EFFECT OF DIETARY SOLUBLE FIBER ON GUT MICROBIOTA IN THE SUGAR GLIDER (*PETAURUS BREVICEPS*): A PILOT STUDY

Lindsey Solden[1], Ellen S. Dierenfeld[2]*, and Kelly Wrighton[1]

[1]*Department of Microbiology, The Ohio State University, Columbus, OH, USA*
[2]*Ellen S. Dierenfeld, LLC, St. Louis, MO, USA*

Introduction

The sugar glider (*Petaurus breviceps*) is an exudativore in nature, eating plant gums, saps, resins, manna, and nectars as well as insect-based honeydew and lerp, with proportions of various ingredients highly dependent on seasonality and locale (Smith, 1982; Howard, 1989). Despite a well-developed cecum that could, in theory, harbor microbial populations with fermentative capabilities (Hume, 1999), a majority of captive gliders are fed diets comprising a high proportion of simple sugars such as nectars and domestic fruits (Dierenfeld et al., 2006). This investigation was undertaken to examine effects of added soluble dietary fiber on gut microbial populations in sugar gliders.

Materials and Methods

Animals

Twelve adult, pair-housed non-breeding gliders were utilized (n=6 study units); four pairs were male-female, with one female and one male pair. Animals (average weight 69.0 ± 9.7 g) were maintained in adjacent coated-wire cages measuring 46 X 46 X 75 cm (WLH), with a plastic catch tray underneath. Enclosures were furnished with flannel-lined hanging pouches, plastic hanging toys, an exercise wheel, and drip bottle drinkers. No special lighting regimen was used; the room was exposed to a normal daylighting cycle (April/May, northern hemisphere) through large windows, and temperature ranged from 18 to 22°C. Locations of cages were rotated weekly to minimize possible effects of light on appetite/activity. Gliders were weighed weekly (as pairs) on a platform scale (Model 3828, Taylor Precision Products, Las Cruces, NM USA) to 0.1 g, and all remained healthy throughout the trial.

Diets

Each glider pair was fed three diets (control, low fiber or high fiber) for a period of one week, with a one-week acclimation period between. Control diets consisted of extruded dry pellets (Glide-R-Chow™, from Pocket Pets™ @www.SugarBears.com) and a powdered supplement (Critter Love® HPW Complete, St. John's, FL); previous intake and digestion trials established dry matter intake (DMI) per pair as ~7 g. Conservative low and high soluble fiber diet treatments (~550 or 800 mg acacia gum + pectin – targeting 7.5 and 10% of DMI , respectively, per glider pair) - were developed by adding known quantities of dry acacia gum and apple pectin to the powdered supplement. Powders were blended with water as per label instructions, a food coloring dye was added to identify different treatments, and the liquid supplements were frozen in ice cube trays to provide consistent meal sizes. The acacia gum product used (Swanson Health Products, Fargo, ND) contained 500 mg per capsule, along with microcrystalline cellulose, magnesium stearate and/or silica and stearic acid, and the apple pectin supplement (Swanson Health Products, Fargo, ND) contained 300 mg pectin per capsule, with a rice flour carrier.

Fecal Collection and Analysis

Fecal samples were collected aseptically on the sixth and seventh days of each diet treatment by lining the cage bottom with autoclaved aluminum foil one hour prior to feeding in the evenings, and collecting fresh droppings, using sterile forceps, into cryotubes within 1-2 hours after feeding commenced. Samples were frozen until overnight shipment to the Ohio State University. DNA was extracted from day seven samples (~0.15 g feces) of each treatment (in duplicate as possible) using a MoBio PowerSoil DNA Isolation Kit following the kit protocol, yielding an average of 60 ng/μl. The V4 region of the 16S rRNA gene was PCR amplified (30 cycles) and sequenced with Illumina MiSeq by Argonne National Laboratory, creating a unique barcode to identify bacteria and archaea present in each fecal sample.

Statistics
Community data for each sample were analyzed using the Bray-Curtis similarity metric and plotted using R (version 3.1.3) or the program Primer (Clarke, 1993). As a result of the conspicuously low abundance of the highly dominant organism of all other samples, cage 5 was removed from further analysis. The average abundance of each OTU per treatment was used to represent the number of OTUs, and all OTUs with an average >0.001% relative abundance were included. Visual interpretations were validated statistically using ANOSIM in Primer (Clarke, 1993) at $P < 0.05$.

Results and Discussion
OTU is an operational definition of a bacterial or archaeal "species" used by microbiologists. Microbiologists refrain from using the term species unless validated using other metrics. Throughout this study, we use an OTU level (>97%) designation to discern different microbes from one another.

Diet and Cage Effects
Using non-metric multidimensional scaling (NMDS), with each colored dot representing the community from a single sample, the distance between each point visually represents the level of similarity between the communities. The high stress of the two NMDS plots (Figures 1 and 2) indicates that the similarity of the samples is not completely represented in two dimensions, and interpretations based solely on this clustering should be avoided. We found that dietary fiber content did not structure the microbial community in feces during the time measured (Figure 1), verified by lack of clustering by fiber treatment (similar symbols did not cluster together) and statistically. Similarly, cages did not impact the structure of the fecal microbiome (Figure 2). The distances within a group of clusters (e.g. filled circles, cage 2) were not visually different from other cages (e.g. filled squares, cage 1); this interpretation was also validated statistically.
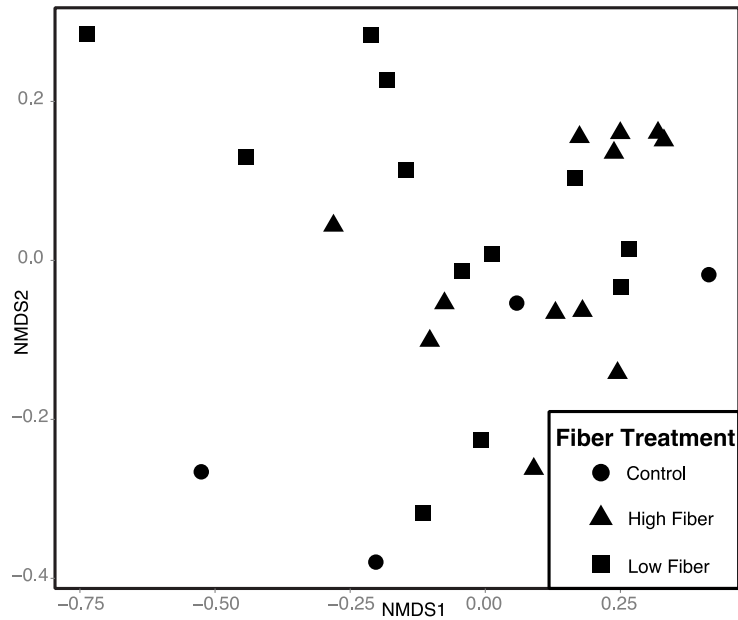
Figure 1. NMDS plot using Bray-Curtis distance for the 16S rRNA gene relative abundance data. Stress: 0.14. The fecal microbial communities (each sample represented by a point) do not cluster by fiber treatment.
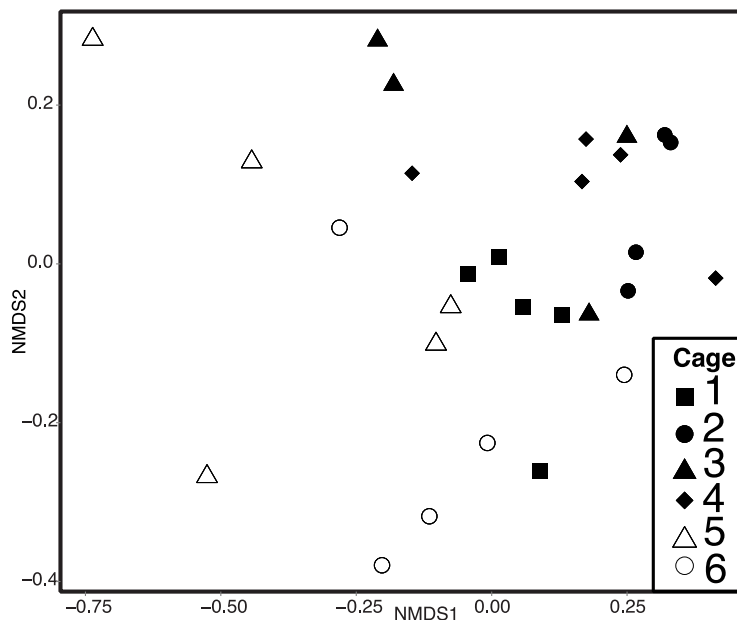

Figure 2. NMDS plot using Bray-Curtis distance for the 16S rRNA gene data set. The fecal microbial communities do not cluster by cage consistently, evidenced by the fact that the samples (points) are not clustered together by symbol.

OTU Overlap
Certain OTUs are shared across treatments (Figure 3). There were on average 153 core microbes shared over all treatments. Fiber treatments shared 70 OTUs; the High Fiber treatment had 22 unique satellite OTUs whereas the Low Fiber treatment had 34 unique satellite OTUs. Notably,

only 1 OTU was identified unique to the baseline treatment that was not below detection in the high or low fiber diets.
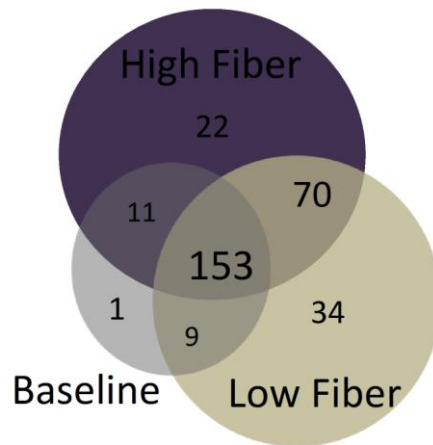

Figure 3. Diagram depicts numbers of OTUs detected in fecal samples from sugar gliders fed a baseline diet, compared with treatments including 7.5 % (Low Fiber) or 10% (High Fiber) added soluble fiber. Numbers are associated with diversity (total), overlap (shared) and unique microbial species identified in the samples.

OTU Change with Fiber Addition
While the entire microbial community failed to show a response to diet, some members of the community did change in abundance with diet (Figure 4). For example, an uncultivated *Bacteroides* sp. increased the most with the high fiber treatment (8.8%; bottom purple bar), followed by a *Succinivibrio* sp. (+2.7% of the high fiber treatment), and a *Faecalibacterium* sp. (+1.5% with high fiber).Typically in microbiome nutritional studies, treatment effects of at least a log-fold change are reported; none of the changes in this study were log-fold, and we cannot say whether or not this is a positive or negative change in the microbiome.
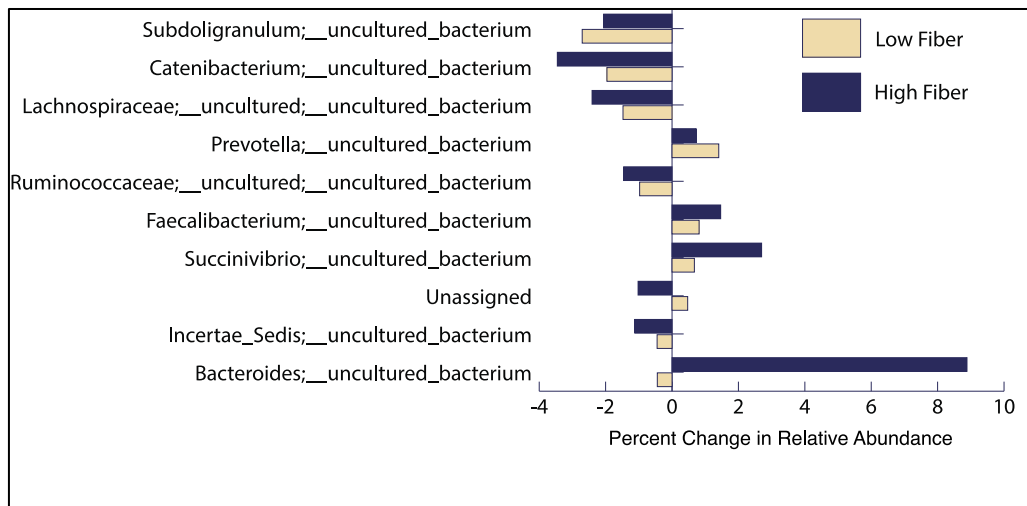
Figure 4. Bar chart representing the change in relative abundance of organisms that most responded to the addition of fiber. An average abundance of each OTU was calculated for each treatment and then compared to baseline.

With recent advancements from the Human Microbiome Project, we have learned that diet alters our gut microbiome and plays a role in our gastrointestinal health (David, 2013, Clemente, 2012). However the role of diet in gut health of many captive animals in zoos and private settings remains uncharacterized, as do normal microflora and optimal levels and types of dietary fiber. Here we explore the effect that increased fiber in the diet may have on the fecal microbial communities of sugar gliders. The inclusion of highly fermentable gel-forming dietary fibers at concentrations ranging from ~5 to 20% of dry matter has been associated with health benefits in a variety of animal and human models, impacting lipid metabolism, glucose tolerance, and absorption of minerals such as Ca and Mg (Levart *et al.*, 1991; Howard *et al.*, 1995). Altered gut microbial populations linked with fermentable dietary fiber are associated with suppression of potentially pathogenic bacteria as well as enhanced cecal mucosal tissue development (Howard *et al.*, 1995).

Captive sugar gliders may also benefit from diets containing higher soluble fiber than currently fed. Addition of up to 10% dietary fiber (as pectin plus gum Arabic) did not alter intakes or fecal consistency. Although microbial changes due to diet treatment were not of a statistically significant magnitude in this pilot study, genomes of closely related microbial genera to those taxa displaying change (Figure 4) contain the potential for fibrolytic activity (including beta-glucosidase, pectinesterase, endoglucanase, and beta-xylosidase). Assuming these enzymes may also be associated with microbial taxa identified in these animals, we speculate that sugar gliders may indeed have the microbial and anatomical capacity for increased fermentation of soluble dietary fibers. Longer trial periods, and/or increased levels in fiber treatments, could result in greater impact on the microbiome which, in turn, may improve digestive physiology and overall health in this species.

Conclusions
1) The most dominant Bacteria and Archaea were shared across the three treatments, with slight changes in abundance. Notably these are also dominant genera observed in fecal samples from other mammals, including:

- a *Bacteroides* sp. (uncultured bacterium from the Bacteroidaceae family)
- a *Prevotella* sp. (uncultured bacterium from the Prevotellaceae family) and
- a *Subdoligranulum* sp. (uncultured bacterium from the Ruminococcaceae family)

2) In this study, we could not statistically discern differences in fecal microbiomes by diet or by cage, although it was clear that one set of samples contained outliers, thus were not included in the analysis. Exclusion was largely attributed to a significant decrease in the most dominant organism found in all other samples.

3) All treatments shared 153 core microbial members, but certain microorganisms were only detected on specific diets.

4) Small changes in abundance of some specific microorganisms in response to diet treatment compared to baseline were observed; due to the limited magnitude of change, we cannot say whether the microbiome is negatively or positively affected. It is possible that longer treatments or increased fiber levels or types could result in greater impact on the microbiomes of the sugar glider.

Literature Cited

Clarke, KR (1993) Non-parametric multivariate analyses of changes in community structure. *Austral J Ecol* 18:117-143.

Clemente JC, Ursell LK, Parfrey LW, and Knight R (2012) The impact of the gut microbiota on human health: an integrative view. *Cell* 148(6):1258-70.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, and Turnbaugh PJ (2013) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505(7484):559-563.

Dierenfeld ES, Thomas D, and Ives R (2006) Comparison of commonly used diets on intake, digestion, growth, and health in captive sugar gliders (*Petaurus breviceps*). *J Exotic Mammal Med Surgery* 15(3):218–24.

Howard J (1989) Diet of *Petaurus breviceps* (Marsupialia:Petauridae) in a mosaic of coastal woodland and heath. *Austral Mammal* 12:15–21.

Howard MD, Gordon DT, Garleb KA, and Kerley MS (1995) Dietary fructooligosaccharide, xylooligosaccharide and gum arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats. *J Nutr* 125: 2604-2609.

Hume ID (1999) Omnivorous marsupials. In: Marsupial Nutrition. Cambridge: Cambridge Press, p. 115.

Levrat M-A, Behr SR, Rémésy C, and Demigné C (1991) Effects of soybean fiber on cecal digestion in rats previously adapted to a fiber-free diet. *J Nutr* 121: 672-678.

Smith AP (1982) Diet and feeding strategies of the marsupial sugar glider in temperate Australia. *J Anim Ecol* 51:149–66.