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BRIEF REPORT

Fiber-Bound Nitrogen in Gorilla Diets: Implications for Estimating Dietary Protein Intake of Primates

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Protein is essential for living organisms, but digestibility of crude protein is poorly understood and difficult to predict. Nitrogen is used to estimate protein content because nitrogen is a component of the amino acids that comprise protein, but a substantial portion of the nitrogen in plants may be bound to fiber in an indigestible form. To estimate the amount of crude protein that is unavailable in the diets of mountain gorillas (*Gorilla beringei*) in Bwindi Impenetrable National Park, Uganda, foods routinely eaten were analyzed to determine the amount of nitrogen bound to the acid-detergent fiber residue. The amount of fiber-bound nitrogen varied among plant parts: herbaceous leaves 14.5 ± 8.9% (reported as a percentage of crude protein on a dry matter (DM) basis), tree leaves (16.1 ± 6.7% DM), pith/herbaceous peel (26.2 ± 8.9% DM), fruit (34.7 ± 17.8% DM), bark (43.8 ± 15.6% DM), and decaying wood (85.2 ± 14.6% DM). When crude protein and available protein intake of adult gorillas was estimated over a year, 15.1% of the dietary crude protein was indigestible. These results indicate that the proportion of fiber-bound protein in primate diets should be considered when estimating protein intake, food selection, and food/habitat quality. Am. J. Primatol. 70:690–694, 2008.

Key words: plant protein; ADIN; nutritional ecology; ape nutrition

INTRODUCTION

Nitrogen (N) is used to estimate protein content because it is an easily measurable component of the amino acids in protein. Crude protein is typically estimated by multiplying the amount of N in a plant by 6.25, because many domesticated plant and animal proteins are known to contain about 16% nitrogen [Van Soest, 1994]. Many authors have estimated the protein content of primate foods on this basis, but few have considered the fact that not all of this crude protein is available [but see Conklin-Brittain et al., 1999; Curtis, 2003; Milton & Dintzis, 1981; Silver et al., 2000; Yeager et al., 1997]. In domesticated plant species, 60–80% of the crude protein is ''true protein'' that is composed of amino acids [Van Soest, 1994]. First, some of the nitrogen may be bound to the lignified plant cell wall (i.e., fiber-bound N) that is resistant to digestion by animal and microbial enzymes. Second, nitrogen can be bound to secondary compounds, particularly tannins, and thus rendered indigestible. Third, plants contain significant amounts of nonprotein nitrogen in secondary compounds (i.e., alkaloids, glucosinolates, and cyanides), in nucleic acids or ammonia, or as products of amino acid catabolism. Because the 6.25 coefficient is inaccurate for non-amino forms of N, some studies have proposed adjustments to the conversion factor to account for N-containing fractions other than true protein [Levey, 2000; Milton & Dintzis, 1981]; but Conklin-Brittain et al. [1999] suggested that conversion factors might underestimate available protein (AP). There is considerable inter- and intraspecific variability in plant N fractions that cannot be accounted for by a single conversion factor [Van Soest, 1994]. In addition, protein digestibility is affected by an animal's digestive anatomy, body size, diet quality, and physiological status; therefore, adjustments must be both species- and diet-specific. Nutritionists have persisted in using the 6.25 coefficient to convert N to crude protein for ration formulation as amino acid profiles generally are not available. The utility of

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the crude protein approximation could be improved by using estimates of fiber-bound nitrogen to predict crude protein availability [Conklin-Brittain et al., 1999].

The objective of this analysis is to examine the significance of estimating fiber-bound protein in evaluating protein quality of primate diets. Using gorilla diets as an example, we report the amounts of fiber-bound nitrogen in foods eaten, and estimate the amount of dietary crude protein intake that is unavailable owing to the fact that it is bound to fiber. The nutritional content of foods eaten by gorillas (*Gorilla* sp.) from several research sites across Africa was reviewed by Rothman et al. [2006b]. The diet of the mountain gorillas (*Gorilla beringei*) in Bwindi Impenetrable National Park, Uganda, is primarily composed of herbaceous leaves (about 67% wet weight), but pith, fruit, bark, and decaying wood are also consumed [Rothman et al., 2007; Stanford & Nkurunungi, 2003]. Herbaceous leaves eaten by Bwindi gorillas are higher in crude dietary protein than other foods [Rothman et al., 2006a]. In some months, the percentage of fruit eaten exceeds 40% of total intake by weight and dietary crude protein concentration is reduced at this time [Rothman et al., 2008]. However, no studies have reported the amount of fiber-bound protein, without which we do not know how much dietary protein is available to gorillas. To determine the amount fiber-bound nitrogen in gorilla foods and their diets, we collected and analyzed food items eaten by the Bwindi mountain gorillas. This analysis provides a means to consider the importance of fiber-bound protein and should be of value to studies evaluating the significance of protein to primates.

MATERIALS AND METHODS

We studied a group of gorillas in Bwindi Impenetrable National Park (0°53′–1°08′S, 29°35′–29°50′E), southwestern Uganda. Details of plant collections and behavioral methods used to estimate nutrient intake are available in Rothman et al. [2008].

To estimate AP and unavailable protein, we analyzed 72 gorilla foods following the protocol outlined by Licitra et al. [1996]. First, we measured the N in all samples using a Leco FP-528 combustion analyzer (St. Joseph, Michigan) and multiplied the N by 6.25 to estimate the crude protein content. Then, we removed the fiber-bound unavailable N using an acid-detergent fiber (ADF) procedure, which primarily measures the amounts of cellulose and lignin, but also includes any N bound to these fiber fractions [Van Soest et al., 1991]. The ADF analysis was performed using an ANKOM A200 fiber analyzer using filter bags (Macedon, NY). The total N in the acid-detergent residue was measured using the Kjeldahl procedure, which gives similar results to the combustion method [Etheridge et al., 1998]. This N fraction is the acid-detergent-insoluble nitrogen (ADIN). The ADIN multiplied by 6.25 is used to estimate the acid-detergent-insoluble crude protein (ADICP). The AP was determined by subtracting the ADICP from the crude protein concentration of the samples. All results are presented on a dry matter (DM) basis [Shreve et al., 2006].

As we were analyzing hundreds of samples for crude protein to determine the composition of the gorilla diet [i.e., Rothman et al., 2007, 2008], we used combustion analysis to estimate crude protein. The combustion procedure uses less hazardous chemicals, smaller amounts of plant material and is faster than the Kjeldahl procedure. We used Kjeldahl procedures in the same laboratory to estimate the N in the fiber residue as our combustion analyzer could not accommodate fiber residues. Both Kjeldahl or combustion analysis can be used to estimate N contents and provide similar results [Etheridge et al., 1998; Rothman, unpublished data].

To ensure that ADIN was not affected by heat, the plants in this study were dried in a cool, dark area that did not exceed 22°C. Drying at temperatures above 60°C significantly increases the amount of nitrogen in the ADF residue, as amino acids react with sugar to create browning in the Maillard reaction [Van Soest & Mason, 1991]. The ADIN content of plants dried at high temperatures or in the sun will be substantially overestimated.

The ADIN estimates the amount of fiber-bound N in foodstuffs and may include some tannin–protein complexes and Maillard products [Van Soest, 1994]. The ADIN has been a good predictor of N indigestibility in many studies of domestic ruminants [Huhtanen & Hristov, 2001; Van Soest, 1994; Van Soest & Mason, 1991] and has been used to establish the guidelines for nutrient requirements of domestic animals published by the National Research Council [2001a,b] and in other commonly used domestic animal models of feed utilization and requirements [Sniffen et al., 1992]. However, there is some question whether ADIN is completely indigestible. Most of this controversy stems from whether Maillard products resulting from heat treatment that are recovered in the ADIN are degradable or not [Nakamura et al., 1994; also discussed in National Research Council, 2001a,b; Van Soest, 1994; Van Soest & Mason, 1991]. Formation of Maillard products from heat treatment is unlikely if the samples are dried appropriately, but without comprehensive feeding trials with wild primates and native diets, it is impossible to know whether ADIN is indigestible for all primates. Nevertheless, the substantial data for domestic animals suggest that ADIN is a good indication of unavailable protein.

The concentrations of ADICP were compared among plant parts using a Kruskal Wallis test with the multiple comparison procedures that were based

on Dwass, Steel, Critchlow-Fliger pairwise rankings [Hollander & Wolfe, 1999].

Our research protocol did not require approval from Cornell University's Institutional Animal Care and Use Committee (IACUC) because it did not involve more than simple field observations of the gorillas. All research conducted during this study complied with the regulations of the Government of Uganda and was conducted with the permission of the Uganda Wildlife Authority and the Uganda Council for Science and Technology.

RESULTS AND DISCUSSION

Across plant parts, we found variation in ADICP content and in the portion of the crude protein that was ADICP (Table I). The portion of crude protein in gorilla foods that was unavailable varied from 14.5% DM (herbaceous leaves) to 85.2% DM (decaying wood). Over 12 months, adult gorilla diets varied in AP content (Fig. 1).

The protein requirement of gorillas is not known, but human men require about 1.8 g of protein per kg of metabolic body mass, and lactating women require 2.8 g per kg [Food and Nutrition Board of the National Academy of Science, 2005]. The nutritional requirements of domestic swine are well established and may provide a reference for gorillas as they reach similar body mass and, like gorillas, are hindgut fermenters [Rothman et al., 2008]. Both adult female lactating pigs and adult male breeding pigs require 4.1 g of protein per kg of metabolic body mass [National Research Council, 1998]. The AP intake by adult gorillas exceeded the requirements of both humans and swine; adult males ate 10.5 g per kg of metabolic body mass, and adult females ate 15.7 g per kg [DM intake and body weight following Rothman et al., 2008], suggesting that gorillas were not protein-limited. This was probably due to the consumption of large quantities of herbaceous leaves [Rothman et al., 2007], which were high in crude protein and low in ADICP, and together resulted in a high AP content (Table I). Foods high in ADICP, and thus low in AP, were probably selected for other nutrients. For example, the small amount of crude protein in decaying wood was almost entirely bound, but gorillas probably ate wood because it contains sodium [Rothman et al., 2006c]. Similarly, fruits are likely eaten for their high sugar content rather than for protein [Rothman et al., 2006a]. Variation in ADICP among plant parts may arise from variation in the types and amounts of lignin and their associated N in different plant parts.

Estimation of the digestibility of crude protein in captive western gorilla diets through digestion trials indicated that the apparent digestibility of crude protein was 72 and 79% during two phases of the study [Remis & Dierenfeld, 2004]. As the hindgut of gorillas contains symbiotic bacteria that digest fiber [Frey et al., 2006], feces contain microbes that are composed of N. Accordingly, in an examination of the digestibility of protein through digestion trials a careful assessment of the origin of N in the feces is needed. If the origin of fecal N is not examined [as in Remis & Dierenfeld, 2004], microbial N present in the feces may confuse estimations of dietary protein digestibility. Although AP in the diets of Bwindi gorillas appeared to be adequate, primates in other

TABLE I. The Mean Percentage of (a) Acid-Detergent-Insoluble Crude Protein (ADICP) and (b) ADICP Expressed as a Percentage of Total Crude Protein (CP) That Is ADICP in Gorilla Foods With Comparison to Foods Eaten by Other Primates at Different Research Sites

Plant part	*n*	(a) ADICP ± sd	(b) % of CP bound ± sd
Mountain gorilla (*Gorilla beringei*)[1]			
Tree bark	6	$3.6^{ac} \pm 1.1$	$43.8^{ad} \pm 15.6$
Whole fruit/ flowers	15	$3.0^{abc} \pm 1.3$	$34.7^{acd} \pm 17.8$
Herbaceous leaves	28	$2.7^{abc} \pm 1.4$	$14.5^{b} \pm 8.9$
Mature tree leaves	7	$2.3^{abc} \pm 1.0$	$16.1^{bc} \pm 6.7$
Pith/peel/stem	10	$1.9^{b} \pm 0.7$	$26.2^{abc} \pm 16.2$
Decaying wood	6	$4.9^{c} \pm 3.3$	$85.2^{d} \pm 14.6$
Various primates in Kibale, Uganda[2]			
Fruit skin/ husk	4	5.5 ± 2.4	50.8 ± 16.3
Ripe pulp	41	3.2 ± 1.4	35.8 ± 24.4
Ripe seed	6	3.1 ± 2.9	28.4 ± 19.6
Unripe pulp	22	2.9 ± 1.1	38.6 ± 27.0
Unripe seed	8	2.6 ± 1.9	22.8 ± 20.4
Mature leaves	6	2.4 ± 0.3	8.7 ± 1.8
Young leaves	36	3.3 ± 2.1	17.9 ± 17.7
Proboscis monkey (*Nasalis larvatus*)[3]			
Mature leaves	16	2.9	27.9
Young leaves	16	3.6	34.6
Black howler monkey (*Alouatta pigra*)[4]			
Young leaves	54	5.5 ± 3.0	28.6 ± 19.0
Mature leaves	32	4.6 ± 2.4	28.5 ± 16.2
Fruits	19	3.0 ± 1.1	41.9 ± 17.3
Flowers	20	5.5 ± 3.1	32.0 ± 14.4
Hose's langur (*Presbytis hosei*)[5]			
Leaves	10	7.4 ± 3.7	46.2
Flowers	2	8.4 ± 2.2	84.0
Seeds	9	5.8 ± 3.8	53.2
Snub-nose monkey (*Rhinopithecus brelichi*)[5]			
Leaves	49	5.3 ± 2.4	37.9
Fruits	5	1.5 ± 1.2	26.3

All analyses are reported on a dry matter basis. Different lowercase letters denote statistical significance at $P < 0.05$.
[1]This study.
[2]Conklin-Brittain et al. [1999].
[3]Yeager et al. [1997].
[4]Silver et al. [2000].
[5]Nijboer et al. [1997].

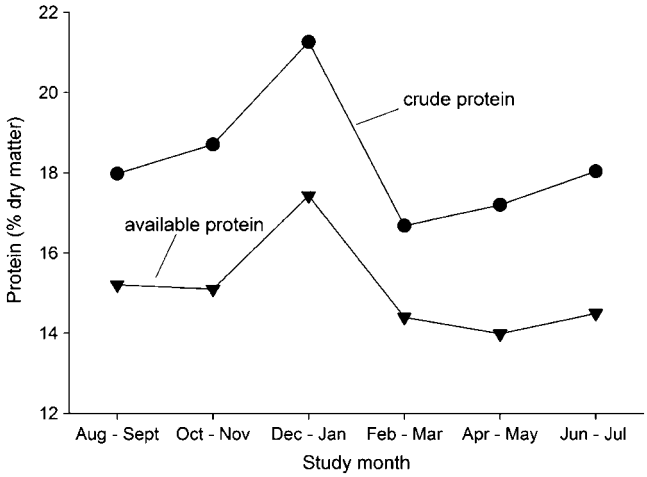

Fig. 1. Crude protein (●) and the maximum amount of available protein (▼) as a percentage of dry matter in the diets of adult gorillas ($n = 8$) in Bwindi Impenetrable National Park, Uganda, over 1 year. Concentrations of crude protein as per Rothman et al. [2008].

habitats may be limited by protein. Estimating ADICP is important in understanding the maximum amount of protein that is potentially available to them. For example, the mean protein content of young leaves eaten by proboscis monkeys was only 10% crude protein DM, 35% of which was fiber-bound, leaving only 6.5% AP [Yeager et al., 1997], which may only marginally meet protein requirements.

Better understanding of dietary protein adequacy and protein metabolism of primates requires a more rigorous approach to dietary characterization. Estimating the amount of *available* proteins in primate foods and diets, instead of *crude* protein, is an important first step. Such improvements promise new insights into primate-feeding strategies and the ecological determinants of primate biomass.

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