American Journal of Primatology 64:323–335 (2004)

RESEARCH ARTICLE

Use of Total Dietary Fiber Across Four Lemur Species (*Propithecus verreauxi coquereli*, *Hapalemur griseus griseus*, *Varecia variegata*, and *Eulemur fulvus*): Does Fiber Type Affect Digestive Efficiency?

J.L. CAMPBELL[1]*, C.V. WILLIAMS[2], AND J.H. EISEMANN[1]
[1]*Interdepartmental Nutrition Program and Department of Animal Science, North Carolina State University, Raleigh, North Carolina*
[2]*Duke University Primate Center, Durham, North Carolina*

In vivo digestibility and transit of two experimental diets were compared across four lemur species for which gastrointestinal morphology and preliminary data on physiology differ: *Varecia variegata* (VV), *Eulemur fulvus* (EF), *Propithecus verreauxi* (PV), and *Hapalemur griseus* (HG). Since free-ranging groups consume varied amounts of slowly fermentable insoluble fiber (IF) and rapidly fermentable soluble fiber (SF), differences in digestibility may be related to variation in the fiber types consumed. To investigate this, two diets were designed to provide 28% of dry matter (DM) as total dietary fiber (TDF). The ratio of IF/SF (g/g) differed across the diets (12.15:1 for the IF diet, and 3.76:1 for the IF/SF diet). The DM digestibility (DMD) of both diets differed across species: DMD was lower for EF and VV (approximately 56–58%), and higher for PV (72%) and HG (76%). The fiber digestibility results were as follows: TDF digestibility was similar for VV and EF (23% and 28%), higher for PV (56%), and highest for HG (66%). IF digestibility was lower for VV and EF (20% and 28%), and higher for PV and HG (53% and 62%). The transit times (TTs) of the two markers Cr and Co were similar (approximately 3.5 hr for VV and EF, 25 hr for PV, and 30 hr for HG). The mean retention times (MRTs) showed the same trend. The results from these captive groups suggest there are large differences in digestive efficiency that are likely related to the varied fiber composition of the free-ranging diet, and the amount of time the digesta are retained in the gut. Am. J. Primatol. 64:323–335, 2004. © 2004 Wiley-Liss, Inc.

Key words: fiber utilization; lemurs; total dietary fiber; insoluble fiber; soluble fiber; primate nutrition

INTRODUCTION

Information about food items consumed in the wild suggests that Coquerel's sifaka (*P. v. coquereli*), the gentle gray lemur (*H. g. griseus*), the ruffed lemur

*Correspondence to: Jennifer L. Campbell, Delaware Valley College, 700 East Butler Ave., Doylestown, PA 18901. E-mail: jenny.campbell@devalcol.edu

Received 31 July 2003; revision accepted 16 July 2004

DOI 10.1002/ajp.20081
Published online in Wiley InterScience (www.interscience.wiley.com).

© 2004 Wiley-Liss, Inc.

(*V. variegata*), and the brown lemur (*E. fulvus*) consume foods that differ in the amount of total dietary fiber (TDF) present, as well as in the ratio of insoluble fiber (IF) to soluble fiber (SF). Free-ranging *P. v. coquereli* groups seasonally consume large amounts of leaves, which are high in IF relative to other plant parts [Lambert, 1998; Richard, 1977]. *H. g. griseus* consumes the young leaf bases and branch shoots of bamboo, a monocotinous plant that is also high in IF [Tan, 1999]. *V. variegata* consumes a frugivorous diet, and although fruit has less fiber overall than other plant parts, it has a higher ratio of IS to IF, owing to the presence of pectins and gums [Britt, 2000; Lambert, 1998]. Free-ranging *E. fulvus* consume a varied diet, and leaf and fruit consumption differs daily and seasonally [Overdorff, 1993]. Thus, *E. fulvus* consumes a diet that varies in type of fiber as well as quantity. Because these differences may be related to differences in digestive physiology, perhaps captive diets should vary not only in fiber quantity but also in fiber type.

''Fiber'' is a general term used to describe the structural polysaccharides and lignin found in plant cell walls. Mammals do not possess the enzymes necessary to hydrolyze the bonds between monomeric units, and thus are dependent upon microorganisms to make the carbohydrate available to them. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) are fiber categories that are commonly utilized to quantify the fiber content of forages and determine the digestive efficiency of fiber utilization among animals. Both represent IF (i.e., fiber that is not soluble in water). These types of fiber are slowly fermented by microbial action. NDFs include the carbohydrates cellulose and hemicellulose, and the noncarbohydrate lignin, whereas ADFs include cellulose and lignin. By comparison, SFs, such as pectins and gums, are rapidly fermentable; however, they have not been quantified in NDF and ADF analyses [Van Soest, 1994]. TDF, as described by Prosky et al. [1984, 1992], includes both soluble (SF) and insoluble (IF) components, and can be partitioned into these constituents. Therefore, TDF analyses may provide more information about foods (such as fruits, nuts, and seeds) that vary in terms of fiber type [Southgate, 1991].

In fact, since lemurs consume a variety of plant parts, they may vary in their capacity to process the different fiber types. When assessing fiber utilization for these species, it may be appropriate to consider the TDF, IF, and SF content of their food. For instance, it has been shown that *V. variegata* cannot maintain body weight on a diet with moderately high IF levels as measured by NDF [Edwards & Ullrey, 1999a]; however, if the fiber were a mixture of IF and SF, the results might differ. *E. fulvus* may be moderate in its ability to process TDF, based on its wild diet. In direct contrast are the *Propithecus* and (possibly) *Hapalemur* species, which, based on their feeding ecology, are likely to be better able to process and utilize greater amounts of IF in their captive diet than the other species.

The overall goal of this study was to better characterize the efficiency of TDF utilization across four lemur species that vary in dietary profile, and to determine the extent to which fiber type (IF and SF) could affect the results. This was accomplished through determination of digestibility and marker transit while animals were fed two diets that possessed the same quantity of TDF but differed in dietary fiber profile. In order to compare our results with those of previous studies in which NDF and ADF were used to assess fiber use, we also measured these components in the current study. This information can be used to refine captive diets and to gain a better understanding of fiber utilization across lemur species.

MATERIALS AND METHODS

Animals and Housing

The Duke University Institutional Animal Care and Use Committee approved the use of animals for this project. Six animals from each of four species (*Hapalemur griseus* (HG), *Varecia variegata* (VV), *Eulemur fulvus* (EF), and *Propithecus verreauxi coquereli* (PV)) were selected from the colony at Duke University Primate Center (DUPC; Durham, NC). Selection was based on animal availability; however, no juvenile or geriatric animals were included in the study. All of the selected animals had been housed in male/female pairs prior to the study. None of the females used were pregnant or lactating during the experiment. During the diet-adjustment phase of the project, the animals were housed as pairs in their standard DUPC cages, which ranged in size from 2.7 $\times$ 1.9 $\times$ 3.4 (L $\times$ W $\times$ H) m to 3.5 $\times$ 4.1 $\times$ 6.9 m in size. During the 2 weeks of the adaptation and collection periods, the animals were housed individually in stainless steel collection cages ranging in size from 0.9 $\times$ 0.9 $\times$ 0.9 m to 0.9 $\times$ 0.9 $\times$ 1.8 m. These cages are used by the DUPC for temporary housing; therefore, all of the animals used in the study had been housed in them before. While the pairs were separated, they were caged adjacent to one another and separated by wire mesh that allowed for some contact in order to minimize the stress of isolation. The animals were separated from urine and feces by a rubber-coated grate. A fine screen was placed over the stainless steel collection pan to separate urine from feces and thus minimize contamination. The animals were fed twice daily and provided fresh water at all times.

Experimental Diets

The diet was a combination of biscuit (90% of DM) and produce (10% of DM). The fiber present in the IF diet was primarily IF at a ratio of 12.15 g IF:1 g SF. The IF/SF diet was a combination of IF and SF at a ratio of 3.76 g IF:1 g SF. The main ingredients used to manufacture the diets are shown in Table I. Soy hulls and oat hulls were the primary sources of IF, and citrus pulp and beet pulp were the primary sources of SF in the diets. The biscuit portion of the diet was prepared at Purina Mills Inc. (St. Louis, MO). After the ingredients were mixed, a biscuit was extruded. This was similar in form to the regularly-fed commercial diet, which is also offered in an extruded biscuit. The biscuit was offered daily with a combination of locally available produce routinely fed at DUPC. The quantities were as follows: carrot and sweet potato at 6.6% of the total DM, and either kale or apple for the remaining 3.3% of the diet. Table I indicates the nutrient concentrations of the two diets used.

Experimental Design

The design was single reversal, and all animals were tested on each diet. Cage limitations necessitated the division of animals into two groups, with all four species represented in each group. All animals in each group underwent the entire protocol together. Because the animals were housed as pairs during the adaptation phase leading up to the collection period, diets were randomly assigned to pairs, not individual animals. This resulted in an unbalanced design such that four animals (two pairs) within a species were placed in one group, while two (one pair) were placed in the other. As a result, when the group with only one pair was undergoing a collection period, only one diet was being tested for that species. Once animals were assigned to groups, they were transitioned

TABLE I. Ingredient Composition and Analyzed Crude Protein and Fiber Composition of the Two Experimental Diets*

	IF[a]	IF/SF[b]
Ingredient		
Ground corn	229.5	238.5
Oat hulls	175.5	–
Beet pulp	–	99.0
Citrus pulp	–	87.3
Soy hulls	189.7	191.7
Dehulled soymeal	261.0	245.1
Produce mix[c]	100.0	100.0
Vitamin/mineral mix[d]	44.3	38.3
Component		
Crude protein	181.4	181.0
Neutral detergent fiber	306.1	259.9
Acid detergent fiber	174.3	153.7
Total dietary fiber	282.5	280.8
Insoluble fiber	264.9	228.9
Soluble fiber	21.8	60.9

*Based on 90% biscuit and 10% produce diet ratio. Values are expressed as g/kg of diet dry matter.
[a]Insoluble fiber diet (ratio of insoluble: soluble fiber is 12.15g:1g).
[b]Mixed fiber diet (ratio of insoluble: soluble fiber is 3.76g:1g).
[c]Produce was a combination of equal parts sweet potato and carrot and either kale (*P. verreauxi*) or apple (*E. fulvus, H. griseus*, and *V. variegata*).
[d]Vitamin/mineral mix met or exceeded NRC nonhuman primate recommended intakes [NRC, 1978].

onto one of the experimental diets over a 1-week period. Once the animals were completely transitioned, they consumed the experimental diet for 30 days, after which they were brought inside to the experimental cages. The animals were given 7 days to adjust to housing conditions, and on day 8 the animals were dosed with markers to measure the transit of both the liquid and particulate phase of digesta through the gastrointestinal tract. Feed intake was measured, and stool and urine output were collected for 7 days, after which animals were released into their original cages and transitioned to the second experimental diet. Following a 1-week transition, the animals consumed the second experimental diet for 30 days before they were returned to the research cages for another collection period. The total feed intake and fecal output were then used to calculate the following variables: dry matter (DM), nitrogen (N), TDF, IF, SF, NDF, and ADF digestibility.

Transit Marker and Digestibility Collection Protocol

Marker passage and apparent digestibility collections were run concurrently. Two markers, chromium and cobalt, were used to measure the passage of the solid and liquid phases of the digesta, respectively. The chromium, which was prepared according to the method of Uden et al. [1980], was mordanted to the fiber particles present in the two experimental diets. The Cr concentrations were 14.2 mg/g and 15.11 mg/g of mordanted fiber for the IF and IF/SF diets, respectively. The dosage of Cr-mordanted fiber was 0.1 g of Cr-mordant per kg of BW. The Cr-mordant was mixed into 5–10 g of pulverized biscuit and combined with 5 mL of mango nectar and water to form a ''dough ball'' that was offered to

each animal prior to their morning meal. After the animals consumed this marker, they were given 1 mL of cobalt-EDTA (0.02 mg Co/mL) in liquid form by mouth via a 3-cc syringe.

After the markers were administered, the animals were watched for the first 6 hr, and any stools produced were collected and frozen at –20°C for later analysis. From 6 hr through the first 24 hr, stools were collected every 3 hr. Stools were then collected every 6 hr for the next 3 days, and every 12 hr for the last 3 days. Subsamples of the food offered, as well as any refusals that remained each day, were collected and the amounts were recorded for each animal over the 7-day period. These were also frozen at –20°C for later analysis. All urine specimens were collected daily and frozen at –20°C for later analysis of Cr and Co concentrations to check for possible marker absorption. We determined marker recovery by quantifying the total fecal and urine Cr and Co, and calculating the percentage recovered as a factor of the original dose.

Chemical Analyses for Determination of Digestibility

We determined the DM of the feed, refusals, and feces by freeze-drying the samples to a constant weight. Feed, refusal, and fecal N was determined via Kjeldahl analysis [Association of Official Analytical Chemists, 1990]. The NDF and ADF of feed, refusals, and feces were determined with the use of a modified version of the Van Soest procedure [Van Soest, 1967] developed by Ankom Technologies (Fairport, NY). The TDF, IF, and SF were determined by the methods outlined by Prosky et al. [1984, 1992].

Cr and Co Determination and Transit Analysis

The concentrations of Cr and Co in feces and urine were determined via neutron activation analysis at the Nuclear Services Facility at North Carolina State University [Kennelly et al., 1982]. The samples were irradiated for 12 hr, and radioactivity was quantified on a germanium detector (ORTEC, Oak Ridge, TN) after 1 month of radioactive decay to eliminate background radiation. The radioisotopes used to determine the Co and Cr concentrations in the samples were Co-60 and Cr-51, respectively. The transit time (TT) of the markers was recorded as the time of first appearance in the feces, and the mean retention time (MRT) was calculated with the following equation:

$$\mathrm{MRT\,(h)} = (\Sigma \mathrm{M_i T_i})/\Sigma \mathrm{M_i}$$

Where $\mathrm{M_i}$ is the amount of marker excreted in the i-th defecation at time $\mathrm{T_i}$ [Caton et al., 2000].

Statistical Analysis

The data were analyzed as a completely randomized design by means of the PROC MIXED procedure in SAS, a procedure that is recommended for unbalanced designs (SAS Statistical Software, Cary, NC). Species, diet, and species $\times$ diet were fixed effects in the model, and animal (species) and period were both random effects. Means were estimated by the least squared means procedure, and the PDIFF procedure was used to determine differences among or between means. The level of significance was $P < 0.05$.

RESULTS

DM Intake, DM Digestibility (DMD), and N Digestibility

The initial and final body weights are listed in Table II. Although most animals on the research diets maintained body weight, some animals with low DM intakes lost weight during the research protocol. Animals with very low intakes (one EF, one VV, and two PVs) were removed from the study. DM intake (expressed per kilogram of body weight) was highest for EF ($P < 0.05$). DM intake values for PV and VV were similar to each other and lower than in EF, and intermediate in HG (Table III). The average values for DM intake of the animals included in the study were $>2\%$ of body weight for all species. The animals with lower intakes had adequate intakes when they were in regular cages, so low intakes were generally attributed to the stress of indoor housing and separation from cage mates.

DMD differed across diets ($P < 0.05$) and species ($P < 0.0001$; Table III). Only main effects are presented, due to a lack of significant interactions. The DMD was

TABLE II. Initial and Final Weights, Averaged by Species, of Animals Used in the Research Protocol

Species	Initial weight, g	Final weight, g
V. variegata[a]	3720.0 ± 123.7	3524.0 ± 80.6
E. fulvus[b]	2274.0 ± 50.5	2243.2 ± 43.7
P. verreauxi[c]	3372.0 ± 77.2	3420.0 ± 103.6
H. griseus[d]	1042.0 ± 109.7	1058.0 ± 110.4

[a]n = 5.
[b]n = 5.
[c]n = 4.
[d]n = 6.

TABLE III. Dry Matter Intake, Apparent Dry Matter Digestibility, and Nitrogen Digestibility Across Species and Diet *

	Dry matter intake	Dry matter digestibility	Nitrogen digestibility
Species			
V. variegata	22.28 ± 3.0^{A}	55.6 ± 1.4^{A}	65.2 ± 1.9^{A}
E. fulvus	31.76 ± 3.0^{B}	58.6 ± 1.4^{A}	63.7 ± 1.9^{A}
P. verreauxi	20.87 ± 3.2^{A}	71.9 ± 2.0^{B}	66.1 ± 2.0^{A}
H. griseus	25.96 ± 2.8^{AB}	76.3 ± 1.9^{B}	71.6 ± 1.9^{B}
Diet			
IF[a]	25.1^{X}	63.9^{X}	69.0^{Y}
IF/SF[b]	25.4^{X}	67.3^{Y}	64.4^{X}
SEM[c]	2.3	0.9	1.6

*Dry matter intake is expressed as g of dry matter/kg body weight. Apparent dry matter digestibility is expressed on a percentage basis. Nitrogen digestibility is expressed on a percentage basis. Values for species or diet with similar letters within a column do not differ ($P > 0.05$).
[a]IF, insoluble fiber to soluble fiber ratio is 12.15g:1g.
[b]IF/SF, insoluble to soluble fiber ratio is 3.76g:1g.
[c]n = 20.

higher for the IF/SF diet than for the IF diet. The DMD values for VV and EF were similar to each other, and lower than those obtained for HG and PV, which were also similar to each other. Nitrogen digestibility was higher for the IF diet ($P < 0.01$). The values for nitrogen digestibility obtained for HG were higher than those for the other three species, which all had similar values ($P < 0.01$).

NDF, ADF, TDF, IF, and SF Digestibility

Diet differences were observed for NDF ($P < 0.01$), ADF ($P < 0.01$), TDF ($P < 0.0001$), and IF ($P < 0.0001$) digestibility. Again, only the main effects are reported, due to a lack of significant interactions. For these variables, the IF/SF diet had higher digestibility than the IF diet. Species differences were observed across all digestibility variables (Table IV). Results for NDF and ADF digestibility showed the pattern HG > PV > EF > VV, and the range of values was large (35.3–70.8% for NDF digestibility, and 11–68% for ADF digestibility). TDF digestibility was higher for HG than for PV. HG and PV were similar in terms of IF and SF digestibility, but there was a trend toward higher values for HG ($P < 0.10$). VV and EF were similar for TDF, IF, and SF digestibility, but there was a trend toward higher IF digestibility for EF ($P < 0.10$). For TDF, IF, and SF digestibility, the results for VV and EF were all lower than those for HG and PV.

TT, MRT, and Recovery of the Cr and Co Markers

The TT of Co differed across species ($P < 0.0001$). Values for HG were longest, while EF and VV values were similar and shortest. There was a trend toward diet differences ($P < 0.10$), with values for the IF diet longer than for the IF/SF diet (Table V). This may have been due to a trend toward a diet × species interaction ($P < 0.10$) such that HG tended to have longer TTs on the IF diet. Results for the MRT of Co were the same as for the TT, with species differences ($P < 0.0001$). HG had the longest MRTs recorded, while again the EF and VV results were similar and most rapid. For all of these transit results, PV values differed from the other species in that the results fell between those obtained for HG and the other two species.

TABLE IV. Apparent Digestibility of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Total Dietary Fiber (TDF), Insoluble Fiber (IF), and Soluble Fiber (SF) Across Species and Diet*

	NDF	ADF	TDF	IF	SF
Species					
V. variegata	35.3 ± 2.2^{A}	10.8 ± 3.1^{A}	22.7 ± 3.3^{A}	19.7 ± 3.4^{A}	34.1 ± 6.5^{A}
E. fulvus	41.5 ± 2.2^{B}	21.5 ± 3.1^{B}	28.3 ± 3.3^{A}	27.7 ± 3.4^{A}	31.9 ± 6.5^{A}
P. verreauxi	60.1 ± 2.4^{C}	46.9 ± 3.4^{C}	56.0 ± 3.5^{B}	53.1 ± 3.6^{B}	65.4 ± 7.4^{B}
H. griseus	70.9 ± 2.2^{D}	67.8 ± 2.8^{D}	66.2 ± 3.0^{C}	62.3 ± 3.2^{B}	84.3 ± 6.0^{B}
Diet					
IF[a]	47.4^{X}	32.8^{X}	38.2^{X}	34.5^{X}	55.3^{X}
IF/SF[b]	54.7^{Y}	40.7^{Y}	48.4^{Y}	46.9^{Y}	52.5^{X}
SEM[c]	1.3	1.9	2.0	2.2	4.7

*All values are expressed on a percentage basis. Values for diet and species with similar letters within a column do not differ ($P > 0.05$).
[a]IF, insoluble fiber to soluble fiber ratio is 12.15g:1g.
[b]IF/SF, insoluble to soluble fiber ratio is 3.76g:1g.
[c]n = 20.

TABLE V. Transit Time (TT) and Mean Retention Time (MRT), in Hours, of Chromium and Cobalt Across Species and Diet*

	TT Co	TT Cr	MRT Co	MRT Cr
Species				
V. variegata	4.03 ± 1.5^{A}	3.88 ± 1.5^{A}	7.50 ± 3.0^{A}	7.92 ± 2.4^{A}
E. fulvus	3.25 ± 1.5^{A}	3.15 ± 1.5^{A}	10.43 ± 3.0^{A}	7.94 ± 2.4^{A}
P. verreauxi	24.42 ± 1.7^{B}	24.64 ± 1.8^{B}	33.62 ± 3.3^{B}	35.64 ± 2.7^{B}
H. griseus	30.60 ± 1.3^{C}	29.96 ± 1.4^{C}	45.88 ± 2.8^{C}	47.50 ± 2.1^{C}
Diet				
IF[a]	16.95^{X}	16.88^{X}	26.55^{X}	24.94^{X}
IF/SF[b]	14.20^{X}	13.94^{X}	22.17^{X}	24.56^{X}
SEM[c]	1.1	1.1	2.2	1.7

*Values for diet and species with similar letters within a column do not differ ($P > 0.05$).
[a]IF, insoluble fiber to soluble fiber ratio is 12.15g:1g.
[b]IF/SF, insoluble to soluble fiber ratio is 3.76g:1g.
[c]n=20.

The TT of Cr also differed across species ($P < 0.01$; Table V). EF and VV showed the most rapid TTs while those reported for HG were slowest ($P < 0.05$). There was a diet $\times$ species interaction, with the slowest TTs recorded for HG on the IF diet (35.5 ± 2.2 hr) compared to the other species. This likely explains the observed trend ($P < 0.10$) toward a faster TT for the IF/SF diet compared to the IF diet (Table V). The MRT of Cr also differed across species ($P < 0.0001$). HG showed the longest MRT, and values were again similar and most rapid for EF and VV, with values for PV falling between HG and the other two species.

Recovery of Cr and Co across all animals was $90.0\% \pm 24.0\%$ and $76.8\% \pm 14.0\%$, respectively. Over-recovery of Cr may have been due to the fact that animals were housed in stainless steel cages, which can contain up to 20% chromium [Lula, 1986]. In fact, urine values were not included in the recovery totals for Cr because it is likely that acidification of the urine resulted in liberation of Cr from the stainless steel. Since urine was separated from feces, acidification of the urine should not have affected fecal recovery of Cr; however, the animals were exposed to stainless steel, which may have resulted in the over-recovery.

DISCUSSION

Animals in the study generally consumed less food when housed in the research cages. This was likely related to both a reduction in activity levels and increased stress due to the cage conditions [Schneider & Flatt, 1975]. In most cases, intakes increased to adequate levels after the first few days, and then remained consistent throughout the collection period. Since quantification of intake and fecal output necessitates that animals be separated for the duration of the adjustment and collection period, some degree of stress was unavoidable. However, visual, auditory, and olfactory contact between pairs should have reduced this stress. While a marked reduction in feed intake can cause an increase in digestibility and a decrease in transit, stress itself can cause the opposite effect [Schneider & Flatt, 1975], which makes it difficult to assess the impact of either factor on the results.

Two types of fiber analysis were used in this study: the NDF-ADF system outlined by Van Soest [1967], and the TDF system outlined by Prosky et al. [1984, 1992]. The NDF-ADF system was used to allow for comparison with data from published work. The rationale for measuring TDF, IF, and SF was that diets consumed by free-ranging lemurs most likely differ not only in the quantity of fiber in the diet, but also in the fiber type (IF and SF), and that these differences in consumption may relate to differences in efficiency of SF and/or IF utilization. The measured NDF and IF values obtained for each diet differed (Table I); however, this difference was consistent across the two diets. In both experimental diets, the IF amount determined was approximately 15% less than the NDF value. This resulted in measured NDF intakes that were approximately 15% greater than IF intakes for all animals in the study. Theoretically, NDF and IF should reflect measurement of the same dietary components (hemicellulose, cellulose, and lignin). Some studies, however, have indicated that measured NDF and IF values can differ under some conditions [Marlett, 1988]. The most common problem is inadequate removal of resistant starch and protein from NDF samples, which results in an overestimation of NDF [Robertson & Van Soest, 1981]. Overestimation can also be due to inadequate removal of SF in high-SF diets [Marlett, 1988]. Heat-stable amylase has been added to the updated van Soest procedure in an effort to remove the excess starch and improve accuracy, but this still may not be adequate for some high-starch diet items. In the TDF-IF-SF procedure, both heat-stable amylase and protease enzymes are used, and a further nitrogen determination step ensures that all nitrogen present has been corrected for in the final calculation.

The NDF and IF values obtained for feces did not differ, which adds some support to the claim that dietary NDF was overestimated. Digestion within the animal effectively removed the resistant starch and/or protein. If NDF intakes were overestimated, but NDF in the feces were not, this would result in an overestimation of NDF digestibility and is the likely explanation for higher NDF digestibility results. This suggests that in these types of analyses (particularly when high starch and protein feed ingredients are used), the IF digestibility values are more reliable than those obtained for NDF, and should be considered with greater emphasis in species comparisons. Further, measurement of TDF is also useful because it provides a measurement for all of the fiber components in a diet combined.

Estimated TT and MRT values across both the particle (Cr-mordant) and the liquid phase (Co-EDTA) markers did not differ. The results for PV were similar to those observed by Campbell et al. [1999]. Edwards and Ullrey [1999b] also reported no large differences in the passage of two markers given to *Alouatta* sp. fed either a 24% or 39% NDF diet. However, the current results differ from those published by Caton et al. [2000] for *Galago moholi*, an omnivorous prosimian that appears to selectively retain the liquid phase of digesta for fermentation. In most herbivores that possess a large cecum for processing fiber, the MRT of a fluid marker is generally much greater than that of a particle marker [Stevens & Hume, 1995]. Of the species tested in this experiment, PV in particular possesses both a large, sacculated cecum and a long, spiraled colon. This suggests that selective retention might be a factor; however, it may not have been observed because of the dosage-time and collection pattern in this study. After dosage of the markers, samples were rarely collected between the hours of 8 P.M. and 6 A.M. because the animals generally did not produce feces at late-night collection times. Dosage of markers in the evening may improve accuracy in future marker-passage studies.

The results from this study confirm that a wide variety of digestive strategies accompany the equally wide range of dietary profiles observed in these lemurs. The differences observed also compare well with the database of information on all herbivorous species [Stevens & Hume, 1995]. Some data were not expected–particularly the results for HG. The gastrointestinal anatomy of HG differs from that of other lemur species in that the entire gut is short relative to body length, and the cecum is blunt and rounded rather than lengthy and haustrated. The colon also differs in that it is shortened and well haustrated [Campbell et al., 2000]. The results for DMD, IF, and SF digestibility were similar for HG and PV, but values for HG were higher for N, NDF, ADF, and TDF digestibility (the NDF values are likely overestimates, however, due to the overestimation of NDF in the feed). This suggests that their capacity for dietary processing is similar to, if not slightly greater than, that of PV. Both NDF and IF digestibility results were also higher in comparison to data obtained from other primates that possess a postgastric fermentation chamber and consume a fibrous diet (*Alouatta* sp., 44.8% NDF digestibility when fed a 24% NDF diet [Edwards & Ullrey, 1999b]). In fact, within the primates, the values obtained for HG show similarities only to Colobine primates, which utilize a pregastric fermentation chamber to process dietary fiber (77.1% NDF digestibility when fed a 24% NDF diet [Edwards & Ullrey, 1999b]). Only one other study has reported NDF digestibility values for HG [Klein, 1991], and the values were similar to those obtained in this study.

Published TTs of plastic markers fed to captive HG [Overdorff & Rasmussen, 1995] were similar to the TTs of both markers used in this study. When these data are examined together with the digestibility results, it seems clear that HG differs greatly from the other lemurs in physiological as well morphological adaptations to its bamboo diet. Information about patterns of transit within the tract may explain why residence times are longer for HG despite the dramatic differences in gastrointestinal tract morphology compared to PV.

Given the high amounts of IF in the folivorous diet of PVs, we expected that the digestibility and transit values for PV would be similar to or greater than those for HG. For some variables, however, PV was less efficient than HG, and marker passage times were shorter. Digestibility values obtained for PV in a previously published study in which animals were fed a 33% NDF diet [Campbell et al., 1999] were lower than those reported in this study. In the previous study, results for DMD, crude protein, NDF, and ADF digestibility were 65%, 64%, 41%, and 35%, respectively. This difference could be related to the different ingredient composition of the biscuits used in the two trials. Ground soybean hulls and dehulled soybean meal were the first two ingredients in the commercially available biscuit used in the previous study (Mazuri Leaf-Eater Primate Mini-Biscuit #5672; Purina Mills, St. Louis, MO), while ground corn and dehulled soymeal were the first two ingredients in both of the experimental diets used in the present study. The NDF and ADF digestibility results obtained for PV in both experiments were still lower than those observed for HG. The results for PV are higher than reported data from other primates, such as *Alouatta* sp., that utilize postgastric fermentation to process fibrous diets [Edwards & Ullrey, 1999b], but again this could be attributable to differences in biscuit composition.

Values for DMD, N digestibility, and digestibility of the fiber components (TDF, IF, and SF) were similar for VV and EF. These data are interesting in that feeding-ecology data suggest that EF may consume more fiber in its wild diet compared to VV. An examination of the gastrointestinal anatomy revealed a degree of similarity that may help to explain these results [Campbell et al., 2000; Hill, 1953]. Moreover, in vitro fermentation trials showed that bacteria present in

fecal innoculum collected from EF were not any more efficient at processing fiber than bacteria in VV [Campbell et al., 2002]. The only data that supported the possibility that EF might be more efficient at fiber utilization than VV were the results for NDF and ADF digestibility. It is possible that EF uses fiber more efficiently than VV, but the conflicting results from the two fiber analysis methods used in this study make it difficult to make this claim. Given the limitations of the NDF methodology, the IF results are most likely the more accurate measurement of IF digestibility. Further research using diets that differ to a greater degree in fiber content and type may clarify whether EF is better able to utilize the IF portion of its diet than VV.

Since intakes were similar for both diets, palatability values did not appear to differ. However, the IF/SF diet was generally more digestible than the IF diet across most measured variables, even though TT and MRTs were similar. Thus, the increase in digestibility was not due to longer residence time. The ingredients present in the IF/SF diet were more rapidly processed and thus better utilized by the animal. This may have been due to the fact that a significant portion of the IF/SF diet was beet pulp and citrus pulp, ingredients that are more rapidly fermented than the oat hulls in the IF diet [Bourquin et al., 1996]. These diet differences generally did not result in diet $\times$ species interactions, which would have indicated among-species differences in fiber utilization across the two diets. Again, if the fiber types differed more dramatically between the two diets, then perhaps species differences related to fiber source would have been observed.

The diets used in this project were formulated to be similar in amount but different in composition of TDF. Both diets possessed approximately 28% TDF, and the high IF diet had a fiber ratio of 12.75:1 IF to SF. The mixed-fiber diet (IF/SF) had a fiber ratio of 3.75:1 IF to SF. The estimated lower limit of IF in the diet was set by the inclusion of PV, a species that appears to require a captive diet that is at least moderately high ($>$25% IF) in IF in order to maintain optimal gastrointestinal health (DUPC medical records). The upper limit was set by VV, a species that has experimentally been shown to be unable to maintain body weight when fed diets that are moderately high in IF [Edwards & Ullrey, 1999a]. This limited the degree to which the experimental diets could differ. Despite this limitation, this research provides solid species comparisons and evidence for large differences in digestive efficiency and fiber utilization. VV and EF were generally similar in their ability to process these diets, and HG and PV both showed an enhanced degree of fiber utilization that is comparable to other primates known to consume diets high in IF. To characterize fiber utilization in HG more clearly, their digestive efficiency should be investigated further by feeding them diets that are high in IF, as well as diets that differ in particle size and/or fiber source. Now that species comparisons have been made using similar diets, trials that focus on diets that differ based on specific aspects of feeding ecology can further define the relationship between diet, anatomy, and physiology that is unique to each species.

ACKNOWLEDGMENTS

We thank the DUPC for supporting this project, and Purina Mills, Inc., for providing the experimental diets. We also thank Kelly Glenn, Bill Hess, Emily Dill, and other primate technicians for their assistance in coordinating the care and use of the animals during the research protocol. In addition, we thank Samantha Trull and Gauri Pradhan for their assistance in animal care, data collection, and analysis.

REFERENCES

Association of Official Analytical Chemists. 1990. Official methods of analysis. 15th ed. Washington, DC: AOAC.

Bourquin LD, Titgemeyer EC, Fahey GC. 1996. Fermentation of various dietary fiber sources by human fecal bacteria. Nutr Res 16:1119–1131.

Britt A. 2000. Diet and feeding behavior of the black and white ruffed lemur (*Varecia variegata variegata*) in Betampona Reserve, eastern Madagascar. Folia Primatol 71: 133–141.

Campbell JL, Eisemann JH, Glander KE, Crissey SE. 1999. Intake, passage, and digestibility of a commercially designed diet by two Propithecus species. Am J Primatol 48:237–246.

Campbell JL, Eisemann JH, Williams CV, Glenn KM. 2000. Description of the gastrointestinal tract of five lemur species: Propithecus tattersalli, Propithecus verreauxi coquereli, Varecia variegata, Hapalemur griseus, and Lemur catta. Am J Primatol 52:133–142.

Campbell JL, Williams CV, Eisemann JH. 2002. Fecal innoculum can be used to determine the rate and extent of in vitro fermentation of dietary fiber sources across three lemur species that differ in dietary profile: *Varecia variegata, Eulemur fulvus*, and *Hapalemur griseus*. J Nutr 132: 3073–3080.

Caton JM, Laws M, Cunningham C. 2000. Digestive strategy of the south-east African lesser bushbaby, Galago moholi. Comp Biochem Phys A 127:39–48.

Edwards MS, Ullrey DE. 1999a. Effect of dietary fiber concentration on apparent digestibility and digesta passage in nonhuman primates. I. Ruffed lemurs (*Varecia variegata variegata* and *V. v. rubra*). Zoo Biol 18:529–536.

Edwards MS, Ullrey DE. 1999b. Effect of dietary fiber concentration on apparent digestibility and digesta passage in nonhuman primates. II. Hindgut and foregut-fermenting folivores. Zoo Biol 18:537–549.

Hill WCO. 1953. Primates, comparative anatomy and taxonomy. I. Strepsirhini. Edinburgh: Edinburgh Press. 798 p.

Kennelly JJ, Turner BV, Apps MJ. 1982. Determination of non-radioactive liquid phase markers, Co-EDTA and Cr-EDTA, by neutron activation analysis. J Sci Food Agric 233:1235–1243.

Klein M. 1991. Digestibility of dietary fiber and passage rate in brown lemurs and gentle lemurs. Master's thesis, Duke University, Durham, NC. 55 p.

Lambert JE. 1998. Primate digestion: interactions among anatomy, physiology, and feeding ecology. Evol Anthropol 7: 8–20.

Lula RA. 1986. Stainless steel. Ohio: American Society for Metals. 173 p.

Marlett J. 1988. Analysis of fiber in human foods. In: Kritchevsky D, Bondfield C, Anderson JW, editors. Dietary fiber: chemistry, physiology, and health effects. New York: Plenum Press. p 31–48.

National Research Council. 1978. Nutrient requirements of nonhuman primates. Washington, DC: National Academy Press. 83 p.

Overdorff DJ. 1993. Similarities, differences, and seasonal patterns in the diets of *Eulemur rubriventer* and *Eulemur fulvus* in the Ranomafana National Park, Madagascar. Int J Primatol 14:721–753.

Overdorff DJ, Rasmussen MA. 1995. Determinants of nighttime activity in ''diurnal'' lemurid primates. In: Alterman L, editor. Creatures of the dark. New York: Plenum Press. p 61–84.

Prosky L, Asp NG, Furda I, Devries JW, Schweizer TF, Harland BF. 1984. Determination of TDF in foods, food products, and total diets: interlaboratory study. J Assoc Off Anal Chem 67: 1044–1052.

Prosky L, Asp NG, Schweizer TF, Devries JW, Furda I. 1992. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. J Assoc Off Anal Chem 75:360–367.

Richard A. 1977. The feeding behavior of *Propithecus verreauxi*. In: Clutton-Brock TH, editor. Primate ecology: studies of feeding and ranging behavior in lemurs, monkeys, and apes. London: Academic Press. p 71–96.

Robertson JB, Van Soest PJ. 1981. The detergent system of analysis and its application to human foods. In: James WPT, Theander O, editors. The analysis of dietary fiber in food. New York: Marcel Dekker. 276 p.

Schneider BH, Flatt WP. 1975. The evaluation of feeds through digestibility experiments. Athens: University of Georgia Press. 423 p.

Southgate DAT. 1991. Determination of food carbohydrates. 2nd ed. Essex: Elsevier Press. 232 p.

Stevens CE, Hume ID. 1995. Comparative physiology of the vertebrate digestive system. Cambridge: Cambridge University Press. 400 p.

Tan CL. 1999. Group composition; home range size and diet of three sympatric bamboo lemur species (genus *Hapalemur*) in Ranomafana National Park, Madagascar. Int J Primatol 20:547–566.

Uden P, Colucci PE, Van Soest PJ. 1980. Investigation of chromium, cerium, and cobalt as markers in digesta: rate of passage studies. J Sci Food Agric 31:625–632.

Van Soest PJ. 1967. Development of a comprehensive system of feed analysis and its application to forages. J Anim Sci 26: 119–127.

Van Soest PJ. 1994. Nutritional ecology of the ruminant. Ithaca, NY: Cornell University Press. 476 p.