Deconstructing Protein in the Diet and Biomass of Colobine Primates

Katarina D. Evans [1,2] · William J. Foley [3] · Colin A. Chapman [4,5,6,7] ·
Jessica M. Rothman [1,2,8,9]

Abstract

Dietary protein is often considered a factor that limits the growth of primate populations. The ratio of crude protein (CP) to fiber in common mature leaves in a forest reliably predicts colobine biomass across Africa and Asia. This relationship is puzzling because CP of mature leaves is notably high in some forests, including Kibale National Park, Uganda, where mature leaves have a higher protein concentration (*ca.* 18% CP on a dry matter basis) than what is required for primate growth, maintenance, and reproduction. We used an *in vitro* assay that incorporates the role of fiber and tannins to assess the biological relevance of CP in the leaves that comprise the diets of colobines (*Colobus guereza*, *Piliocolobus tephrosceles*) in Kibale (N = 37 spp. mature leaves, 39 spp. young leaves). Relative to CP, available (digestible) protein (AP) concentrations were 50% lower in mature leaves and 38% lower in young leaves, and protein-to-fiber ratios were lower when incorporating AP. Surprisingly, leaf AP did not differ among sites within Kibale, and AP at all sites exceeded primate protein requirements. Future research, particularly in protein-rich forests like Kibale, should consider focusing on fiber, energy, and other nutritional factors to assess the relationship between colobine biomass and leaf nutritional quality. Our study expands on the way that primate ecologists measure protein, integrates several traits into a single currency of AP, and contributes to conservation management plans.

Keywords Folivore · Nitrogen availability · Nutritional ecology · Polyethylene glycol · Tannins

✉ Katarina D. Evans
  kevans@gradcenter.cuny.edu

Extended author information available on the last page of the article

Introduction

Animal densities are often variably distributed across landscapes, and nutritional factors have been suggested to explain this variation (DeGabriel *et al.* 2009, 2014; Donati *et al.* 2017; Fimbel *et al.* 2001; Ganzhorn *et al.* 2017; Moore and Foley 2005; Waterman *et al.* 1988; Windley *et al.* 2016a). Nitrogen, as a proxy for protein, is typically implicated in nutritional models to predict animal abundance as it is often environmentally limited (Ganzhorn *et al.* 2017; White 2012). However, traditionally used measures of crude protein (CP) may not be biologically relevant given factors that affect the digestibility of protein in animal diets.

Several factors, but particularly concentrations of tannins and fiber, can reduce the digestibility of dietary CP by herbivores. Many different classes of tannins are ubiquitous in tree leaves and can form insoluble complexes with plant proteins, rendering them largely indigestible (Hagerman *et al.* 1992; Marsh *et al.* 2019; Robbins *et al.* 1987). Furthermore, the presence of protein bound to cell wall constituents (fiber) reduces access of digestive enzymes and may limit the availability of protein (McKey *et al.* 1981; Milton 1979; Rothman *et al.* 2008). Protein digestibility is also affected by digestive processes that differ across taxa, such as foregut fermentation (Cork 1996; Matsuda *et al.* 2019). These factors indicate that CP is not equivalent to, and likely overestimates, available (digestible) protein (AP) (Milton 1979; Milton and Dintzis 1981; Oftedal 1991).

In studies of both frugivorous (Felton *et al.* 2009b) and folivorous primates (Hanya and Bernard 2015; Matsuda *et al.* 2017; Righini *et al.* 2017), as well as other folivores (DeGabriel *et al.* 2009; McArt *et al.* 2009), a focus on AP, rather than CP, has revealed insights into protein intake that would have otherwise gone undetected. For example, higher AP of *Eucalyptus* foliage is correlated with greater reproductive success and growth rates of young in common brushtail possums (*Trichosurus vulpecula*) in tropical Australia (DeGabriel *et al.* 2009). Greater reproductive success is also correlated with higher AP of the principal browse plants of moose (*Alces alces*) in Alaska (McArt *et al.* 2009). While researchers have examined the limitations of traditional CP estimates in primate ecology (e.g., Conklin-Brittain *et al.* 1999; Ganzhorn 1988; Milton and Dintzis 1981), much remains unknown about the combined effects of tannins and fiber on the availability of protein in primate foods.

Despite the often high protein concentrations in the tree leaves of their habitats, numerous colobines prefer foods with high protein-to-fiber ratios, including African colobines (*Piliocolobus tephrosceles*: Mowry *et al.* 1996, Wasserman and Chapman 2003; *Colobus guereza*: Chapman *et al.* 2004, Fashing *et al.* 2007, Wasserman and Chapman 2003; *Colobus satanas*: McKey *et al.* 1981; *Procolobus verus*: Oates 1988) and Asian colobines (*Presbytis rubicunda*: Davies *et al.* 1988, Matsuda *et al.* 2013; *Presbytis melalophos*: Davies *et al.* 1988; *Presbytis johnii*: Oates *et al.* 1980; *Presbytis entellus*: Kar-Gupta and Kumar 1994; *Nasalis larvatus*: Matsuda *et al.* 2013, 2017, Yeager *et al.* 1997). These findings suggest that protein and fiber are important nutritional determinants of colobine food selection (Rothman *et al.* 2021). Moreover, the ratio of protein to fiber in mature leaves explains a significant part of the variance in the biomass of colobines and other folivorous primates in populations across continents, regions, and sites within the same forest (Chapman and Chapman 1999; Davies

1994; Fashing *et al*. 2007; Ganzhorn 1992, 2002; Oates *et al*. 1990; Wasserman and Chapman 2003; Waterman *et al*. 1988). In addition, measurements of CP and fiber in mature leaves predict the biomass of red colobus (*Piliocolobus tephrosceles*) and black-and-white colobus monkeys (*Colobus guereza*) across sites within Kibale National Park, Uganda (hereafter Kibale) (Chapman *et al*. 2002). Furthermore, protein-to-fiber ratios of mature leaves from Kibale, surrounding stable forest fragments, and previous data available from around the globe, explain most (87%) of the variance in the biomass of black-and-white colobus monkeys (Chapman *et al*. 2004).

While the protein-to-fiber model is generally a useful predictor of colobine biomass, it is unclear why protein is a limiting factor in colobine populations (Ganzhorn *et al*. 2017; Oftedal 1991). Folivorous primates require only *ca*. 4–7% protein (on a dry matter (DM) basis; 85% digestibility) for growth and maintenance and 8% for reproduction as a proportion of their total intake; when accounting for reduced digestibility due to tannins, these estimates increase to 7–11% for growth and maintenance and 14% for reproduction (DM basis) (Oftedal 1991). However, the leaves that primates eat contain an average of 12–16% CP (National Research Council 2003; Oftedal 1991). It has also been suggested that the model does not properly account for tannins and that available nitrogen or AP are better indicators of nutritional quality (DeGabriel *et al*. 2014; Wallis *et al*. 2012). In addition, the ratio of AP to non-protein energy (Johnson *et al*. 2017) and mineral content (Rode *et al*. 2003) influence variation in colobine biomass. Furthermore, other nonnutritional factors contribute to colobine abundance including predation (Watts and Mitani 2002), disease risk (Bonnell *et al*. 2010), and social factors (Chapman and Pavelka 2005; Yeager and Kirkpatrick 1998).

Our study examines the AP in leaves eaten by red colobus (*Piliocolobus tephrosceles*) and black-and-white colobus monkeys (*Colobus guereza*) in Kibale National Park, Uganda. We test the hypothesis that AP is a more biologically relevant measure than CP in the diets of colobus monkeys. We predict that 1) AP is less than CP in colobine foods; 2) young leaves have more AP than mature leaves; 3) the difference between CP and AP is higher in mature than young leaves; 4) tannins reduce digestibility of protein; 5) colobines exhibit a feeding preference for tree species that have relatively high concentrations of AP; 6) feeding preference is highest for foods containing the smallest differences between CP and AP; 7) measures of AP lower estimates of the protein-to-fiber ratio of colobine foods; 8) there is site-specific variation in the difference between CP and AP concentrations, with sites containing the highest colobine biomasses in Kibale (Chapman *et al*. 2002) exhibiting the highest concentrations of AP and the smallest differences between CP and AP; and 9) sites within Kibale vary in the extent that tannins affect AP with sites containing the highest colobine biomasses exhibiting the lowest effects of tannins.

Methods

We conducted this study in Kibale National Park, Uganda, a moist evergreen tropical forest (795 km²) in western Uganda, east of the foothills of the Ruwenzori mountains (0°13–0°41N and 30°19–30°32E) (Chapman and Lambert 2000; Struhsaker 2010)

(Fig. 1). Kibale has one of the most diverse and extensive populations of primates in Africa and includes large populations of both black-and-white colobus (*Colobus guereza*) and red colobus (*Piliocolobus tephrosceles*) (Chapman *et al.* 2005).

Plant Sampling and Analysis

We analyzed mature leaves and young leaves from tree species ($N = 37$ spp. mature leaves, 39 spp. young leaves; Electronic Supplementary Material [ESM] Table SI) that are most abundant at different sites within Kibale, as well as those
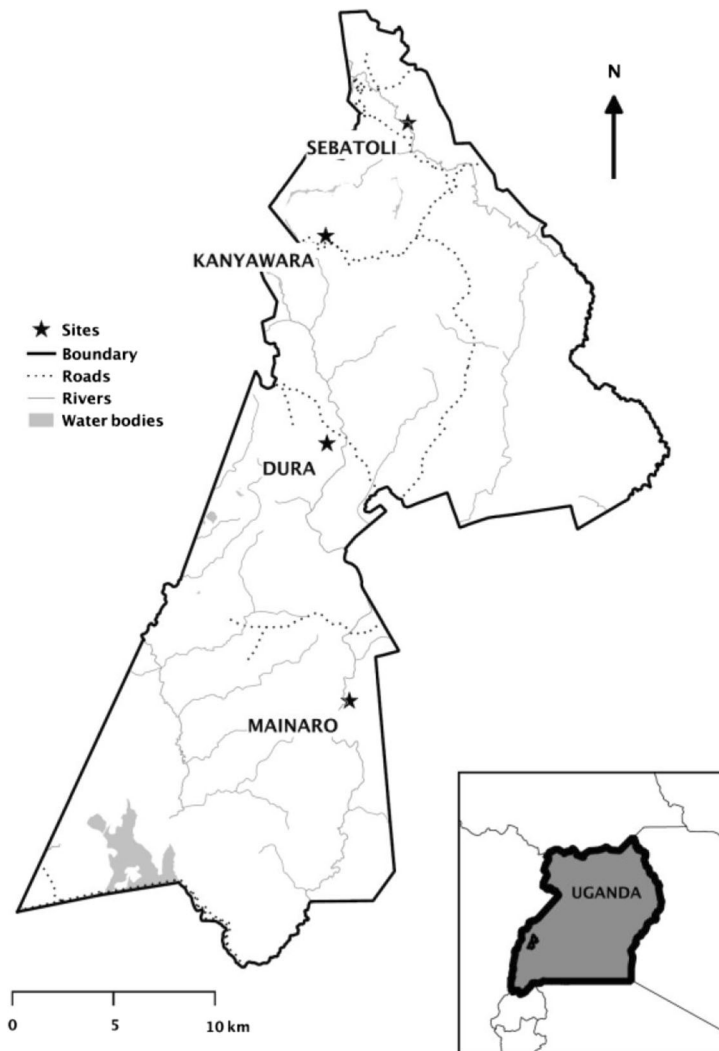

Fig. 1 A map of Kibale National Park, Uganda, showing the location of the four study sites (Mainaro, Sebatoli, Dura, Kanyawara) from which mature and young leaves were collected from April to August 2008.

that constitute the predominant food source of the colobine diet (Chapman *et al.* 2002). While the protein-to-fiber model has been based solely on mature leaves, we also included young leaves in our analysis because young leaves constitute the largest proportion of the colobine diet in Kibale (Ryan *et al.* 2013; Struhsaker 2010).

We collected leaves from the lower part of the tree crown using a tree pruner and dried them in a Nesco food dehydrator at 40–50°C to constant weight. We then milled leaves in a Wiley Mini Mill fitted with a 1 mm screen. After storing the dried and milled leaves in polythene bags with silica gel, we transported them to the Nutritional Ecology Laboratory at Hunter College, New York for analysis. We found no evidence of mold or moisture (Rothman *et al.* 2012) in any sample upon analysis.

Nutritional Analysis

We measured AP based on a modification of a previously described method (DeGabriel *et al.* 2008). This method implements a two-stage *in vitro* digestion to determine the effects of both fiber and tannins on the digestibility of CP in leaves. The *in vitro* assay emulates digestion in mammalian herbivores, wherein a fungal cellulase that is widely used in *in vitro* digestibility experiments with ruminants digests fiber and an acid pepsin subsequently digests protein. We assessed the effect of tannins on protein digestibility by conducting the assay in the presence and absence of polyethylene glycol (PEG) 4000 in a Tris base buffer solution prior to incubations with cellulase and pepsin. PEG preferentially binds to tannins and releases protein from tannin–protein complexes, thus rendering more protein available for digestion (Makkar 2003). The assay was designed to emulate digestion in a hindgut fermenting herbivore where acid digestion precedes fermentative digestion (DeGabriel *et al.* 2008). We reversed the order of these steps to emulate the digestive processes of colobine monkeys in which fermentative digestion precedes acid digestion in the forestomach. Earlier studies have used *in vitro* digestibility assays to evaluate primate foods (Choo *et al.* 1981) but without a focus on protein. In this study, we used a fungal cellulase to emulate digestion but this may not be similar to the foregut of colobines or the hindguts of animals under study.

For each leaf sample that we collected and processed for analysis of AP, we weighed 0.8050 ± 0.0050 g of dry, ground plant material into four preweighed ANKOM F57 fiber filter bags (ANKOM Technology, Macedon, NY), two of which were digested in the presence of PEG 4000 and two of which were digested in the absence of PEG 4000. We incubated half of the samples in 50 mL/bag of 0.05 M Tris-base buffer solution (Trizma® Sigma-Aldrich) (6.057 g of Trizma® base + 1 L of distilled water, pH = 7.1) for 24 h at 37°C, while we incubated the other half of the samples in 50 mL/bag of 33.33 g L^{-1} PEG 4000 in 0.05 M Tris-base buffer solution (pH = 7.1) for 24 h at 37°C. We then incubated samples in 70 mL/bag of the cellulase solution (6.25 g of cellulase + 6.8 g of sodium acetate + 2.9 mL of glacial acetic acid + 1 L of distilled water, pH = 4.8) for 48 h at 37°C. Following the cellulase step, we incubated samples in 70 mL/bag of 2.00 g of pepsin in 1 L of 0.1 N HCl (8.212 mL of HCl + 1 L

of distilled water, pH = 1.0) for 24 h at 37°C. After each step of the assay, we completely washed samples under hot tap water and subsequently under distilled water. With the completion of all incubation stages, we dried samples in the oven at 50°C for 30 min, desiccated them for another 30 min, and recorded dry weights. Using a Leco Truspec Nitrogen Analyzer (Leco Corporation, St. Joseph, MI), we determined nitrogen concentration of the residues in all bags and in an undigested sample of each leaf. We determined CP concentrations of the samples using a conversion factor of 6.25 × N.

To compare our AP results with studies of hindgut fermenters and previous studies (DeGabriel *et al.* 2008; McArt *et al.* 2009), we reversed the order of this protocol for a subset of samples (N = 39 samples in duplicate). We found no significant differences according to protocol order using a paired *t*-test (mean difference = 0.051, $t = 0.235$, df = 38, $P = 0.815$).

Data Analysis

We determined nitrogen digestibility using the following formula (DeGabriel *et al.* 2008):

$$\text{N digestibility} = \frac{100 \times (\text{Nleaf} - \text{Nres})}{\text{Nleaf}}$$

wherein Nleaf is the amount of N (mg of DM) in the original leaf and Nres is the amount of N (mg of DM) in the residue of each bag following digestion. The amount of digestible N (% DM) can be calculated by multiplying the N digestibility (%) by the concentration of N in the original sample (g per 100 g of DM). We scaled this number by 6.25 to estimate CP (instead of nitrogen), and AP (instead of available nitrogen). All samples were analyzed on a DM basis.

The decrease in AP that is due to the presence of tannins can be represented by the increase in protein that is rendered available for digestion by the inclusion of PEG. To assess the effects of tannins, we created the following formula:

$$\%\text{Decrease in AP due to tannins} = \frac{\text{AP(PEG)} - \text{AP(no PEG)}}{\text{AP(PEG)}}$$

To understand how our measurements of AP alter the existing protein-to-fiber model, we compared ratios of protein to fiber, using CP estimates, with those calculated using AP. We extracted leaf fiber concentrations from a previous subset of data (Chapman *et al.* 2002) for mature leaves from the four different sites in Kibale (Dura, Kanyawara, Mainaro, and Sebatoli). We compared protein-to-fiber ratios using three different measurements of protein: CP extracted from the previous subset of data (Chapman *et al.* 2002), CP from our analysis, and AP from our analysis.

To assess feeding preference, we created an index,

$$\text{Feeding preference} = \frac{\%\text{Feeding time}}{\text{Density}}$$

using values for percentage of time spent feeding and densities of tree species extracted from the previous subset of data (Chapman *et al.* 2002). We

subsequently assessed the relationship between AP and feeding preference using the subset of our samples for which we had both nutritional and feeding preference data.

Statistical Analysis

We conducted all statistical analyses in R version 3.6.2. Given the nonnormal distribution of the data, we used nonparametric analyses. We used Wilcoxon rank sum tests with continuity corrections to examine 1) CP vs. AP in both mature and young leaves, 2) CP in mature vs. young leaves, 3) AP in mature vs. young leaves, and 4) AP not accounting for tannins (no PEG) vs. AP accounting for tannins (PEG) in both mature and young leaves. We used Kruskal–Wallis rank sum tests to determine the relationships between 1) feeding preference and concentrations of both CP and AP, as well as feeding preference and differences between CP and AP, in both mature and young leaves; 2) sites within Kibale (with varying colobine biomasses) and concentrations of both CP and AP, as well as sites and differences between CP and AP in both mature and young leaves; and 3) differences in the extent to which tannins decrease AP across sites for both mature and young leaves. To assess the relationship between the CP-to-fiber ratio and concentrations of AP in mature leaves both not treated and treated with PEG, we ran a linear regression analysis. We visually assessed regression diagnostic plots (residuals vs. fitted, normal Q–Q, scale-location) to confirm that our model did not violate linear regression assumptions.

Ethical Note

This research was non-invasive and did not involve research on any animal subjects. Permission to conduct the research was given by the Uganda Wildlife Authority. The authors declare that they have no conflict of interest.

Data Availability The data sets analyzed during the current study are available from the corresponding author on reasonable request.

Results

Available Protein vs. Crude Protein

We found that concentrations of AP were significantly lower than concentrations of CP in both mature and young leaves (Tables I and II; Fig. 2; ESM Table SII).

Digestibility of Mature vs. Young Leaves

Consistent with our prediction, we found that mature leaves across species contained reduced concentrations of both CP and AP compared to young leaves

Table I Crude protein and available protein (on a dry matter basis) in mature and young leaves collected April–August 2008 from four study sites (Dura, Kanyawara, Mainaro, Sebatoli) in Kibale National Park, Uganda. Estimated proportional dietary protein requirements of folivorous primates: 7–11% (*growth and maintenance*); 14% (*reproduction*) (National Research Council 2003; Oftedal 1991)

	Mature leaves (N = 37 spp.)	Young leaves (N = 39 spp.)
Crude protein (mean ± SD %)	18.09 ± 4.38	23.06 ± 5.90
Available protein (mean ± SD %) without PEG (not accounting for tannins)	9.11 ± 5.05	14.25 ± 6.71
Available protein (mean ± SD %) with PEG (accounting for tannins)	10.62 ± 5.05	15.91 ± 6.49

(Table I; Fig. 2; ESM Table SII). We found significant differences in CP for mature vs. young leaves (mature leaves < young leaves) and AP for mature vs. young leaves (mature leaves < young leaves) (Table II). In line with our prediction, relative to CP, concentrations of AP were 50% lower in mature leaves and 38% lower in young leaves (Fig. 2).

Effects of Tannins: PEG vs. Non-PEG

Accounting for tannins by the inclusion of PEG in the analysis increased concentrations of AP, though effects were subtle (Fig. 2), demonstrating that biologically active tannins are low in Kibale foods. The concentrations of AP in mature and young leaves

Table II Results of Wilcoxon rank sum tests investigating available protein vs. crude protein, protein in mature leaves vs. young leaves, and the effects of tannins on protein digestibility; data collected April–August 2008 from four study sites (Dura, Kanyawara, Mainaro, Sebatoli) in Kibale National Park, Uganda

		W	P	N	
Crude protein vs. available protein				N_{AP}	N_{CP}
Mature leaves	Without PEG	3088	< 0.0001	99	99
	With PEG	2982	< 0.0001	97	99
Young leaves	Without PEG	3661	< 0.0001	122	122
	With PEG	3473	< 0.0001	122	122
Mature leaves vs. young leaves				N_{ML}	N_{YL}
Crude protein		899.5	< 0.0001	99	122
Available protein	Without PEG	992.5	< 0.0001	99	122
	With PEG	928	< 0.0001	97	122
Effect of tannins (without PEG vs. with PEG)				$N_{non-PEG}$	N_{PEG}
Mature leaves		1349	0.066	99	97
Young leaves		1845.5	0.131	122	122

Without PEG: not accounting for tannins; with PEG: accounting for tannins. N = number of trees.
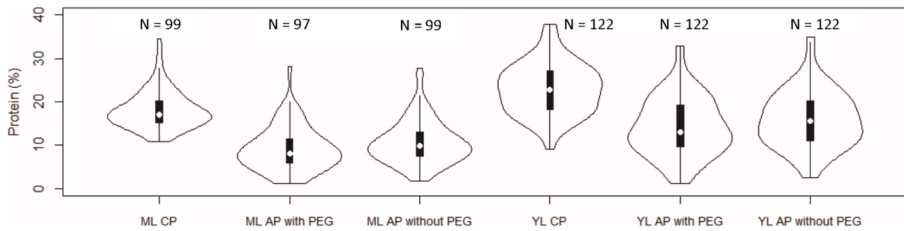
Fig. 2 Concentrations of crude protein (CP) and available protein (AP) in mature leaves (ML) and young leaves (YL) collected April–August 2008 from study sites (Dura, Kanyawara, Mainaro, Sebatoli) in Kibale National Park, Uganda. All values expressed relative to dry matter. N = number of trees.

did not differ significantly in the presence of PEG compared to those in the absence of PEG (Table II).

There is large variation across tree species in the concentration of PEG binding (0–88% decrease in AP due to the presence of tannins, ESM Table SIII). *Mimusops bagshawei* had the most biologically active tannins in both mature and young leaves, with an 82% and 88% decrease, respectively, in AP due to tannins. Mature leaves of *Cordia africana* and *Ficus exasperata* contained highly biologically active tannins, whereas *Aningeria altissima* and *Myrianthus arboreus* contained highly biologically active tannins in young leaves. *Bequertiodendron oblanceolatum*, *Blighia unijugata*, *Cynometra alexandri*, *Lynchodiscus cerospermus*, *Polyscias fulva*, and *Pseudospondias microcarpa* mature leaves and *Celtis mildbraedii* and *Chionanthus africanus* young leaves had no tannins or, if they did, they were not biologically active tannins.

Relationship Between Available Protein and Feeding Preference

We did not find that colobines exhibit a feeding preference for CP or AP in mature or young leaves (N = 77 trees of 10 species for which we had nutritional and feeding preference data; mature leaves: $\chi 2$ = 18, df = 18, P > 0.45 for all comparisons, N = 34 trees; young leaves: $\chi 2$ = 19, df = 19, P > 0.45 for all comparisons, N = 43 trees). However, notable observations include higher concentrations of both CP and AP, particularly in young leaves, for *Celtis gomphophylla* at Dura, which has a preference index of 0.43 compared to the same species at Mainaro, which has a preference index of 0.18 (ESM Table SII). Conversely, *Chrysophyllum* has a higher preference index at Sebatoli (0.52) where there are lower concentrations of both CP and AP compared Mainaro (0.11) where the same species has higher concentrations of both CP and AP (ESM Table SII). Moreover, we did not find support for our prediction that feeding preference is highest in foods containing the smallest differences between CP and AP for mature leaves (without PEG: $\chi 2$ = 16, df = 17, P = 0.544; with PEG: $\chi 2$ = 18, df = 18, P = 0.456) or young leaves

(without PEG: $\chi2$ = 19, df = 19, P = 0.457; with PEG: $\chi2$ = 19, df = 19, P = 0.457).

Effect of Available Protein on Protein-to-Fiber Ratios

As predicted, we found that AP lowers the mean protein-to-fiber ratio of mature leaves (Fig. 3). Our calculated CP-to-fiber ratios were comparable to those previously found in Kibale (Chapman *et al.* 2002) at all sites and AP-to-fiber ratios were lower than CP-to-fiber ratios (Fig. 3). In addition, our study revealed positive relationships between CP-to-fiber ratios and concentrations of AP in mature leaves not treated (linear regression: R^2 = 0.68, F = 120.9, df = 56, P < 0.0001) and treated with PEG (R^2 = 0.69, F = 127.9, df = 56, P < 0.0001). These relationships are significant using non-parametric analyses as well (Spearman's rank correlation: CP/F and AP without PEG: r_s = 0.78, P < 0.0001; CP/F and AP with PEG: r_s = 0.82, P < 0.0001).
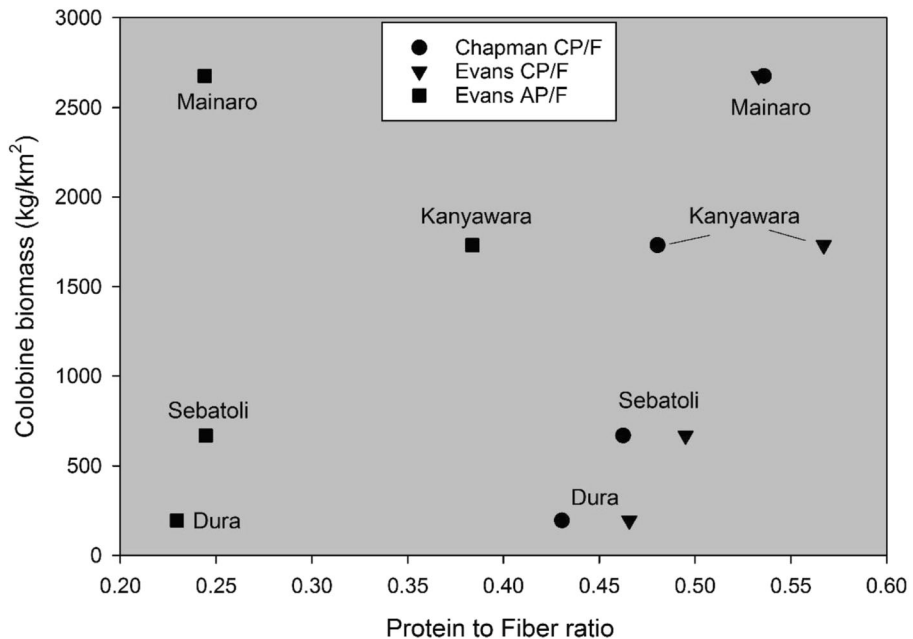

Fig. 3 Protein-to-fiber (P/F) ratios using measures of crude protein (CP) and available protein (AP) without PEG from mature leaves collected from April to August 2008 at study sites in Kibale National Park, Uganda: Dura, Kanyawara, Mainaro, Sebatoli. Evans CP/F and AP/F comprise data derived from this analysis and Chapman CP/F data derived from Chapman *et al.* (2002). From this study, Dura: CP/F = 0.46, AP without PEG/F = 0.23, AP with PEG/F = 0.26; Sebatoli: CP/F = 0.49, AP without PEG/F = 0.24, AP with PEG/F = 0.30; Mainaro: CP/F = 0.53, AP without PEG/F = 0.24, AP with PEG/F = 0.29; Kanyawara: CP/F = 0.57, AP without PEG/F = 0.37, AP with PEG/F = 0.39.
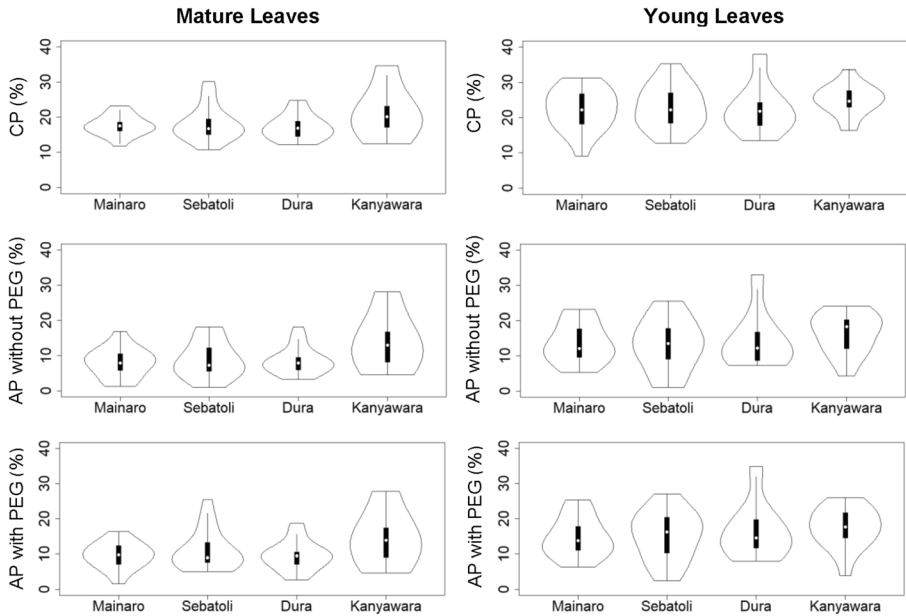
Fig. 4 Crude protein (CP) and available protein (AP) with and without PEG in mature and young leaves for study sites (Mainaro, Sebatoli, Dura, Kanyawara) in Kibale National Park, Uganda collected from April to August 2008.

Differences in Protein by Site

We found no significant difference in CP across sites for mature or young leaves (Fig. 4; Table III; ESM Table SII). Similarly, we did not find significant differences in concentrations

Table III Results of Kruskal-Wallis rank sum tests investigating differences in protein across sites; data collected April–August 2008 from four study sites (Dura, Kanyawara, Mainaro, Sebatoli) in Kibale National Park, Uganda

		$\chi2$	df	P	N
Mature leaves	Crude protein	2.08	3	0.557	99
	Available protein without PEG	2.39	3	0.496	99
	Available protein with PEG	1.69	3	0.639	97
	Difference between crude protein and available protein without PEG	3.48	3	0.324	99
	Difference between crude protein and available protein with PEG	2.10	3	0.551	97
	With PEG vs. without PEG	4.23	3	0.238	97
Young leaves	Crude protein	2.95	3	0.400	122
	Available protein without PEG	2.84	3	0.416	122
	Available protein with PEG	2.29	3	0.514	122
	Difference between crude protein and available protein without PEG	1.17	3	0.760	122
	Difference between crude protein and available protein with PEG	2.24	3	0.524	122
	With PEG vs. without PEG	2.82	3	0.420	122

Without PEG: not accounting for tannins; with PEG: accounting for tannins. N = number of trees.

of AP across sites for mature or young leaves, when analyzed with or without PEG (Table III). We also did not find that sites vary significantly in differences between CP and AP for mature or young leaves (Table III).

Differences in the Effect of Tannins Across Sites

Contrary to our prediction that sites containing the highest colobine biomasses exhibit the lowest effect of tannins, we did not find significant differences in the extent to which tannins decrease the availability of protein across sites, for either mature or young leaves (Table III).

Discussion

Our data show that traditional crude measures dramatically overestimate digestible protein in the diets of colobine primates. The *in vitro* digestibility assay originally described (DeGabriel *et al.* 2008) accounts for two factors that reduce protein digestibility: fiber and tannins. This assay integrates CP and the effects of both tannins and fiber on the digestibility of CP into a single biologically relevant measure. In our study, there was a considerable difference between CP and AP in both mature and young leaves, suggesting that CP is a gross overestimate of dietary protein.

The concentrations of AP in young leaves exceed both the thresholds for reproduction and growth of folivorous primates, but the AP for mature leaves falls around the expected protein threshold (Table I), indicating that the protein digestibility of mature leaves may partially explain the reliability of the protein-to-fiber model that predicts colobine biomass on continental scales across Asia and Africa. However, young leaves constitute the predominant portion of the colobine diet and research over six decades suggests that mature leaves have never been a considerable part of the colobine diet (Chapman and Chapman 1999; Rothman and Chapman *unpubl. data*; Ryan *et al.* 2013; Struhsaker 2010). Thus, protein in mature leaves may be irrelevant when estimating smaller scale differences among sites. Three of the four sites in this study had almost identical amounts of CP and AP (Fig. 4); in fact, mature leaves at these sites only varied from a mean of 17.2 to 18.2% CP and 8.0 to 8.9% AP (without PEG), which is surprising given that intraspecific variation in leaf protein of trees is so high (Chapman *et al.* 2003). More perplexing, Kanyawara, the site with the highest CP and AP, did not have the highest biomass of colobines. Altogether, our results indicate that the biological mechanism for the utility of the protein-to-fiber model is not a result of limited protein in Kibale (Table I). Thus, fiber, and perhaps different components of digestible fiber (hemicellulose, cellulose), likely play a stronger role than protein in the model predicting colobine biomass in Kibale. This is likely also the case in Kakamega Forest, Kenya, wherein black-and-white colobus monkeys ate foods based on fiber content (not CP) and fiber played a much greater role in the rate of intake of different food items (Fashing *et al.* 2007). In addition, nutrient balancing affects colobine foraging decisions; the ratio of macronutrients to fiber, as well as available protein to nonprotein energy, is important (Dunham and Rodriguez-Saona 2018; Johnson *et al.* 2017).

Our results support the logic that protein is only selected when it is limited in the environment (DeGabriel *et al.* 2014; Ganzhorn *et al.* 2017; Oftedal 1991; Rothman *et al.* 2008). A meta-analysis of 24 studies of food selection in leaf-eating primates

showed that protein was selected only when it was low in the respective forests (Ganzhorn *et al.* 2017). The CP in Kibale leaves has been previously described as high compared to other Asian and African forests (Chapman *et al.* 2004; Oates *et al.* 1990; Waterman *et al.* 1988), even compared to agricultural crops, and Kibale has some of the highest densities of colobines in the world (Chapman *et al.* 2005; Oates *et al.* 1990). Our results indicate that the young leaves in Kibale have higher levels of both CP and AP (Table I) than the young leaves eaten by both red leaf monkeys (*Presbytis rubicunda*) in Danum Valley, Borneo (Hanya and Bernard 2015) and proboscis monkeys (*Nasalis larvatus*) in Sabah, Malaysia (Matsuda *et al.* 2017), confirming that Kibale has higher protein concentrations in the environment than the Asian colobine sites studied. In addition, compared to young leaves in Kibale (Table I), those eaten by hindgut-fermenting folivorous howler monkeys (*Alouatta pigra*) in El Tormento, Mexico were lower in AP (Righini *et al.* 2017).

In this study, we aimed to unravel the biological significance of CP in colobine diets through the use of AP. Both condensed and hydrolysable tannins impact protein digestibility (Hagerman *et al.* 1992; Marsh *et al.* 2019; Robbins *et al.* 1987) and the structure of these tannins is important. Based on our analysis of AP with and without PEG, the difference between CP and AP in mature leaves was higher than that of young leaves demonstrating that the protein binding effects of tannins are more prevalent in mature leaves than young leaves. However, many of the tree species we analyzed did not appear to have high concentrations of tannins and we found that they do not limit protein digestibility for colobines in Kibale, as we had predicted and as others have noted (Oates *et al.* 1990). Using a limited dataset from a previous study in Kibale, the impact of tannins on CP digestibility of leaves from sites in multiple continents was found to be highly variable with Kibale showing minimal effects but other sites in Australia and Bolivia showing large differences (Wallis *et al.* 2012); our data are in support of these findings.

Our study shows that protein, crude or available, is not the primary factor influencing colobine biomass in Kibale. For Kibale and other sites with high leaf protein, we need to refocus our emphasis to other macronutrients and energy as drivers of food selection. Minerals may also be important as indicated by the fact that sodium is an important factor in movement patterns of black-and-white colobus (Harris and Chapman 2007).

Although PEG has been used to study the effects of tannins on protein digestibility in many domestic and a few wild herbivores, it has been used only in a few studies of primate diet selection (Felton *et al.* 2009b; Hanya and Bernard 2015; Matsuda *et al.* 2017; Righini *et al.* 2017). It has been noted that more data are needed to understand exactly what types of tannins PEG is capable of binding because differences in the tannin profile of plants could affect the interpretation of AP assays (Windley *et al.* 2016b). In addition, it has recently been shown that variation in AP was related to tannin structure in *Eucalyptus* (Marsh *et al.* 2019), but a similar study in a more diverse system, such as Kibale, would help to better identify these links.

Nutritional ecology studies can provide insights into population demography (DeGabriel *et al.* 2014), which have important implications for conservation, but these studies must carefully consider the mechanisms behind and consequences of these models (Wallis *et al.* 2012). Studies that examine the effects of AP on folivore populations could benefit from an increased understanding of the consequences of protein, if any, on reproduction, as has been shown in other herbivorous mammals

(DeGabriel *et al.* 2009, 2014; McArt *et al.* 2009). For example, AP was strongly correlated with reproductive success in brushtail possums (*Trichosurus vulpecula*); possums with higher quality trees had increased fitness and faster growing offspring (DeGabriel *et al.* 2009). However, Australian ecosystems are protein deficient and, therefore where protein is limited, it will cause variation in mammal abundance (DeGabriel *et al.* 2014; Ganzhorn *et al.* 2017).

Understanding dietary requirements and how they intersect with population demography of primates and other animals can contribute to constructing more effective conservation plans (Rothman 2015). Identifying the nutritional needs of endangered or threatened species, and the plant species best providing those needs, allows conservationists to protect specific forest areas and tree species, identify the best trees to use in restoration efforts, and determine the trees to be protected in logging operations if it is to have the least possible impact (Chapman *et al.* 2004, 2020; Felton *et al.* 2009a; Rothman *et al.* 2012). Our study demonstrates the utility of AP and we hope that more primate ecologists will consider using AP instead of CP in future studies of primate nutritional ecology.

Author Contributions JMR, CAC, and WJF originally formulated the idea. JMR and CAC provided materials and performed the fieldwork. KDE performed the labwork and analyzed the data. KDE wrote the manuscript and JMR, CAC, and WJF critically evaluated and revised the manuscript.

References

Bonnell, T. R., Sengupta, R. R., Chapman, C. A., & Goldberg, T. L. (2010). An agent-based model of red colobus resources and disease dynamics implicates key resource sites as hot spots of disease transmission. *Ecological Modelling, 221*, 2491–2500. https://doi.org/10.1016/j.ecolmodel.2010.07.020.

Chapman, C. A., Bicca-Marques, J. C., Dunhan, A. E., Fan, P., Fashing, P. J., *et al.* (2020). Primates can be a rallying species to promote tropical forest restoration. *Folia Primatologica*, 1–19. https://doi.org/10.1159/000505951.

Chapman, C. A., & Chapman, L. J. (1999). Implications of small scale variation in ecological conditions for the diet and density of red colobus monkeys. *Primates, 40*, 215–232. https://doi.org/10.1007/BF02557712.

Chapman, C. A., Chapman, L. J., Bjorndal, K. A., & Onderdonk, D. A. (2002). Application of protein-to-fiber ratios to predict colobine abundance on different spatial scales. *International Journal of Primatology, 23*, 283–310. https://doi.org/10.1023/A:1013831511405.

Chapman, C. A., Chapman, L. J., Naughton-Treves, L., Lawes, M. J., & McDowell, L. R. (2004). Predicting folivorous primate abundance: Validation of a nutritional model. *American Journal of Primatology, 62*, 55–69. https://doi.org/10.1002/ajp.20006.

Chapman, C. A., Chapman, L. J., Rode, K. D., Hauck, E. M., & McDowell, L. R. (2003). Variation in the nutritional value of primate foods: Among trees, time periods, and areas. *International Journal of Primatology, 24*, 317–333. https://doi.org/10.1023/A:1023049200150.

Chapman, C. A., & Lambert, J. E. (2000). Habitat alteration and the conservation of African primates: A case study of Kibale National Park, Uganda. *American Journal of Primatology, 50*, 169–186. https://doi.org/10.1002/(SICI)1098-2345(200003)50:3<169::AID-AJP1>3.0.CO;2-P.

Chapman, C. A., & Pavelka, M. S. (2005). Group size in folivorous primates: Ecological constraints and the possible influence of social factors. *Primates, 46*, 1–9. https://doi.org/10.1007/s10329-004-0093-9.

Chapman, C. A., Struhsaker, T. T., & Lambert, J. E. (2005). Thirty years of research in Kibale National Park, Uganda, reveals a complex picture for conservation. *International Journal of Primatology, 26*, 539–555. https://doi.org/10.1007/s10764-005-4365-z.

Choo, G. M., Waterman, P. G., McKey, D. B., & Gartlan, J. S. (1981). A simple enzyme assay for dry matter digestibility and its value in studying food selection by generalist herbivores. *Oecologia, 49*, 170–178. https://doi.org/10.1007/BF00349185.

Conklin-Brittain, N. L., Dierenfeld, E. S., Wrangham, R. W., Norconk, M., & Silver, S. C. (1999). Chemical protein analysis: A comparison of Kjeldahl crude protein and total ninhydrin protein from wild, tropical vegetation. *Journal of Chemical Ecology, 25*, 2601–2622. https://doi.org/10.1023/A:1020835120701.

Cork, S. J. (1996). Optimal digestive strategies for arboreal herbivorous mammals in contrasting forest types: Why koalas and colobines are different. *Australian Journal of Ecology, 21*, 10–20. https://doi.org/10.1111/j.1442-9993.1996.tb00581.x.

Davies, A. G. (1994). Colobine populations. In A. G. Davies & J. F. Oates (Eds.), *Colobine monkeys: Their ecology, behaviour and evolution* (pp. 285–310). Cambridge: Cambridge University Press.

Davies, A. G., Bennett, E. L., & Waterman, P. G. (1988). Food selection by two South-east Asian colobine monkeys (*Presbytis rubicunda* and *Presbytis melalophos*) in relation to plant chemistry. *Biological Journal of the Linnean Society, 34*, 33–56. https://doi.org/10.1111/j.1095-8312.1988.tb01947.x.

DeGabriel, J. L., Moore, B. D., Felton, A. M., Ganzhorn, J. U., Stolter, C., et al. (2014). Translating nutritional ecology from the laboratory to the field: Milestones in linking plant chemistry to population regulation in mammalian browsers. *Oikos, 123*, 298–308. 10.1111/j.1600-0706.2013.00727.x.

DeGabriel, J. L., Moore, B. D., Foley, W. J., & Johnson, C. N. (2009). The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology, 90*, 711–719. https://doi.org/10.1890/08-0940.1.

DeGabriel, J. L., Wallis, I. R., Moore, B. D., & Foley, W. J. (2008). A simple, integrative assay to quantify nutritional quality of browses for herbivores. *Oecologia, 156*, 107–116. https://doi.org/10.1007/s00442-008-0960-y.

Donati, G., Santini, L., Eppley, T. M., Arrigo-Nelson, S. J., Balestri, M., et al. (2017). Low levels of fruit nitrogen as drivers for the evolution of Madagascar's primate communities. *Scientific Reports, 7*, 1–9. https://doi.org/10.1038/s41598-017-13906-y.

Dunham, N. T., & Rodriguez-Saona, L. E. (2018). Nutrient intake and balancing among female *Colobus angolensis palliatus* inhabiting structurally distinct forest areas: Effects of group, season, and reproductive state. *American Journal of Primatology, 80*, e22878. https://doi.org/10.1002/ajp.22878.

Fashing, P. J., Dierenfeld, E. S., & Mowry, C. B. (2007). Influence of plant and soil chemistry on food selection, ranging patterns, and biomass of *Colobus guereza* in Kakamega Forest, Kenya. *International Journal of Primatology, 28*, 673–703. https://doi.org/10.1007/s10764-006-9096-2.

Felton, A. M., Felton, A., Lindenmayer, D. B., & Foley, W. J. (2009a). Nutritional goals of wild primates. *Functional Ecology, 23*, 70–78 https://www.jstor.org/stable/40205503.

Felton, A. M., Felton, A., Raubenheimer, D., Simpson, S. J., Foley, W. J., et al. (2009b). Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behavioral Ecology, 20*, 685–690. https://doi.org/10.1093/beheco/arp021.

Fimbel, C., Vedder, A., Dierenfeld, E., & Mulindahabi, F. (2001). An ecological basis for large group size in *Colobus angolensis* in the Nyungwe Forest, Rwanda. *African Journal of Ecology, 39*, 83–92. https://doi.org/10.1111/j.1365-2028.2001.00276.x.

Ganzhorn, J. U. (1988). Food partitioning among Malagasy primates. *Oecologia, 75*, 436–450. https://doi.org/10.1007/BF00376949.

Ganzhorn, J. U. (1992). Leaf chemistry and the biomass of folivorous primates in tropical forests: Test of a hypothesis. *Oecologia, 91*, 540–547. https://doi.org/10.1007/BF00650329.

Ganzhorn, J. U. (2002). Distribution of a folivorous lemur in relation to seasonally varying food resources: Integrating quantitative and qualitative aspects of food characteristics. *Oecologia, 131*, 427–435. https://doi.org/10.1007/s00442-002-0891-y.

Ganzhorn, J. U., Arrigo-Nelson, S. J., Carrai, V., Chalise, M. K., Donati, G., et al. (2017). The importance of protein in leaf selection of folivorous primates. *American Journal of Primatology*, *79*, 1–13. https://doi.org/10.1002/ajp.22550.

Hagerman, A. E., Robbins, C. T., Weerasuriya, Y., Wilson, T. C., & McArthur, C. (1992). Tannin chemistry in relation to digestion. *Journal of Range Management, 45*, 57–62. https://doi.org/10.2307/4002526.

Hanya, G., & Bernard, H. (2015). Different roles of seeds and young leaves in the diet of red leaf monkeys (*Presbytis rubicunda*): Comparisons of availability, nutritional properties, and associated feeding behavior. *International Journal of Primatology, 36*, 177–193. https://doi.org/10.1007/s10764-015-9819-3.

Harris, T. R., & Chapman, C. A. (2007). Variation in diet and ranging of black and white colobus monkeys in Kibale National Park, Uganda. *Primates, 48*, 208–221. https://doi.org/10.1007/s10329-006-0036-8.

Johnson, C. A., Raubenheimer, D., Chapman, C. A., Tombak, K. J., Reid, A. J., & Rothman, J. M. (2017). Macronutrient balancing affects patch departure by guerezas (*Colobus guereza*). *American Journal of Primatology, 79*, 1–9. https://doi.org/10.1002/ajp.22495.

Kar-Gupta, K., & Kumar, A. (1994). Leaf chemistry and food selection by common langurs (*Presbytis entellus*) in Rajaji National Park, Uttar Pradesh, India. *International Journal of Primatology, 15*, 75–93. https://doi.org/10.1007/BF02735235.

Makkar, H. P. (2003). *Quantification of tannins in tree and shrub foliage: A laboratory manual*. Dordrecht: Kluwer Academic.

Marsh, K. J., Wallis, I. R., Kulheim, C., Clark, R., Nicolle, D., et al. (2019). New approaches to tannin analysis of leaves can be used to explain in vitro biological activities associated with herbivore defence. *New Phytologist*, *225*, 488–498. 10.1111/nph.16117.

Matsuda, I., Chapman, C. A., & Clauss, M. (2019). Colobine forestomach anatomy and diet. *Journal of Morphology, 280*, 1608–1616. https://doi.org/10.1002/jmor.21052.

Matsuda, I., Clauss, M., Tuuga, A., Sugau, J., Hanya, G., et al (2017). Factors affecting leaf selection by foregut-fermenting proboscis monkeys: New insight from *in vitro* digestibility and toughness of leaves. *Scientific Reports*, *7*, 42774. In *10.1038/srep42774*.

Matsuda, I., Tuuga, A., Bernard, H., Sugau, J., & Hanya, G. (2013). Leaf selection by two Bornean colobine monkeys in relation to plant chemistry and abundance. *Scientific Reports, 3*, 1873. https://doi.org/10.1038/srep01873.

McArt, S. H., Spalinger, D. E., Collins, W. B., Schoen, E. R., Stevenson, T., & Bucho, M. (2009). Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska. *Ecology, 90*, 1400–1411. https://doi.org/10.1890/08-1435.1.

McKey, D. B., Gartlan, J. S., Waterman, P. G., & Choo, C. M. (1981). Food selection by black colobus monkeys (*Colobus satanas*) in relation to plant chemistry. *Biological Journal of the Linnean Society, 16*, 115–146. https://doi.org/10.1111/j.1095-8312.1981.tb01646.x.

Milton, K. (1979). Factors influencing leaf choice by howler monkeys: A test of some hypotheses of food selection by generalist herbivores. *The American Naturalist, 114*, 362–378. https://doi.org/10.1086/283485.

Milton, K., & Dintzis, F. R. (1981). Nitrogen-to-protein conversion factors for tropical plant samples. *Biotropica, 13*, 177–181. https://doi.org/10.2307/2388122.

Moore, B. D., & Foley, W. J. (2005). Tree use by koalas in a chemically complex landscape. *Nature, 435*, 488–490. https://doi.org/10.1038/nature03551.

Mowry, C. B., Decker, B. S., & Shure, D. J. (1996). The role of phytochemistry in dietary choices of Tana River red colobus monkeys (*Procolobus badius rufomitratus*). *International Journal of Primatology, 17*, 63–84. https://doi.org/10.1007/BF02696159.

National Research Council (2003). *Nutrient requirements of nonhuman primates*. Washington, DC: National Academies Press.

Oates, J. F. (1988). The diet of the olive colobus monkey, *Procolobus verus*, in Sierra Leone. *International Journal of Primatology, 9*, 457–478. https://doi.org/10.1007/BF02736220.

Oates, J. F., Waterman, P. G., & Choo, G. M. (1980). Food selection by the South Indian leaf monkey, *Presbytis johnii*, in relation to plant chemistry. *Oecologia, 45*, 45–56. https://doi.org/10.1007/BF00346706.

Oates, J. F., Whitesides, G. H., Davies, A. G., Waterman, P. G., Green, S. M., et al. (1990). Determinants of variation in tropical forest primate biomass: New evidence from West Africa. *Ecology*, *71*, 328–343. 10.2307/1940272.

Oftedal, O. T. (1991). The nutritional consequences of foraging in primates: The relationship of nutrient intakes to nutrient requirements. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 334*, 161–170. https://doi.org/10.1098/rstb.1991.0105.

Righini, N., Garber, P. A., & Rothman, J. M. (2017). The effects of plant nutritional chemistry on food selection of Mexican black howler monkeys (*Alouatta pigra*): The role of lipids. *American Journal of Primatology, 79*, 1–15. https://doi.org/10.1002/ajp.22524.

Robbins, C. T., Hanley, T. A., Hagerman, A. E., Hjeljord, O., Baker, D. L., *et al.* (1987). Role of tannins in defending plants against ruminants: Reduction in protein availability. *Ecology, 68*, 98–107. https://doi.org/10.2307/1938809.

Rode, K. D., Chapman, C. A., Chapman, L. J., & McDowell, L. R. (2003). Mineral resource availability and consumption by colobus in Kibale National Park, Uganda. *International Journal of Primatology, 24*, 541–573. https://doi.org/10.1023/A:1023788330155.

Rothman, J. M. (2015). Nutritional geometry provides new insights into the interaction between food quality and demography in endangered wildlife. *Functional Ecology, 29*, 3–4. https://doi.org/10.1111/1365-2435.12320.

Rothman, J. M., Chapman, C. A., & Pell, A. N. (2008). Fiber-bound nitrogen in gorilla diets: implications for estimating dietary protein intake of primates. *American Journal of Primatology, 70*, 690–694. https://doi.org/10.1002/ajp.20540.

Rothman, J. M., Chapman, C. A., & Van Soest, P. J. (2012). Methods in primate nutritional ecology: A user's guide. *International Journal of Primatology, 33*, 542–566. https://doi.org/10.1007/s10764-011-9568-x.

Rothman, J. M., DePasquale, A., Evans, K. D., & Raboin, D. L. (2021). Colobine nutritional ecology. In I. Matsuda, J. Teichroeb, & C. Greuter (Eds.), *The colobines: Natural history, behaviour and ecological diversity*. Cambridge: Cambridge University Press. (in press).

Ryan, A. M., Chapman, C. A., & Rothman, J. M. (2013). How do differences in species and part consumption affect diet nutrient concentrations? A test with red colobus monkeys in Kibale National Park, Uganda. *African Journal of Ecology, 51*, 1–10. https://doi.org/10.1111/j.1365-2028.2012.01346.x.

Struhsaker, T. T. (2010). *The red colobus monkeys: Variation in demography, behavior, and ecology of endangered species*. Oxford: Oxford University Press.

Wallis, I. R., Edwards, M. J., Windley, H., Krockenberger, A. K., Felton, A., *et al.* (2012). Food for folivores: Nutritional explanations linking diets to population density. *Oecologia, 169*, 281–291. https://doi.org/10.1007/s00442-011-2212-9.

Wasserman, M. D., & Chapman, C. A. (2003). Determinants of colobine monkey abundance: The importance of food energy, protein and fibre content. *Journal of Animal Ecology, 72*, 650–659. https://doi.org/10.1046/j.1365-2656.2003.00736.x.

Waterman, P. G., Ross, J. A. M., Bennett, E. L., & Davies, A. G. (1988). A comparison of the floristics and leaf chemistry of the tree flora in two Malaysian rain forest and the influence of leaf chemistry on populations of colobine monkeys in the Old World. *Biological Journal of the Linnean Society, 34*, 1–32. https://doi.org/10.1111/j.1095-8312.1988.tb01946.x.

Watts, D. P., & Mitani, J. C. (2002). Hunting behavior of chimpanzees at Ngogo, Kibale National Park, Uganda. *International Journal of Primatology, 23*, 1–28. https://doi.org/10.1023/A:1013270606320.

White, T. C. (2012). *The inadequate environment: Nitrogen and the abundance of animals*. Berlin: Springer-Verlag.

Windley, H. R., Barron, M. C., Holland, E. P., Starrs, D., Ruscoe, W. A., & Foley, W. J. (2016a). Foliar nutritional quality explains patchy browsing damage caused by an invasive mammal. *PloS ONE, 11*, e0155216. https://doi.org/10.1371/journal.pone.0155216.

Windley, H. R., Wigley, H. J., Ruscoe, W. A., Foley, W. J., & Marsh, K. J. (2016b). The use of polyethylene glycol in mammalian herbivore diet studies: What are we measuring? *Journal of Chemical Ecology, 42*, 523–532. https://doi.org/10.1007/s10886-016-0709-8.

Yeager, C. P., & Kirkpatrick, R. C. (1998). Asian colobine social structure: Ecological and evolutionary constraints. *Primates, 39*, 147–155. https://doi.org/10.1007/BF02557727.

Yeager, C. P., Silver, S. C., & Dierenfeld, E. S. (1997). Mineral and phytochemical influences on foliage selection by the proboscis monkey (*Nasalis larvatus*). *American Journal of Primatology, 41*, 117–128. https://doi.org/10.1002/(SICI)1098-2345(1997)41:2<117::AID-AJP4>3.0.CO;2-%23.

Affiliations

Katarina D. Evans [1,2] · **William J. Foley** [3] · **Colin A. Chapman** [4,5,6,7] · **Jessica M. Rothman** [1,2,8,9]

[1]   Anthropology Program, Graduate Center, City University of New York, New York, NY, USA

[2]   The New York Consortium in Evolutionary Primatology (NYCEP), New York, NY, USA

[3]   Research School of Biology, Australian National University, Canberra, ACT, Australia

[4]   Department of Anthropology, Center for the Advanced Study of Human Paleobiology, George Washington University, Washington, DC 20037, USA

[5]   Department of Anthropology, McGill University, Montreal, QC, Canada

[6]   School of Life Sciences, University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa

[7]   Shaanxi Key Laboratory for Animal Conservation, Northwest University, Xi'an, China

[8]   Department of Anthropology, Hunter College of the City University of New York, New York, NY, USA

[9]   Uganda Wildlife Authority, Kampala, Uganda