Notes and records

Assessing dietary protein of colobus monkeys through faecal sample analysis: a tool to evaluate habitat quality

Colin A. Chapman[1,2]*, Terry Webb[3], Raime Fronstin[1], Michael D. Wasserman[1] and Ada Mayte Santamaria[1]

[1]Department of Zoology, University of Florida, Gainesville, FL 32611, [2]Wildlife Conservation Society, 185th Street and Southern Boulevard, Bronx, NY 10460 and [3]Metro Zoo Miami, One Zoo Boulevard, 12400 S.W. 152nd Street, Miami, FL 33177, U.S.A.

Understanding determinants of animal abundance has become increasingly vital as ecologists are asked to apply their knowledge to assist conservation biologists construct informed management plans for endangered species. With respect to primates the importance of these theoretical issues has become increasingly critical as human development continues to threaten their habitats (Chapman & Peres, 2001). African forest primates are of particular concern as one estimate suggests that the amount of forest remaining is approximately 1,490,000 km^2 or 55% of the original area in Central Africa, 190,000 km^2 or 28% of the area in West Africa, and 70,000 km^2 or 28% of the original area of East Africa (Martin, 1991). However, understanding and predicting factors that determine abundance of specific primate species has proved extremely difficult. Numerous studies of forest primates have revealed a high degree of inter-site variation in density (Oates *et al.*, 1990; Chapman & Chapman, 2002), but there have been few direct tests of general hypotheses proposed to account for this variation. Notable exceptions are studies of folivorous primates. Milton (1979) proposed that the protein-to-fibre ratio was a good predictor of leaf choice. By measuring overall mature leaf acceptability as the ratio of protein-to-fibre, several subsequent studies have found positive correlations between colobine biomass and this index of leaf quality at local (Ganzhorn, 1992; Chapman & Chapman, 2002) and regional scales (Oates *et al.*, 1990). Unfortunately, applying our understanding of the importance of protein and fibre to the conservation of small-bodied folivores is presently difficult because of the need to quantify the nutritional quality of many potential food items. In addition, obtaining, drying, and processing these food items is difficult and time consuming.

Here we evaluate the suitability of using the protein content of colobus monkey faecal samples as an index of the protein content in foods eaten. This method would substitute the tedious task of collecting many potential food items with the relatively easy task of collecting faecal samples. Previous studies of lagomorphs (Sinclair, Krebs & Smith, 1982) and ruminants (Bredon, Harker & Marshall, 1963; Mould & Robbins, 1981) have documented a positive correlation between the protein content of foods and faecal protein; however, there are a number of potentially important differences in the digestive physiology of these animals and colobus monkeys. We made this evaluation through a series of feeding trials on Angolan black-and-white colobus (*Colobus angolensis*), in which we altered the protein content of their foods. Subsequently, we described the protein content of faecal samples of populations of Abyssinian black-and-white (*C. guereza*) and red colobus (*Piliocolobus tephrosceles*) found in or near Kibale National Park, Uganda. Samples were collected from a nutritionally stressed population, living in a forest fragment, and an unstressed population from within the national park.

Methods

To experimentally examine whether the protein content of the foods ingested correlated to the protein content of faecal samples, *C. angolensis* from Miami Metro Zoo were fed three pelletted diets that differed in protein levels (10%, 16%, and 20% on a dry matter basis). The low and high protein diets were specially made by Purina Mills and matched the regular zoo diet (medium protein diet) as closely as possible with respect to other nutritional components. The group's diet was also supplemented with fruits, vegetables, and tree leaved from trees located in their enclosure. These supplementary dietary components were kept constant among trials. The average protein

*Correspondence: Colin A. Chapman, Anthropology Department and McGill School of Environment, 855 Sherbrooke St West, McGill University, Montreal, QC, Canada, H3A 2T7.
E-mail: colin.chapman@mcgill.ca

content of these supplementary foods was 10.9%. Faecal collections were first made for 3 days from the animals while on their regular zoo diet. They were then given the high protein diet and after 3 days on this diet, samples were collected for 3 days. After collection the group was returned to its regular diet for 3 weeks followed by the low protein diet with collections made after 3 days for 3 days. Faecal samples were collected, placed in ziplock bags, and frozen. Special care was taken to ensure that faecal samples were free of urine contamination.

Protein (nitrogen) content was assessed using Kjeldahl procedures (Horowitz, 1970; Goering & van Soest, 1970). Samples were digested using a modification of the aluminium block digestion procedure of Gallaher, Weldon & Futral (1975). The digestion mix contained 1.5 g of 9 : 1 K_2SO_4 : $CuSO_4$, and digestion was conducted for at least 4 h at 375°C using 6 ml of H_2SO_4 and 2 ml H_2O_2. The nitrogen in the digestate was determined by semiautomated colorimetry (Hambleton, 1977). Measuring total nitrogen provides an estimate of crude protein (protein levels = N × 6.25; Maynard & Loosli, 1969). A better conversion factor for tropical leaves may be 4.4 (Milton & Dintzis, 1981). We used the 6.25 factor so that our results would be comparable with those of previous studies (Oates *et al.*, 1990). Duplicates were run on each day's samples and the measurement error was always <1%. In addition, variance among days was small (overall average protein 24.7 ± 0.44 SE).

Collections were also made from *C. guereza* and *P. tephrosceles* from groups within or near Kibale National Park, Uganda (795 km^2; 0.13′–0.41′N and 30.19′–30.32′E; Chapman & Lambert, 2000). Mean annual rainfall in the region is 1741 mm (1990–2002). Groups that were likely experiencing very different levels of dietary stress were selected. One group of both species in an unlogged-protected area of the national park was selected to represent conditions with little dietary stress. In contrast, two groups in a forest fragment were selected to represent a nutritionally stressed population. This population had experienced a significant increase (320%) in population density between 1995 and 2000 because immigration from neighbouring fragments were cleared. This fragment is part of a community-based conservation project that we established in 1993. It is protected to provide an attraction to tourists who camp at the lake, thus the forest in the fragment has not been degraded. Our assessment that the fragment population was nutritionally stressed is supported by the fact that at the time of collection none of the adult females had infants, while in Kibale 63% of the adult females in our *P. tephrosceles* study group had infants and 25% of the females in the *C. guereza* group had infants. Collections were made between May and July 2002, encompassing the end of the wet season and start of the dry season. Samples collected on different days were not considered independent, as colobus monkeys can have retention times up to 38 h (Kay & Davies, 1994), and thus they were pooled.

Results and discussion

As predicted, faecal protein content of the *C. angolensis* fed a high protein diet was higher than the content from the same animals fed a low or medium protein diet (Fig. 1). However, the animals produced faecal material with higher protein content when on the low protein diet compared with the medium protein diet. Faecal protein consists of unabsorbed nitrogen which comes primarily from the undigested cells that remain if the lignin fractions is high, bound protein (tannins and salivary protein), and protein from microbial protein that the animals did not absorb. In the zoo trials, tannins could not have played a role as they were absent from the diet. Furthermore, the lignin content among feeds used in the different trails was very similar. Thus, the source of the variation is likely a result of variations in microbial populations. Our results

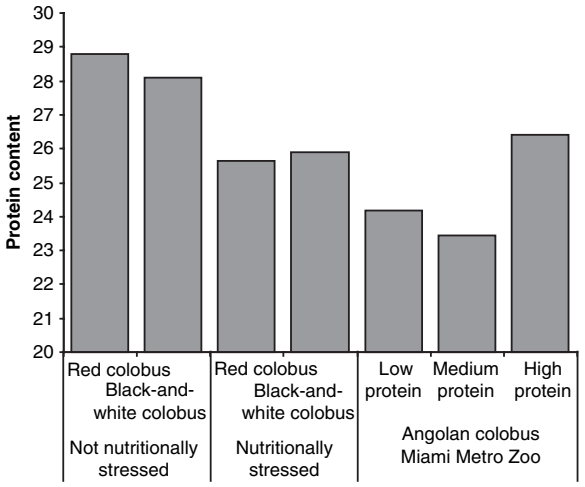

Fig 1 Faecal protein content from red colobus (*Piliocolobus tephrosceles*) and black-and-white colobus (*Colobus guereza*) from the undisturbed forest in Kibale National Park, Uganda (not stressed), from a forest fragment near Kibale (nutritionally stressed), and from Angolan black-and-white colobus (*C. angolensis*) from the Miami Metro Zoo fed diets with three different protein levels

suggest that the increase in the microbial populations is non-linear. Kay & Davies (1994) suggest that if dietary nitrogen is insufficient, relative to fermentable energy, to maintain microbial activity, colobines are capable of secreting blood urea into saliva or diffusing it across the rumen wall to add to the rumen ammonia pool. This will allow additional microbial protein to be synthesized, encourage fibre digestion, and increase the microbial population. It could also result in an increase in the amount of microbial protein found in faecal material and account for the non-linear pattern observed here.

As predicted, the faecal material collected from *P. ephrosceles* and *C. guereza* from the undisturbed forest of Kibale had higher faecal nitrogen content than that collected from the forest fragment population thought to be experiencing dietary stress (Fig. 1). Additionally, the two species had very similar faecal nitrogen contents within a given setting.

These results suggest that measuring faecal nitrogen content can portray differences in protein content of the foods ingested if the differences are large, but not if the differences are small. It seems likely that the colobus are employing a physiological strategy, such as that suggested by Kay & Davies (1994), to respond to low protein diet. Studies of primate digestion are few and the more general field of digestive ecology is still in its infancy (Glander, 1982; Lambert, 1998). The results found here suggest that quantifying faecal nitrogen levels may be a useful way of assessing habitat quality for colobines; however, before applying these ideas a greater understanding of colobine digestive physiology will be required.

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