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NUTRITIONAL COMPOSITION HAS LIMITED IMPACT ON THE FORAGING PATTERNS OF NORTHERN PLAINS GRAY LANGURS (*SEMNOPITHECUS ENTELLUS*)

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ABSTRACT: Along with the feeding behaviour, understanding the nutritional requirements of a threatened species can aid in effective conservation strategies. This study aimed to analyze whether the nutritional properties of food items correlate with the time spent feeding in Northern Plains Gray Langurs (*Semnopithecus entellus*). We studied eight groups of Langurs living in the non-forested area of Jessore, Bangladesh, from September 2012 to August 2013. Data on time spent feeding was collected through 10-minute continuous focal animal sampling from a total of 303 observation hours. Meanwhile, food items consumed by langurs, were collected from their habitat and afterwards, nutritional properties were analyzed from the food science laboratory of the Institute of Nutrition and Food Science (INFS) and the Center for Advanced Research in Sciences (CARS), University of Dhaka. The results showed that there were marked differences in protein, fat, carbohydrate, ash, and moisture content among food items of different plant species. We correlated the nutritional properties of analyzed food items with the time spent feeding on those particular food items. None of the nutritional content in food items significantly correlates with the langurs' feeding time spent on particular food items. The findings suggest that langurs exhibit opportunistic feeding behaviour, prioritizing accessibility over macronutrient contents. Additionally, other factors such as food availability, seasonal variation, secondary metabolites, and social dynamics may play a critical role in shaping langurs feeding patterns. Therefore, future research on ecological and physiological factors, along with the other nutritional composition, such as fiber content and secondary metabolites, should be considered in order to better understand langurs' dietary decisions.

Key words: Feeding, Nutritional properties, Proximate analysis, Food selection, Langurs

INTRODUCTION

Most non-human primates (hereafter only primates) are highly selective feeders and spend considerable proportion of their time and energy searching for

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preferred foods (Hughes 2009). The food choices of primates mainly depend on two principal factors: 1) the nutritional or deterrent content of the particular plant part (Barton and Whiten 1994, Jaman and Huffman 2011) and 2) its relative spatial and temporal availability (Leighton 1993, Stevenson 2004). Several other factors such as body size or gut morphology also contribute to the food selection in primates (Jildmalm *et al.* 2008).

Studies have demonstrated positive correlations between the nutrient contents of a given food item and the preferences displayed by primates (Conklin-Brittain *et al.* 1998, Milton 1998). Conversely, several researchers reported that the food selection of primates correlates negatively with the contents of plant secondary compounds such as phenolics, alkaloids, or tannins that inhibit digestion of proteins and polysaccharides (Glander 1982, Wrangham *et al.* 1998). A possible means to obtain information on whether nutrients might affect primate food selection is to observe the animal throughout its active period and analyze the nutritional properties of its food.

The proportions of leaf and non-leaf foods in the diet of langur in different habitats vary according to plant species diversity, seasonality, and the nutritional quality of the leaves (Davies and Oates 1994). Nutritional ecology might be useful for understanding many aspects of behaviour ecology of primate species and could be a valuable tool in primate conservation. In addition, the nutritional condition of animals is determined by physiological conditions and the input and output of energy via various activities (Coelho 1986). Various approaches have been used to assess nutritional condition, including measurements of morphometric variables such as body mass and body dimensions. Body mass is one of the most popular indices of nutritional condition in non-human primates (Smith and Jungers 1997). Fluctuating ecological conditions such as physical environment (e.g., temperature) and food resource conditions (e.g., availability) may influence the nutritional status of animals (Smith and Jungers 1997). Moreover, the variations in feeding budgets of langur are responsible for the availability of different food items (Khatun *et al.* 2018). Along with the ecological perspective, it is also similarly important to consider whether their food preferences are directly or indirectly linked to the nutritional qualities of the selected food items.

Feeding is one of the vital factors for the survival of any species and therefore, highly considerable issue for the conservation of this species concerned. Besides feeding, fulfilment of nutritional demand is another key factor for species survival. Understanding the nutritional requirements and allocations of time spent feeding of a threatened species may help for the development of conservation strategies and management policies. Thus, the present study endeavored to analyze whether Northern Plains Gray Langurs

(*Semnopithecus entellus*) correlate with the nutrient content of their food items and the time devoted to feeding on those particular food items.

MATERIAL AND METHODS

Study area and subjects: The study was conducted from September 2012 to August 2013 in Keshabpur (22^{0}54'29.71" N, 89^{0}13'9.18" E) and Manirampur (23^{0}1'0" N, 89^{0}14'0" E) Upazila of Jessore district, located in the southwest region of Bangladesh. Annual temperatures in the two areas range between a minimum of 11.2 ^{0}C in January and a maximum of 37.1 ^{0}C in June. Annual rainfall during the study period was 1537 mm. The topography of these areas is mainly open plains. The vegetation is dominated by economically important crops and plantations. There are fragmented patches of natural vegetation like herbs and shrubs found mostly in the fallow lands. There were eight groups of Northern Plains Gray Langur found during this study. Group size varied from 11 to 29 individuals and each group had a definable home range.

Sample collection and preparation for nutritional analysis: We collected behavioural data from each langur group using 10-minute continuous focal animal sampling methods (Altmann 1974, Bateson and Martin 2021). During that study period, we collected 1818 focal samples, a total of 303 hours of behavioural data. These data showed that the langur groups consumed different parts from 54 plant species (Rahman *et al.* 2015). Out of these utilized plant species, we filtered out the top ten plant species (*Musa sapientum, Dalbergia sisoo, Acacia nilotica, Mangifera indica, Tamarindus indica, Syzygium cumini, Carissa carandan, Spondius dulcis, Corchorus capsularis* and *Zizyphus mauritiana*), based on the highest time spent feeding. Due to the time and financial constraints, we selected only ten species for nutritional analysis. However, these ten plant species constitute about 45% of the total feeding time spent by langur (Rahman *et al.* 2015). We also identified the food items of the selective plants for further nutritional analysis, which are the leaves of the selected plants except *Musa sapientum*. The other food item was fruit of *Musa sapientum.*

For nutritional analysis, plant food items were collected during the months when they were eaten, as well as during months when they were not eaten, then composite the two samples to avoid seasonal variation of nutrient composition. The weight of fresh sample was taken just after the collection of plant items and kept it on black cloth (for heat absorption) for sun dry. This procedure varies species to species depending on plant's nature and moisture. An average, it took 3-4 days to complete sundry process. These dry samples were kept in zip lock plastic packs. In the end, these sun-dried samples were prepared for freeze

Table 1. Nutritional properties of leaf items (except *Musa sapientum*) of ten preferred plant species consumed by langur

Name of consumed plants	Family	English name	Time spent in minute (percentage)	Moisture (%/ 100g)	Ash (%/ 100g)	Protein (%/ 100g)	Fat (%/ 100g)	Carbohy drate (%/100g)	Total energy Kcal/100g
Musa sapientum	Musaceae	Banana	331.68 (8.36)	75.43	2.51	3.84	0.33	17.89	87.96
Dalbergia sissoo	Fabaceae	Indian Rosewood	272.4 (6.86)	56.91	7.47	16.45	0.29	18.88	124.82
Acacia nilotica	Fabaceae	Acacia	222.5 (5.61)	7.96	4.95	19.05	0.68	67.36	338.52
Mangifera indica	Anacardiaceae	Mango	220.67 (5.56)	20.1	13.37	9.46	0.14	56.93	265.06
Tamarindus indica	Fabaceae	Tamarind	151.87 (3.83)	22	7.41	13.78	5.34	51.47	296.97
Syzygium cumini	Meliaceae	Berry	134.17 (3.38)	5	5.72	9.7	3.62	75.96	374.75
Carissa carandan	Apocynaceae	Crane Berry	129.47 (3.26)	64	9.49	10.03	1.32	15.16	100.99
Spondius dulcis	Anacardiaceae	Hog Plum	124.02 (3.12)	59.65	11	13.6	0.12	15.63	102.28
Corchorus capsularis	Malvaceae	White Jute	97.75 (2.46)	64.53	9.41	14.64	1.50	9.92	92.58
Zizyphus mauritiana	Rhamnaceae	Indian Jujube	77.75 (1.96)	55.45	0.3	4.44	0.39	39.42	180.46

drying where -54 ^{0}C to -55 ^{0}C temperature was maintained for 24 to 30 hours. For these instances, a freeze dryer (Ilshin lab company ltd.) in Center for Advanced Research in Sciences (CARS), University of Dhaka was used. After completion of freeze drying, the dried samples were further weighted then grinded by blender and kept air tied ziplock packed and preserved it for next analysis.

Most of the nutrient analysis methods selected for this study were basically approved by the Association of Official Analytical Chemists (AOAC) International. These methods were established in the food science laboratory of the Institute of Nutrition and Food Science (INFS), University of Dhaka. Analyses were performed with homogenate sample in a repeated manner. Repeated analyses from the same homogenate (from extraction to analysis) validate the homogeneity of the sample and repeated analyses of the same extract validate instrument precision. Variations in the nutritional value of protein, fat and ash of leaf items were evaluated.

Methods for proximate analysis: Proximate composition of each sample of food item was determined in duplicate estimations and the mean value was recorded. The Moisture content was determined by initial weight minus the dried sample weight. The moisture-free samples were charred and heated to 600 ^{0}C until a constant weight was achieved, the residue being quantified as ash (AOAC 2000). The protein content was determined by Kjeldahl method No 984.13 (AOAC 2000, 17th) modified in INFS's laboratory at a micro scale. After acid digestion in Buchi Digest Systemk-437 equipped with a Buchi Scrubber, B-414, samples were distilled in Buchi Distillation Unit, K-350. Released nitrogen was trapped in 0.1N sulfuric acid and back titrated with 0.1N sodium hydroxide to estimate the total nitrogen which was converted to protein by multiplying with 6.25.

The Soxhlet method is recognized by AOAC as the standard method for crude fat analysis. The crude fat from the dried sample was estimated by the semi-continuous solvent extraction procedure (Soxhlet method), described in method no. 991.36 of AOAC 2000. The fat was extracted from the dried sample using petroleum ether (40-60 boiling range) as a solvent. The fiber is another content of proximate analysis, but due to the limited funding and laboratory facilities, we could not determine the fiber contents of the samples. As the fibers are the indigestible portion of carbohydrates (cellulose, lignin, etc.), we merged fiber contents with carbohydrates and the carbohydrate content of each sample is determined by the following formula. Carbohydrates (%) = 100− (Moisture + Protein + Fat + Ash). The energy value of each food item was calculated based on their content of crude protein, fat and carbohydrate using a formula described by Crisan and Sands (1978) as follows: Energy value (kcal/100 g) = (2.62 × % Protein) + (8.37 × % Fat) + (4.2 × % Carbohydrate).

A multiple linear regression and a simple linear regression analysis were conducted to determine whether any single nutritional property of food items influences in time spent feeding or the total energy content of the food item influences the time spent feeding, respectively. These analyses were completed using R (Version 4.0.4, R Core Team). Tests were two-tailed with a 5% level for significance ($p < 0.05$). For data visualization, we used 'ggplot2' package in R.

RESULTS AND DISCUSSION

Among the ten plant species analyzed, *Acacia nilotica* leaves have the highest protein content (19.05%), followed by *Dalbergia sissoo*, *Corchorus capsularis*, and others. Similarly, *Tamarindus indica* leaves contain the highest fat content (5.34%), followed by *Syzygium cumini*, *Corchorus capsularis* and others. *Syzygium cumini* exhibits the highest carbohydrate content (75.96%), followed by *Acacia nilotica*, *Mangifera indica*, and others.

In contrast, *Musa sapientum*, *Spondias dulcis*, and *Corchorus capsularis* have the lowest protein, fat, and carbohydrate contents, respectively. The ash content is highest in *Mangifera indica* and lowest in *Zizyphus mauritiana*. Regarding moisture content, *Musa sapientum* has the highest, whereas *Syzygium cumini* has the lowest.Finally, *Syzygium cumini* provides the highest total energy per 100 grams of leaf, while *Musa sapientum* has the lowest (Table 1).

The multiple regression analysis for time spent feeding in relation to the nutritional content of food items was not statistically significant ($R^2 = 0.12$, $F(4,5) = 0.16$, $p = 0.947$). Similarly, regression analysis for time spent feeding in relation to total energy also showed no significant effect ($R^2 = 0.003$, $F(1,8) = 0.03$, $p = 0.868$). Furthermore, none of the analysed nutritional properties significantly influenced the feeding time of langurs (Table 2, Figure 1).

Table 2. Output of the multiple linear regression model for the function of time spent feeding

Predictors	Estimate	Std. Error	t value	p-value
Intercept	189.78	106.71	1.77	0.135
Protein	1.28	7.78	0.16	0.875
Fat	-16.74	21.78	-0.76	0.477
Carbohydrate	0.34	1.57	0.22	0.834
Ash	-2.53	9.77	-0.25	0.806

The analysis of nutritional content in various plant species reveals marked differences in protein, fat, carbohydrate, ash, and moisture content, which can influence their potential use as food resources. *Acacia nilotica* leaves are noted for their high protein content, while *Tamarindus indica* leaves have the highest

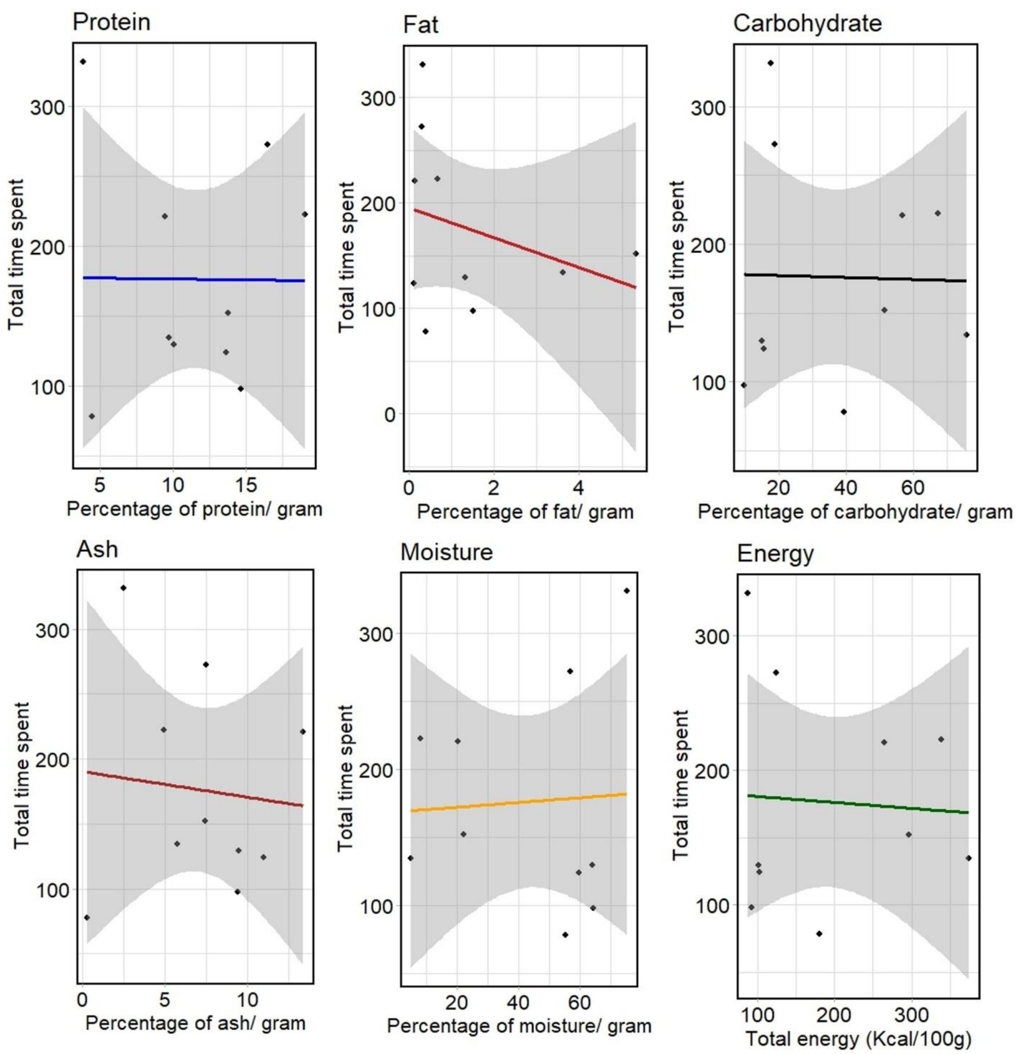

Fig. 1. The relationship between nutritional properties of food items from selected plant species and time spent feeding on them. The shaded area in each plot represents the confidence interval at 95%.

Fat content, and *Syzygium cumini* leads in carbohydrate content. These variations highlight the diverse nutritional profiles of plant species, which can be leveraged for different dietary needs and applications. However, the relationship between these nutritional properties and time spent feeding of langurs appears to be non-significant, suggesting other factors may influence feeding time. Among the preferred fodder plants, *Acacia nilotica, Dalbergia sissoo* and *Corchorus capsularis* leaves exhibit a greater protein content. This aligns with studies that highlight the importance of protein-rich leaves in primate diets, as they are often selected for their nutritional value (Nakagawa 2003, Ulappa *et al.* 2014). Protein is a critical nutrient for growth and maintenance, particularly in juvenile and female langurs, as observed in gorillas (Rothman *et al.* 2008). The presence of fat in leaves is relatively rare and may indicate a strategy to attract specific herbivores (Bryant *et al.* 1992). *Syzygium cumini*, with its high fat content, could be a preferred food source for langurs, as fat is a dense energy source. Carbohydrates are a primary energy source for many herbivores, and their abundance in these species may make them a staple in langur diets (Rogers *et al.* 1990, Popovich *et al.* 1997). *Corchorus capsularis*, despite its low carbohydrate content, is consumed less frequently, suggesting that other factors, such as fiber-rich or excessive moisture content, or secondary metabolites, may influence its selection (Ulappa *et al.* 2014). Ash content often reflects the mineral composition of plant materials, which can influence its palatability and nutritional value (Baranga 1982). *Musa sapientum*, with the highest moisture content, may be more appealing during dry seasons when water is scarce, as observed in other primate species (Carlson *et al.* 2013).

However, the study results suggest that the nutritional composition of food items does not significantly influence the time langurs spend for feeding and foraging. Despite variations in the amount of protein, fat, carbohydrate, energy, and ash content across the ten plant species analysed, the amount of nutritional content of the plant food items showed no significant relationship between these nutritional factors and feeding time on the particular plant parts. This result indicates that langurs do not necessarily allocate more feeding time on particular food items with higher macronutrient or energy content, suggesting that their foraging decisions may be influenced by other factors beyond simple nutritional quality (Lambert and Rothman 2015). It also suggests that other ecological, behavioural or social factors may play a more critical role in determining feeding patterns (Hladik and Chivers 1978).

One possible explanation is that langurs may prioritize food availability and accessibility over nutritional composition. In natural environments, primates often exhibit opportunistic feeding behaviour, consuming what is readily available rather than selectively choosing food items based on their macronutrient content (Chaves and Bicca-Marques 2016). The seasonal variation in food resources may further influence their choices, leading them to consume optimal foods when preferred options are scarce (Jaman *et al.* 2010). Additionally, factors such as leaf toughness, fiber content, and secondary metabolites (e.g., tannins, alkaloids) may affect palatability and digestibility, potentially overriding the influence of macronutrient composition on feeding time (Chapman *et al.* 2002, Jaman *et al.* 2010, Hickmott *et al.* 2021).

Furthermore, social and behavioural factors could also play a crucial role in determining feeding duration. Competition for food resources, group dynamics and predator avoidance strategies may influence how long langurs spend feeding on a particular food item (Pruetz 1999). In group-living primates, feeding time is often constrained by social interactions, with dominant individuals sometimes monopolizing preferred food sources while subordinates feed opportunistically (Pruetz 1999, Jaman and Huffman 2011). Similarly, vigilance against potential predators might lead langurs to limit their feeding time, regardless of the nutritional content of their food.

The lack of a significant relationship between total energy content and feeding time further supports the idea that langurs do not strictly optimize their feeding behaviour based on caloric intake. Instead, they may prioritize factors such as digestive efficiency, food processing time or even taste preferences (Milton 1984). Previous research on primate feeding ecology has highlighted the importance of gut physiology and digestion rates in determining food choices, with some species preferring easily digestible foods over those that provide high caloric or protein content but require prolonged digestion (Milton 1981, Chivers and Hladik 1984).

CONCLUSION

This study highlights the complexity of langur feeding ecology. While plant species exhibit significant variation in macronutrient content, particularly protein, fat, and carbohydrates, these nutritional properties do not significantly affect the time langurs spend in feeding. This may suggest they did not select a plant targeting any particular nutrient content, rather opportunistically select plants that are available in their range areas. This further suggests that nutritional value alone does not drive foraging decisions. Instead, other ecological and behavioural factors such as food availability, digestibility, secondary metabolites, and social dynamics appear to play more critical roles.

To better understand langur dietary strategies, future research should incorporate a broader range of variables, including fiber content, plant toughness, anti-nutritional compounds, seasonal availability, and patterns of social interaction during feeding. Longitudinal studies across different habitats and seasons would also help clarify how langurs adapt their foraging behaviour to environmental changes. Such comprehensive approaches are essential for informing conservation strategies and habitat management for these ecologically important primates.

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