Vietnamese Journal of Primatology (2009) 3, 29-36

The chemistry of eaten and uneaten leaves by Delacour's langurs (*Trachypithecus delacouri*) in Van Long Nature Reserve, Vietnam

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Key words: Delaour's langur, food choice, Vietnam, limestone langurs, soils, leaf chemistry

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Summary

Several morphological, physiological, and ecological factors influence colobine leaf selection, but nutritional factors, especially leaf protein and digestibility, are among the most powerful. In particular, the protein/fiber ratio of leaves has repeatedly been supported as a strong indicator of leaf selection across for African and Asian colobines. However, the influence of leaf chemistry on food choice has not yet been analyzed for the limestone langurs of Southeast Asia, six taxa found in close association with limestone karst. Vegetation on limestone differs greatly in species composition and structure relative to forests with other xeric and edaphic conditions, and therefore we wanted to determine if protein/fiber ratio holds as an important determinant of food choice in limestone habitats.

We collected instantaneous focal animal data on Delacour's langurs (*Trachypithecus delacouri*) on a 265 hectare limestone karst block in Van Long Nature Reserve, Vietnam from August 2007-July 2008. We collected leaf samples that were eaten (n=50) and uneaten (n=49) by focal animals. Samples were analyzed for protein, fiber, condensed tannins, phenolics, water, and ash content. Among eaten leaves, we also made chemical comparisons between young vs. mature leaves. The protein/fiber ratio was higher in eaten versus uneaten leaves (p = 0.0003), but not significantly different for any other comparison.

The Delacour's langur population at Van Long does not seem to be nutritionally stressed and the population is increasing, and therefore Van Long Nature Reserve offers the best chance for long-term survival of this species

Thành phần hóa học của các lá cây là thức ăn và không là thức ăn của voọc mông trắng (*Trachypithecus delacouri*) ở KBTTN Vân Long, Việt Nam

Tóm tắt

Có nhiều yếu tố ảnh hưởng đến sự lựa chọn lá cây làm thức ăn của các loài voọc như các yếu tố hình thái, sinh lý và các yếu tố sinh thái, nhưng các yếu tố dinh dưỡng, đặc biệt là chất protein và khả năng tiêu hóa của lá cây là những yếu tố tác động mạnh mẽ nhất. Đặc biệt, tỷ lệ giữa hàm lượng protein và chất xơ chứa trong lá thường được xem là chỉ thị mạnh của sự lựa chọn lá cây của các loài voọc châu Á và châu Phi. Tuy nhiên, chưa có nghiên cứu về ảnh hưởng thành phần hóa học của lá cây đến sự lựa chon thức ăn của các loài voọc núi đá ở Đông Nam Á, 6 bậc phân loại có mối quan hệ chặt chẽ với sinh cảnh núi đá caxtơ. Thành phần loài và cấu trúc của thảm thực vật núi đá khác nhiều so với các khu rừng có các điều kiện khô hạn và thổ nhưỡng khác. Vì vậy, chúng tôi muốn xác định xem liệu tỷ lệ protein/chất xơ có là yếu tố quan trọng quyết định sự lựa chọn thức ăn ở các sinh cảnh núi đá.

Chúng tôi đã thu thập các số liệu của voọc mông trắng (*Trachypithecus delacouri*) bằng phương pháp quan sát ngẫu nhiên con vật trọng tâm tại khu vực núi đá 265 ha ở KBTTN Vân Long, Việt Nam, từ 8/2007 đến 7/2008. Chúng tôi thu thập các mẫu lá cây mà các thể voọc quan sát ăn (n=50) và không ăn (n=49) để phân tích hàm lượng các chất protein, chất xơ, tannin đậm đặc, phenolics, nước và tro. Trong số các là cây vo ọc ăn, chúng tôi so sánh thành phần hóa hoạc của các lá non với các lá trưởng thành. Tỷ lệ protein/chất xơ là cao hơn ở các lá voọc ăn so với các lá voọc không ăn (p=0,0003), nhưng không có sự khác biệt đáng kể đối với các chỉ số so sánh khác.

Quần thể voọc mông trắng ở Vân Long có vẻ không bị ức chế về dinh dưỡng và đang gia tăng, vì vậy, KBTTN Vân Long là cơ hội tốt cho sự tồn tại lâu dài của loài này.

Introduction

For three decades, protein/fiber ratio has been recognized as a good predictor of leaf choice for relatively small mammalian herbivores, including primates (Milton, 1979). While an optimal level of fiber is needed to regulate the emptying of the colobine forestomach, fiber is inversely related to digestibility (Waterman & Kool, 1994). Chapman et al. (2002) list several studies that have supported the importance of protein and fiber in colobine leaf selection (McKey et al., 1981; Davies et al., 1988) and others that support colobine selection for easily digestible material (Oates et al., 1980; Waterman & Choo, 1981). As leaves age, they contain less protein and more fiber and lignin, and therefore young leaves are generally more digestible than mature ones (Baranga, 1986). The importance of protein and fiber in colobine leaf choice is further emphasized by the robust link between mature leaf protein/fiber ratio and colobine biomass across Africa and Asia (Waterman & Kool, 1994).

While protein and fiber levels are of paramount importance in leaf selection, various secondary compounds may also influence selection. Phenolics are the parent group of tannins, hydrophilic polymeric phenols that precipitate starch and proteins, lower nitrogen availability, lower nutrient quality, and reduce digestion (Rhoades & Cates, 1976). Tannins sometimes have beneficial effects in the diet by decreasing bloat (a foaming of digesta in the forestomach) and binding to, precipitating, and detoxifying alkaloids (Cork & Foley, 1991; Glander, 1994), but condensed tannins bind proteins, and there by negatively influence food choice, (Feeny, 1976; Coley & Barone, 1996).

Despite extensive studies of nutritional dietary ecology among colobines, few data exist for the six limestone langur taxa of Southeast Asia. Detailed studies of feeding ecology in the wild have only been conducted on *Trachypithecus leucocephalus* (Huang Chengming et al., 2000; Li Zhaoyuan et al., 2003; Li Zhaoyuan & Rogers, 2006) and *Trachypithecus delacouri* (Workman, in review), but the relationship between plant chemistry and food selection has not yet been considered for any of these species. This omission has important implications given that vegetation on limestone differs greatly in species composition and structure relative to forests with other xeric and edaphic conditions (Sterling et al., 2006). Vegetation on karst is notoriously stunted, with many grasses, lithophytic plants, shrubs and small trees (Li Zhaoyuan et al., 2003; Day & Chenoweth, 2004; Liu Zaihua et al., 2004). This stunted vegetation, coupled with the presumed soil conditions (thin, highly alkaline, sandy, dry, low in mineral nutrients) has been assumed to reflect plants whose leaves are well-defended by defensive compounds because they are growth-limited (Sterling et al., 2006).

The largest population of wild Delacour's langurs lives at Van Long Nature Reserve (VLNR) in northern Vietnam. A recent study at VLNR (Workman, in review) recorded VLNR Delacour's langurs as highly folivorous, eating 78% foliage annually: 59.3% young leaves and leaf buds, 20.4% mature leaves, 9.2% unripe fruit, 5.1% flowers and flower buds, 0.6% seeds, 0.3% stems, 0.1% ripe fruit, and 5% unclassified items. Young leaves contributed the greatest percentage to the diet during all months and seasons. Having a radiation of leaf-eating monkeys that are so highly folivorous and found in close association with limestone habitat creates a new opportunity to reassess the degree of variation in colobine diets and food selection. In this study, we wanted to determine how leaves eaten by Delacour's langurs differ in protein, fiber, and phenolics (especially tannins) with uneaten leaves. We then compare the chemistry of leaves eaten and uneaten by Delacour's langurs with those eaten and uneaten by *Colobus guereza* in Kakamega Forest, Kenya (Fashing et al., 2007).

Methods

Study site

Research was conducted at the Dong Quyen karst mountain of Van Long Nature Reserve (20º20'55"N, 105º48'20"E) in Ninh Binh Province, northern Vietnam. This habitat is characterized by limestone massifs fragmented by shallow wetlands. Dong Quyen Mountain is a 265 hectare massif that rises from 1 m to 328 m elevation (Fig. 1, 2). There is no dominant plant family or species on Dong Quyen and vegetation is comprised of woody trees and shrubs (43.5%), herbs (25.4%), climbers (29.7%), and grasses (1.4%) (Workman & Nguyen The Cuong, unpublished data). During the study period, the mean maximum temperature was 31ºC, and the mean minimum was 13ºC (*n*=394, range=9-37ºC). Total annual rainfall was 1375.62 mm, with 89% falling between May-October.

Plant sample collection and plant chemistry

Both eaten and uneaten plant samples were collected for chemical analysis. We collected samples from plants fed on by focal individuals that CW observed. We collected additional feeding samples on non-focal individuals that LVD observed. Instantaneous focal animal sampling data were collected from August 2007-July 2008 (*n*=21,012 minutes; 200 days). We observed Delacour's langur adult males, adult females, females with dependent young, and subadults in seven groups, but we focused on three groups which were most easily observed (*n*=28). We made a concerted effort to rotate age/sex groups daily. Details of the behavioral sampling methods have been described previously (Workman, in review).

Plant feeding samples were collected at the end of a morning or evening observation session, when groups had moved out of view. Most plants were short enough that tree-climbing was usually not necessary. A sample from the plant that was eaten (e.g. the young leaves of a small tree) was collected as well as a matching phenophase sample from a plant that was next to the eaten plant, but not consumed. If there was a plant of the same species close by, a sample from that plant was collected. While the species consumed is reported here, the focus was less on the plant species eaten than on the plant individual that was eaten.

Intraspecific variability in the nutritional content of primate foods has been well documented (Chapman et al., 2003), but the aim of this study was not to determine the nutritional content of certain species on which Delacour's langurs fed, but rather on the differences between plant individuals at a given time. The steep topography of the karst habitat precluded us from accessing

Fig. 1. The Dong Quyen karst mountain of Van Long Nature Reserve, the locality of the study. Photo: T. Nadler.

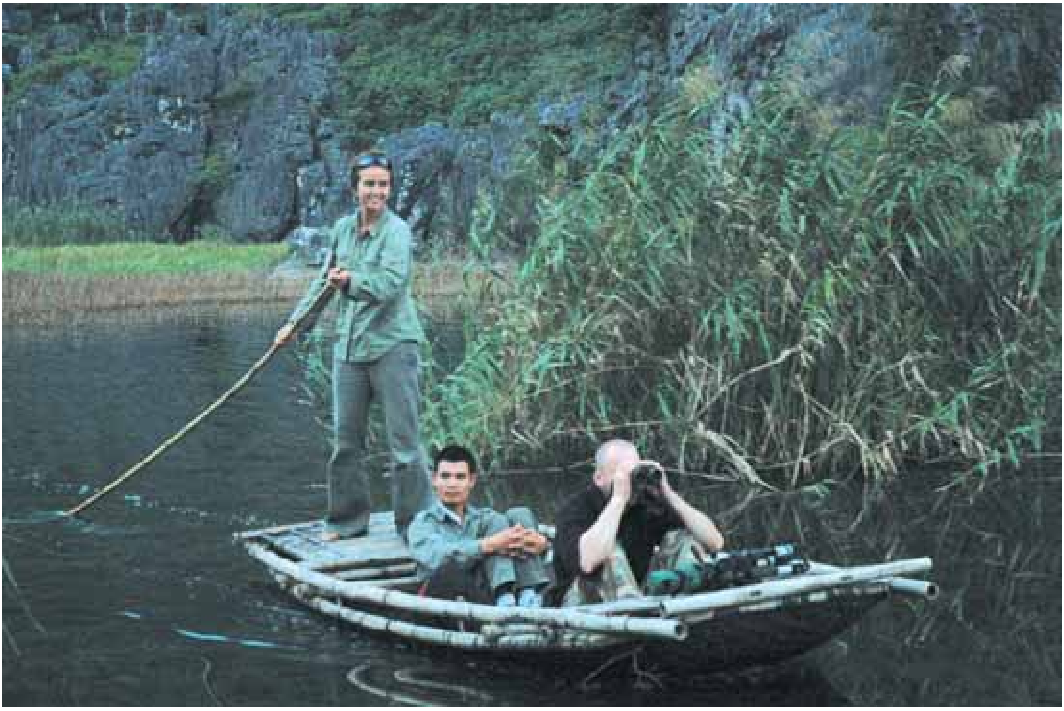

Fig. 2. The area of the study is only accessible by boat. Photo: T. Nadler.

certain areas and therefore we realize that our sample is limited to those plants which we could safely access. Eaten and uneaten samples had fresh masses between 80 g and 1047 g, with a mean of 535 g and SD of 116 (*n*=50 eaten samples (40 young leaves, 10 mature leaves; *n*=49 uneaten samples (39 young leaves, 10 mature leaves).

Samples were transported in bags to the Van Long Nature Reserve Headquarter and weighed within two hours of collection, dried in the shade over a period of days or weeks (depending on weather), and then kept at room temperature until analysis. Dried samples were taken to Ms. Lan Anh at the Hanoi University of Science for transport to one of two testing facilities: 1) Food and Chemical Microbiology and Food Testing Laboratory of Quality Assurance and Testing Center Number, Hanoi; 2) National Institute of Animal Husbandry, Hanoi. Eaten and uneaten samples were analyzed for crude protein, neutral detergent fiber (NDF), condensed tannin, total phenolics, crude ash, and water content. Samples were ground dry in a Wiley laboratory mill and passed through a 1 mm wire screen (Chapman et al., 2002). All samples were dried to a constant weight of 100ºC and all results are provided on a dry matter basis.

Crude protein content was assessed using the Kjeldahl method (Horowitz, 1970). Total nitrogen content was first measured and then used to estimate the crude protein level (protein content = nitrogen * 6.25: Maynard & Loosli, 1969). Fiber (Neutral detergent fiber (NDF)) was measured by following the methods described in van Soest (1963). NDF is a measure of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin. NDF was measured because it is a more reliable measure of the fibrous component in the diet (compared to ADF) but we realize that not having ADF makes our results difficult to compare with other studies. Total phenolics were analyzed by the Folin-Denis method (Swain & Hillis, 1959). Tannins were determined using the KMnO4 titration method (Tempel, 1982).

Statistical analyses

Differences in plant chemistry (crude protein, NDF, total phenolics, condensed tannins, water, ash) were analyzed between two groups. First, we compared eaten leaves (*n*=50) vs. uneaten leaves (*n*=49). Second, from August 2007-July 2008, young leaves dominated the Delacour's langurs' diet monthly, seasonally, and annually. We therefore compared young leaves (n = 40) vs. mature leaves (*n*=10). All differences were analyzed using a non-parametric Wilcoxon test in R 2.7.1 software (the equivalent of the non-parametric Mann-Whitney U test).

Results

Eaten versus uneaten leaves

Leaves eaten by Delacour's langurs (*n*=50) had a higher protein/fiber ratio than leaves not selected (*n*=49) (mean = 0.42 +/- 0.18 vs. mean = 0.31 +/- 0.16; $p < 0.0004$) (Table 1). Leaves eaten and uneaten were not statistically different in protein (mean = 12.04 +/- 6.88 vs. mean = 10.58 +/- 5.82; $p = 0.13$), fiber (mean = 32.47 +/- 18.62 vs. mean = 38.18 +/- 21.09; $p = 0.06$), total phenolics (mean = 2.13 +/- 2.17 vs. mean = 1.52 +/- 1.68; p = 0.09), tannins (mean= 6.36 +/- 4.81

Table. 1. Mean values of eaten (*n* = 50) and uneaten leaves (*n* = 49). Standard deviations in parentheses, * significant $p < 0.0004$.

	Eaten leaves	Uneaten leaves
CP/F*	0.42 (0.18)	0.31 (0.16)
Protein	12.04 (6.88)	10.58 (5.82)
Fiber	32.47 (18.62)	38.18 (21.09)
Tannins	6.36 (4.81)	4.81 (3.92)
Phenolics	2.13 (2.17)	1.52 (1.68)
Water	80.88 (5.74)	78.92 (8.53)
Ash	16 (6.97)	19.53 (9.18)

vs. mean = 4.81 +/- 3.92; p = 0.08), water (mean = 80.88 +/- 5.74 vs. mean = 78.92 +/- 8.53; p = 0.36), or ash content (mean = 16 +/- 6.97 vs. mean = 19.53 +/- 9.18; p = 0.11).

Leaf stage differences in plant chemistry

Samples of young (n=40) and mature leaves (n=10) eaten by Delacour's langurs did not differ in their content for any of the analyzed constituents: protein (mean = 12.13 =/- 6.92 vs. mean = 11.94 +/- 6.74; p = 0.91), fiber (mean = 34 +/- 19.53 vs. mean = 26.17 +/- 13.28; p = 0.22), total phenolics (mean = 2.25 +/- 2.38 vs. mean = 1.55 +/- 0.87; p = 0.75), condensed tannins (mean = 6.01 +/- 4.74 vs. mean = 7.79 +/- 5.1; p = 0.28), water (mean = 81.19 +/- 5.51 vs. mean = 79.61 +/- 6.75; p = 0.57), ash (mean = 16.24 +/- 6.89 vs. mean = 16.04 +/- 7.82; p = 0.88), protein/fiber ratio (mean = 0.41 +/- 0.19 vs. mean = 0.46 +/- 0.16; p = 0.53).

Discussion

The leaves eaten by Delacour's langurs at Van Long contain less than half the protein of leaves eaten by guerezas at Kakamega, Kenya (Table 2) (Fashing et al., 2007). Fiber content of leaves eaten by Delacour's langurs is also less than that of guerezas. At Kakamega, protein content was the primary factor determining whether or not guerezas consumed specific leaf items, with eaten leaves at or above a protein threshold of 14% dry matter (Fashing et al., 2007). At Van Long, protein content did not differ between eaten and uneaten leaf items. Further, protein content of eaten leaves averaged 12% dry matter, with several leaf items containing protein levels far below that.

Table. 2. Neutral detergent fiber (NDF) and protein levels for young leaves (YL) and mature leaves (ML) consumed by *Colobus guereza* and *Trachypithecus delacouri*.

Species	NDF	Protein	Plant part	Citation
Colobus guereza	48.0	23	mean- leaves 3.5	Fashing et al. (2007)
Trachypithecus delacouri	38.0	12.2	mean- YL	this study
	33.4	10.4	mean- ML	

While low compared to guerezas at Kakamega, Delacour's langurs are meeting the 7-11% protein (of dry matter) that primates need for maintenance and growth (Oftedal, 1991). Delacour's langurs at VLNR are also eating leaf items which are above the critical protein needed for ruminants to maintain positive nitrogen balance (4-8% dry weight) (Milton, 1979). Oftedal (1991) states that primate populations need to consume protein at 14% dry matter to sustain reproduction. Delacour's langurs are not quite meeting this threshold; however, the population on Dong Quyen Mountain at VLNR has doubled in 9 years (~35 langurs in 2000, ~ 70 langurs in 2009). It appears, therefore, that langurs are not limited in sustaining reproduction and are possibly receiving additional protein from food sources that we did not sample. Further, because Van Long has both evergreen and deciduous species, it is possible that - as at Kibale National Park, Uganda - the forest never reaches a very low nutrient value and colobines are not nutritionally stressed, allowing for quick population rebound (Baranga, 1986).

At Kibale, *Colobus guereza* chose young leaves that had more protein and higher protein/fiber ratios than mature leaves, although the two leaf stages did not differ in secondary compound content (Chapman et al., 2004). The preference for leaves with higher protein/fiber ratios also held at Van Long and young leaves had slightly higher protein content than mature leaves, although the difference was not significant. Further, our study supports the contention that tannin concentrations may be of minor significance to primates (Oates et al., 1980).

Future studies should collect community-level plant data at Van Long to facilitate colobine biomass comparisons across Africa and Asia. However, explaining primate biomass using the protein/fiber ratio will be misleading if populations are not at carrying capacity (Chapman et al., 2004). For the monophyletic limestone langur species of northern Vietnam, southern China, and eastern Laos, intense hunting pressure precludes a solely ecological explanation of their distribution and abundance on karst habitats. A similar ecological conundrum for African primate communities has been addressed by Struhsaker (1999). He notes that the present-day distribution of many species may be the artifact of recent hunting, rather than the result of resource base selective pressures. Given this consideration a focus on proximate factors influencing langur food choice seems appropriate.

Acknowledgements

We thank the Management Board of the Hoa Lu-Van Long Nature Reserve and Cuc Phuong National Park for permission to conduct this research. We gratefully acknowledge advice, support, and friendship from the Endangered Primate Rescue Center, especially Tilo Nadler and Nguyen Thi Thu Hien. Thank you to Ms. Nguyen Thi Lan Anh for conducting the plant chemical analyses. Thank you to Mr. Nguyen The Cuong and Mr. Nguyen Manh Cuong for assisting in botanical identifications. Dr. Ken Glander provided mentorship during the collection of these data and useful comments on earlier versions of this manuscript. We also thank Dr. Barth Wright for useful suggestions and discussions about these data. This project was supported by funding from the National Science Foundation, National Geographic Society, and the Margot Marsh Biodiversity Fund.

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