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Diet Analysis of Sympatric Colobine Monkeys from Bako National Park, Sarawak, Borneo

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ABSTRACT

Habitat quality and abundant of food resources are among the key factors influencing the continued existence of primates in the wild. Although much has been studied on primate habitats and their diets, little is known about the nutritional value of the colobines' foods. This study aimed to assess the dietary nutrient compositions of two sympatric colobine monkeys, *Trachypithecus cristatus* and *Nasalis larvatus*, in Bako National Park using proximate analysis of faecal, leaf and fruit samples of eight dominant tree species in Bako NP. Five nutrient parameters, namely crude protein, crude fat, crude fibre, ash, phosphorus, and energy content, were choosen to assess the nutritional demands of the monkeys in the wild. The faecal samples showed significantly higher percentage of crude fibre (27.58%) in *N. larvatus* compared to *T. cristatus*. In contrast, crude fat (8.52%), ash content (1.79%) and phosphorus (5.76 mg/g) were found to be significantly higher in the faecal samples of *T. cristatus* than in *N. larvatus*. The nutrient composition of leaves samples from the tree species consumed by *N. larvatus* and *T. cristatus* showed a significantly higher percentage of crude protein (14.56%) in *Barringtonia asiatica* (sea poison tree) and higher ash (13.70%) in *Morinda citrifolia* (Indian mulberry). Meanwhile, nutrient composition in fruit samples showed highest percentage of crude fibre (32.58%) and crude fat (12.35%) in *Calophyllum inophyllum (*Alexandrian laurel), whereas higher phosphorus (5.76%) and energy (24.26 kJ) were recorded in *Ceriops tagal* (Yellow mangrove). The higher crude fiber detected in *N. larvatus'* faecal samples compared to *T. cristatus* may indicates that *N. larvatus* experiences lower digestibility as they are incapable of completely digesting the tough leaves or fruits. This study provides useful information for the conservation and management of these primate species especially on their dietary requirements in captivity or in a new habitat.

Keywords: Food intake, nutrition, proboscis monkey, silvered langur

INTRODUCTION

Primates are found predominantly across the tropical regions around the world. They play an integral role in the tropical ecosystems, for example as seed predators and dispersers (Haurez *et al*., 2015). However, primates have different nutritional needs when selecting the types and quantities of food they consume (Felton *et al*., 2009). Each species or individual's nutritional requirement depends on their body size, metabolism, daily behaviour, and type of digestive system (Parra, 1978; Milton, 1993). Adequate food and specific diet components are essential for the primate's survival within their habitat (Wiafe, 2015). Thus, understanding primate nutritional composition is crucial to assess species ecology, such as behaviour and foraging strategies (Moges & Balakrishnan, 2014).

Primates' dietary selection is an important aspect that needs to be addressed effectively to conserve them in their natural habitat (Chapman *et al*., 2020). Consequently, determining the nutritional value of the diet of wild primates can promote a better management of primates nutritional needs in captivity (Felton *et al*., 2009). Faecal analyses can provide important nutritional information on primates' diet, especially in areas with high tourist visitation that may disrupt their natural feeding behaviour (McGrew *et al.*, 2009).

Bako National Park (Bako NP), situated about 24 km away from Kuching town, is Sarawak's most visited national park. This is mainly due to the unique habitats within the park and the opportunity to observe fauna within the vicinity of the park's headquarters, especially *Trachypithecus cristatus* (silvered langur), *Nasalis larvatus* (Proboscis monkey) and *Macaca fascicularis* (Long-tailed macaque). Despite their common occurrence in Bako NP, no recent studies have been conducted on their nutritional needs. Additionally, this provides opportunities to conduct studies on various aspects of these primates, including population size, behaviour, genetic diversity, and diet (Laman *et al*., 2007; Kombi & Abdullah, 2016; Mazlan *et al*., 2019).

The *Hibiscus tiliaceus* (Coast cottonwood), *Buchanania* sp. (Otak Udang tree), *Vitex pubescens* (Leban tree), *Pandanus* sp. (Pandan), and *Nephrolepis biserrata* (Paku Kubok) are the diets documented for *T. cristatus* (Laman *et al.*, 2007). *Trachypithecus cristatus* has a large sacculated and ruminant-like stomach with microbial fermentation capability that are vital for their folivorous diet (Strasser & Delson, 1987). The genus *Trachypithecus* including *T. crisatus* has a larger gastrointestinal tract than *Presbytis* species, allowing *T. cristatus* to consume more leaves and less fruit (Caton, 1999; Chivers, 2001). The diet of *T. cristatus* comprises 91% leaves and 9% fruits (Hock & Sasekumar, 1979).

Nasalis larvatus in Bako NP was observed to consume mainly seeds of *Syzygium* spp. and *Garcinia* spp. In mangrove forests, *N. larvatus* mostly prefers young leaves of *Sonneratia alba, Calophyllum inophyllum* and *Terminalia catappa* (Kombi & Abdullah, 2013; Budeng, 2014). *Nasalis larvatus* was also previously recorded to consume termites as a protein source in their diet (Matsuda, 2008). Their digestive system can be divided into compartments with various microbes that can neutralise the toxin from leaves, fruits and seeds (Nowak, 1999). As a result, *N. larvatus* has a large potbelly that weighs about a quarter of their total body weight. Furthermore, *N. larvatus* prefer unripe fruits and seeds over young leaves due to the high crude fibre content and easily digested carbohydrates found in fruits compared to young leaves (Davies *et al*., 1988).

Faecal analysis of wild *N. larvatus* showed less fibre than in captivity due to food resources of wild *N. larvatus* being dominated by leaves with higher digestibility that containing less fibre (Yeager *et al*., 1997; Matsuda *et al*., 2013; Matsuda *et al*., 2017; Matsuda *et al*., 2018). However, this information can be expected to vary across species, habitat, and depending on the availability of food resources. We hypothesised that faeces of *T. cristatus* has higher ratio of crude protein but lower fibre contents than *N. larvatus* due to their digestitive limitation based on their leaves selection. Therefore, this study is designed to increase the body of knowledge about the nutritional need of *T. cristatus* and *N. larvatus* in Bako NP through the analyses of their faecal samples and food resources.

MATERIALS AND METHODS

Study Area

Bako NP covers 2,727 hectares and is the first Sarawak's national park that was gazetted in 1957 (Figure 1; Ashton, 1971; Hazebroek & Abang-Kasim, 2000). Sarawak Forestry Corporation has listed Bako NP as one of the four protected areas in Borneo for *T. cristatus* and *N. larvatus* (Hazebroek & Abang-Kasim, 2000; Nijman & Meijaard, 2008). Given that the location of the park is only about 30 minutes away from Kuching Town and can be easily accessed by boat, extensive biodiversity studies have been conducted in the park. For example, bats (Khan *et al*., 2007; Naharuddin *et al*., 2015), rodents, other mammals, aves, herpetofauna and insect (Dzulhelmi & Abdullah, 2009; Zahidin *et al*., 2016), flora (Katagiri *et al*., 1991; Bukhori *et al*., 2018).

Sample Collections

Fresh faecal samples of each colobine monkey were collected and stored in a falcon tube. The faecal samples for each species were pooled together according to species assuming the colobine monkeys in Bako NP that moved together in the same troop consumed food at the same place or tree. The selected leaves and fruits were collected from trees that were regularly visited and consumed by these primates (Laman *et al*., 2007; Kombi & Abdullah, 2013; Budeng, 2014; Kombi & Abdullah, 2016; Wan-Azman *et al*., 2021). Based on the recent observations, eight tree species were indentified as food tree species of the primates. These food tree species were divided into two categories depending on the food items eaten by the primates i.e., fruits: *Ceriops tagal* (Yellow mangrove), *Dillenia suffruticosa* (Shrubby dillenia), *C. inophyllum (Alexandrian Laurel)*, and leaves: *Pongamia pinnata* (Pongam), *Terminalia catappa* (Singapore almond), *H. tiliaceus* (Coast cottonwood), *Barringtonia asiatica* (Sea poison tree) and *Morinda citrifolia* (Indian mulberry) (Figure 2).

Nutrient Composition Analyses

Proximate analyses were conducted to analyse the nutritional composition of the faecal samples from *Nasalis larvatus* and *Trachypithecus cristatus* follows the standard set by the Association of Official Analytical Chemists (AOAC), 1980. The samples were analysed for dry matter, ash, crude protein, crude fat, crude fibre, phosphorus and energy at the Protein Laboratory, Faculty of Resource Science and Technology, UNIMAS. This study adopted two analyses methods by Moges and Balakrishnan (2014).

These include ash content analysis (Furnance Elite Thermal Systems Limited) determined through the ash percentage and analysis of the crude protein using the Kjeldahl method and distillation (Buchi Labortechnik AG K-350/K-355). The crude fat analysis was performed using Soxhlet Extraction Mantle (SN: 2042), the crude fibre analysis was performed using Gerhardt Fibrebag System and phosphorus was analysed using spectrometer, whereby the graph showing the absorbance against the concentration of phosphorus was plotted to calculate the regression equation (Boaz & Smetana, 1996).

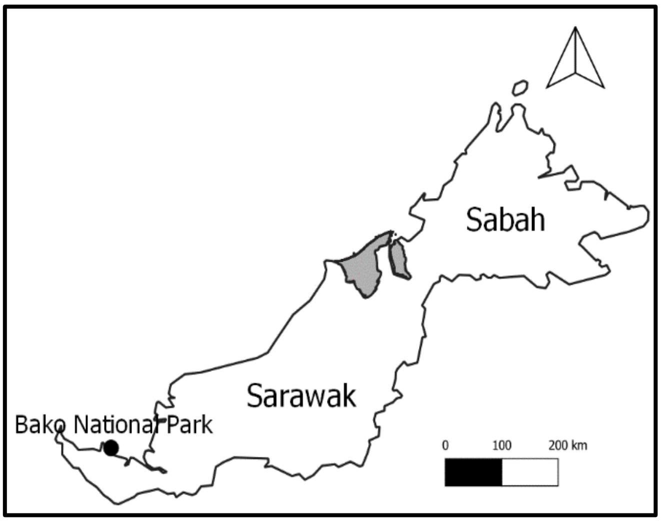

Figure 1. Map of the study area showing the sampling location at Bako National Park in Sarawak (Map was prepared using QGIS version 3.4.2.)

Figure 2. Leaves and fruits from plant species ingested by *Nasalis larvatus* and *Trachypithecus cristatus* in Bako NP, Sarawak; (a) *Terminalia catappa*; (b) *Hibiscus tiliaceus*; (c) *Barringtonia asiatica;* (d) *Morinda citrifolia*; (e) *Calophyllum inophyllum*; (f) *Pongamia pinnata*; (g) *Dillenia suffruticosa* and (h) *Ceriops tagal*

Energy analysis was conducted using a Bomb Calorimeter (IKA C2000 basic). A total of 100 g of each samples were used for each nutrient analysis conducted and each sample analysis was conducted with three replicates.

Data Analysis

Chemical composition were calculated following Kassim *et al*. (2017). A normality check was performed using Kolmogorov-Smirnov test to fulfil the normality assumption of the ANOVA. The mean differences in crude protein, crude fibre, crude fat, ash, phosphorus and energy between *N. larvatus* and *T. cristatus* were compared statistically using one-way ANOVA. The significance level was set at $p<0.05$. All statistical tests were conducted using the Statistical Package for Social Science (SPSS) software version 24.

RESULTS

Nutrient Composition in Faecal Samples

The crude protein composition was the same in *N. larvatus* and *T. cristatus* (Table 1). However, the percentages of crude fibre and minerals were higher in *N. larvatus* (27.58% and 6.08%, respectively). Crude fat and phosphorus were higher in *T. cristatus* with 8.52% and 5.76%, respectively.

Nutrient Content in Leaf and Fruit Samples

Table 2 listed the ratios of crude protein, crude fibre, crude fat, ash, phosphorus and energy in the tree species consumed by *T. cristatus* and *N. larvatus*. The proximate analyses showed that the highest proportion of crude protein was found in *B. asiatica* (15.06% and the lowest was in *D. suffruticosa* (1.43%). The highest fibre content in samples of leaf and fruit consumed by *T. cristatus* and *N. larvatus* was found in *C. tagal* (32.45%) whereas the lowest was in *P. pinnata* (7.36%). *Calophyllum inophyllum* had the highest fat content (12.35%), and *M. citrifolia* had the highest ash content (13.70%). The highest content of phosphorus was found in *C. tagal* (5.76 mg/g), while the lowest was found in *C. inophyllum* (1.07 mg/g). In terms of the value of energy, *C. tagal* had the highest energy content (24.26 kJ), and the lowest was recorded for *D. suffruticosa* (15.84 kJ).

DISCUSSION

Faecal Sample Analysis

The nutritional intake of *N. larvatus* and *T. cristatus* from Bako National Park were successfully determined using faecal sample analyses. The highest value of crude fibre in faecal samples was found in *N. larvatus*. This is due to the fact that *N. larvatus* was found to ingest more *C. inophyllum* than *T. cristatus*.

Calophyllum inophyllum has the greatest crude fibre values when compared to other fruits and leaves ingested by the two species. The time for daily rumination by *N. larvatus* will increase when the fibre content of the ingested diet increases (Lauper *et al*., 2013). Hence the higher fibre content has been found to cause longer feeding time during the day.

Nasalis larvatus is a foregut digester that allows them to re-chew large particles and they clears their foregut quickly from any digested particles (Matsuda *et al.,* 2011). They can consume tougher leaves more than *Trachypithecus* because tougher leaves consists of high neutral-detergent fibre (NDF) and low digestibility (Matsuda *et al.,* 2017). Neutral detergent fibre (NDF) is the essential element that consists of hemicellulose and cellulose, which helps better digestion of animals (Albayrak *et al*., 2011). For the crude fat in faecal samples, the highest was found in samples from *T. cristatus* (8.52%). This shows that, *T. cristatus* contains more gastrointestinal microbiome namely Firmicutes than *N. larvatus* (Clayton *et al*., 2018; Koo *et al*., 2019). In addition, this species' stomach contains sodium chloride, which helps in digesting cellulose and neutralises leaf toxins as well as harsh tannins (Phillipps & Phillipps, 2016).

The ratio of crude protein and dietary fibre is used to describe the quality of primates (Felton *et al*., 2009). The leaf-eating primates experience digestive restrictions by choosing leaves that were high in protein but low in fibre (Davies *et al*., 1988; Waterman *et al.,* 1988). Thus, *N. larvatus* and *T. cristatus* showed low nutritional composition value in the faecal sample.

Table 1. Nutrient contents of faecal samples of *Nasalis larvatus* and *Trachypithecus cristatus* from Bako National Park, Sarawak

Species	Crude protein (%)		Crude fibre (%)		Crude fat (%)		Ash (%)		Phosphorus (mg/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Trachypithecus cristatus	3.44	0.03	19.17	0.11	8.52	0.44	1.79	0.00	5.76	0.09
Nasalis larvatus	3.41	0.06	27.58	0.07	6.56	0.70	6.08	0.90	4.80	0.16

Table 2. Nutrient contents of leaves and fruits samples from eight plant species from Bako National Park, Sarawak

Species	Common name	Crude protein (%)		Crude fibre (%)		Crude fat (%)		Ash (%)		Phosphorus (mg/g)		Energy (kJ)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ceriops tagal (fruits)	Yellow mangrove (tagal)	6.08	0.04	32.45	0.34	1.81	0.02	4.19	0.05	5.76	0.00	24.26	0.13
Dillenia suffruticosa (fruits)	Shrubby dillenia (simpor gajah)	1.43	0.07	8.67	0.26	NA	NA	NA	NA	NA	NA	15.84	0.20
Pongamia pinnata (leaves)	Pongam (kacang kayu laut)	5.58	0.06	7.36	0.29	NA	NA	NA	NA	NA	NA	19.35	0.14
Terminalia catappa (leaves)	Singapore almond (ketapang)	12.55	0.22	9.43	0.24	1.54	0.03	NA	NA	NA	NA	15.63	0.07
Calophyllum inophyllum (fruits)	Alexandrian Laurel (penaga laut)	3.09	0.03	32.58	0.04	12.35	0.04	4.20	0.07	1.07	0.04	17.58	0.13
Hibiscus tiliaceus (leaves)	Coast cottonwood (baru laut)	12.90	0.09	16.12	0.02	4.46	0.20	7.90	0.05	2.33	0.25	18.11	0.05
Barringtonia asiatica (leaves)	Sea poison tree (putat laut)	15.06	0.01	18.29	0.12	11.41	0.13	6.45	0.08	1.49	0.35	19.14	0.05
Morinda citrifolia (leaves)	Indian mulberry (mengkudu liar)	14.56	0.19	12.72	0.19	NA	NA	13.70	0.05	0.90	0.01	16.72	0.02

NA: Result not available

These colobine monkeys have a higher nutritional value of fibre contents than the other elements which influenced the digestion capacity in their gut (Rose *et al*., 2015). As seen in this study, the proportion of crude protein was lower than the proportion of crude fibre. This is because crude fibre cannot be digested naturally by the folivorous primates (Nijboer, 2006). However, *N. larvatus* and *T. cristatus* do not experience digestive limitation based on their leaves selection because these primates mostly consume low crude protein leaves that are rich in crude fibre. These leaves are more nutritious and easier to digest due to their low fibre content (Davies *et al*., 1988; Waterman *et al*., 1988). The mineral content (ash) was found to be highest in samples from *N. larvatus* (6.08%) indicating that *N. larvatus* has incomplete non-digestible food. However, colobine monkeys have a suitable digestive system for the foregut fermentation system (Power *et al*., 2019). This might be due to the lower transit time of the food items passing through the gut that has led to incomplete digestion of the food. *Trachypithecus cristatus* showed a greater value of phosphorus in faecal samples because they were observed to feed on *C. tagal* in Bako National Park regularly, indicating that they have a poor calcium intake in their diet (Yeager *et al*., 1997).

Leaf and Fruit Samples Analysis

The proximate analysis showed that crude protein was highest in *B. asiatica* (15.06%). It is believed that young leaves of *B. asiatica* can be beneficial to relieve stomach ache (World Health Organization, 2009). Since primates do contract intestinal parasite that can cause stomach pain, *B. asiatica* can help to indirectly relieve this symptoms (Li *et al*., 2019). On the other hand, the highest percentage of crude fibre is from *C. inophyllum* (32.58%). This plant contains osteogenic compounds in their nuts as a potential source of developing anti-osteoporosis remedies (Wei-Hsien *et al.*, 2015). The occurrence of loss of bone density and strength in primates is accompanied by changes in bone dynamics (Legrand *et al*., 2003). Therefore, *C. inophyllum* can be regarded as an essential element in *N. larvatus* and *T. cristatus* diet. This aids in reducing osteoporosis effects which is critical for their foraging activity. *Callophyllum inophyllum* contains 62.21 - 64.57% of unsaturated fatty acid (Saddhu & Kivade, 2016). A high content of unsaturated fatty acids in dietary intake promotes better digestion and absorption of foods (Cetingul & Yardimci, 2008). *Morinda citrifolia* had the highest value of mineral content compared to the other fruit and leaf samples. According to Chunhieng *et al*. (2004), *M. citrifolia* has mineral content of 8.4% of dry matter. *Morinda citrifolia* is another important diet for colobine monkeys and is proven to maintain overall good health which can boosts the immune system (Brown, 2012; Manjula *et al.*, 2016). The highest phosphorus composition was recorded in *C. tagal*. *Ceriops tagal* has displayed good cytotoxicity against colon cancer and helps to treat cancer malignancies (Mukhtar *et al*., 2017). *Ceriops tagal* also gave the highest energy value (24.26 kJ). Each 0.5 g of sample provides 24.26 kJ of energy to the primates. This finding is also supported by Qadri and Jamil (1993) that shows *C. tagal* (fruits) contains the highest energy value of 0.81 kJ per 0.5 g of samples.

In brief, the *T. catappa*, *H. tiliaceus*, *B. asiatica* and *M. citrifolia* are good food sources of foods for the colobine monkeys in Bako National Park. They supplied various nutritional content. However, *C. tagal*, *D. suffruticosa* and *C. innophyllum* were poor quality foods for the primates, with a low crude protein amount and a high percentage of crude fibre that provides little nutritional value.

CONCLUSION

In summary, faecal samples analysis of *N. larvatus* showed higher values of crude fibre and mineral. Faecal samples of *T. cristatus* had a high values of crude fat and phosphorus. However, the highest percentage of the nutrient composition were as follows: crude protein - *B. asiatica,* crude fibre - *C. tagal,* crude fat - *C. inophyllum,* ash or mineral - *M. citrifolia*, phosphorus - *C. tagal* and energy - *C. tagal* respectively. Results from this study provide the baseline information for habitat monitoring as well as the necessary diet for managing these primate species especially in captivity.

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