05).

When comparing the two age groups there was a significant positive correlation (SpearmanP<0.0004), meaning that the food preferences were similar between the two youngsters and the two adults.

Table 2. Food items and their rank order of preference

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Criterion 1** | **Vide** | **Lily** | **Bi** | **Ester** | **Criterion 2** | **∑V+L+B+E** |
| Apple | 103 | 102 | 101 | 106 | Apple | 412 |
| Sweetpotato | 88 | 85 | 94 | 102 | Sweetpotato | 369 |
| Melon | 88.5 | 96 | 77 | 85.5 | Melon | 347 |
| Beetroot | 89.5 | 85 | 89.5 | 49.5 | Beetroot | 313.5 |
| Carrot | 75 | 61 | 67 | 76.5 | Carrot | 279.5 |
| Egg | 25 | 69 | 78.5 | 64 | Egg | 236.5 |
| Eggplant | 36.5 | 37 | 24.5 | 28.5 | Eggplant | 126.5 |
| Pumpkin | 39.5 | 25.5 | 26.5 | 31 | Pumpkin | 122.5 |
| Cucumber | 30.5 | 27.5 | 25.5 | 28 | Cucumber | 111.5 |
| Tomato | 34 | 21.5 | 25.5 | 30 | Tomato | 111 |
| Cabbage | 25 | 27 | 26 | 31.5 | Cabbage | 109.5 |
| Meal worm | 25.5 | 23.5 | 25 | 27.5 | Meal worm | 101.5 |

## **4.3 Food preference rankings and nutritional content**

Table 3 displays the Spearman rank-order correlation statistics. The food preference rankings displayed by the lemurs were highly significantly correlated with the content of carbohydrates and sucrose (Spearman, p<0.01). This was true both when the four lemurs were considered separately and when they were considered as a group. The food preference rankings did not correlate significantly with any other macro- or micronutrient. Here, too, this was true both for the individual lemurs and for the group.

Table 3. Spearman rank-order correlations between food preference rankings and the nutritional values of the food items.

|  |  |  |
| --- | --- | --- |
|  | **r** | **p** |
| **Macronutrients** |  |  |
| Water | -0.28 | 0.36 |
| Total nitrogen | -0.33 | 0.29 |
| Protein | -0.33 | 0.28 |
| Fat | -0.43 | 0.16 |
| Carbohydrates | **0.72** | **0.007** |
| Energy kcal | 0.29 | 0.35 |
| Energy kJ | 0.29 | 0.35 |
| Starch | 0.20 | 0.51 |
| Dietary fiber (NSP) | 0.28 | 0.38 |
| **Individual sugars** |  |  |
| Glucose | 0.23 | 0.47 |
| Fructose | 0.26 | 0.40 |
| Sucrose | **0.69** | **0.01** |
| **Fatty acids** |  |  |
| Saturated | -0.32 | 0.30 |
| Mono-unsaturated | -0.47 | 0.11 |
| Poly-unsaturated | -0.37 | 0.23 |
| Cholesterol | -0.29 | 0.36 |
| **Minerals** |  |  |
| Na | 0.12 | 0.70 |
| K | -0.17 | 0.60 |
| Ca | -0.17 | 0.60 |
| Mg | 0 | 1.0 |
| P | -0.34 | 0.29 |
| Fe | -0.26 | 0.42 |
| Cu | 0.10 | 0.76 |
| Zn | -0.09 | 0.78 |
| Cl | -0.15 | 0.63 |
| Mn | -0.10 | 0.80 |
| Se microg | -0.33 | 0.30 |
| I microg | -0.40 | 0.19 |
| **Vitamins** |  |  |
| Retinol | -0.29 | 0.36 |
| Carotene | 0.11 | 0.73 |
| Vitamin D | -0.29 | 0.36 |
| Vitamin E | -0.20 | 0.53 |
| Thiamin | -0.38 | 0.22 |
| Riboflavin | -0.13 | 0.69 |
| Niacin | -0.43 | 0.16 |
| Vitamin B6 | -0.36 | 0.25 |
| Vitamin b12 | -0.29 | 0.36 |
| Folate | 0.13 | 0.70 |
| Pantothenate | -0.35 | 0.26 |
| Biotin | -0.20 | 0.54 |
| Vitamin C | 0.19 | 0.55 |

# Discussion

The results of this study demonstrate that zoo-housed ring-tailed lemurs display marked preferences for certain types of food that are part of their daily diet, and that these food preferences significantly correlate positively with the total content of carbohydrates and the content of sucrose of the food items used.

## **5.1 Evaluation of the method used**

The two-choice test is widely used to assess food preferences, both with primates and non-primate mammals. An advantage of this method is that it allows some control over the state of hunger of the animals. Further, due to its binary nature it provides a clear-cut decision for one of the two simultaneously presented food options. Observation of the consumptive behaviour in free-ranging animals can be open to interpretation and does not allow any control with regard to the animals’ motivational status.

Recently, two studies used a touchscreen to assess food preferences in non-human primates instead of using real food items (Huskisson *et al*., 2020; Hopper *et al*., 2019). This new method has the advantage that any experimenter bias can be excluded. However, it has the disadvantage that the food items are only presented visually, without the somatosensory and olfactory channels being available to the animals. It is well known that smell and touch are important for the food choices of primates (Laska *et al*., 2007). Using a touchscreen only makes it possible to record an animal’s decision for one of the two food items whereas the method used in the present study also allows the experimenter to record behaviours such as hesitating of an animal or the duration of time that an animal takes until it decides for one of the two food options, or whether a chosen food item is consumed completely or not. A touchscreen plus an automated food delivery also requires a technical system which includes the risk of malfunction.

One aspect that may have affected the lemurs’ choices in the present study is the ripeness of the food items. It has previously been shown that ripeness of fruits may affect food choices of primates (Redford *et al*., 1984). Care was therefore taken only to present food items of the same degree of ripeness.

It has previously been shown that novelty of food items may also affect choice behaviour (Fragaszy *et al*., 1997). To make sure that this factor would not affect the lemurs’ choices, care was taken to only use foods that were regularly fed to the lemurs at the zoo and thus familiar to them.

## **5.2 Carbohydrates, sugar and total energy content**

The lemurs’ food preferences correlated significantly with the content of carbohydrates and sucrose but not with the total energy content. This suggests that ring-tailed lemurs are not opportunistic feeders with regard to energy gain but rather seek to meet their requirements of metabolic energy by preferring foods that are high in soluble carbohydrates. This is in line with the findings of Gould & Gabriel (2014) who reported that free-ranging lemurs include large amounts of Zedrak (*Melia azedarach*) fruits as well as fruits of several *Ficus* species into their diet, both containing high levels of carbohydrates.

Ring-tailed lemurs have been reported to be one of the most adaptable and ecologically flexible lemur species with the ability to adjust to food resource seasonality (Sauther, 1998; Gould, 2006). Due to their dietary flexibility they are able to consume a wide variety of foods and even poor-quality food items (Gemmell & Gould, 2008; LaFleur & Gould, 2009). According to Dierenfeld & McCann (1999) *Lemur catta* has a slower gut passage time than other, more frugivouros lemur species, such as *Lemur fulvus* or *Varecia variegata*, and they have a more developed gastrointestinal tract to deal with plant fiber. Even though free-ranging lemurs have shown a high degree of adaptability to different habitats and thus to different compositions of available food they still seem to aim at optimizing their intake of carbohydrates and sugars (Yamashita, 2008).

## **5.3 Proteins and lipids**

The food preferences of the lemurs in this study did not significantly correlate with the content of protein in any of the food items. The items with the highest protein content were mealworm and egg. Meal worm were not preferred over any if the items, but egg were preferred over six items. According to O’Mara (2012) wild ring-tailed lemurs can catch and consume insects but it is not seen in all populations. The presence of insects is also highly seasonal, Soma (2006) reports that *Lemur catta* were observed to spend up to 15.7% of their feeding time foraging on insects such as cicadas and caterpillars.

Mertl-Millhollen *et al*. (2003) reported that during a year of drought *Lemur catta* were travelling to and fed from tamarind trees near water sources, and the fruits and leaves showed a significantly higher level of protein and water compared to trees in open and dryer areas. The authors’ predictions were that the lemurs would stay in their home range, even though there might be trees with higher nutritional content nearer to water sources, but they were proven wrong. The results of their study show that ring-tailed lemurs travel to trees with higher levels of protein and sugar-rich fruits, however, they still continue to defend their territorial border.

Comparatively little is known about the importance of lipids as macronutrients in the diet of primates. One possible reason for this may be that plant material consumed by non-human primates only rarely contains amounts of lipids that would markedly contribute to the ingested total energy of a primate. Animal matter consumed by primates, in contrast, may contain considerable amounts of lipids but information as to the lipid content of e.g arthropods consumed by primates in the wild is sparse.

The meal worms presented in the present study, for example, contain 21.9 g of lipids per 100 g of weight, and thus more than 40 times more lipids compared to the most lipid-rich fruit included in the present study. Nevertheless, the lemurs did not display any preferences for this lipid-rich and therefore energy-rich food item. One possible explanation for this somewhat unexpected finding is that *Lemur catta* might have only a limited ability to effectively digest lipids and thus to exploit this source of energy. Spider monkeys were found to be opportunistic feeders with regard to meeting their energy requirements but a study using two-choice food preference tests (Laska *et al*., 2000) showed that their most favorite one among 12 types of food tested were avocados – the food with the highest lipid content of all alternatives.

## **5.4 Vitamins and minerals**

The food preferences of the lemurs in the present study did not correlate with the content of any of the micronutrients considered. Since *Lemur catta* eat soil to meet their sodium requirements (Hanlon & Wilson, 2010) it was interesting to assess if their food preferences correlated with sodium content in the food items. No significant correlation was found, and this can be an indicator that the lemurs’ diet contained enough sodium to meet their need for it.

It is well-established that animals may display marked preferences for certain micronutrients if their diet is lacking them or if the diet contains too little of them over a prolonged period of time (Yeager *et al*., 1997; Fashing *et al*., 2007). Animals then often develop a craving for food items that contain the missing nutrient. The finding that the lemurs did not display any significant preferences for any of the micronutrients that were included in the analyses in the present study, suggests that the diet fed to the lemurs was balanced so that the lemurs did not need to compensate any nutrient deficiencies by displaying preferences for food items that contain high amounts of the deficient micronutrient. Spontaneous nutritional anemias in primates are much less common today because of the wide-spread use of nutritionally complete primate biscuits and pellets (Crissey & Pribyl, 2000).

## **5.5 Water**

The results of the present study show that the lemurs’ food preferences did not correlate with the content of water. This should not be surprising since wild ring-tailed lemurs do not depend on finding water-rich food items to meet their water requirements, but have been reported to regularly drink from open water sources (Wilson & Hanlon, 2010). The lemurs in the present study were always provided with ad libitum fresh water from a drinking bowl.

Food preference tests in squirrel monkeys (Laska, 2001) and in spider monkeys (Laska *et al*., 2000) found a significant negative correlation between food preferences and water content. This suggests that water content may indeed play a role in food selection in these two primate species. In this context it is interesting to note that both *Saimiri sciureus* and *Ateles geoffroyi* meet their water requirements by consuming juicy fruits rather than by drinking from open water sources. The same negative correlation between food preferences and water content has been reported in a frugivorous rodent, the paca (Laska *et al*, 2003).

## **5.6 Comparison with other species**

The findings of the present study are similar to the results of previous studies. The food preferences of pigtailed macaques (Laska 2001) and white-handed gibbons (Jildmalm *et al*., 2008) did also significantly correlate with the total carbohydrate content but not with total energy, lipid, protein or vitamin and mineral content. In contrast, the food preferences of spider monkeys (Laska *et al*., 2000), capuchin monkeys (Visalberghi, 2003) and pacas (Laska *et al*., 2003) did correlate with the total energy content independent of the energy source.

A possible explanation for these different strategies that animals may adopt to meet their requirements of metabolic energy might be that sympatric species have adapted and developed different preferences for sources of metabolic energy to avoid competition for foods. To be able to survive and reproduce animals must find their own niche of specific food items (MacKinnon & MacKinnon, 1980). Most primate species include a higher or lower proportion of fruits in their diet (Nevo *et al*., 2018) and it is reasonable to assume that there is a competition for this food resource. Therefore, it is reasonable to assume that frugivorous species have developed different preferences for food items and the nutrient composition in them.

## **5.7 Diet of *Lemur catta* in the wild compared to in zoos**

The diet of *Lemur catta* in the wild has been the focus of a variety of studies and the knowledge about their diet is abundant. In general, feeding animals in zoos with the exact diet they would eat in the wild is usually impossible and this can result in several problems. Zoo-housed lemurs in human care face a variety of nutrition-related health issues such as obesity, diabetes, hepatic iron storage disease and reproduction problems (Britt *et al*., 2015). Diets provided for lemurs in zoos can markedly differ in their nutritional composition from their natural diets and knowledge about their feeding ecology is crucial to be able to understand their nutritional needs. Since the diet of zoo-housed ring-tailed lemurs often consists of commercial fruits and vegetables and different primate pellets and cookies it is not similar to their natural diet (Britt *et al*., 2015). However, the populations of wild *Lemur catta* are highly fragmented due to deforestation (Gabriel, 2013) and the ring-tailed lemur is reported as one of the one of the most ecologically flexible species (LaFleur & Sautner, 2015) and therefore the diets of different populations can differ markedly in their composition.

The lemurs in the present study are only allowed to be outside and feed on leaves and grass in the warmer summer months, during winter they are held inside due to cold climate. In this way their diet changes during the year as fresh non-commercial plants are not available all year around. Wild ring-tailed lemurs are reported to consume up to 51.4% mature leaves during the wet season and 25.7% during the dry season (Soma, 2006). It is difficult and costly to provide the zoo-housed lemurs in countries with winter climate with the amount of leaves their wild conspecifics feed on. This could imply that some populations of zoo-housed lemurs consume a less fibrous diet resulting in several health issues. Increasing the fibre content and reducing the sugar- or starch-rich food items has been reported to result in several benefits for both animal welfare, health and reproduction success (Britt *et al*., 2015).

The lemurs in the present study were particularly “picky” in their food choices compared to lemur groups at other zoos (Lundholm, personal communication). It is difficult to decide exactly why that is. Possible explanations for food “pickiness” could be that the animals are provided too much food in general so they can choose to only feed on a certain, preferred types of food. The group composition could also affect “pickiness” since small groups of lemurs provided a large amount of food results in less food competition. Animals could also learn to avoid certain types of food if they had a bad experience from that particular type of food (Duncan & Gordon, 1999).

There could also be other explanations for “pickiness”. The lemurs in the present study were fed a diet with a high proportion of sugar-rich fruits and vegetables until two years before the start of the study. Early life experience of foods can affect the food acceptance in later life (Ventura & Worobey, 2013). However, two animals of the present study were infants and had always been fed a fruit-free diet. Although the results between all four individuals did not differ significantly there was a trend that the two younger individuals, Lilly and especially Vide, ate a wider variety of food items than the older ones, Bi and Ester. It is interesting to note that individuals being used to feeding on several types of vegetables are not as “picky” as the ones used to eat carbohydrate-rich foods. This knowledge could have important implications when composing diets for zoo-housed ring-tailed lemurs in the future.

## **5.8 Wild fruits compared to cultivated fruits**

Cultivated fruits have been grown for human consumption and are selectively bred and cultivated to appeal to human tastes (Schwitzer *et al*., 2009). This modern cultivation emerged in fruits that are high in beneficial nutrients such as carbohydrates, and low in fiber and in plant secondary compounds (Milton 2000). Frugivorous primates in zoos and wildlife parks are usually provided with a large proportion of cultivated fruits and in captive environments these easily digestible, low-fibre and high-sugar fruits can be the cause of a variety of health problems (Schwitzer *et al*., 2009). Obesity, heart diseases, diabetes, cancer and problems with reproduction are all examples that have been reported for several primate species on a fruit-rich diet (Seppelt & Zunft 2000; Videan *et al*. 2007; Schaaf & Stuart 1983). Britt *et al.* (2015) also reported that zoo-housed ring-tailed lemurs displayed less aggressive behaviours towards each other when fruits were removed from their diet. Schwitzer *et al*. (2009) suggests that commercial varieties of vegetables that more resemble wild fruits in their nutrient composition, are more suitable for frugivorous primates kept in zoos and wildlife parks.

Although the fruits and vegetables used in the present study are different from the wild fruits consumed by the lemurs in their natural habitat, using cultivated fruits helps to identify nutrients that correlate positively with their food preferences. This would be difficult, if possible at all, when using non-cultivated fruits because with those the food choices of the animals are likely to be determined by avoiding noxious compounds rather than by the attractiveness of beneficial compounds.

## **5.9 Implications for the welfare of zoo-housed lemurs**

Even though the lemurs in the present study showed marked food preferences for fruits with high levels of carbohydrates and sucrose this does not mean they should be provided with a diet high in carbohydrates. However, the results of this study imply that ring-tailed lemurs have a strong preference for carbohydrate-rich fruits and vegetables which at least needs to be considered when composing the diet for zoo-housed lemurs. Since vegetables such as cabbage, cucumber, tomato and eggplant were nearly never eaten, the more carbohydrate-rich ones such as sweet potato and carrot can be used. This is in line with the conclusion of Schwitzer *et al*. (2009) who suggest that some cultivated vegetables are more similar in their composition to the wild fruits consumed by free-ranging lemurs.

This was the first study on food preferences and nutrient composition in *Lemur catta* and it would therefore be useful to perform a follow-up study on another group of individuals to assess how representative the present findings are for this species. Since the diet of wild ring-tailed lemurs can differ according to season and food availability in the wild there might also be a difference between zoo populations of *Lemur catta*.

Also, studies on food preferences and nutrient composition have so far mainly focused on species that are more or less frugivorous. It should therefore be interesting to study primates with other dietary specializations, such as insectivorous or folivorous species. The method used in the present study has been shown to be a useful tool to assess food preferences and if applied to other primate and non-primate species it could give further important information about food choices in animals.

## **5.10 Conclusion**

The results of the present study show that zoo-housed ring-tailed lemurs display marked food preferences, when presented with 12 different food options, in relation to nutrient composition. The lemurs’ food preferences correlated significantly with the content of carbohydrates and sucrose. The food preferences did not correlate with any of the other macro- or micronutrients. Due to the low number of tested individuals the results of the present study preclude more generalizing conclusions about food preferences in zoo-housed lemurs. The knowledge about animals’ food preferences might give important information on how to improve their welfare in zoos.

# Societal & ethical considerations

The experiments reported here comply with the *American Society of Primatologists’ Principles for the Ethical Treatment of Primates,* with the *European Union Directive on the Protection of Animals Used for Scientific Purposes* (EU Directive 2010/63/EU), and also with current Swedish animal welfare laws. As varying both the composition and the mode of presentation of the diet of zoo-housed animals is part of the environmental enrichment efforts that zoos are required to perform by law, the present study did not need an extra ethical approval.

The lemurs in the present study participated on a strictly voluntary basis. The lemurs could leave the test set-up at any time and the data collection depended on the cooperation of the lemurs. All the food items were familiar to the lemurs and approved by the caretakers and zoologist at the zoo.

An increased understanding of the food preferences of zoo-housed primates can help to alleviate nutrition-related problems such as obesity and other health problems. Knowledge about the nutritional choices of endangered species such as *Lemur catta* may contribute to conservation efforts. Providing zoo-housed ring-tailed lemurs with a balanced and healthy diet is crucial for their welfare and to obtain stable zoo-populations. Comparative studies on food preferences and nutrient composition may also increase our understanding of basic mechanisms underlying ingestive behaviour. This, in turn, may even contribute to human health problems such as obesity and diabetes.

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# References

Britt, S., Cowlard, K., Baker, K., Plowman, A. (2015). Aggression and self-directed behaviour of captive lemurs is reduced by feeding fruit-free diets. *Journal of Zoo and Aquarium Research,* 3, 52–8.

Chivers, D. J. (1998). Measuring food intake in wild animals: Primates. *Proceedings of the Nutrition Society*, 57, 321–332.

Coiner-Collier, S., Scott, R. S., Chalk-Wilayto, J., Cheyne, S. C., Constantino, P., Dominy, N. J., Elgart, A. A., Glowacka, H., Loyola, L. C., Ossi-Lupo, K., Raguet-Schofield, M., Talebi, M. G., Sala, E. A., Sieradzy, P., Taylor, A. B., Vinyard, C. J., Wright, B. W., Yamashita, N. & Vogel, E. R. (2016). Primate dietary ecology in the context of food mechanical properties. *Journal of Human Evolution*, 98, 103-118.

Crissey, S. & Pribyl, L. (2000). A review of nutritional deficiencies and toxicities in captive new world primates. *International Zoo Yeabook,* 37, 355-360.

Dierenfeld, E. S. & McCann, C. M. (1999). Nutrient composition of selected plant species consumed by semi free-ranging Lion-tailed macaques (*Macaca silenus*) and Ring-tailed lemurs (*Lemur catta*) on St. Catherines Island, Georgia, U.S.A. *Zoo Biology*, 18, 481-494.

Duncan, A. J. & Gordon, I. J. (1999). Habitat selection according to the ability of animals to eat, digest and detoxify foods. *Proceedings of the Nutrition Society*, 58, 799-805.

Fashing, P. J., Dierenfeld, E. S. & Mowry, C. B. (2007). Influence of plant and soil chemistry on food selection, ranging patterns and biomass of *Colobus guereza* in Kakamega forest, Kenya. *International Journal of Primatology*, 28, 673-703.

Fragaszy, D., Visalberghi, E. & Galloway, A. (1997). Infant tufted capuchin monkeys’ behaviour with novel opportunism, not selectivity. *Animal Behaviour*, 53, 1337-1343.

Freeland, W. J. & Janzen, D. H. (1974). Strategies in herbivory by mammals: the role of plant secondary compounds. *The American Naturalist*, 108, 269-289.

Gabriel, D. (2015). Habitat use and activity patterns as an indication of fragment quality in a strepsirrhine primate. *International Journal of Primatology*, 34, 388-406.

Gemmell, A. & Gould, L. (2008). Microhabitat variation and its effects on dietary composition and intragroup feeding interactions between adult female Lemur catta during the dry season at Beza Mahafaly special reserve, southwestern Madagascar. *International Journal of Primatology*, 29, 1511–1533.

Glander, K. E. (1982). The impact of plant seconary compounds on primate feeding behavior. *Yearbook of Physical Anthropology*, 25, 1-18.

Gould, L. (2006). Lemur catta ecology: what we know and what we need to know. In: L. Gould & M. L. Sauther (Eds) *Lemurs: Ecology and adaptation.* Springer.

Gould, L & Gabriel, D. N. (2014). Wet and dry season diets of the endangered Lemur catta (ring-tailed lemur) in two mountainous rocky outcrop forest fragments in south-central Madagascar. *African Journal of Ecology*, 53, 320-330.

Hopper, L. M., Egelkamp, C. L., Fidino, M. & Ro, S. R. (2019). An assessment of touchscreens for testing primate food preferences and valuations. *Behavior Research Methods*, 51, 639–650.

Huskisson, S. M., Jacobson, S. L., Egelkamp, C. L., Ross, S. R. & Hopper, L. M. (2020). Using a touchscreen paradigm to evaluate food preferences and response to novel photographic stimuli of food in three primate species (Gorilla gorilla gorilla, Pan troglodytes, and Macaca fuscata). *International Journal of Primatology*.

Jildmalm, R., Amundin, M. & Laska, M. (2008). Food preferences and nutrient composition in captive white-handed gibbons, Hylobates lar. *International Journal of Primatology*, 29, 1535-1547.

Laska, M. (2001). A comparison of food preferences and nutrient composition in captive squirrel monkeys, Saimiri sciureus, and pigtail macaques, Macaca nemestrina. *Physiology and Behavior*, 73, 111-120.

Laska, M., Freist, P. & Krause, S. (2007). Which senses play a role in non-human primate food selection? *American Journal of Primatology*, 69, 282-294.

Laska, M., Hernandez Salazar, L.T. & Rodriguez Luna, E. (2000). Food preferences and nutrient composition in captive spider monkeys, Ateles geoffroyi. *International Journal of Primatology*, 21, 671-683.

Laska, M., Luna Baltazar, J.M. & Rodriguez Luna, E. (2003). Food preferences and nutrient composition in captive pacas, Agouti paca (Rodentia, Dasyproctidae). *Mammalian Biology*, 68, 31-41.

LaFleur, M. & Gould, L. (2009). Feeding outside the forest: the importance of crop raiding and an invasive weed in the diet of gallery forest ring‐tailed lemurs (Lemur catta) following a cyclone at the Beza Mahafaly special reserve, Madagascar. *Folia Primatologica*, 80, 233–246.

LaFleur, M. & Sauther, M. L. (2015). Seasonal feeding ecology of Ring-tailed temurs: A comparison of spiny and gallery forest habitats*. Folia Primatologica*, 86, 25-34.

MacKinnon, J. R. & MacKinnon, K. S. (1980). Niche differentiation in a primate community. In D. J. Chivers (Eds.), *Malayan forest primates*. Springer.

Milton, K. (2000). Back to basics: why foods of wild primates have relevance for modern human health. *Nutrition*, 16, 481–483.

Mowry, C. B., Decker, B. S. & Shure, D. J. (1996). The role of phytochemistry in dietary choices of Tana River red colobus monkeys (Procolobus badius rufomitratus). *International Journal of Primatology*, 17, 63.

Nevo, O., Razafimandimby, D., Jeffrey, J. A. J., Schulz, S. & Ayasse, M. (2018). Fruit scent as an evolved signal to primate seed dispersal. *Science Advances*, 4, eaat 4871.

O’Mara, M. (2012). Development of feeding in ring-tailed lemurs. Doctoral dissertation. Arizona state university.

Redford, K. H., Bouchardet Da Fonseca, G. A. & Lacher, T. E. Jr. (1984). The relationship between frugivory and insectivory in primates. *Primates*, 25, 433-440.

Sauther, M. L. (1998). Interplay of phenology and reproduction in ring‐tailed lemurs: implications for ring‐tailed lemur conservation. *Folia Primatologica*, 69, 309–320.

Schaaf, C. D. & Stuart, M. D. (1983). Reproduction of the mongoose lemur (Lemur mongoz) in captivity. *Zoo Biology*, 2, 23–38.

Schwitzer, C., Polowinsky, S.Y. & Solman, C. (2009). Fruits as foods – common misconceptions about frugivory. In M. Clauss, A. L. Fidgett, J. M. Hatt, T. Huisman, J. Hummel, G. Janssen, J. Nijboer, & A. Plowman (Eds.), *Zoo Animal Nutrition IV* (pp. 131-168). Filander Verlag, Fürth.

Seppelt, B. & Zunft, H. J. F. (2000). Sensitivität und Präferenz zum Süßgeschmack unter langfristig verändertem Kohlenhydratverzehr. *Ernährungs-Umschau*, 47, 4–9.

Simmen, B., Peronny, S., Jeanson, M., Hladik, A. & Marez, A. (2006). Diet quality and taste perception of plant secondary metabolites by Lemur catta. In A. Jolly, N. Koyama, H. Rasamimanana, & R. W. Sussman (Eds.), *Ring-tailed lemur biology* (pp 187-207). Springer.

Stephens, D. W. & Krebs, J. R. (1986). *Foraging theory*. Princeton University Press.

Stevenson, P. R. (2003). Fruit choice by woolly monkeys in Tinigua National Park, Colombia. *International Journal of Primatology*, 25, 367-381.

Soma, T. (2006). Tradition and novelty: Lemur catta feeding strategy on introduced tree species at Berenty Reserve. In A. Jolly, R. W. Sussman, N. Koyama, H. Rasamimanana. (Eds.), *Ringtailed lemur biology* (pp 141-159). Springer.

Souci, S.W., Fachmann, W. & Kraut, H. (1989). *Food composition and nutrition tables*. Stuttgart: Wissenschaftliche Verlagsgesellschaft.

Ventura, A. K. & Worobey, J. (2013). Early influences on the development of food preferences. Current Biology, 23, 401-408.

Videan, E. N., Fritz, J. & Murphy, J. (2007). Development of guidelines for assessing obesity in captive chimpanzees (Pan troglodytes). *Zoo Biology*, 26, 93–104.

Visalberghi, E., Sabbatini, G., Stammati, M. & Addessi, E. (2003). Preferences towards novel foods in Cebus apella: the role of nutrients and social influences. *Physiology and Behavior,* 80, 341-349.

Wrangham, R., Conklin-Brittain, N. L. & Hunt, K. D. (1998). Dietary response of chimpanzees and cercopithecines to seasonal variation in fruit abundance. I. Antifeedants. *International Journal of Primatology*, 19, 949-970.

Yamashita, N. (2008). Chemical properties of the diets of two lemur species in southwestern Madagascar. *International Journal of Primatology*, 29, 339-364.

Yeager, C. P., Silver, S. C. & Dierenfeld, E. S. (1997). Mineral and phytochemical influences on foliage selection by the Proboscis monkey (Nasalis larvatus). *American Journal of Primatology*, 41, 117-128.