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In Vitro Fermentation of Dietary Carbohydrates Consumed by African Apes and Monkeys: Preliminary Results for Interpreting Microbial and Digestive Strategy

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Abstract Primates derive significant nutritional benefits from hosting symbiotic, fermenting microbes in their gut, including the provision of energy from short-chain fatty acids (SCFAs). We measured fermentation parameters in fecal samples of 4 hindgut fermenting species (*Gorilla gorilla*, *Pan troglodytes*, *Papio hamadryas*, *Cercopithecus neglectus*) and 1 foregut fermenting species (*Colobus guereza*) to determine whether differences in SCFA profiles exist between the 2 digestive systems. We analyzed SCFA profiles, culture pH, and methane from fecal samples collected at the North Carolina Zoological Park. Results indicate that the captive individuals exhibit high total SCFA concentrations, with a particularly high acetate-to-propionate ratio. Methane levels were highest in the 2 ape species, and lowest in *Colobus guereza* and *Cercopithecus neglectus*. Total SCFA concentrations were similar between wild and captive-born *Gorilla gorilla*, although concentrations of acetate were significantly greater —and butyrate concentration and methane production significantly lower— in wild-caught gorillas relative to captive-born. These results are consistent with data on diet and digestive retention times among Catarrhini and have implications for our understanding of the interactions among diet, body mass, digestion, and how monogastric cercopithecines can consume either similar or greater levels of fiber than larger-bodied apes.

Keywords Cercopithecinae · Colobinae · *Colobus* · Diet · *Gorilla* · Nutritional ecology · *Pan* · *Papio* · Short-chain fatty acids

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Introduction

Mammalian herbivore clades can be characterized on the basis of where in the gastrointestinal tract most alloenzymatic (microbial) fermentation of dietary carbohydrate occurs (Alexander 1993; Chivers 1994; Clauss *et al*. 2007; Edwards and Ullrey 1999; Kay and Davies 1994; Stevens and Hume 1995; Van Soest 1996). So-called foregut fermenters have evolved an alloenzymatic system facilitating microbial action on plant foods before entering acid and enzyme-producing regions of the gut; microbes reside in 1 to several sections of a sacculated stomach (Clauss *et al*. 2007; Parra 1978; Penry 1993). Foregut fermentation exists as a strategy both with and without commensurate rumination (McDonald and Warner 1975; Van Soest 1982). Hindgut fermenters autoezymatically digest plant foods first in the stomach and small intestine via endogenous enzymes before the food bolus enters the large intestine; fermenting microbes reside in either a modified caecum or enlarged colon, or both (Chivers and Hladik 1980; Kay and Davies 1994; Parra 1978; Penry 1993). Among primates, hindgut fermentation is the ancestral state. Foregut fermentation without rumination has evolved only among the Colobinae (Kay and Davies 1994; Milton 1998; Matsuda *et al.* 2011).

Microbe communities residing in either a modified stomach or expanded regions of the large intestine comprise hundreds of bacterial, protozoan, and fungal species that metabolize nutrients (carbohydrates and, to a lesser extent, amino acids) from plant foods ingested by the vertebrate host using them for ATP and carbon (Kay and Davies 1994; Stevens and Hume 1995). Researchers have studied the digestive microbiology of domesticated ruminating ungulates, e.g., sheep (*Ovis aries*) and cattle (*Bos taurus*), intensively for many decades; this literature is our primary source for understanding foregut fermentation systems (Bryant and Burkey 1953; Blaxter *et al*. 1956; Goering and Van Soest 1970; Kay and Davies 1994). Research on hindgut fermentation has focused on domesticated pigs (*Sus domestica*) and horses (*Equus caballus*) and —more recently— the utility of intestinal bacteria for human health (Dethlefsen *et al*. 2006; Gropper *et al*. 2008; Titgemeyer *et al*. 1991; Walker and Buckley 2006).

Regardless of where gastrointestinal microbes reside in the gut, the primary advantage of their presence is that they allow the host to use plant fiber (structural carbohydrates) as a source of energy in the form of fatty acids (Bergman 1990; Campbell *et al*. 2002; Clauss *et al*. 2007; Kay and Davies 1994). These acids comprise the short-chain fatty acids (SCFAs) acetate, butyrate, and propionate, and longer-chain acid lactate, which is converted to acetate and propionate by lactate-metabolizing bacteria. SCFAs impact the host in a number of beneficial ways, including gastrointestinal cell proliferation, reduction of luminal pH (which facilitates nutrient absorption) and providing substrate for cellular energy (Backhed *et al*. 2005; Gorbach 1990; Walker and Buckley 2006). For example, butyrate may be absorbed via a Na^+/H^+ and or a K^+/H^+ exchange and —in colon fermenters— serves as an ATP source for colonic epithelial cells (Backhed *et al*. 2005; Bergman 1990; Milton 1998; Milton and McBee 1983). However, despite the fact that SCFAs contribute substantially to a primate's energetic balance, with notable exceptions (Campbell *et al*. 2002; Clemens and Phillips 1980; Milton and McBee 1983; Schmidt *et al*. 2005; Ushida *et al*. 2006), microbial metabolism and it impact on

wild, nonhuman primate feeding biology and digestive strategy remain poorly described.

Microbial fermentation and digestive efficiency are often discussed in light of information on mammal body size. Fermentation takes time, and larger mammals have larger guts with putatively longer digestive retention times. It is thus generally held that larger body mass is advantageous to herbivorous mammals because of mass-specific energy requirements (MR) to gut capacity (GC) ratios (MR/GC) and associated fermentation levels: smaller mammals have relatively higher energetic requirements, shorter retention times, and low levels of fermentation, whereas larger herbivorous mammals have relatively lower energetic requirements, longer retention times, and high levels of fermentation (Cork and Foley 1991; Demment and van Soest 1983; Kay 1985; Kay and Davies 1994; McNab 2002; Parra 1978; Van Soest 1996). However, these generalizations do not appear to hold among Catarrhini (Lambert 1997, 1998, 2002a). Indeed, both cercopithecoid subfamilies have relatively longer retention times than order-of-magnitude larger apes despite relatively higher energetic requirements for their body mass. This would have been predicted for folivorous, foregut-fermenting colobines, but not for cercopithecines. Recent analyses suggest that a poor correlation between digestive retention times and body mass is true for other mammal species as well (Clauss *et al*. 2007; Steuer 2010).

We here evaluate total production of SCFAs among primates differing in body mass by an order of magnitude, e.g., 6 kg vs. >100 kg. We measured *in vitro* fermentation parameters from fecal samples of 5 captive primate species, including 4 autoenzymatic, hindgut-fermenting species (*Gorilla gorilla*, *Pan troglodytes*, *Papio hamadryas*, *Cercopithecus neglectus*) and 1 alloenzymatic, foregut-fermenting species (*Colobus guereza*). We present data on acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, and methane concentrations, with a goal of broadening our understanding of digestive and feeding biology among Catarrhini, the only primate infraorder comprising a lineage with adaptations for foregut fermentation (Colobinae), as well as species with the primitive primate condition of hindgut fermentation (Cercopithecinae, Hominoidea).

Methods

Sampling

All subjects were in good health, and the experimental protocol was established in concordance with zoo enclosure maintenance. We collected fresh fecal samples from 5 African Catarrhini species housed at the North Carolina Zoological Park (Ashboro, NC) including 2 pongids, *Gorilla gorilla gorilla* (western lowland gorilla) and *Pan troglodytes schweinfurthii* (common chimpanzee); 2 cercopithecines, *Papio hamadryas* (hamadryas baboon) and *Cercopithecus neglectus* (DeBrazza's guenon); and 1 colobine, *Colobus guereza* (black-and-white colobus monkey) (Table I). Previous research on digestive physiology on multiple catarrhine species (Lambert 2002a; *unpubl. data*) has demonstrated that although defecation can occur throughout the night and day, more defecation takes place in the morning (06:00–09:00 h) than at any other time of a 24-h period ($\chi^2=29.3$; $p<0.1$; df=7). Thus, NC Zoo keepers and

Table I Catarrhine focal subjects from which fecal samples were collected at the North Carolina Zoo, July 2007: Number of adults, adult sex ratio, and number of adults born in captivity and in the wild

Species	Adult *N*	Male/female	Captive born/wild born
Gorilla gorilla gorilla	5	2:3	3:2
Pan troglodytes schweinfurthii	14	5:9	10:4
Papio hamadryas	20	8:12	20:0
Cercopithecus neglectus	2	1:1	2:0
Colobus guereza	2	1:1	2:0

J. E. Lambert collected all fecal samples for the focal subjects over the course of a 90-min morning period (07:00–08:30 h) in a single day (July 23, 2007). NC Zoo keepers rotated sampling efforts from one enclosure to another; after sufficient fecal sample was collected for a species (typically within 15 min), effort shifted to another species, until all 5 species had been sampled in a single period of collection effort. Previous work in the ruminant fermentation laboratory at North Carolina State University (directed by V. Fellner) suggested that *ca.* 0.5 kg of fecal sample is sufficient for culturing fecal inoculum, which is the approximate equivalent of 1 quart-sized Ziploc® bag. Thus, sample collection concluded after 1 quart-sized Ziploc® bag was filled and collected per species. We collected fecal samples from adults within 30 min of defecation (typically sooner); duration depended on availability of trained NC Zoo staff and how quickly a keeper could safely enter the animal enclosure. Because our research addressed questions regarding species-level adaptations, we pooled samples taken from captive-born individuals to obtain 1 inoculum for that species. In addition, we collected representative fecal samples from wild-born *Gorilla gorilla* and compared them with samples from the captive-born *Gorilla gorilla*.

Although previous work has demonstrated that several strictly anaerobic bacteria species have the ability to thrive after minimal exposure to air (via lowering the reduction–oxidation potential), exposure to aerobic conditions is generally detrimental and can result in bacteria oxidation and die-off (Uesugi and Yajima 1978). Fecal bacteria have the potential to be exposed to air immediately after defection. Thus, we used controlled anaerobic methods to minimize exposure to air during sample collection, transportation, manipulation, and maintenance, although some amount of aerobic exposure cannot be ruled out. Following Campbell *et al*. (2002), we placed fresh fecal samples into quart-sized, waterproof plastic Ziploc® bags, sealed them, and then placed them into a second Ziploc® bag. We placed each bag immediately into a 37°C air-tight thermos, then put this thermos into a larger insulated box for immediate transport to the ruminant fermentation laboratory at North Carolina State University. All samples arrived to the laboratory for analysis within 3 h of defecation. In addition to fecal samples, we also collected a sample of each species' diet of plant material and extruded food (Tables II and III). We used this foodstuff to provide an appropriate growth medium in the fermentation tubes (see Laboratory Procedure). Note that although diet was not completely standardized, dietary differences can be indirectly assessed: reported millimolar (mM) SCFA concentrations reflect substrate differences, while SCFA molar percentages reflect microbial activity.

Table II Primate species, group composition, and diets offered to the focal subjects providing fecal samples for *in vitro* fermentation assay

Species	Diet (g/indv)	
	Chow formulation	Fresh plant parts
Colobus guereza	81 g Marion Zoological Leaf Eater Diet®	228 g Vegetative plant parts
	163 g Mazuri Growth & Reproductive Diet®	161 g Fruit
		162 g Modified root
Cercopithecus neglectus	83 g PMI LabDiet Fiber Plus® (5049)	50 g Vegetative plant parts
	75 g Marion Zoological Leaf Eater Diet®	161 g Fruit
		88 g Modified root
Papio hamadryas	126 g PMI LabDiet Fiber Plus® (5049)	152 g Vegetative plant parts
	126 g Marion Zoological Leaf Eater Diet®	267 g Fruit
		119 g Modified root
Pan troglodytes	272 g PMI LabDiet Fiber Plus® (5049)	657 g Vegetative plant parts
	272 g Marion Zoological Leaf Eater Diet®	680 g Fruit
		250 g Modified root
Gorilla gorilla	80 g PMI LabDiet Fiber Plus® (5049)	1460 g Vegetative plant parts
	780 g Marion Zoological Leaf Eater Diet®	1928 g Fruit
		708 g Modified root

Laboratory Procedure

We processed all samples immediately on arrival to the laboratory. Following Williams *et al.* (2005), we used a batch system in which fecal samples (inoculum) were used to inoculate culture bottles. We used glass incubation bottles with rubber septums and crimp tops to prevent escape of fermentation products and gases. The incubation bottles contained the test substrate (food for microbes) and the fecal inoculum. The test substrates consisted of a blended sample of the sample primate diet. We placed both the extruded diet and plant parts on aluminum pans, freeze-dried the food, and then ground it through a 1-mm screen. We added the completely mixed/blended substrate into the inoculation bottles and added a total of 0.5 g of substrate to each culture bottle. The composition of the medium we used to culture the microbes is listed in Table IV.

After thoroughly mixing the fecal samples (pooled by species), we diluted (1:5) each sample in a previously warmed (37°C) anaerobic dilution solution (Bryant and Burkey 1953), which we then blended for 15 s in a Waring blender. We filtered the blended, diluted feces through 4 layers of cheesecloth, and sealed the filtrate in 250-ml bottles under CO_2. We inoculated the appropriate sample and blank serum bottles (containing 30 ml of medium and 0.5 g of substrate) with 5 ml of diluted feces. This solution provided a 1.4:100 dilution of the substrate: 0.5 g of substrate/(30 ml of medium+5 ml of diluted feces). We flushed the bottles with CO_2, capped them with rubber stoppers, sealed them with crimp tops, and placed the bottles in a water bath (37°C). Blank bottles (in duplicate) contained 5 ml of diluted feces and 30 ml of medium but no substrate. Control bottles (in duplicate) contained 0.5 g substrate and

Table III Macronutrient content of diets, including extruded diet and the vegetative parts (leaves, petioles), reproductive parts (fruits, seeds), and modified roots of domesticated plant species

Food category	Food type	Gross energy (kcal/g)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	NDF (%)	ADF (%)
Extruded	Marion Zoological Leaf Eater Diet®	3.1	22.6	5.23	11.1	23.3	14.5
Extruded	Mazuri Primate Growth & Reproductive Diet®	2.7	22.0	7.2	8.0	17.5	9.3
Extruded	PMI LabDiet Fiber-Plus Monkey Diet®	3.0	21.2	5.0	9.5	21.5	12.1
Vegetative plant part (leaves and petioles)	Celery, raw (*Apium graveolens*)	3.83	14.0	2.6	31.7	15.7	12.6
	Parsley, raw (*Petroselinum crispum*)	24.1	6.4	0.94	26.9	21.1	17.8
	Romaine lettuce, raw (*Lactuca setiva*)	4.06	31.8	3.9	33.4	16.3	14.1
	Spinach, raw (*Spinacia oleracea*)	3.99	34.0	4.2	32.1	20.1	11.7
Reproductive plant part (fruits and seeds)	Apple, raw with peel (*Malus sylvestris*)	4.2	1.2	2.2	16.8	10.2	6.0
	Banana, raw with peel (*Musa sapientum*)	4.17	4.0	1.9	9.3	5.4	
	Green snap beans, raw (*Phaseolus vulgaris)*	4.19	18.7	1.2	34.9	21.9	18.3
	Peas, raw (*Pisum sativum*)	1.24	25.6	1.3	—	14.3	8.1
Modified plant root	Carrot, raw (*Daucus carota*)	4.04	8.4	1.6	—	9.7	8.9
	Sweet potato, raw with peel (*Ipomoea batatas*)	4.14	6.1	1.1	11.0	20.0	4.9
	White potato, raw with peel (*Solanum tuberosum*)	4.14	9.7	0.5	—	7.5	2.5

Data from NRC (2003) and from guaranteed analyses from Mazuri®, Marion Zoological®, and PMI Nutrition International LabDiet®. Nutritional content of extruded diet expressed as percent of ration; nutritional content of plant diet expressed as percent of dry matter (100% DM).

Table IV Composition of medium

Component	Concentration in medium
Solution A[a]	330.0 ml/L
Solution B[b]	330.0 ml/L
Trace mineral solution[c]	10.0 ml/L
Water-soluble vitamin mix[d]	20.0 ml/L
Folate-biotin solution[e]	5.0 ml/L
Riboflavin solution[f]	5.0 ml/L
SCFA mix[g]	0.4 ml/L
Resazurin[h]	1.0 ml/L
Distilled water	296.0 ml/L
Yeast extract	0.5 g/L
Trypticase	0.5 g/L
Aa_2CO_3	4.0 g/L
Cyesteine HCl-H_2O	0.5 g/L

[a] Composition (g/L): NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2{\cdot}H_2O$, 0.16; $MgCl_2{\cdot}6H_2O$, 0.06; $CoCl_2{\cdot}6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4.

[b] Composition: K_2HPO_4, 2.7 g/L.

[c] Composition (mg/L): EDTA (disodium salt), 500; $FeSO_4{\cdot}7H_2O$, 200; $ZnSO_4{\cdot}7H_2O$, 10; $MnCl_2{\cdot}4H_2O$, 3; H_3PO_4, 30; $CoCl_2{\cdot}6H_2O$, 20; $CuCl_2{\cdot}2H_2O$, 1; $NiCl_2{\cdot}6H_2O$, 2; $Na_2MoO_4{\cdot}2H_2O$, 3.

[d] Composition (mg/L): thiamin-HCl, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B_{12}, 0.2.

[e] Composition (mg/L): folic acid, 10; biotin, 2; NH_4HCO_3, 100.

[f] Composition (mg/L): riboflavin, 10 mg/L in 5 mM HEPES.

[g] Composition: 250 ml/L each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methylbutyrate.

[h] Composition: Resazurin, 1 g/L in distilled water.

30 ml of medium but no fecal sample. We used duplicate blank and control bottles for all fermentation time periods.

We ran all substrates in triplicate; i.e., we weighed each treatment diet into 3 separate bottles for each time period. This resulted in a total of 15 bottles for each treatment diet and a total of 75 bottles for the batch of 5 treatment diets. We incubated all bottles, i.e., treatment diets and corresponding controls and blanks, at 37°C for 0, 6, 12, or 48 h. After incubation, we removed bottles from the water bath and placed them on ice. We repeated this experimental protocol 3 times to provide for replication. We withdrew gas samples immediately and analyzed them for methane by gas chromatography. After methane analysis, we measured pH and then sampled 4-ml aliquots of unstirred fluid from each bottle in preparation for the SCFA analysis.

Statistical Analysis

We performed multivariate analysis according to a randomized complete block design using the Proc Mixed procedure of SAS (SAS Inc., Cary, NC). The Proc Mixed linear model permits data to exhibit correlation and nonconstant variability; in

contrast to standard linear models, it models both the means of the data as well as their variances and covariances.

We pooled fecal samples from each individual belonging to the same species to provide 1 inoculum for each species. This was also used as our "blocking" variable. The model used was as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where α_i is the effect of treatment and the β_j is the block effect. Treatments were the different species and we blocked for the variation among fecal samples that we obtained from different individuals within each species. We incubated all treatments on 3 separate occasions, resulting in 3 replications. We analyzed data from captive and wild-born gorillas as a separate data set using the same model described in the preceding text; treatments were wild vs. captive. Treatment means are reported in the tables, and superscripts indicate significance across all possible treatment comparisons.

Results

Fermentation profiles across the various time periods are reported in Tables V through VIII. As expected, SCFAs at time 0 (Table V) were low and reflect minimal fermentation due to rapid inhibition of microbial cultures. At 6 h, both total and individual SCFAs increased, with the increase due primarily to an increase in acetate concentration (Table VI). Propionate concentrations remained low in fecal samples from *Colobus guereza* and *Cercopithecus neglectus* and increased for *Papio hamadryas*, *Pan troglodytes*, and *Gorilla gorilla* cultures. Butyrate was highest in fecal samples from *Cercopithecus neglectus*, followed by *Pan troglodytes* and *Gorilla gorilla*; the cultures of *Papio hamadryas* and *Colobus guereza* had the lowest but similar butyrate at 6 h. The isoacids were highest and similar for the cultures of *Colobus guereza* and *Cercopithecus neglectus*; cultures of *P. hamadryas*, *Pan troglodyt*es, and *Gorilla gorilla* had similar concentrations of the isoacids. There was a substantial increase in methane in fecal samples from *Papio hamadryas*, *Pan troglodytes*, and *Gorilla gorilla* when compared to cultures of *Colobus guereza* and *Cercopithecus neglectus*. At 12 h of incubation, SCFAs and methane increased further but the increase was greater in cultures from *Colobus guereza* and *Cercopithecus neglectus* than from *Papio hamadryas*, *Pan troglodytes*, and *Gorilla gorilla* (Table VII).

At the end of 48 h, fermentation had reached a maximum in fecal samples from *Papio hamadryas*, *Pan troglodytes*, and *Gorilla gorilla* (Table VIII), as evidenced by only a slight increase in total SCFAs and methane after 12 h of incubation (Table VII). SCFAs and methane increased only slightly at 48 h in cultures from *Cercopithecus neglectus* fecal samples. Cultures from *Colobus guereza*, however, exhibited a substantial increase in both SCFAs and methane. The concentration of the isoacids remained higher in the cultures from *Colobus guereza* and *Cercopithecus neglectus* at the later incubation periods (12 and 48 h) but the difference was less when compared to the cultures of *Papio hamadryas*, *Pan troglodytes*, and *Gorilla gorilla* at similar times.

Cultures of fecal samples from wild-born *Gorilla gorilla* exhibited a slower rate of fermentation when compared to cultures from the fecal samples of captive-born

Table V Concentration of SCFAs, methane and pH in culture bottles containing feces from captive primate species after 0 h of *in vitro* fermentation

	Colobus guereza	*Cercopithecus neglectus*	*Papio hamadryas*	*Pan troglodytes*	*Gorilla gorilla*	SE
Total SCFAs (mM)	9.29	6.83	10.24	13.28	12.11	0.85
Individual SCFAs (mM)						
Acetate	4.16	1.91	4.00	6.01	5.31	0.53
Propionate	0.41	0.25	1.38	1.62	1.54	0.19
Isobutyrate	1.23	1.42	1.16	1.32	1.23	0.18
Butyrate	1.35	1.23	1.61	2.00	1.81	0.09
Isovalerate	1.08	1.01	1.04	1.16	1.12	0.04
Valerate	1.06	1.02	1.05	1.16	1.09	0.04
mol%						
Acetate (A)	44.60	28.04	39.01	45.22	43.66	2.28
Propionate (P)	4.48	3.48	13.48	12.26	12.57	1.13
Isobutyrate	13.29	20.66	11.36	9.94	10.29	2.23
Butyrate	14.60	17.95	15.71	15.04	14.97	0.44
Isovalerate	11.61	14.79	10.20	8.77	9.39	0.57
Valerate	11.43	14.98	10.24	8.76	9.12	0.48
A:P	10.02	8.07	2.90	3.74	3.55	0.70
Methane (nmol/ml)	17.93	17.47	49.60	40.59	47.08	4.34
pH	6.53	6.60	6.57	6.50	6.47	0.05

G. gorilla (Tables IX and X). This was evident by the fact that captive-born fecal cultures resulted in greater total SCFA concentrations at 6 h ($p=0.001$, $F=67.95$) and 12 h ($p=0.015$, $F=16.87$). However, at the end of 48 h, total SCFA concentration was similar between captive- and wild-born *Gorilla gorilla*.

Fecal samples from the subject primate species exhibit high (65.07–89.00 mM) total SCFA concentrations, within the range expected for ruminant ungulates (Tables VIII–XI). Overall, fecal samples from all focal species exhibit a high acetate-to-propionate ratio (A:P). The concentration of acetate (mol%) was highest in samples from *Colobus guereza* and lowest in those from *Cercopithecus neglectus*; samples from *Gorilla gorilla*, *Pan troglodytes*, and *Papio hamadryas* exhibit intermediate levels of acetate concentrations. Concentration of propionate was highest in samples from *Papio hamadryas* (35.93 mol%) and lowest in those from *Cercopithecus neglectus*; *Gorilla gorilla*, *Pan troglodytes*, and *Colobus guereza* exhibit intermediate levels of propionate concentrations. A:P ratios range from 1.3 (47:21, *Papio hamadryas*) to 2.7 (61:23, *Colobus guereza*).

Results demonstrate that acetate concentrations in the primate subjects are not associated with more concentrated butyrate or methane. *Cercopithecus neglectus* had an unexpectedly high ($p<0.0001$, $F=467.01$) butyrate concentration (29.09 mol%), but acetate is not proportionately higher (46.62 mol%). Butyrate concentration (mol%) was lowest for *Colobus guereza* (9.92 mol%) and *Papio hamadryas* (10.81 mol%); they were intermediate for *Gorilla gorilla* (11.08 mol%) and *Pan troglodytes* (12.15 mol%).

Table VI Concentration of SCFAs and methane and pH in culture bottles containing feces from captive primate species after 6 h of *in vitro* fermentation

	Colobus guereza	*Cercopithecus neglectus*	*Papio hamadryas*	*Pan troglodytes*	*Gorilla gorilla*	SE	df	*F* value
Total SCFAs (mM)	26.16[a]	23.95[a]	49.03[b,f]	54.08[b,f]	64.89[c,e]	3.13	4	65.67
Individual SCFAs (mM)								
Acetate	19.73[a,f,g]	13.96[a,f,h]	24.96[e,h]	29.68[e,g,h]	36.6[b,f,h]	2.34	4	28.10
Propionate	0.77[a,g]	2.63[a,h]	16.21[b,g]	14.29[b,f,h]	16.67[b,e]	0.69	4	251.51
Isobutyrate	1.28[g]	1.27[g]	1.31	1.28[g]	1.40[h]	0.05	4	2.90
Butyrate	2.13[a]	3.89[b]	4.03[b]	6.11 [c]	7.41[d]	0.18	4	268.20
Isovalerate	1.13[a,g]	1.10[a,e]	1.19[f,h]	1.19[f,h]	1.28[b,e]	0.02	4	17.79
Valerate	1.09[a]	1.10[a]	1.33[b]	1.52[c]	1.51[c]	0.03	4	122.57
mol%								
Acetate (A)	75.29[a]	58.25[b,g]	50.73[c,e,g]	54.88[b,h]	56.40[b, f]	1.32	4	102.69
Propionate (P)	2.94[a]	10.96[b]	33.12[c]	26.42[d]	25.71[d]	0.51	4	1171.98
Isobutyrate	4.97[a]	5.31[a]	2.69[b]	2.36[b]	2.16[b]	0.23	4	84.37
Butyrate	8.21[a]	16.25[b]	8.27[a]	11.32[c]	11.42[c]	0.44	4	111.69
Isovalerate	4.39[a]	4.61[a]	2.45[b]	2.20[b]	1.98[b]	0.25	4	50.83
Valerate	4.21[a]	4.61[a]	2.73[b]	2.82[b]	2.33[b]	0.23	4	38.32
A:P	25.65[a]	5.32[b]	1.53[c]	2.08[c]	2.19[c]	0.55	4	692.71
Methane (nmol/ml)	14.47[a]	92.36[a]	951.40[b]	1712.52[c]	1792.41[c]	39.91	4	908.84
pH	6.27[a]	6.23[a,e]	6.12[b,f,g]	6.03[b,h]	6.07[b]	0.03	4	23.63

[a,b,c,d] Species means differ at $p<0.001$.

[e,f] Species means differ at $0.001<p<0.01$.

[g,h] Species means differ at $0.01<p<0.05$.

Methane concentration was lowest in *Colobus guereza* (183.61 nmol/ml) and *Cercopithecus neglectus* (591.63 nmol/ml) and highest in *Gorilla gorilla* (2943.25 nmol/ml) and *Pan troglodytes* (2863.80 nmol/ml); methane levels in *Papio hamadryas* were intermediate at 1116.72 nmol/ml. It appears that the colobine in this study and the smaller of the 2 cercopithecines (*Cercopithecus neglectus*) conserve more of their fermentation energy in short-chain volatile fatty acids and reduce energy loss in methane.

Total SCFA levels were similar in fecal samples from wild-caught and captive-born *Gorilla gorill*a (Tables IX and X), although concentrations of acetate were significantly greater ($p=0.002$, $F=48.72$) in fecal samples from wild-caught gorillas. Butyrate concentration ($p=0.001$, $F=73.47$) and methane production ($p=0.0002$, $F=175.94$) were significantly less in fecal samples from wild-caught individuals relative to captive-born.

Discussion

Several caveats to these results must be noted. First, subjects were fed their normal zoo diet per zoo protocol to minimize stress and disruption. Thus, diets among species were

Table VII Concentration of SCFAs and methane and pH in culture bottles containing feces from captive primate species after 12 h of *in vitro* fermentation

	Colobus guereza	*Cercopithecus neglectus*	*Papio hamadryas*	*Pan troglodytes*	*Gorilla gorilla*	SE	df	*F* value
Total SCFAs (mM)	57.14^{a,e}	45.82^{a,f}	83.24^{b,e}	79.31^{b,h}	69.32^{b,f,i}	3.12	4	49.63
Individual SCFAs (mM)								
Acetate	32.98 ^{a,e,h}	24.38^{b,f}	42.70^{a,c,f}	46.83^{c,f}	38.94^{a,e,i}	1.97	4	39.51
Propionate	14.45^{a,e,h}	10.42^{a,f}	26.71^{c}	20.02^{b,e}	16.79^{b,f,i}	0.85	4	103.32
Isobutyrate	1.43^{e,g}	1.27^{a,f}	1.55^{b,f,g}	1.51^{b,g}	1.39^{e}	0.03	4	20.88
Butyrate	5.96^{a,e}	7.53^{f}	9.04^{b,e}	7.30^{f}	9.01^{b,e}	0.36	4	25.38
Isovalerate	1.21^{a,e,h}	1.12^{a,i}	1.40^{b,e,h}	1.33^{b,f,i}	1.29^{b,f,i}	0.03	4	31.56
Valerate	1.11^{a}	1.09^{a}	1.85^{b}	2.34^{c}	1.91^{b}	0.06	4	173.02
mol%								
Acetate (A)	57.73^{a}	53.20^{b,e,h}	51.28^{b,i}	59.04^{a,e}	56.14^{a,f}	0.78	4	33.92
Propionate (P)	25.28^{a,e}	22.78^{a,f,h}	32.08^{b}	25.24^{a,e}	24.23^{a,i}	0.59	4	74.63
Isobutyrate	2.51^{a,e}	2.78^{a,f}	1.87^{b}	1.90^{b}	2.01^{b}	0.07	4	59.99
Butyrate	10.43^{a,e}	16.42^{b}	10.86^{d}	9.20^{a,f}	13.00^{c}	0.30	4	173.34
Isovalerate	2.11^{a,e}	2.44^{b}	1.69^{c,h}	1.67^{c,e}	1.86^{c,f,i}	0.05	4	71.30
Valerate	1.95^{a,e}	2.38^{b,h}	2.22^{f,i}	2.95^{c,h}	2.75^{c,i}	0.07	4	70.41
A:P	2.29^{a}	2.35^{a}	1.60^{b}	2.34^{a}	2.32^{a}	0.09	4	24.24
Methane (nmol/ml)	50.10^{a,e}	448.20^{a,f}	1397.97^{b}	2218.69^{c}	2790.82^{d}	86.50	4	356.21
pH	6.20^{a}	6.27^{a}	6.22^{a}	6.00^{b}	6.00^{b}	0.03	4	36.38

^{a,b,c,d} Species means differ at $p<0.001$.

^{e,f,g} Species means differ at $0.001<p<0.01$.

^{h,i} Species means differ at $0.01<p<0.05$.

not standardized and different dietary substrates can yield different SCFA profiles (Bergman 1990; Campbell *et al.* 2002; Schmidt *et al.* 2005). However, dietary composition was very similar across species; meals were prepared in the same commissary using produce from a single source, and differences in diet are mainly due to the proportion of each ingredient (Tables II and III). Second, the sample sizes of the species are small (in 2 cases $N=2$) and fecal samples from each individual belonging to a species were pooled in a batch design to provide 1 inoculum. Although sample sizes and batch design are in line with those of similar studies (Campbell *et al.* 2002; Costa *et al.* 1989; Schmidt *et al.* 2005), this sampling protocol precludes intra- vs. interspecific analysis of variance. Finally, although the focal subjects included a foregut fermenting species (*Colobus guereza*), we evaluated fecal material only and not pregastric liquid, given the prohibitive impact this sampling protocol would have had on the well-being of the subjects. Although ideally both regions of the gut would have been evaluated, 2 recent analyses mitigate concern. First, Mauricio *et al.* (2001) compared cattle pregastric liquid with fecal samples and demonstrated that bacterial and SCFA profiles are similar regardless of the region of the gut from which they are derived. More recently, Ley *et al.* (2008) evaluated gut microbiota among 166 mammal species; all analyses were undertaken using fecal samples. Despite

Table VIII Concentration of SCFAs and methane and pH in culture bottles containing feces from captive primate species after 48 h of *in vitro* fermentation

	Colobus guereza	*Cercopithecus neglectus*	*Papio hamadryas*	*Pan troglodytes*	*Gorilla gorilla*	SE	df	*F* value
Total SCFAs (mM)	78.89[j]	65.07[h]	87.11[i]	80.69[j]	89.00[i]	7.30	4	2.98
Individual SCFAs (mM)								
Acetate	49.40[h]	30.14[e,i]	41.87[g]	44.64[h]	51.45[f]	5.13	4	4.59
Propionate	18.21[a,h]	13.72[a,e,i]	31.28[b]	20.33[a,f]	21.81[a,f,i]	1.43	4	41.67
Isobutyrate	1.52	1.43	1.46	1.49	1.51	0.07	4	0.54
Butyrate	7.84[a,h]	17.35[b]	9.41[a,i]	9.77[a,i]	9.79[a,i]	0.58	4	67.01
Isovalerate	1.37[h,i]	1.30[h]	1.47j	1.36[h,i]	1.43[i]	0.05	4	3.50
Valerate	1.63[a]	1.20[a]	1.61[a]	3.11[b]	3.01[b]	0.16	4	61.80
mol%								
Acetate (A)	61.16[a,e,h]	46.62[b]	48.04[b]	55.19[a,f]	57.59[a,i]	1.18	4	52.10
Propionate (P)	23.26[a,e,h]	21.34[a,i]	35.93[b]	25.25[c,f]	24.58[c,i]	0.51	4	269.33
Isobutyrate	1.92[h]	2.17[a,e,i]	1.68[b,i]	1.85[f,i]	1.71[b,i]	0.07	4	12.12
Butyrate	9.92[a,h]	26.09[b]	10.81[a,e]	12.15[c,f,h]	11.08[a,i]	0.36	4	593.16
Isovalerate	1.73[i]	1.98[h]	1.69[i]	1.69[i]	1.63[i]	0.09	4	3.73
Valerate	2.08[a]	1.86[a]	1.85[a]	3.87[b,h]	3.42[b,i]	0.18	4	76.98
A:P	2.65[a,e,h]	2.19[a,f]	1.34[b]	2.19[a,f]	2.35[a,i]	0.09	4	58.91
Methane (nmol/ml)	183.61[a,e]	591.63[a]	1116.72[a,f]	2863.80[b]	2943.25[b]	212.46	4	84.24
pH	5.90[h]	6.00[e]	5.77[h]	5.57[f,i]	5.60[f,i]	0.08	4	10.20

[a,b,c, d] Species means differ at $p<0.001$.

[e,f,g] Species means differ at $0.001<p<0.01$.

[h,i,j] Species means differ at $0.01<p<0.05$.

differences in fermentation systems, colobines clustered with *Papio*, *Gorilla*, *Pan*, and *Pongo* in their gut microbiota arrays, corroborating earlier results that bacterial and SCFA profiles are similar regardless of the region of the gut from which they are sampled. In short, we urge readers to view these results as preliminary and with caution. Nonetheless, given the paucity of information on digestive biology of nonhuman primates more generally, we view these data as valuable and useful for increasing our understanding of primate digestive strategy and feeding biology.

An evaluation of the data presented here in light of information stemming from the literature on wild and captive catarrhines (with domesticated cattle and one platyrrhine species for comparison) suggests that fecal samples from colobine and cercopithecine species produce relatively high total SCFA concentrations *in vitro*, even when compared to *in vitro* samples from the much larger-bodied pongid species (Table XI). Also, patterns of methane production differ among the monkeys and *Pan troglodytes*. *Colobus guereza* and *Cercopithecus neglectus* especially seemed to conserve more of their fermentation energy in SCFAs and reduce the amount of dietary energy lost in methane, while *Pan troglodytes* produced the most methane of the 5 species. The low methane produced by the primate species overall relative to ruminating ungulates, and particularly *Cercopithecus neglectus*, suggest very

Table IX Concentration of SCFAs and methane and pH in culture bottles containing feces from either captive (CAP) or wild born (WILD) gorillas at 0 and 6 h of *in vitro* fermentation (n=3); includes standard error (SE)

	0 h					6 h				
	CAP	WILD	SE	df	*F* value	CAP	WILD	SE	df	*F* value
Total SCFAs (mM)	12.11	13.48	1.22	1	1.26	64.89[c]	41.09[d]	2.89	1	67.95
Individual SCFAs (mM)										
Acetate	5.31[e]	7.51[f]	0.76	1	8.35	36.61[c]	24.11[d]	2.15	1	33.66
Propionate	1.54	1.02	0.28	1	3.41	16.67[a]	10.32[b]	0.55	1	134.15
Isobutyrate	1.23	1.23	0.05	1	0.02	1.40	1.38	0.09	1	0.09
Butyrate	1.81	1.59	0.13	1	0.13	7.41[a]	2.79[b]	0.21	1	486.20
Isovalerate	1.12	1.08	0.03	1	2.68	1.28[c]	1.17[d]	0.02	1	29.68
Valerate	1.09	1.07	0.04	1	0.29	1.51[c]	1.32[d]	0.03	1	41.17
mol%										
Acetate (A)	43.66[c]	55.56[d]	1.70	1	48.72	56.40	58.61	1.21	1	3.39
Propionate (P)	12.57[e]	7.56[f]	1.40	1	12.80	25.71	25.14	0.66	1	0.73
Isobutyrate	10.29	9.13	0.63	1	3.42	2.16[c]	3.36[d]	0.20	1	35.22
Butyrate	14.97[a]	11.80[b]	0.37	1	73.47	11.42[a]	6.81[b]	0.20	1	542.57
Isovalerate	9.39	8.00	0.69	1	4.03	1.98[c]	2.85[d]	0.13	1	48.98
Valerate	9.12	7.95	0.56	1	4.35	2.33[c]	3.23[d]	0.11	1	63.91
A:P	3.55[c]	7.37[d]	0.47	1	67.49	2.19	2.34	0.11	1	1.71
Methane (nmol/ml)	47.1[a]	25.7[b]	1.6	1	175.9	1792.4[a]	926.0[b]	38.5	1	507.7
pH	6.47	6.47	0.05	1	0.00	6.07[c]	6.18[d]	0.02	1	24.50

[a,b] Within time, species means differ at $p<0.001$.

[c,d] Within time, species means differ at $0.001<p<0.01$.

[e,f] Within time, species means differ at $0.01<p<0.05$.

efficient capture of otherwise lost energy. *Cercopithecus neglectus* exhibits a very high acetate and butyrate concentration and very low methane production, a pattern that is similar to Colobinae in terms of acetate, but not similar to ruminants. Generally, acetate-producing microbes have a slower generation time, and hence longer retention times would be favorable for the growth and survival of acetate-producing organisms. However, increased acetate would mean greater H_2 generation as well. It is not inconceivable that the increased acetate under these circumstances is due to the interconversion from methane. In ruminants, fermentation with high acetate production results in greater hydrogen output, e.g., for every mol of acetate 8 mol of hydrogen are released. A low partial pressure of hydrogen is critical for continued generation of acetate and for the oxidation reactions that conduct fermentation. Thus, hydrogen disposal/removal is essential for normal fermentation to continue. In ruminants, the major route of hydrogen disposal is methane. Based on these preliminary results, we speculate that there may be alternative routes of hydrogen disposal, and that these data may provide indirect evidence for acetogenic bacteria. Clearly, this speculation requires further investigation.

Table X Concentration of SCFAs and methane and pH in culture bottles containing feces from either captive (CAP) or wild born (WILD) gorillas at 12 and 48 h of *in vitro* fermentation (n=3); includes standard error (SE)

	12 h					48 h				
	CAP	WILD	SE	df	F value	CAP	WILD	SE	df	F value
Total SCFAs (mM)	69.32[e]	54.10[f]	3.70	1	16.87	89.00	86.02	9.25	1	0.10
Individual SCFAs (mM)										
Acetate	38.94	33.05	2.71	1	4.73	51.45	53.07	6.29	1	0.07
Propionate	16.79[c]	13.21[d]	0.74	1	23.56	21.81	22.06	1.95	1	0.02
Isobutyrate	1.39[e]	1.29[f]	0.03	1	12.15	1.51	1.44	0.09	1	0.56
Butyrate	9.01[a]	3.76[b]	0.26	1	9.01	9.79[c]	5.81[d]	0.75	1	27.81
Isovalerate	1.29[c]	1.20[d]	0.02	1	27.93	1.43	1.31	0.07	1	3.48
Valerate	1.91[a]	1.59[b]	0.02	1	170.55	3.01[e]	2.33[f]	0.20	1	11.77
mol%										
Acetate (A)	56.14[c]	61.01[d]	0.93	1	27.56	57.59[e]	61.70[f]	1.13	1	13.22
Propionate (P)	24.23	24.43	0.31	1	0.43	24.58	25.66	0.45	1	5.93
Isobutyrate	2.01[e]	2.40[f]	0.11	1	12.66	1.71	1.68	0.09	1	0.16
Butyrate	13.00[a]	6.98[b]	0.26	1	528.57	11.08[a]	6.74[b]	0.48	1	81.24
Isovalerate	1.86[e]	2.24[f]	0.12	1	9.48	1.63	1.52	0.10	1	1.11
Valerate	2.75	2.95	0.15	1	1.74	3.42[e]	2.71[f]	0.21	1	11.35
A:P	2.32	2.50	0.07	1	6.86	2.35	2.40	0.08	1	0.47
Methane (nmol/ml)	2790.8[c]	1834.1[d]	135.0	1	50.21	2943.3[e]	2114.0[f]	202.0	1	16.85
pH	6.00[c]	6.08[d]	0.02	1	25.00	5.60	5.67	0.07	1	1.00

[a,b] Within time, species means differ at $p<0.001$.

[c,d] Within time, species means differ at $0.001<p<0.01$.

[e,f] Within time, species means differ at $0.01<p<0.05$.

Data presented here on fermentation profiles are consistent with data on diet and digestive retention times among Catarrhini and may have implications for our understanding of the interactions among diet, body mass, digestion, and how monkeys can consume either similar or greater levels of fiber than larger-bodied apes (Lambert 2002a, 2007a, b). Wherever African apes have been studied in comparison to sympatric cercopithecoids, apes are characterized by their feeding emphasis on plant foods relatively lower in fiber fractions and plant secondary metabolites, while monkey diet tends to include high levels of fiber and toxins, e.g., leaves, seeds, bark (Beeson 1989; Chapman *et al.* 2002; Conklin-Brittain *et al*. 1998; Doran-Sheehy *et al*. 2009; Gautier-Hion 1988; Lambert 2002a, b; Maisels 1993; Remis 2002, 2003; Remis and Dierenfeld 2004; Richard *et al.* 1989; Rogers *et al*. 2004; Rudran 1978; Struhsaker 1978; Wrangham *et al*. 1998). This is to be expected for colobines whose derived stomach anatomy and digestive strategy facilitate the consumption of high-fiber foods, but not Cercopithecinae. In other work, Lambert (1998, 2002a, 2007a, b) has argued that this dietary difference between cercopithecid monkeys and apes and unexpected departures from Basal Metabolic Rate-based body mass predictions of dietary quality can be explained by patterns of digestive kinetics. Both cercopithe-

Table XI Total SCFA concentration (mM) and individual SCFAs (mol%) in all reported[1–12] wild and captive primate species

Species	Total SCFA concentration (mM)	Individual SCFAs (mol%)		
		Acetate	Propionate	Butyrate
WILD				
Procolobus verus [1]	230			
Colobus guereza [1]	107–434			
Cercopithecus aethiops [2]	190–229			
Cercopithecus mitis [2,3]	122–199			
	138–180			
Papio cynocephalus [3]	95–170			
Pan troglodytes [4]	44	31.4	10	2.9
Alouatta palliata [5]		93.7	5.9	0.4
CAPTIVE				
Trachypithecus cristatus [6]	95–133	47–56	24–26	10–18
Semnopithecus entellus [6]	89–233	46–50	22–23	14–23
Colobus guereza [7]	53–65			
Colobus guereza [8]	79	61	23	10
Cercopithecus neglectus [8]	65	47	21	26
Papio hamadryas [8]	87	48	36	11
Pan troglodytes [8]	81	55	25	12
Gorilla gorilla [8]	89	58	25	11
Pongo abelii [9]		61.5–67.0	22–26.5	9.5–13.5
Bos taurus (cattle) [10]	96–210	48–74	14–28	7–18
Ovis aries (sheep) [11]	70–140	40–66	19–40	9–15

Domesticated cattle and sheep are provided for comparison.

[1] Ohwaki *et al*. (1974); [2] Brourton *et al*. (1991); [3] Clemens and Phillips (1980); [4] Ushida *et al*. (2006); [5] Milton and McBee (1983); [6] Bauchop and Martucci (1968); [7] Kay *et al*. (1976); [8] Lambert and Fellner (this study); [9] Schmidt *et al*. (2005); [10] van Soest (1994).

coid subfamilies have relatively longer digestive retention times than the much larger African apes (Lambert 2002a, 2007a, b; Remis and Dierenfeld 2004). Again, this is not a surprising finding among the Colobinae; however, cercopithecines are not derived in their gastrointestinal anatomy (Caton 1998). Indeed, most recent literature suggests a poor correlation between animal body mass and digestive retention time, a pattern first noted in primates (Lambert 1998) and, more recently, in mammals more generally (Clauss *et al*. 2007) and even sauropod dinosaurs (Steuer 2010). Longer digestive retention times result in higher levels of fermentation that in turn increases the value of a food to its consumer (Lambert 1998; Milton 1984, 1986, 1993; Remis 2002). *Cercopithecus neglectus*, in particular, is noteworthy for yielding very high levels of acetate and butyrate, producing low methane and thus efficient capture of otherwise lost energy, and also having among the longest mean digestive retention time relative to body mass in primates (Lambert 2002a). That

SCFA concentrations were generally similar in fecal samples from smaller primate species relative to larger species suggests similar digestive efficiency; i.e., being smaller is not necessarily a disadvantage for consuming a diet high in plant fiber. The relationships among wild dietary quality, digestive strategy, methane production, SCFA profiles, and body mass in primates certainly warrant further investigation.

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