Published in final edited form as:
J Chem Ecol. 2013 March ; 39(3): 447–449. doi:10.1007/s10886-013-0258-3.

Soil Consumed by Chacma Baboons is Low in Bioavailable Iron and High in Clay

Paula A. Pebsworth,
Section of Social Systems Evolution, Primate Research Institute, Kyoto University, Inuyama, Japan

Gretchen L. Seim,
Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA

Michael A. Huffman,
Section of Social Systems Evolution, Primate Research Institute, Kyoto University, Inuyama, Japan

Raymond P. Glahn,
Robert Holley Center for Agriculture and Health, Ithaca, NY 14853, USA

Elad Tako, and
Robert Holley Center for Agriculture and Health, Ithaca, NY 14853, USA

Sera L. Young
Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA

Sera L. Young: sera.young@cornell.edu

Abstract

Despite widespread consumption of soil among animals, the role of geophagy in health maintenance remains an enigma. It has been hypothesized that animals consume soil for supplementation of minerals and protection against toxins. Most studies determine only the total elemental composition of soil, which may not reflect the amount of minerals available to the consumer. Our aim was to test these hypotheses by evaluating the bioavailability of iron in soil consumed by chacma baboons, using a technique that simulates digestion and adsorption. Our results indicate that, despite variation in absolute iron concentration of soil samples, actual iron bioavailability was low while clay content was quite high. This suggests that iron supplementation is unlikely to be the primary motivation for geophagy in this population, and that detoxification is a plausible explanation. This study demonstrates that more research on bioavailability and clay composition is needed to determine the role geophagy plays in health maintenance.

Keywords

Geophagy; Iron absorption; Caco-2 cells; Nonhuman primates

Introduction

Geophagy, the consumption of soil, is widespread among animals and has been documented in 93 nonhuman primate species (NHP) (Rowe and Myers, 2012; Young et al., 2011). There

Correspondence to: Sera L. Young, sera.young@cornell.edu.

are two main hypotheses concerning the physiological purpose of geophagy: mineral supplementation and gastrointestinal protection (Young et al., 2011). A recent review of published accounts of geophagy among NHPs found a similar number of studies that claim to support either of these hypothesis (Young et al., 2011). However, none of these studies conclusively supports one hypothesis over the other. Firstly, assessment of bioavailability has been rare; total elemental composition of consumed soil is typically reported without consideration of bioavailability (Young et al., 2010), which is considered to be vastly over-estimated (Wilson, 2003). To date, only five *in vitro* studies have evaluated the bioavailability of geophagic soil using techniques that consider alimentary biochemistry, and each of these has limitations (Young, 2010). Second, in studies on geophagy, iron bioavailability in soil that NHPs actually consume has never been determined. Therefore, our objective in this study was to use the most precise *in vitro* methodology to assess total iron and iron bioavailability of geophagic soils actually consumed by a NHP, the chacma baboon (*Papio hamadryas ursinus*).

Methods and Materials

Soil samples were collected from Wildcliff Nature Reserve, Western Cape, South Africa, a site where chacma baboons frequently engage in geophagy (Pebsworth et al., 2012). During an 18 month study, video camera traps continually monitored baboon geophagy; subsequently these images were scored to determine patterns of soil consumption (Pebsworth et al., 2012). Soil pH, particle size, major and trace components, and clay minerals present were analyzed at the Central Analytical Facility, University of Stellenbosch, South Africa. Soil pH of a 50 ml water extract of 10 g of soil was measured with a Metrohm pH meter. Particle size distribution was determined using the hydrometer technique (Van der Watt, 1966). Using bulk material, major and trace elements were measured by X-ray fluorescence (Axios 2.4 kWatt Rh X-ray tube, PANalytical B.V., Almelo, NL). Minerals present in the clay fraction were determined by X-ray diffraction (X'Pert multi-purpose diffractometer fitted with a Cu tube, PANalytical B.V., Almelo, NL). Total iron concentration in samples was determined at Cornell University, Ithaca, NY, USA, by an inductively coupled argon-plasma/atomic emission spectrophotometer following wet ashing (ICAP-AES Thermal Jarrell Ash Trace Analyzer, Jarrell Ash Co., Franklin, MA, USA).

Iron bioavailability was assessed with an *in vitro* digestion/Caco-2 cell culture model (Glahn et al., 1998) at Cornell University. To contextualize total iron and bioavailability of iron in geophagic soil, an iron-rich test "meal" of white bean (WB) was analyzed for comparison. Before analysis, geophagic soil (0.1 g) and white bean (0.5 g) samples were ground to a powder; less geophagic material was used because 0.5 g of soil clogged the membrane used to simulate gastric digestion.

To simulate gastric digestion, samples were mixed with saline buffer, brought to a pH of 2, and then incubated on a rocker at 37 °C for 1 h in the presence of pepsin. Intestinal digestion was simulated by adjusting the contents to pH7 and incubating at 37 °C for 2 h with pancreatic enzymes and bile salts. The simulated intestinal digestion was carried out in the upper chamber of a two-chamber system, created by fitting the bottom of a Transwell insert ring with a 15000 Da molecular weight cut-off membrane (Spectra/Por 2.1, Spectrum Medical, Gardena, CA, USA). This system allows iron from the digested samples to diffuse into the lower chamber containing the Caco-2 cells. The cells are then able to take up the soluble iron in proportion to its bioavailability. The Caco-2 cells were seeded at a density of 50,000 cells/cm^2, and the experiment was conducted 13 d post-seeding.

After intestinal digestion, the inserts were removed, and the cells were further incubated at 37 °C for 24 h to allow ferritin to form. The cell monolayer was harvested. Ferritin concentration of the cell suspension, an indicator of iron uptake, was measured by using an immunoassay (FER-IRON II Ferritin assay, Ramco laboratories, Houston, TX, USA). Cell protein concentration was determined with a colorimetric assay (Bio-Rad DC Protein assay, Bio-Rad, Hercules, CA, USA). The index of iron bioavailability used was the ratio of cell ferritin to cell protein concentration. All measurements were performed in triplicate. Data were analyzed in Excel by using paired *t-tests*. Mean iron concentration and ferritin responses were compared between soil samples, between soil samples and WB, and between all samples and the blank. Significance was defined as $P<0.05$.

Results

At the most frequented geophagy site, baboons demonstrated a clear preference for white soil over equally accessible pink soil (Pebsworth et al., 2012). X-ray diffraction revealed that both soil samples were composed of kaolinite (7AÁ clays) and illite with minor amounts of goethite, montmorillonite, and hydrated halloysite making up part of the generic "bentonite" clay that is common in this geological area (Pebsworth, unpublished data). While major elements and pH were similar, particle size and total iron and iron bioavailability differed greatly (Table 1). While the white sample contained 42.1 % clay, the pink sample contained only 22.5 % clay.

Total iron concentration in pink soil was higher ($P= 0.037$, Fig. 1) than in white soil (16317.2 ppm and 3087.7 ppm, respectively). The pink soil's mean ferritin response was higher ($P=0.037$, Table 1, Fig. 1) than that of the white soil; both were higher than the blank (3.8 μg ferritin/mg cell protein). However, when compared to WB, the ferritin responses of both soils were much lower ($P< 0.001$), despite their higher concentrations of iron (Table 1, Fig. 1).

Discussion

Although the samples were similar in many respects, including alkalinity, the preferred geophagic soil (white) was lower in total and bioavailable Fe than the less preferred pink soil. Furthermore, despite the high iron content, the iron in geophagic soils is less bioavailable than that in a test meal (WB) with lower total iron content. Together, these data suggest that it is unlikely that Fe supplementation motivates geophagy. Further, these data emphasize the importance of reporting on total elemental composition and bioavailability; total Fe content alone can be misleading.

A second difference between the samples is that while both soil samples contain clay, the clay content of the preferred white soil was almost twice that of the avoided pink soil. This observation lends support to the hypothesis that geophagy may protect against harmful pathogens and chemicals by adsorbing them or preventing their passage through the intestinal lumen.

We encourage the evaluation of both iron bioavailability and clay content in all future research on the role of soil in health maintenance for NHP.

Acknowledgments

We acknowledge Mary Bodis and Pei-Pei Chang for assistance with laboratory analyses. PAP thanks Jennifer Giddy, the executive director of Wildcliff Nature Reserve and CapeNature for the opportunity to conduct research in South Africa, Wilderness Wildlife Trust, and the Cooperation Research Program of Primate Research Institute, Kyoto University, for financial support. She further thanks Jeannine McManus and Kate Muller of Landmark

Foundation for support in the field. SLY was supported by the Thrasher Research Fund. SLY was supported by NIH K01MH09902. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health.

References

Glahn RP, Lee OA, Yeung A, Goldman MI, Miller DD. Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. J Nutr. 1998; 128:1555–1561. [PubMed: 9732319]

Pebsworth PA, Bardi M, Huffman MA. Geophagy in chacma baboons: Patterns of soil consumption by age class, sex, and reproductive state. Am J Primatol. 2012; 74:48–57. [PubMed: 21969111]

Rowe, N.; Myers, M., editors. All the World'sPrimates. Primate Conservation Inc; Charlestown RI: 2012. www.alltheworldsprimates.org

van der Watt H. Improved tables and simplified procedure for soil particle size analysis by the hydrometer method. S Afr J Ag Sci. 1966; 9:911–916.

Wilson MJ. Clay mineralogical and related characteristics of geophagic materials. J Chem Ecol. 2003; 29:1525–1547. [PubMed: 12921434]

Young SL. Pica in pregnancy: New ideas about an old condition. Annu Rev Nutr. 2010; 30:403–422. [PubMed: 20420523]

Young SL, Sherman PW, Lucks JB, Pelto GH. Why on Earth? Evaluating hypotheses about the physiological functions of human geophagy. Q Rev Biol. 2011; 86:97–120. [PubMed: 21800636]

Young SL, Wilson MJ, Hillier S, Delbos E, Ali SM, Stoltzfus RJ. Differences and commonalities in physical, chemical and mineralogical properties of Zanzibari geophagic soils. J Chem Ecol. 2010; 36:129–140. [PubMed: 20063204]

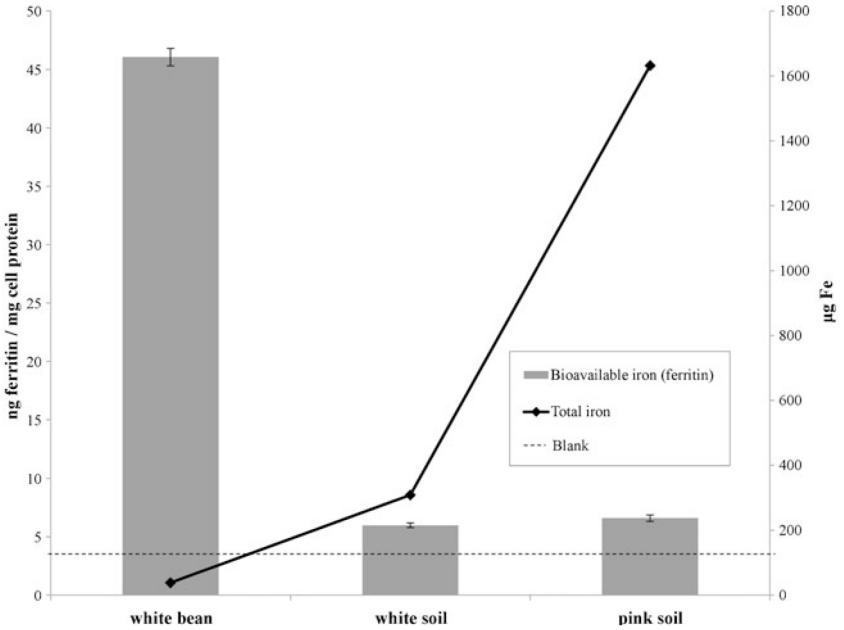

Fig. 1.

Total iron and iron bioavailability in geophagic soil samples and a test meal, white bean (WB). Total iron and ferritin formation values are means±SEM, *N*=3. Total iron is calculated from ICP data and iron bioavailability is based on from ferritin data from Caco-2 experiments. “Blank” refers to cell ferritin levels in wells without WB or soil

Table 1

Physical characteristics of white (preferred) and pink geophagic soil samples consumed by chacma baboons

Sample Characteristics		White sample	Pink sample	White bean
Particle Size (%)	Sand (2.0–0.05 mm)	2.2	4.4	
	Silt (0.05–0.002 mm)	55.7	73.1	
	Clay (<0.002 mm)	42.1	22.5	
pH		10.1	10.3	
X-Ray Fluorescence major components (wt,%)	Al_2O_3	23.0	22.2	
	CaO	0.1	0.1	
	Cr_2O_3	0.0	0.0	
	K_2O	4.2	4.1	
	MgO	0.7	0.6	
	MnO	0.0	0.0	
	Na_2O	0.8	1.4	
	P_2O_5	0.0	0.0	
	SiO_2	63.0	62.3	
	TiO_2	1.1	1.1	
Iron, Total & Bioavailable	(ppm)	308.8 *	1631.7 *^	76.8
	ng Ferritin/mg cell protein	6.0 **	6.6 **^	46.1

* Significantly higher (P<0.05) than white bean

^ Significantly higher (P<0.05) than white geophagic sample

** Significantly lower (P<0.05) than white bean