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Research article

Diet impacts the structure and function of the bacterial community in the gastrointestinal tract of a sloth bear

Andrew Loudon[1,2], Kadie Anderson[3], Katherine Krynak[4], Zhongtang Yu[5] and Patricia Dennis[1,6]

[1]*Cleveland Metroparks Zoo, 3900 Wildlife Way, Cleveland, OH, 44109, USA.*
[2]*Department of Biology, Vancouver Island University, Nanimao, British Columbia, Canada V9R 5S5 .*
[3]*Point Defiance Zoo and Aquarium, 5400 N. Pearl St, Tacoma, WA 98407, USA.*
[4]*Ohio Northern University, 525 S. Main St, Ada, Ohio 45810, USA.*
[5]*The Ohio State University, 281 W Lane Ave, Columbus, OH 43210, USA.*
[6]*The Ohio State University College of Veterinary Medicine, 1920 Coffey Rd, Columbus, OH 43210, USA.*

Correspondence, Andrew Loudon, email; andrew.loudon@viu.ca

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Abstract

Sloth bears *Melursus ursinus* are a threatened species that have a high incidence of hepatobiliary adenocarcinoma in human care. The diet of sloth bears under human care differs greatly from that consumed by wild sloth bears. Wild sloth bears consume a diet comprised predominantly of insects and wild fruits that is likely higher in fat and protein and lower in dietary starch compared to sloth bear diets in zoos. One male sloth bear was experimentally fed with a diet that was lower in dietary starch than its traditional diet. Overall, the faecal bacterial community of this sloth bear was dominated by 12 operational taxonomic units (OTUs) of bacteria (each having a greater than 1% relative abundance). The most predominant OTU was assigned to the genus *Turicibacter* within the class Erysipelotrichia. The relative abundances of some bacterial taxa changed when the lower carbohydrate diet was fed while bacterial OTU richness did not change. Of the 12 predominant OTUs, 8 shifted in relative abundance between the two diets. The lower carbohydrate diet also resulted in increased faecal branched-chain volatile fatty acids, which is indicative of increased protein digestion and amino acid fermentation, a decrease in faecal acetate to propionate ratio and an increase in pH. These results encourage further dietary changes to better mimic the wild sloth bear diet with the purpose of improving captive sloth bear health.

Introduction

Sloth bears *Melursus ursinus* are endemic to the subcontinent of India and are listed as threatened in the wild according to the International Union for Conservation of Nature (IUCN) (Dharaiya et al. 2020). Sustainable and healthy populations of sloth bears in human care are necessary to ensure a future for this species within the Association of Zoos and Aquariums (AZA). Within AZA, sloth bears experience high mortality from disease, in particular biliary adenocarcinoma (Anderson et al. 2018). Diet has been proposed as a factor affecting the development of this disease, possibly resulting from some component of sloth bear diet in human care promoting or not inhibiting carcinogenesis (Arnhold et al. 1995). Recent studies in other species have examined the role of diet and the gut microbiome in various chronic diseases including inflammatory bowel disease, diabetes and cardiovascular disease (Singh et al. 2017). Gut microbiome dysbiosis may also promote tumorigenesis resulting in cholangiocarcinoma (Rao et al. 2021). Gut microbial dysbiosis leads to dysfunction of the intestinal barrier resulting in transfer of intestinal bacteria and lipopolysaccharides into the liver via the portal vein. Chronic inflammation and alterations in bile acid metabolism promote tumorigenesis (Rao et al. 2021).

Diet affects gastrointestinal bacterial communities (David et al. 2014) and their production of volatile fatty acids (VFA) as fermentation products (Ríos-Covián et al. 2016). In zoos, the sloth bear diet mostly consists of commercial fruits, vegetables and a commercial pelleted dog food or exotic bear food (AZA Bear Taxon Advisory Group 2019). Wild sloth bear diet consists of insects—primarily ants and termites—vegetation and wild fruits (Joshi et al. 1997; Khanal and Thapa 2014; Laurie and Seidensticker 1977; Mewada 2015; Palei et al. 2014, 2020; Rather et al. 2020; Sukhadiya et al. 2013). Fruits that sloth bears may consume in the wild are nutritionally different from the domesticated fruits that are commonly used as feed in zoos: commercially available fruit generally has less fibre and more sugars (Milton 1999). Overall, zoo diets for sloth bears are likely higher in dietary starch and lower in protein and fat than the diets of wild sloth bears. This report examines the effects on the gut microbiota of a diet lower in simple carbohydrates, particularly starch, and higher in protein and fat than the traditional zoo diet.

The bacteria in the bear gastrointestinal tract ferment indigestible food and produce VFA (Schwab et al. 2009). VFA perform many beneficial functions, such as serving as energy sources, reducing or attenuating gastrointestinal inflammation and influencing the immune system of the host (Ríos-Covián et al. 2016). In brown bears *Ursus arctos*, different bacterial communities and varying VFA profiles are associated with their seasonally variable diet (e.g. primarily berries versus vegetation in sampled stool) (Schwab et al. 2009). The gastrointestinal bacterial communities of brown bears are linked to host metabolism (Sommer et al. 2016). The gut microbiota of hibernating brown bears has reduced levels of Firmicutes and Actinobacteria as well as increased levels of Bacteroidetes compared to the gut microbiota populations found in bears during the summer. The gut microbiota of hibernating bears is associated with higher serum cholesteryl esters, triglycerides and free cholesterol compared to gut microbiota populations from bears during the summertime. Overall, Sommer et al. (2016) concluded that seasonal variation in brown bear diet is associated with seasonal changes in the gut microbiota and metabolism. Additionally, colonisation of the bear microbiota into germ-free mice resulted in the mice exhibiting similar metabolic features, suggesting that the seasonal differences in bear microbiota contribute to seasonal metabolic differences (Sommer et al. 2016).

A large cross-taxa study on mammalian gastrointestinal bacterial communities demonstrated differences in faecal bacterial communities between animals in human care and those in the wild (McKenzie et al. 2017). One of the patterns identified is less relative abundance of Prevotella in mammals in human care, which the authors attributed possibly to an increase in protein in the captive diet relative to the wild, although they did not have the necessary diet data to test this idea directly. With regard to the bacterial phylum Firmicutes, the authors found that captive mammals have lower relative abundance of Clostridia and much higher Bacilli than wild mammals. The authors also found a general pattern that within the bacterial phylum Proteobacteria, there was an increase in Gammaproteobacteria amongst captive mammals. The authors conclude that dietary changes for mammals in human care can have important consequences (McKenzie et al. 2017). A comparative study of Andean bears *Tremarctos ornatus* also revealed differences in gastrointestinal bacterial communities between bears under human care and those in the wild; the gastrointestinal bacterial community of the bears in human care had a lower predicted function for carbohydrate fermentation than that of wild bears (Borbón-García et al. 2017). A lower predicted carbohydrate fermentation function is likely the result of the lack of complex carbohydrates in the diet consumed by the bears in human care.

Sloth bears diverged from other bears roughly six million years ago (McLellan and Reiner 1994), and their digestive system and symbiotic bacteria have likely evolved to effectively utilise their natural diet. A study of the gut microbiome of 60 diverse mammalian species from zoos and the wild found that host diet and phylogeny influence bacterial diversity and that bacterial communities co-diversified with their mammalian hosts (Ley et al. 2008). The objective of the present study was to examine the effect of diet change on the gastrointestinal bacterial community and fermentation profiles in a single sloth bear housed at the Cleveland Metroparks Zoo. The content of simple carbohydrates, specifically dietary starch, in the diet was decreased to more closely mimic the diet of wild bears. This study sought to examine how a single sloth bear's gastrointestinal bacterial community and VFA production changed when the bear was fed a lower carbohydrate diet.

Methods

One male sloth bear at the Cleveland Metroparks Zoo was experimentally fed a lower carbohydrate diet—specifically lower in dietary starch—that differed from the traditional diet, which served as the baseline of the experiment. The sloth bear was fed daily throughout the study. The traditional diet consisted of 1.1 kg Mazuri Exotic Canine Diet (Mazuri 5MN2), 0.2 kg Purina Dog Chow Light & Healthy Dog Food (Purina Mills), 150 g apple, 160 g raisins, 45 g peanut butter and 85 g mealworms *Tenebrio molitor*. To reduce the simple carbohydrates in the diet, the pelleted foods (Mazuri Exotic Canine Diet and Purine Dog Chow Light & Healthy Dog Food) were replaced with a commercially available pelleted food made without grains (EVO Grain-Free Turkey and Chicken Formula for Cat and Kitten dry food). The lower carbohydrate diet consisted of 1.1 kg EVO Grain-Free Turkey and Chicken Formula for Cat and Kitten dry food (EVO Mars Petcare), 150 g apple, 160 g raisins, 45 g peanut butter and 85 g mealworms. The commercial pelleted feeds were analysed for nutritional content by wet chemistry procedures through Dairy One Forage Laboratory (Dairy One, 730 Warren Road, Ithaca, New York 14850). The nutritional content and differences between the pelleted feeds offered in the two diets are shown in Table 1. The entire diet was consumed daily. Faecal samples were collected once a week for five weeks while the sloth bear was fed the traditional diet followed by a three-week transition period between the diets where no sampling took place. Samples were then collected for 19 weeks while the lower carbohydrate diet was fed. While dietary interventions have been documented to alter gut microbiota within days, few studies have examined the effect of dietary changes over longer periods. Habitual diet of the host is thought to be the primary influence on core gut microbial populations (Leeming et al. 2019) so faecal gut microbiota were monitored over several months. The daily allowance of the bear's diet was divided into morning and afternoon meals, with food scattered on-exhibit to promote foraging behaviour. Faecal samples during each diet phase were collected once a week from the floor of the exhibit. Faecal samples were placed into sterile plastic collection bags, air was pressed out and the bag was sealed. Faecal samples were collected within 4 hours of defecation. Once collected, samples were frozen at −80°C until DNA extraction and VFA analyses. Five faecal samples during the traditional zoo diet period and thirteen samples from the lower carbohydrate diet were analysed. DNA was extracted from faecal samples in the laboratory at Ohio Northern University using the Qiagen DNeasy PowerSoil Kit per manufacturer's protocol. To determine bacterial community structure the V4 region of the 16S rRNA gene was sequenced (2 × 250 paired-end sequencing) on the Illumina MiSeq platform (University of Michigan's Center for Microbial Systems) using the dual-indexing sequencing strategy

Table 1. Diet nutrient composition (based on dry matter)

Nutrient	Initial diet %	Experimental diet %	Difference %
Crude protein	34.8	54.8	20.0
Crude fat	15.0	25.8	10.8
Lignin	1.7	2.1	0.4
Water soluble carbohydrates	1.7	2.0	0.3
Simple sugars	2.0	2.1	0.1
Ash	8.6	7.5	-1.1
Acid detergent fibre	5.2	3.3	-1.9
Neutral detergent fibre	16.8	11.0	-5.8
Starch	24.0	9.3	-14.7
Non-fibre carbohydrates	24.8	0.8	-24.0

(Kozich et al. 2013). Amplicon libraries were prepared according to Illumina's Protocol for Preparing Libraries for Sequencing on the MiSeq (part# 15039740 Rev. D) for 2nM or 4nM libraries by the University of Michigan's Center for Microbial Systems. FASTQ files were deposited to NCBI GENBANK (accessions SAMN13323524–SAMN13323544; Bioproject PRJNA590263).

To form operational taxonomic units (OTUs) of bacteria, paired-end sequences were combined using QIIME 1.9.1 (Caporaso et al. 2010) and OTUs were formed using UCLUST (Edgar 2011) at a 97% threshold. Taxonomy was assigned using the SILVA 132 database in QIIME. To ensure accurate results the following steps were taken: 1) any OTUs that were classified as chloroplast or as unassigned taxonomy were removed, 2) OTUs with fewer than 50 total reads were removed, 3) OTUs that were only present in one sample were removed and 4) any chimeras, as determined with VSEARCH, were removed (Rognes et al. 2016). Samples were rarefied to 21,000 sequences per sample for alpha and beta diversity analyses. This number was chosen to ensure maximum sequence coverage while keeping all of the samples; multiple rarefactions in QIIME were run to test whether this adequately observed diversity. The BLAST sequence analysing tool (Madden 2013) was used to clarify the resultant de novo OTUs.

Bray-Curtis similarity was used to analyse bacterial community similarity between the two diets and an Adonis test performed using Phyloseq (McMurdie and Holmes 2013) and Vegan (Oksanen et al. 2015). Results were visualised using a nonmetric multidimensional scaling (NMDS) plot and differences in dispersion were tested for using Vegan. To test for differences in alpha diversity Chao1 was calculated and a nonparametric independence test performed. To determine which bacteria are different between the two diet conditions DESeq2 was used (Love et al. 2014), which tests for differences between treatments for each bacterium and adjusts for multiple comparisons.

Volatile fatty acids in the faecal samples were analysed using gas chromatography (GC) at the Ohio State University as reported in Zhou et al. (2011). Briefly, three parts of sterile water were added to one part of each faecal sample based on weight and the pH was determined immediately after mixing. Samples were then centrifuged at 3,000g for 10 minutes. The supernatant was collected and acidified with 25% meta-phosphoric acid (1:4 volume ratio). Samples were kept at 4°C until further processing. Samples were then centrifuged at 3,000g for 15 minutes before adding 2-ethybutyric acid as the internal standard and stored at −20°C until GC analysis. To test if diet affected bacterial community function a nonparametric independence test was performed on VFA and pH data using the coin package in R (Hothorn et al. 2008).

Results

From the 21 faecal samples from the single sloth bear, 516,435 sequences were obtained, which were grouped into 178 OTUs. Samples were rarefied to 21,000 sequences per sample; there were 113 OTUs within the dataset after rarefaction. Multiple rarefactions were run in QIIME (Caporaso et al. 2010) and samples reached the asymptote by 21,000 sequences, indicating that the communities had been adequately sampled to observe most of the bacterial diversity within these communities. OTU denovo560, which was abundant and frequent when the lower carbohydrate diet was fed, was assigned to the genus *Epulopiscium*, which is found in the gut of marine tropical fishes (Angert and Clements 2004). Since this genus of bacteria is unlikely to be in the gastrointestinal tract of a sloth bear, a BLAST search was performed to find the most similar sequences. All of the top 100 sequence hits within NCBI were identical to denovo560; all were detected in the environment, wastewater or mammalian gastrointestinal tracts (cattle and humans). Furthermore, within the Ribosomal Database Project, species of *Epulopiscium* are listed as being within the genus *Cellulosilyticum* (Cole et al. 2014). The taxonomy for denovo560 is likely a misnomer within the SILVA database.

Bacterial community compositions were different between the traditional zoo diet and lower carbohydrate diet (df=1:19, F=7.77, R2=0.29, P<0.001), and there was no difference in dispersion between the diets (F=2.78, P=0.11; Figure 1a). Alpha diversity did not differ but approached significance for increasing when the lower carbohydrate diet was fed compared to the traditional zoo diet (Z=−1.89, P=0.059; Figure 1b). Changes in the relative abundances of individual bacteria were detected when the lower carbohydrate diet was fed. Of 113 OTUs, 40 significantly changed in abundance between the treatments, with 19 increasing and 21 decreasing with the lower carbohydrate diet; many of these are rare OTUs. To better understand which bacteria changed with diet, bacteria that had an average relative abundance of greater than 1% in one of the diet treatments were focused on. This threshold has been applied in previous studies to characterise common bacteria (Li et al. 2012). Twelve OTUs were considered common (greater than 1% relative abundance), ten of which

Table 2. Bacterial operational taxonomic units (OTUs) each with a relative abundance greater than 1% in at least one of the two diets. Each OTU represents a unique bacterial variant. Mean bacterial OTU percentage for each diet treatment is presented, in addition to log2 fold change and adjusted P-value. OTUs are ranked by the log2 fold change between the two dietary treatments: this represents the fold change in relative abundance between treatments.

OTU	Traditional zoo	Lower carbohydrate	log2 fold change	stat	P value	Taxonomy
denovo560	0.0000	0.0585	-10.567395	-8.5234325	<0.001	Firmicutes, Clostridia, Clostridiales, Lachnospiraceae, *Epulopiscium*
denovo3970	0.0000	0.0267	-9.227	-8.229	<0.001	Firmicutes, Clostridia, Clostridiales, Lachnospiraceae, *Cellulosilyticum*
denovo3043	0.0001	0.0085	-6.532	-6.582	<0.001	Firmicutes, Clostridia, Clostridiales, Clostridiaceae, *Clostridium* sensu stricto
denovo3882	0.0414	0.1996	-1.456	-3.301	0.004	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, *Terrisporobacter*
denovo4977	0.0480	0.0841	-0.127	-0.214	0.830	Firmicutes, Clostridia, Clostridiales, Clostridiaceae, *Clostridium* sensu stricto
denovo867	0.3154	0.3370	0.814	2.370	0.048	Firmicutes, Erisipelotrichia, Erysipelotrichales, Erysipelotrichaceae, *Turicibacter*
denovo868	0.1903	0.1646	0.908	1.871	0.095	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, *Romboutsia*
denovo838	0.0338	0.0311	0.940	1.387	0.199	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, *Romboutsia*
denovo3930	0.0235	0.0022	4.241	6.050	<0.001	Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, Burkholderiaceae, *Burkholderia pseudomultivorans*
denovo987	0.0119	0.0000	9.163	5.118	<0.001	Firmicutes, Clostridia, Clostridiales, Clostridiaceae, *Clostridium* sensu stricto
denovo4174	0.0173	0.0000	11.122	4.490	<0.001	Firmicutes, Bacilli, Lactobacillales, Streptocoocaceae, *Streptococcus*
denovo3253	0.0999	0.0000	13.700	5.045	<0.001	Firmicutes, Clostridia, Clostridiales, Clostridiaceae, *Sarcina*
denovo927	0.1313	0.0000	13.888	7.543	<0.001	Proteobacteria, Gammaproteobacteria, Enterobacteriales, Enterobacteriaceae, Escherichia, *Shigella*

are in Firmicutes and two are in Proteobacteria (Table 2). Of the common bacteria, three increased in abundance, five decreased in abundance under the lower carbohydrate diet and four were unchanged in abundance (Table 2).

The faecal propionate, iso-butyrate, branched-VFA, and iso-valerate increased and the acetate-propionate ratio (A:P ratio) decreased when the sloth bear consumed the lower carbohydrate diet (Table 3). The concentrations of total VFA, acetate, valerate and butyrate did not differ between the two diets. A higher, more neutral faecal pH (Z=−2.22, P=0.026) resulted from the lower carbohydrate diet compared to the traditional zoo diet (Figure 2).

Table 3. Faecal volatile fatty acids (VFA) profiles (mM/gram feces) that were measured between the traditional diet and lower carbohydrate diet. Samples are ranked by P-value. () = standard deviation.

VFA	Traditional zoo	Lower cabohydrate	Z	P
A:P (ratio)	28.77 (2.69)	10.88 (2.18)	4.30	<0.001
Propionate	0.4 (0.16)	1.5 (0.7)	-2.78	0.01
Iso-butyrate	0.1 (0.07)	0.32 (0.15)	-2.62	0.01
Branched-VFA	0.29 (0.22)	0.74 (0.31)	-2.58	0.01
Iso-valerate	0.2 (0.14)	0.42 (0.17)	-2.35	0.13
Total VFA	14.2 (6.1)	19.7 (7.2)	-1.48	0.17
Acetate	11.4 (4.7)	15.4 (5.7)	-1.36	0.17
Valerate	0.13 (0.06)	0.16 (0.06)	-0.89	0.37
Butyrate	1.95 (1.1)	1.92 (0.78)	0.06	0.95

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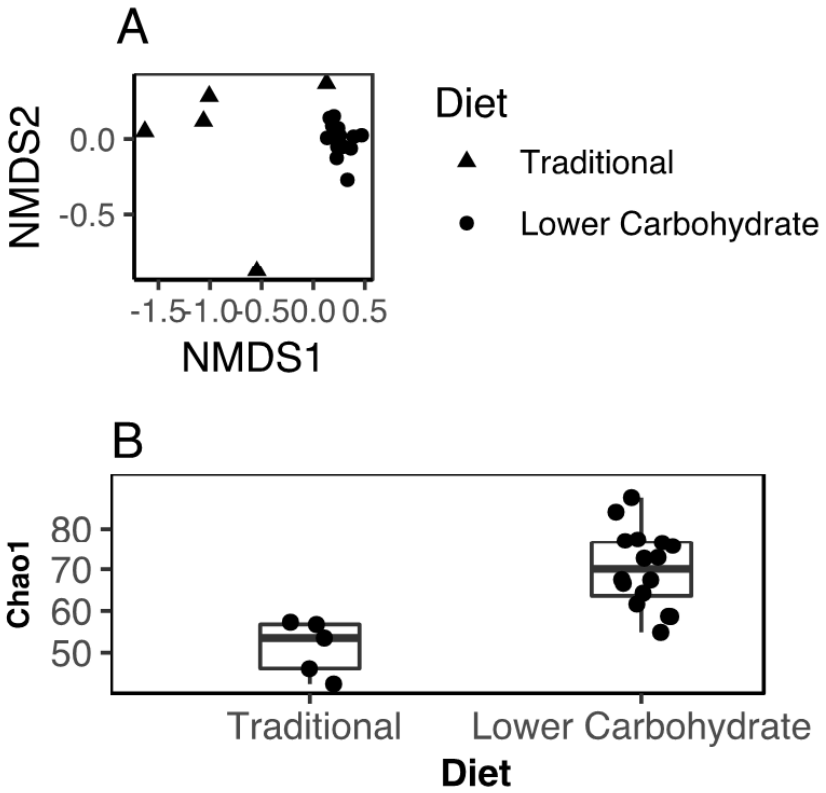

Figure 1. Nonmetric multidimensional scaling (NMDS) plot showing the comparison of the overall bacterial communities between the two diets (A) and Chao1 richness estimate (B). The NMDS plot has a stress of 0.11. Alpha diversity, measured as Chao1, increases with the lower carbohydrate diet.

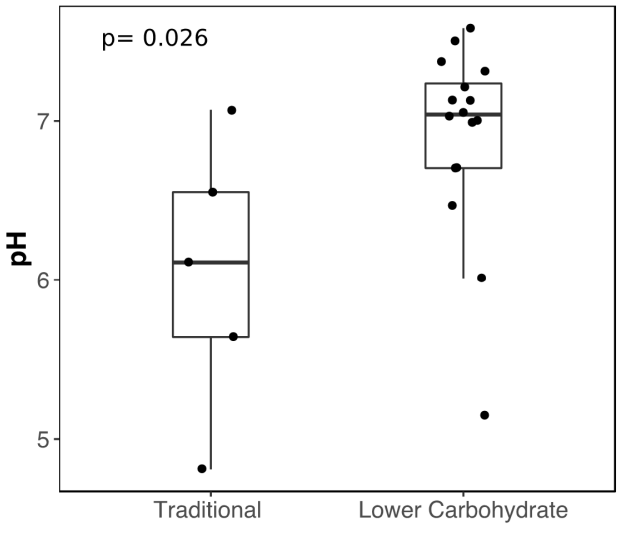

Figure 2. Fecal pH values corresponding to the two diets.

Discussion

Gastrointestinal bacterial community composition shifted when the sloth bear was fed a lower carbohydrate diet and there was a trend in increased alpha diversity. Diet is a major contributor to the composition of gastrointestinal bacterial communities, and bacterial communities predictably and quickly change with dietary changes in humans (David et al. 2014). The importance of diet is likely explained by bacterial communities being strongly shaped by environmental filtering (Mazel et al. 2018), where only some bacteria are able to live in the niche space, which, in this case, is a gastrointestinal tract (Kraft et al. 2015). The niche space is influenced by diet; therefore, a change in diet will change the nutritional niche space that is available for bacteria. In this study, the effect of a lower carbohydrate diet on gut bacterial community composition and VFA in a sloth bear was examined. The traditional zoo diet contained less crude fat and protein but more starch, non-fibre carbohydrates and fibre than the lower carbohydrate diet (Table 1). This lower carbohydrate diet was chosen to more closely mimic the diet consumed by wild sloth

bears that consists of primarily ants and termites and seasonally wild fruit. A primarily insect and fruit-based diet would contain little dietary starch. While protein and fat levels became more similar to the composition of termites, starch reduction was the major aim of the dietary change in this study. The aim of the study was to examine the effect of reduced dietary starch on the gut microbiome of the sloth bear. This study did not attempt to replicate the levels of chitin found in the diets of wild sloth bears as chitin was not a component of the traditional zoo diet. Chitin is an aminopolysaccharide polymer that is an important component of the insect exoskeleton. Myrmecophagous species may degrade chitin via chitinolytic gut bacteria or by chitinases, which break down chitin by hydrolysing glycoside bonds (Tabata et al. 2018), but the degree to which myrmecophagous species ferment or utilise dietary chitin is unknown.

There were more bacterial OTUs when the sloth bear was fed the lower carbohydrate diet (Figure 1b). This coincided with a faecal pH closer to neutral (from ~6 to ~7; Figure 2). The trend in alpha diversity may be explained by a more neutral pH, or possibly by the new diet having more diversity in substrates. For example, increased alpha diversity was associated with a more neutral pH, compared to a more acidic pH in in vitro assays that simulated the human gastrointestinal tract (Ilhan et al. 2017). Increases in alpha diversity have been associated with high protein/low carbohydrate diets in dogs fed a high meat/low grain diet compared to a grain-rich diet (Jackson and Jewell 2019). There was also a difference in faecal pH in the dogs on the different diets, with high meat/low grain pH closer to neutral (6.34 versus 5.98 on grain-rich diet) (Jackson and Jewell 2019). In contrast, in raw diets in domestic cats *Felis catus*, the faecal pH decreased in a fibre-added diet compared to a raw meat diet (high protein/high fat) (Butowski et al. 2019); however, alpha diversity did not differ significantly between the raw diet and the higher fibre diet.

The bacterial communities in this study are simple; there were 12 bacteria that had a relative average abundance of over 1% and 10 of these changed in abundance (Table 2). The most abundant bacterium is *Turicibacter*, which was stable within the gut of the bear and did not significantly change in relative abundance. *Turicibacter* is frequent and abundant in Asian black bears *Ursus thibetanus* (Song et al. 2017) and is a common bacterium found in animal gastrointestinal tracts (Goodrich et al. 2016; Xue et al. 2015). Furthermore, *Turicibacter* may be associated with animals that eat insects since it is enriched in giant anteaters *Myrmecophaga tridactyla*, compared to sloths which are a sister taxon (Folivora) that does not eat insects (Delsuc et al. 2014). At the family level there was an increase in Lachnospiraceae (denovo3970; *Cellulosilyticum*). This may be in response to the shift from a pelleted diet with a corn and rice base in the traditional zoo diet to the lower carbohydrate diet which is grain-free and contains pea fibre and cranberries. Xyloglucan is the most common hemicellulose in the primary cell wall of most dicotyledonous plants and non-graminaceous monocotyledonous plants (Williams et al. 2017) and is found in both peas and cranberries. In vitro faecal fermentation studies showed increased Lachnospiraceae with xyloglucan as compared to pectin (Cantu-Jungles et al. 2019). In a study of rats, dietary pea fibre increased the abundance of Lachnospiraceae in the gut microbiome (Hashemi et al. 2017). The grain-free aspect of the lower carbohydrate diet may more closely mimic the wild diet of sloth bears than the traditional zoo diet, as the plant material in the diet of wild sloth bears is primarily fruits of dicotyledonous plants, which would be lower in dietary starch than graminaceous plants such as corn and rice. The hemicellulose found in the pea fibre and cranberries also more closely mimics that of wild fruits than do the grains found in the traditional diet.

All but two of the common bacteria in this study are in the bacterial phylum Firmicutes; this group is associated with high-fat diets that are associated with the Western diet in humans (Tomova et al. 2019). The function of these bacteria, and whether they are beneficial, commensal or pathogenic to sloth bears is unknown.

The products of bacterial communities changed when the sloth bear ate the lower carbohydrate diet. VFA was quantified as a proxy of bacterial fermentation. The acetate: propionate ratio decreased from ~29 to ~11 in the lower carbohydrate diet, which was the result of an increase in propionate. An increase in propionate would not be expected if bacteria were producing propionate by fermenting fibre since the lower carbohydrate diet has less fermentable fibre. A:P ratios have previously been linked to gastrointestinal health (Wolever et al. 1991) and are often used to compare treatment groups (den Besten et al. 2013). The sloth bear in this study had a high A:P ratio compared to polar *Ursus maritimus*, black *Ursus americanus* and brown *Ursus arctos* bears (Schwab and Gänzle 2011). The actual increase of propionate was approximately four-fold; however, the concentration was low compared to the total amount of VFA. The exact substrates that are present in each diet are unknown, and it is possible that there is an increase in a substrate in the lower carbohydrate diet that was lower in abundance or absent from the initial diet. The pea fibre and cranberries in the grain-free EVO component of the lower carbohydrate diet may explain both the increase in Lachnospiraceae as well as the increase in propionate. Propionate is a VFA produced by the fermentation of xyloglucan, a hemicellulose found in peas and cranberries (Cantu-Jungles et al. 2019). Alternatively, there could be more propionate if there are fewer bacteria that metabolise propionate, since propionate can be used as a single carbon source by bacteria (Suvorova et al. 2012). The lower carbohydrate diet was higher in dietary protein than the traditional diet. This increase in protein, though not the direct aim of the study, could explain the change in faecal pH. A dietary study in dogs, which are also carnivores and have a similar digestive tract to bears, found that dogs transitioned to a higher protein diet had an increase in faecal pH over time (Lin et al. 2022). There was also an increase in branched-VFA, which is not surprising since branched-VFA are primarily produced from protein degradation and subsequent amino acid fermentation (Smith and Macfarlane 1997) and the lower carbohydrate diet had increased protein.

Diet is a vital component of health and providing a proper diet can be challenging when feeding animals in human care. Often, the actual diet of a species is not available commercially or is not practical to formulate, making it challenging to recreate the nutritional components of the wild diet. Although this study only includes one sloth bear, it provides valuable information on the microbial dynamics in the gastrointestinal tract of an endangered species that frequently has health complications in human care (Anderson et al. 2018). Studies with only one subject are not ideal since single subjects could respond atypically; however, it is difficult to have typical sample sizes for endangered species (West et al. 2019). Although it was a single sloth bear, the bacterial communties and VFA were examined over time, which is similar to an early tactic of examining the human microbiome, e.g. where two humans were examined repeatedly over a year (Caporaso et al. 2011). In this study, dietary starch was decreased and fat and protein increased, which is expected to be more similar to the wild diet, without considering fibre or indigestible diet components. This dietary change resulted in a shift in bacterial composition, an increase in colonic pH, greater branched-VFA concentrations and more propionate, which resulted in a lower A:P ratio. Future studies should examine the effects of insects, which are a major component of wild sloth bear diets, on the structure and function

of the gastrointestinal bacterial community. Dietary insects may contribute to the production of VFA as the chitinous exoskeleton is not digested and may be fermented by the gut microbiota. The addition of insects into the diets of dogs increased VFA production (Bosch et al. 2016). A diet more similar to that of wild sloth bears may have beneficial long-term health impacts.

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