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Food preferences and nutrient composition in captive white-faced sakis (*Pithecia pithecia*)

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# 1. Abstract

The aim of the present study was to assess the occurrence of spontaneous food preferences in captive white-faced sakis (*Pithecia pithecia*) and to evaluate possible correlations with nutrient composition. Employing a two-alternative choice test, five white-faced sakis were repeatedly presented with all possible binary combinations of 15 familiar food items. The resulting group rank order of preference was: Peanut > Hazelnut > Avocado > Melon > Egg > Apple > Mealworms > Beetroot > Carrot > Cucumber > Cabbage > Tomato > Sweet Potato > Broccoli > Eggplant. This preference ranking was found to be significantly and positively correlated with the total energy content indicating that the most attractive food items had the highest energy values. The sakis’ food preference was neither significantly correlated with total carbohydrate content nor with protein nor lipid content. Nevertheless, the strongest correlation among these three macronutrients was with lipids and not carbohydrates, which is unusual among frugivorous primates. Additionally, the food preference ranking of the sakis was significantly and negatively correlated with water, carotene and vitamin C content, indicating that the least attractive food items had the highest amounts of these nutrients, and significantly and positively correlated with mono-unsaturated fatty acids, copper, iodine, vitamin E, riboflavin and biotin. These results suggest that white-faced sakis are opportunistic feeders with regard to maximizing their net gain of energy. However, the strong correlation found between food preference and lipid content also suggests that the sakis exploit the energy provided by the lipids contained in seeds.

Keywords: Correlational analysis, Food preferences, Nutrient composition, *Pithecia Pithecia*, Seed predator, White-faced saki

# 2. Introduction

Primates are known to feed on a wide variety of plant and animal matter to meet their nutritional requirements (Coiner-Collier, 2016). Field studies, however, reported that primates do not make dietary choices randomly, but instead are selective in order to maximize their intake of critical nutrients (Chivers, 1998). The food choices of primates are primarily influenced by two factors: the nutritional and/or toxic content of a specific plant or animal (Barton & Whiten, 1994) as well as its relative spatial and temporal availability (Castellanos & Chanin, 1996). Other factors, like body size and gut morphology, have also been found to play a part in food selection in primates even though they are considered evolutionary adaptations to the previously mentioned main factors (Chivers & Hladik, 1980). High concentrations of secondary chemical and toxic compounds in plants are actively avoided and have been reported to be negatively correlated with food choice in several studies (Glander, 1982; Belovsky & Schmitz, 1994). On the other hand, nutritionally valuable compounds, which are desired and searched for, are expected to give rise to possible positive correlations with food choice. Only a few studies in non-human primates have so far focused on assessing possible positive correlations between specific nutrient compositions and food choice (Laska *et al*., 2000; Laska, 2001; Jildmalm *et al*., 2008; Hansell *et al*. 2020). The reason for the higher number of studies that focus on the negative correlations with plant secondary compounds is due to the fact that food items in the wild are composed of a mixture of both aversive plant secondary compounds and attractive nutrients and it is therefore difficult to disentangle whether a wild fruit, for example, is preferred due to its nutrients or due to its low concentration of plant secondary compounds (Glander, 1982). One method to assess potential positive correlations between food choice and nutrient composition is to employ cultivated fruits and vegetables, which are known to have negligible quantities of plant secondary compounds and have well known nutrient compositions (Food Standards Agency, 2002).

Food preferences related to nutritional content have been studied previously in primate and non-primate species. Captive pigtail macaques (*Macaca nemstrina*), white-handed gibbons (*Hylobates lar*) and ring-tailed lemurs (*Lemur catta*) have all been reported to display food preferences that correlated significantly and positively with the contents of carbohydrates (Laska, 2001; Jildmalm *et al*., 2008; Hansell *et al*., 2020). Proteins, lipids, or the total content of energy were not found to be significantly correlated with their food preferences. Accordingly, these three species were considered *selective feeders* with carbohydrates as the preferred source of metabolic energy (Laska, 2001; Jildmalm *et al*., 2008; Hansell *et al*., 2020). In contrast, captive spider monkeys (*Ateles geoffroyi*), squirrel monkeys (*Saimiri sciureus*) and pacas (*Agouti paca*) were all reported to display food preferences that correlated significantly and positively with the total contents of energy (Laska *et al*., 2000; Laska, 2001; Laska *et al*., 2003), irrespective of the source of metabolic energy. Accordingly, these three species were considered *opportunistic feeders* with regard to maximizing their net gain of energy (Laska *et al*., 2000; Laska, 2001; Laska *et al*., 2003). Hence, as previous studies have shown, an animal can use different food-choice strategies to meet its energy requirements.

White-faced sakis (*Pithecia pithecia*) are Neotropical frugivorous primates with a habitat that ranges from dry to seasonally flooded forests across Brazil, Venezuela, French Guiana, Guyana and Suriname (Marsh *et al*., 2021). Their diet comprises a variety of fruits, leaves, insects and flowers (Norconk & Conklin-Brittain, 2004). In contrast to most other frugivorous primate species that may act as seed dispersers, sakis prey heavily upon the seeds of the fruits they consume and are thought to exploit the nutrients contained in them (Veiga *et al*., 2013; Norconk & Conklin-Brittain, 2004). The average nutritional intake of lipids by white-faced sakis is markedly higher than that of other frugivores, mostly due to the ingestion of young seeds that are especially high in lipid content (Norconk *et* al., 2002; Norconk & Conklin-Brittain, 2004). Reports also state that white-faced sakis tend to forage for and select unripe non-succulent fruits, reducing possible dietary stresses (due to seasonal changes in the availability of ripe fruit) and avoiding competition with other sympatric species during periods of preferred food scarcity (Kinzey & Norconk, 1990; Veiga *et al*., 2013; Ledogar *et al*., 2013). As sclerocarpic (hard pericarp fruit and seed exploiting) foragers, their dental morphology (e.g. tusklike lower canines and scooplike wedge lower incisors) is adapted for extraction and mastication of hard unripe fruits and seeds protected by tough outer membranes, and their gut physiology provides the opportunity for fermentation and prolonged digesta retention to process fibrous seeds and leaves (Kinzey & Norconk, 1990; Norconk *et* al., 2002; Ledogar *et al*., 2013; Rosenberger, 2020). Seed predation and the ability to digest fruits of varying maturity helps white-faced sakis maintain a steady diet by reducing the impact of seasonal shortages. However, during the dry season (March-May) they have been reported to feed more heavily on leaves, insects and flowers (Norconk & Conklin-Brittain, 2004; Cunningham & Janson, 2006). A previous study by Norconk and Conklin-Brittain (2004) identified the importance of lipids in the annual diet of Venezuelan white-faced sakis, with this nutrient alone explaining about 50% of the variance in feeding time in the wild. Nevertheless, it is still unknown how lipids and other nutrients may affect food choice behavior in captive white-faced sakis. Thus, the aim of the present study was to assess food preferences in a group of captive white-faced sakis towards a variety of cultivated fruits and vegetables along with other animal source foods that differed significantly in their nutritional content. Correlational analysis was then used to assess whether the resulting food preference ranking correlated with the contents of certain macro- and/or micronutrients of the food items used. My hypothesis was that the white-faced sakis would display a preference for certain food options depending on their nutritional content.

# 3. Materials and Methods

## 3.1 Animals

This study was carried out using five adult white-faced sakis *(Pithecia pithecia)* held at Furuvik Zoo, Gävle, Sweden (Fig. 1). The group comprised a male (Kariakou, 16 years of age) and female (Lisha, 14 years) couple, and their offspring, two females (Elin, 7 years and Anita, 6 years) and one male (Engelbrekt, 10 years). Kariakou was born in La Vallée des Singes (France), Lisha was born in Krefeld Zoo (Germany), and all three offspring were born in Furuvik.

Figure 1. A male (Kariakou holding an avocado seed) and female (Anita) white-faced sakis.

The animals had access to both an outdoor (127$m^{3}$) and an indoor enclosure (633$m^{3}$) at all times and were already accustomed to short-term separation prior to the study.

The sakis’ regular diet consisted of fruits and vegetables, primate extrudate high fiber pellets (from Granovit Zoofeed, Kaiseraugst, Switzerland) and a tamarin cake (from Mazuri Zoo Foods, Witham, Essex, Great Britain), while also having free access to fresh leaves and branches that were placed in the enclosure occasionally. Fruits and vegetables were fed twice each day around 11:00 and 15:00, while water was constantly available in a drinking bowl.

## 3.2 Experimental procedure

Food preferences were assessed using a two-alternative choice test. Each animal was presented with pairs of equally-sized food items, and its choice behavior, i.e. which of the two food items was consumed, was recorded. The animals were tested individually to avoid competition or distraction that could affect an animal's choice behavior.

The animals were separated for an average of 5 test sessions each day, between 07:00 and 17:00. The hour that followed their two daily meals was excluded in order to maximize the animal’s appetite and motivation to participate while avoiding overexposure to a certain fruit or vegetable. Each test session comprised a maximum of 5 pairwise presentations per animal and the position of the food items was pseudorandomized to counterbalance possible side preferences. All 105 possible binary combinations of 15 types of food were presented for a total of 10 times per animal and care was taken to never present a food item that had been part of the previous pair to prevent any bias. All foods were cut to (or presented at) an equal size, approximating cubes with a side length of 2 cm to avoid choice behavior being affected by size differences.

In each session the animals voluntarily entered a testing cage (1 x 2.4 x 3 m) connected to the indoor enclosure through a sliding door. The animals then placed themselves on a wooden platform attached to a metallic mesh that separated the testing cage from the enclosure’s service area (Fig. 2). The food was presented on a 30 x 22 cm cutting board and was covered by a box until its front edge came in contact with the mesh at the height of the wooden platform in order to guarantee that the sakis would be exposed to both food items simultaneously (Fig.3). This was also intended to stimulate their curiosity and successive motivation to participate in the tests. The mesh was wide enough to allow the sakis to fit their hand through and grab hold of the food items (Fig.3).

The 15 different types of food employed were broccoli (*Brassica oleracea var botrytis*), cucumber (*Cucumis sativus*), tomato (*Lycopersicum esculentum*), carrot (*Daucus carota*), eggplant (*Solanum melongena*), beetroot (*Beta vulgaris*), sweet potato (*Ipomoea batatas*),avocado (*Persea americana*), Napa cabbage (*Brassica rapa, subsp. pekinensis*), apple (*Malus pumila*), peanut (*Arachis hypogaea*), honey melon (*Cucumis melo*), hazelnut (*Corylus avellana*), mealworms (i.e. larvae of the mealworm beetle *Tenebrio molitor*), and hard-boiled egg (from chicken, *Gallus gallus*). The rationale for choosing these types of food was that a) all of them are part of the animals’ diet in captivity and thus familiar to the white-faced sakis, b) data for the contents of the macro- and micronutrients in these types of food are available, allowing me to assess possible correlations between food preferences and nutrient contents (Food Standards Agency 2002), and c) they differ markedly in their content of macronutrients and/or micronutrients.

Figure 2. Testing cage separated from the service area through a metallic mesh.

Figure 3. (On the left) Presentation of the food item pair covered by a box on the cutting board. (On the right) Choice behavior displayed by the saki reaching his hand through the mesh and retrieving the preferred food item.

## 3.3 Data analysis

The software program RStudio was used for all statistical tests performed. A total of 5250 choices were recorded, which correspond to the 105 possible binary combinations of the 15 food items presented 10 times to each of the 5 animals. For each food pair presented to the sakis, 1 point was attributed to the food item eaten, whilst the remaining food item was attributed 0. Alternatively, 0.5 points were attributed to both food items when the sakis failed to choose within 10 seconds from the removal of the box covering the food items. Food preference rankings were established both at the individual and at the group level using different criteria (Hansell *et al*. 2020) and tested for statistical significance using the two-tailed binomial test. At the individual level, all choices for each of the 15 different food types were summed, with a theoretical maximum score of 140 points (14 combinations x 10 presentations) for a given food item. At the group level, all individual choices were pooled across the five sakis and summed, resulting in a theoretical maximum score of 700 points (14 combinations x 10 presentations x 5 individuals) for a given food item.

Correlations between the food preference rankings and the contents of macro- and micronutrients were evaluated by calculating Spearman rank-order correlation coefficients rs and tested for statistical significance by computing z-scores. Spearman rank order correlations tests were also performed between the preference rankings of all the different individuals to assess if all five sakis shared similar food preferences. A Spearman rank order correlation test between males and females was also performed to assess if there were sex-related differences in food preference.

# 4. Results

## 4.1 Food Preferences

The summarized data for all the food choices made by the five sakis for all possible combinations of food pairings are shown in Table 1. The group of sakis displayed a significant preference for one of the two food items in 96 out of the 105 total binary combinations. While peanut was significantly preferred over all 14 other possible food items, eggplant was never preferred over any alternative. Hazelnut and avocado were also significantly preferred over all other food items (with the obvious exception of peanut). Although broccoli was significantly preferred over eggplant, and tomato was significantly preferred over broccoli and eggplant, these three food items were never significantly preferred over others.

Table 1. Choice behavior of the five white-faced sakis.

The first value in each table cell 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 and *n.s.* indicates the lack of a significant preference.

## 4.2 Food preference rankings

A summary of all food choices made by the sakis at the individual and group level is shown in Table 2. Peanut, hazelnut and avocado were the three most attractive food items both at the group and individual level. Eggplant and broccoli were among the three least attractive food items for all individuals and at the group level. The five sakis showed similar rankings of preference with only small differences in the order of the 15 food items. Accordingly, all individual rankings of preference were significantly correlated with each other (Spearman rₛ ≥ 0.91, P ˂ 0.05). Food preference rankings were also similar between the two males and the three females. Accordingly, the rankings of preference were significantly correlated between male and female sakis (Spearman rₛ = 0.98, P ˂ 0.05).

Table 2. Total number of choices per food item at the individual and group level.

The food items are listed from most to least attractive according to the group level data.

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## 4. 3 Food preference rankings and nutritional content

Spearman rank-order correlation results between the food preference rankings of the sakis and the nutritional contents of the 15 food items are displayed in Table 3.

At the 1% level of significance (Spearman, P ˂ 0.01), energy was the only nutritional content that was positively correlated with all the individual and group preference rankings (Spearman rₛ = 0.693). The positive correlation with energy means that the most attractive food items had the highest energy values per mass unit.

Also at the 1% level of significance (Spearman, P ˂ 0.01), Carotene and vitamin C were negatively correlated with all the individual and group preference rankings, with the single exception between vitamin C amounts and Engelbrekt’s preference ranking (where P = 0.013). The negative correlations with carotene and vitamin C mean that the least attractive food items had the highest amounts of carotene and vitamin C per mass unit.

At the 5% level of significance (Spearman, P ˂ 0.05), the contents of mono-unsaturated fatty acids, copper, iodine, vitamin E, riboflavin and biotin were all correlated positively with the group preference ranking. Out of the previously listed nutrients, only mono-unsaturated fatty acids, riboflavin and biotin were also significantly and positively correlated with all individual preference rankings (Spearman, P ˂ 0.05).

Also at the 5% level of significance (Spearman, P ˂ 0.05), the content of water was negatively correlated with the group and all individual preference rankings.

In addition to the significant correlations found at the group level, Lisha’s individual preference ranking was also significantly and positively correlated with the lipid content of the food items employed (Spearman rₛ = 0.55, P ˂ 0.05).

Table 3. Spearman rank-order correlations between food preference rankings and nutritional contents of the 15 food items employed in the study.

Values for the statistical measure r may range from +1 (perfect positive correlation) to -1 (perfect negative correlation). Statistically significant correlations are shown in bold typeface.

# 5. Discussion

The results of this study demonstrate that white-faced sakis display marked food preferences in a two-alternative choice testing situation for a selection of food items that are part of their captive diet. These food preferences significantly correlated with energy content but not with the contents of the individual energy-rich macronutrients carbohydrates, proteins, and lipids. The most attractive food items were the ones highest in energy. Additionally, the least attractive food items were the ones with the highest water content.

## 5.1 Macronutrients

The sakis’ food preferences were found to correlate significantly with energy content, suggesting that white-faced sakis are opportunistic feeders with regard to energy gain. This means that the sakis, unlike some other primate species (Jildmalm *et al*., 2008), do not base their food preference on a particular source of metabolic energy, such as carbohydrates, but instead they prefer foods that are high in any kind of energy source which may include carbohydrates, proteins, or lipids. This is illustrated by the top three preferred food items across all individuals (peanut, hazelnut and avocado) being part of the top four food items with the highest energy values. Cucumber, eggplant, tomato and cabbage, in contrast, were the food items with the lowest energy values and accordingly ranked low in the preference rankings.

The low correlation values (rs) and the lack of statistical significance (Spearman, P > 0.05) of the correlations between the sakis’ food preferences and carbohydrate and total sugar content, respectively, suggests that the sakis do not explicitly prefer foods with high values of soluble carbohydrates to meet their metabolic energy requirements. This can be illustrated with the low placement of sweet potato in the sakis’ food preference ranking, which was the food item with the highest carbohydrate content among the 15 tested here (see table 2). These results are consistent with Norconk and Conklin-Brittain’s report (2004) on white-faced sakis in the wild having a lower intake of free simple sugars than other frugivorous primates.

White-faced sakis are thought to meet their metabolic energy requirements through the selection of foods with high proportions of lipids. The food items with the highest amounts of lipids (hazelnut, peanut, mealworms, avocado, and egg, in descending order), all with an at least 10 times higher lipid content than the other food items employed here, are highly placed in the food preference rankings (see table 2). This result is consistent with the Norconk and Conklin-Brittain (2004) study, where Venezuelan white-faced sakis in the wild were reported to exploit seeds for their high lipid content in order to achieve an extraordinarily high average lipid intake. Although the correlation between lipid content and food preference was only statistically significant for Lisha (Spearman rₛ = 0.55, P ˂ 0.05), it is remarkable that the group’s preference ranking correlation value with the lipids (Spearman rₛ = 0.38) was higher than the ones obtained with the carbohydrates and proteins, respectively (Spearman rₛ = 0.16 and rₛ = 0.34). In all other primate species tested so far, the correlation between food preference and macronutrient content was always highest with carbohydrates, even with opportunistic feeders (Laska *et al*., 2000; Laska, 2001; Jildmalm *et al*., 2008; Hansell *et al*. 2020). In contrast, the sakis’ food preference correlation with carbohydrates had the lowest value among the macronutrients, an unprecedented finding between food selection and the content of specific nutrients.

The content of protein was not significantly correlated with any of the sakis’ individual or group preference rankings. The intake of crude protein by white-faced sakis in the wild has been reported to be lower than that of colobines, catarrhine monkeys and chimpanzees (Norconk & Conklin-Brittain, 2004). Additionally, the wide interannual variation in crude protein intake by white-faced sakis is attributed to insect availability (Norconk & Conklin-Brittain, 2004), represented in the present study by mealworms. Although mealworms were the food item with the second highest protein content, they were only placed 7th in the group’s preference ranking. Still, the positive correlation value obtained between the group preference ranking and protein content (Spearman rₛ = 0.34) is due to peanut, hazelnut and egg (part of the 4 food items highest in protein contents) being highly preferred.

The significant negative correlation found between water content and the sakis’ food preference ranking reflects a low preference for food items containing high proportions of water. This finding is in line with the fact that white-faced sakis are accustomed to having free access to water resources such as natural tree cisterns (Cunningham & Janson, 2013). Without having to rely on water-rich food items to meet their water requirements, the sakis can therefore prioritize food items with higher energy values, which are typically low in water content (Food Standards Agency 2002). The three food items with the highest proportion of water (cucumber, tomato and eggplant, in descending order) were also the three food items with the lowest energy value, and accordingly, they were placed low in the preference rankings.

The exploitation of lipids over carbohydrates and proteins is reasonable from an energetic point of view; lipids provide 9 kcal/gram whereas both carbohydrates and proteins provide only 4 kcal/gram (Food Standards Agency, 2002). However, the fact that most frugivorous primate species studied so far rely mainly on carbohydrates as their primary source of metabolic energy, suggests that the effective exploitation of lipids may require anatomical and/or physiological evolutionary adaptations of the digestive system that are only favored under certain selective pressures and which may have acted upon the white-faced sakis.

## 5.2 Vitamins and minerals

It is known that animals are capable of selectively preferring foods that supply certain micronutrients in order to counterbalance the lack of said micronutrient in their diet (Yeager *et al*., 1997; Fashing *et al*., 2007).

 The sakis’ food preference ranking was significantly and positively correlated with several micronutrients, implying that the higher the amount of said micronutrient in the food item, the more preferred it was. With the exception of carotene and vitamin C, which were negatively correlated with the preference ranking and thus not preferred, the sakis actively preferred food items with higher amounts of copper, iodine, vitamin E, riboflavin and biotin. This could perhaps imply a deficit of these micronutrients in the sakis’ zoo diet, given their apparent craving for food that supplies them. However, given the fact that nutritional deficiency-related health problems are rare nowadays, and definitely not apparent in the animals of the present study, these significant correlations found here are most likely a by-product of the sakis’ preference for energy-rich foods (Crissey & Pribyl, 2000). The contents of copper, vitamin E and riboflavin were all significantly positively correlated with the food energy values (Spearman P ˂ 0.05) and negatively correlated with the proportion of water in food (Spearman P ˂ 0.05). On the other hand, the contents of carotene and vitamin C were significantly negatively correlated with the food energy values (Spearman P ˂ 0.05), while carotene was also positively correlated with the proportion of water in food (Spearman P ˂ 0.05).

## 5.3 Comparison with other species

Some of the findings of the present study are in line with those of previous studies performed with a similar method. A significant positive correlation that explains the increasing preference for food items with higher energy values was also found in squirrel monkeys (Laska, 2001), spider monkeys (Laska *et al*., 2000) and pacas (Laska *et al*., 2003). Based on these findings, all of these species were considered to be opportunistic feeders with regard to energy gain. In line with the results for the white-faced sakis reported here, none of the species above had a food preference that significantly correlated with the contents of protein, lipids or carbohydrates, respectively. Additionally, a food preference study performed with tufted capuchins (Visalberghi *et al*., 2003) also found a significant positive correlation that describes the increasing preference for food items with high energy values.

On the other hand, white-handed gibbons (Jildmalm *et al*., 2008), pigtail macaques (Laska, 2001) and ring-tailed lemurs (Hansell *et al*., 2020), did not show a significant preference towards foods that are high in energy value, but instead towards a specific source of metabolic energy, in this case carbohydrates.

The fact that some primate species seem to be opportunistic feeders with regard to their preferred source of metabolic energy whereas others are not, has been speculated to be due to variations in the degree of frugivory of said species (Laska *et al*., 2000; Laska, 2001; Laska *et al*., 2003; Jildmalm *et al*., 2008). Fruit pulp, which represents the bulk of the diets of many frugivores, is known to contain significant amounts of carbohydrates (Norconk & Conklin-Brittain, 2004).

Another proposed explanation for the fact that some primates have a preferred source of metabolic energy while others do not, derives from the avoidance of competition between sympatric species that developed different preferences for different sources of metabolic energy (MacKinnon & MacKinnon, 1980).

The preference for food items with low water content found in the present study was also found in squirrel monkeys (Laska, 2001), spider monkeys (Laska *et al*., 2000) and pacas (Laska *et al*., 2003), and was also thought to be due to the fact that plant food high in water content is usually low in energy values. Although not statistically significant (Spearman P = 0.09), a similar negative correlation between water content and food preference was found in tufted capuchins (Visalberghi *et al*., 2003).

## 5.4 Diet of *Pithecia pithecia* in the wild and in zoos

White-faced sakis are known as frugivorous seed predators, with a diverse diet in the wild composed mostly of fruit pericarp, seeds, flowers, leaves and insects (Ledogar *et al*., 2013). As seed predators, their dental morphology is adapted to allow them to consume mechanically challenging hard and unripe fruits and to extract and masticate hard seeds, expanding their food choices (Kinzey & Norconk, 1990; Norconk *et al*., 2002). This process of exploiting seeds is rather uncommon among frugivorous primates since the majority of them do not take advantage of the nutrients contained within the seeds, but rather defecate them intact and thus serve as seed dispersers (Kinzey & Norconk, 1990). This way, white-faced sakis can avoid possible competition over ripe fruit and minimize food shortages during the dry season.

Since dietary requirements specific to captive white-faced sakis have not yet been identified, the recommendations commonly used for this species are those for New World monkeys in general, gathered in the “Zoo Animal Nutrition Tables and Guidelines” by Jansen and Nijboer (2003). These are often paired with Norconk and Conklin-Brittain’s (2004) data on the nutritional content of an average diet of Venezuelan white-faced sakis in the wild to compose a balanced diet for captive white-faced sakis.

The European Association of Zoos and Aquaria (EAZA) recommends the captive white-faced sakis’ diet to include: concentrate pellets, vegetables, seeds and nuts, insects, browse and multivitamin and/or mineral supplements. They also refer to the possibility of including plant exudates (e.g. Arabic gum) and whole grains and advise against the administration of large amounts of fruits, dairy products and meat products. Additionally, EAZA suggests a diet composed of 20% concentrate feeds (pellets), 5% seeds and nuts, 60% vegetables and 15% other food items, such as browse, insects, fruits, plant exudates and whole grains. Given this existing information, the findings of this present study can help refine and improve the current dietary recommendations.

Cultivated fruits for human consumption have been selectively bred in order to appeal to the human taste. Consequently, they have markedly higher levels of sugar (particularly sucrose) and lower levels of fiber, while also being a poorer source of protein, vitamins, and minerals than their uncultivated counterparts (Dierenfeld, 1997; Milton, 2000). Considering that a part of the diet of sakis in the wild is composed of unripe fruit, the difference in soluble carbohydrates between wild and commercially available fruits is even greater (Ledogar *et al*., 2013; Veiga *et al*., 2013).

 In captive environments, a fruit-based diet can cause nutritional imbalances impacting the animal’s general health and breeding success, and possibly lead to dental problems, overweight issues and diabetes (Schwitzer *et al*., 2008). Therefore, it is recommended to implement a wider variety of vegetables in the diet of frugivorous primates, as they more closely resemble the nutritional profile of non-cultivated fruits which are part of the diet of animals in the wild (Schwitzer *et al*., 2008).

## 5.5 Implications for welfare of white-faced sakis

The sakis in the present study displayed a marked preference for food items with a high energy value (i.e., number of calories per gram), however, their diet should not consist only of highly caloric ingredients. In the wild, this preference is sustainable given the energy that the animals have to invest in foraging and predator awareness for example, but in captive environments the provision of easily digestible, low-fiber and high-sugar foods comes at no energy cost to the sakis.

Even so, the information gathered here regarding the food preference rankings is relevant in the elaboration and update of captive diet catalogs. Although eggplant, broccoli, tomato and cabbage were considered among the least desirable food items, they represent healthy food options for the sakis. Carrot and beetroot can replace these occasionally or be provided in minor daily quantities to increase the diet’s attractiveness.

Given that this is the first study on food preference and nutritional content performed with captive white-faced sakis, a follow up survey with additional white-faced saki populations should be carried out to assess how representative the present results are of this species.

## 5.6 Evaluation of the method used

Given that other factors, apart from the nutritional content of a certain food, can affect an animal’s choice behavior (Laska *et al*., 2007), the two-choice test method was employed in a way to minimize confounding factors.

It is well established that the size of food items in a two-alternative test situation can affect choice behavior (Menzel & Draper, 1965). This was controlled for by presenting both food items with the same approximate size (2 cm length cube), avoiding any potential bias towards a larger piece of food.

Ripeness can alter the aspect, taste and nutritional content of a given food item and has been shown to affect food selection (Food Standards Agency, 2002; Redford *et al*., 1984). Therefore, all food items were carefully chosen so that the degree of ripeness of the food presented would remain consistent throughout the whole study.

Another factor known to influence choice behavior in previous studies was the novelty of the food items presented (Fragaszy *et al*., 1997). Food neophobia, or the fear of trying new foods, is a defense mechanism usually observed when nonhuman primates are presented with novel food items (Johnson, 2000). To control for this, all food items were made familiar to the sakis prior to the start of the study.

The hunger state of animals may also be a potential confounding factor, with both states of ravenous hunger or satiety possibly affecting food choices (Critchley & Rolls, 1996). Therefore, testing sessions were timed so as to present food in periods where a common prandial state could be maintained for all individuals during testing.

It has also been shown that food choice behavior and consumption can vary according to the social context in which food is presented (Visalberghi *et al*., 1998). This confounding factor was controlled for by performing all tests with the animals placed individually in the testing cage. Care was also taken to ensure that all animals were accustomed to and comfortable with short-term separation and displayed no signs of separation stress.

When performing the preliminary tests, I realized that the order in which the animals saw the food items might affect their choice behavior. To overcome this problem, I decided to cover both food items with a cardboard box until they were at a distance that would guarantee their simultaneous visualization. Additionally, by hiding the food items and their respective preparation for presentation, the sakis’ curiosity and interest, as well as their cooperation in the tests, was kept.

Finally, the position in which the food items were placed on the cutting board (right or left) was considered as a possible confounder, affecting the animal’s choice behavior (Chapelain *et al*., 2006). On that account, the food items’ placement was pseudorandomized, thus minimizing the risk of any spontaneous or learned side bias.

By addressing all of these concerns, I feel that it is safe to interpret the results of the present study as a true reflection of the sakis’ food preferences and that these are based on their nutritional differences. The two-alternative choice test employed in the present study is widely used across primate (e.g. squirrel monkey and pig-tailed macaques) and non-primate species (e.g. pacas) (Laska, 2001; Laska *et al*., 2003).

Two recent studies have employed a different method for assessing food preferences in nonhuman primates. They used a touch screen interface instead of using real food items (Huskisson *et al*., 2020; Hopper *et al*., 2019). This method removes the experimenter’s role of presenting and preparing the food items, thereby avoiding any possible bias or unintentional cues. However, since the food items are only presented visually as two-dimensional images, the animal is deprived of somatosensory and olfactory exploration, two important factors in nonhuman primate food selection (Laska *et al*., 2007). Additionally, the results obtained from a touchscreen interface would be restricted to the single decision for one of the food items, neglecting any other additional food choice behaviors, such as hesitation, time taken to decide, or the amount of food that is actually consumed.

Finally, given the present study’s focus on the relation between food choices and nutritional content, it seems appropriate that the animals should have access to the food items at hand, something that the touchscreen interface does not provide.

## 5.7 Conclusion

According to the results of the present study, when provided with 15 different food options, zoo-housed white-faced sakis demonstrate marked food preferences in relation to nutrient composition. The sakis’ food preference was significantly positively correlated with the total energy content and negatively with water content. No specific source of metabolic energy was found to be significantly correlated with food preference, although lipids had the strongest correlation with food preference. This is an unusual finding in frugivores, but also one that supports the idea that white-faced sakis exploit seeds for their nutritional value and their high contents of lipids in particular. While the results of this study do not allow for more generalizing conclusions on food preferences in zoo-housed white-faced sakis given the small number of tested individuals, knowledge of animal feeding preferences provides valuable insight on how animal wellbeing in zoos can be improved.

# 6. Societal and ethical considerations

The experiments reported here comply with the *European Directive on the Protection of Animals Used for Scientific Purposes (EU Directive 2010/63/EU),* the *American Society of Primatologists’ Principles for the Ethical Treatment of Primates,* and also with current Swedish laws. The current study did not require an additional ethical approval because modifying the composition of the diet and its mode of presentation to zoo-housed animals is part of the environmental enrichment initiatives that zoos are required to have by law.

All of the food was familiar to the sakis and had been authorized by the zoo's caretakers. The sakis who took part in this study did so on a completely voluntary basis. The sakis would enter the test set-up of their own free will and were permitted to exit whenever they desired, or the experiment was concluded. Data collection was contingent on their cooperation.

Increased knowledge of the food preferences of zoo-housed primates may aid in the treatment of nutrition-related issues such as obesity and other health issues. The findings of this study are valuable for the creation of diet catalogs for zoo-housed white-faced sakis especially since specific dietary requirements for this species have not yet been identified by EAZA. Providing a balanced and healthy diet to zoo-housed white-faced sakis is critical for their welfare and for maintaining stable captive populations. IUCN Red list has also indicated that the white-faced saki population trend is decreasing in the wild, giving the study of their food preference and the nutritional compositions of their diet greater value.

Comparative studies of food preferences and nutrient composition may also help us better understand the basic mechanisms that underlie eating behavior. This, in turn, could be of service in the study of human health issues like obesity and diabetes.

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