Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/j.1439-0396.2012.01299.x

ORIGINAL ARTICLE

Dietary shifts affect the gastrointestinal microflora of the giant panda (*Ailuropoda melanoleuca*)

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Keywords
gut microbiome, foraging effects, microbial digestion, giant panda

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Received: 30 November 2011;
accepted: 23 February 2012

Summary

Giant pandas exhibit seasonal changes in bamboo plant part preference. The influences on the gastrointestinal tracts (GIT) microbial populations were evaluated during a 14-month period for a pair of adult male and female giant pandas housed at the Memphis Zoo using traditional culturing methods to enumerate eight bacterial groups (total anaerobes, total aerobes (TAR), streptococci (STR), total enterics, *Escherichia coli*, *Bacteroides* spp., lactobacilli and *Clostridium* spp.). Both the male and female pandas altered bamboo consumption behaviours, with a sharp decrease in leaf preference in April 2010 and returning to high levels of leaf preference from June to October, corresponding to significant shifts in the densities of TAR, STR, and lactobacilli and *Bacteroides* spp. These findings indicate seasonal changes in food preference affect the assemblages of microbial populations within the GIT of the giant panda and contribute to a better understanding of the importance of bamboo in this species' foraging strategy.

Introduction

The giant panda's unconventional biology sets it apart from other members of Ursidae. As a highly specialized herbivore, the diet of pandas consists of approximately 99% bamboo (Dierenfeld et al., 1982; Christiansen, 2008). Herbivores typically extract energy from their diets utilizing longer digesta retention times in gastrointestinal tracts (GIT) that are 10–22 times longer than body length and through the selective consumption of large amounts of forage (Schaller et al., 1985; Van Soest, 1996). Although giant pandas exhibit selective consumption of large amounts of forage, their GIT is approximately four times the body length, similar to most carnivores, with a rapid passage of digesta (Schaller et al., 1985; Tarou et al., 2005; Loeffler et al., 2006; Hansen et al., 2010). Giant pandas display preferences for both specific bamboo species and specific portions of the plant (Tarou et al., 2005; Hansen et al., 2010). For example, pandas housed at the Memphis Zoo show a preference for leaves from June to December and shift to preferring culm beginning in February and continuing through May (Hansen et al., 2010). It is unknown how these seasonal changes in bamboo plant part consumption impact the GIT.

In herbivores, the GIT microbiota are involved in critical symbiotic relationships with the host. These organisms are typically anaerobic bacteria, and they are crucial in the digestion and fermentation of fibrous forage. Most bacteria inhabiting the GIT are saccharolytic and utilize the degradation of carbohydrates as a source of energy and carbon, and many can derive energy and carbon from complex

carbohydrate degradation either independently or with assistance from other microbes and do not rely on the availability of simple sugars as substrates (Chesson et al., 1986; Gilbert and Hazlewood, 1993; Hudson and Marsh, 1995; Kato et al., 2004; O'Sullivan et al., 2004). Because of the giant panda's highly fibrous diet and lack of herbivorous digestive specializations, cellulolytic organisms, such as *Clostridium* and *Bacteroides* spp., may play a role in bamboo digestion. Furthermore, complete sequencing of the panda genome shows this species does not contain any homologues for digestive cellulase genes needed to break down structural carbohydrates (Li et al., 2010), but there is evidence of cellulose digestion by the microbiota subsisting in the GIT. The metagenomic approach of Zhu et al. (2011) showed the presence of predictive genes for cellulolytic enzymes in microbes in the GIT. These findings suggest the importance of micro-organisms present in the giant panda GIT with a potential role in microbial digestion of plant material. With its highly fibrous diet and unique digestive physiology, the giant panda may have unique and interesting microbiota that have specialized functions in extracting energy useable by its host.

Dietary changes are an important factor influencing the constant state of flux of GIT microflora populations (Yokoyama and Johnson, 1988; Buddington and Sunvold, 1998; Collins and Gibson, 1999). Altering the availability of some dietary substrates will cause a change in the proportions of GIT micro-organisms and their fermentation products; this is evidenced by a decrease in the proportion of pathogenic organisms accompanied by an increase in beneficial bacteria following feeding of fermentable fibre (Varel and Pond, 1985; Varel et al., 1987; Varel and Yen, 1997; Buddington and Sunvold, 1998). For example, Zentek et al. (2003) evaluated different diets and their effects on microbial populations of bifidobacteria (beneficial) and *Clostridium perfringens* (pathogenic) in canines. Shifts from fibrous diets to protein-rich diets increased *C. perfringens* populations, while shifts from a protein-rich diet to a highly fibrous diet increased bifidobacteria populations (Zentek et al., 2003).

To the authors' knowledge, there have been only three giant panda GIT microflora studies conducted to date. Hirayama et al. (1989) used anaerobic sampling and traditional culturing methods. In contrast, Wei et al. (2007) used 16S rDNA-based screening methods. Zhu et al. (2011) used a metagenomic approach to characterize functional aspects of the microbiota, giving insight into the cellulose metabolism of the microflora. These previous studies only gave 'snapshot' in time characterizations of giant panda GIT flora and did not take into account seasonal or dietary changes that might affect the microbiota. Knowing that pandas undergo temporal shifts in bamboo species consumption and plant part preference along with the fact that diet affects gut microflora and animal health, it is imperative that a complete seasonal profile is generated to better understand the dynamic GIT microflora of these bears.

This study's main objective was to assess the influence of dietary shifts on the gastrointestinal flora of the giant panda. Bamboo part preference data and microflora populations were monitored for fourteen months. By correlating the changes in bacterial populations and the change in foraging behaviour, a comprehensive profile was generated to give greater insight into the relationship between diet and microflora; this also allowed for a better understanding of host–microflora symbiosis.

Materials and methods

Behaviour analysis of bamboo consumption

The study of bamboo consumption behaviour at the Memphis Zoo has been ongoing since the fall of 2003 and was conducted as previously described (Hansen et al., 2010). In brief, behaviour data were collected in 20-min periods in 30-s increments while the bear was feeding on bamboo using an ethogram focusing on foraging behaviours. These behaviours were divided into four main consumption categories: leaf, culm (stalk), other plant part (i.e. shoot or branch) and unknown plant part. For each month, the total consumption behaviours were quantified by time spent consuming specific parts, and each individual behaviour was expressed as a percentage of the total consumption behaviours. Bamboo was harvested from Shelby Farms (Memphis, TN, USA) and Cook Family Farm (North Mississippi). Species of bamboo included *Phyllostachys sulcata*, *P. nuda*, *P. bissetti*, *P. rubromarginata*, *P. glauca*, *P. aurea* and *Pseudosasa japonica*. Other diet enrichments included leaf-eater biscuits (Mazuri), fresh produce (apples, bananas, honeydew, strawberries, blueberries, kiwi and grapes) and dried fruit (pineapple, prunes, apricots, raisins, cranberries, cherries, mangoes, cantaloupe and banana chips) combined were approximately 4% of daily dietary intake for both male and female bears.

Sample collection and analysis

This study consisted of an adult male (Studbook number, 466; age, 14) and female giant panda (Studbook number, 507; age, 12) housed at the Memphis Zoo. The indoor giant panda housing was climate controlled, with an average temperature of 21 °C, and pandas were only allowed into their outside enclosure when temperatures were in the range of 0–27 °C. Faecal samples were collected monthly for 14 months (October 2009–November 2010) for both male and female giant pandas. Samples were collected within 20 min following defecation and placed into an AnaeroPack System anaerobic box, and an AnaeroPack System generator was used to achieve anaerobic conditions (Mitsubishi Gas Chemical Co., Tokyo, Japan). An anaerobic indicator (AnaeroPack Anaero Indicator) was also included to verify that anaerobic conditions were created and maintained. The anaerobic jar, including the sample, was transported on ice to the laboratory and maintained at 6 °C prior to analysis.

Once samples reached the laboratory, they were placed into a Coy anaerobic chamber with an atmosphere of 80% nitrogen, 10% hydrogen and 10% carbon dioxide. Approximately 5 g of sample was processed using a Wiley mill to ensure the entire sample was fully ground. Two grams of ground sample was then added to twenty millilitres of sterile anaerobic diluent (Difco Yeast Extract; BD Biosciences, Sparks, MD, USA) and homogenized using a Fisher PowerGen 125 homogenizer to ensure a homogeneous suspension. From this suspension, tenfold dilutions were prepared using sterile anaerobic diluent to 10^{-8}. Dilutions were plated using the Spiral Autoplater APC 4000. The following bacterial growth media were used: Centers for Disease Control and Prevention (CDC) anaerobic blood agar for total anaerobes (TAN) (Remel), blood agar for total aerobes (TAR) (Remel), Columbia agar for gram-positive aerobic bacteria including streptococci (STR) (Remel), MacConkey agar for total enterics (Remel), eosin methylene blue agar for *Escherichia coli* (BBL), and *Bacteroides* Bile Esculin agar (BBL) for *Bacteroides* (BBE), *Lactobacillus* selective agar (LBS) for lactobacilli and CDC anaerobic blood agar following an ethanol treatment for *Clostridium*. Several media were incubated in the anaerobic chamber at 35 °C including CDC blood agar, LBS agar, BBE agar and CDC ethanol-treated blood agar, while the remaining media were incubated at 37 °C in room atmosphere. At appropriate time periods stated in the Wadsworth manual, plates were removed and colonies were counted (Jousimies-Somer et al., 2002).

Statistical analysis

All statistical analyses were carried out using SAS 9.2 statistical software (Cary, NC, USA). Colony-forming units per gram faecal material (CFU/g) values were log-transformed using PROC UNIVARIATE. The proper covariance structure was sought using Schwarz Bayesian Criterion (BIC) values for several structures, and these values established that a Banded Toeplitz [TOEP(2)] was the correct covariance structure for our model. Using PROC MIXED with a type I test for fixed effects, the model explored interactions between time of year and leaf consumption behaviour (model = time, leaf, time*leaf, time*time, time*time*leaf/H type = 1) at $\alpha = 0.05$. Linear and quadratic fits were performed using PROC REG for both time and leaf consumption behaviour effects. The Pearson correlation was carried out to determine any correlation between microflora and leaf consumption behaviour (PROC CORR). Statistical analyses were also conducted comparing culm consumption to bacterial populations and time of year, but there were no changes in levels of significance when compared to analysis conducted using leaf proportion.

Results

Bamboo consumption behaviour

Dramatic eating behavioural shifts were observed in both pandas. The mean monthly observed bamboo consumption behaviours for both male and female pandas can be seen in Fig. 1. The bears more frequently consumed culm and leaf; other plant part and unknown portion consumption was less frequently observed (male and female combined 14-month average, 3% and 0.4% respectively).

The female bear demonstrated peak leaf consumption from June to October, reducing her proportion of leaves consumed by November 2010 (Fig. 1a). Unlike the male panda, she was not observed consuming any bamboo plant portion exclusively in any month. However, she did display the highest observed culm consumption in April 2010 (approximately 86%). The months June through October displayed the highest observed leaf-eating behaviour for the female, ranging from approximately 50–94% with peak leaf consumption in September. In November 2010, her leaf consumption behaviour returned to 50%.

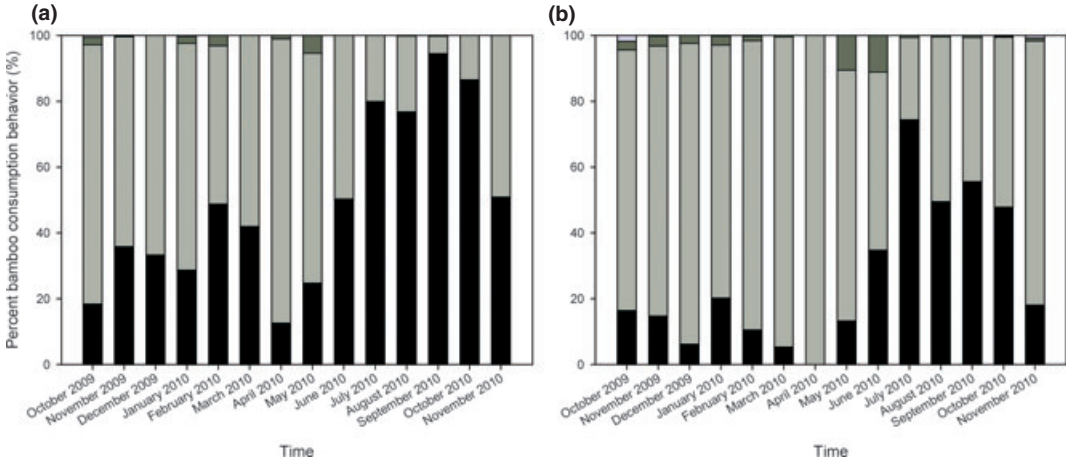

Fig. 1 (a) Mean monthly proportions of bamboo consumption behaviour observed for leaf (■), culm (▨), other (▩) and unknown (□) displayed as a percentage of total feeding observations by the female panda. (b) Mean monthly proportions of bamboo consumption behaviour observed for leaf (■), culm (▨), other (▩) and unknown (□) displayed as a percentage of total feeding observations by the female panda.

The male panda also showed a similar behavioural shift in preference. Although the proportion of bamboo parts consumed was different from the female, he did demonstrate a similar peak leaf consumption from June to October. Throughout most of the year, the male bear had a preference for the culm portion, but in June showed a shift in preference to high leaf consumption, returning to lower values by November 2010 (Fig. 1a). In April 2010, he was observed eating the culm portion exclusively, but in May, he began consuming leaves, reaching his highest level of leaf consumption in July, approximately 75%. August through September observed leaf consumption values ranged from approximately 44–51%, dropping to approximately 20% in November 2010.

Enumeration of microflora

Microflora enumeration for the eight bacterial groups was calculated using colony-forming units per gram faecal material (CFU/g). Data were log-transformed and displayed in Fig. 2. The female's microbial flux displayed a similar trend for TAN, TAR and STR, with the lowest points in November 2009–January 2010 and a relatively constant trend for the remainder of the study (Fig. 2a). However, total enterics (ENT) and *Escherichia coli* (EC) showed a different pattern; these microbes were found at lower levels in October 2009 (10^6), then reaching their highest level in January 2010 (10^8), decreasing to 10^6 in March 2010, and ranging from 10^7 to 10^8 for the remainder of the study. Anaerobe populations for *Bacteroides* spp. (BAC), *Lactobacillus* spp. (LBS) and *Clostridium* spp. (CLO) fluctuate greatly over the 14-month collection period for the female (Fig. 2b). BAC was below the detection limit ($<10^2$) until April 2010 and reached its highest level (10^4) in October 2010. From the initial sampling, levels of LBS were relatively constant at 10^5 until exhibiting a decline in June 2010 (Fig. 2b). CLO also displayed variation with levels ranging from $<10^2$ to 10^6.

In contrast, the male's TAN, TAR, STR, ENT and EC clustered together and followed a similar trend to the female's throughout most of the year with the exception of EC and ENT, which displayed a separation from the other bacterial groups from January to March 2010, showing their lowest levels of 10^3 and 10^5 for EC and ENT respectively (Fig. 2c). Similarly, all other microbial types displayed their lowest levels from November 2009 to February 2010. Anaerobes such as BAC, LBS and CLO fluctuated month to month over the entire study period (Fig. 2d). BAC was below the detection limit ($<10^2$) until May 2010 and reached its highest observed level of 10^4 in October 2010. LBS fluctuated as well, ranging from a high of 10^7 in November 2010 to below detection ($<10^2$) in February 2010 (Fig. 2d). CLO also displayed similar variation to the female panda with levels ranging from $<10^2$ to 10^6 (Fig. 2d).

Relationship between time of year, foraging behaviours and microflora populations

The mixed procedure using a type I test for fixed effects explored any interactions between time of year and leaf consumption. No significant interac-

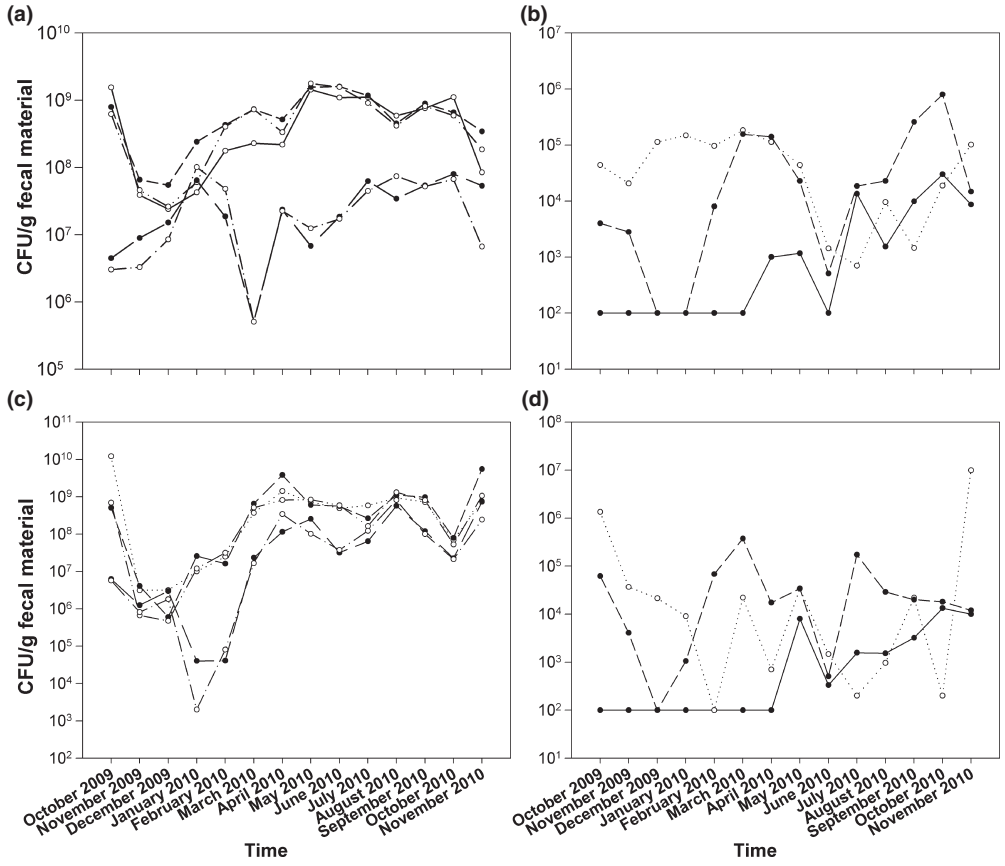

Fig. 2 (a) Mean monthly plots of log-transformed CFU/g values for total anaerobes (TAN; ··○··), total aerobes (TAR; -·-●-·-), streptococci (STR; -··○··-), total enterics (ENT; -·-●-·-) and *E. coli* (EC; -··○··-) for the female panda. (b) Mean monthly plots of log-transformed CFU/g values for *Bacteroides* spp. (BAC; —●—), lactobacilli (LBS; ··○··) and *Clostridium* spp. (CLO; -·-●-·-) for the female panda. (c) Mean monthly plots of log-transformed CFU/g values for total anaerobes (TAN; ··○··), total aerobes (TAR; -·-●-·-), STR (-··○··-), total enterics (ENT; -·-●-·-) and *E. coli* (EC; -··○··-) for the male panda. (d) Mean monthly plots of log-transformed CFU/g values for *Bacteroides* spp. (BAC; —●—), lactobacilli (LBS; ··○··) and *Clostridium* spp. (CLO; -·-●-·-) for the male panda.

tions were observed, demonstrating that the effect from the combination of time of year and leaf consumption does not interact to significantly change microflora populations (data not shown). Linear and quadratic models were used to determine the independent effects from time of year and observed leaf-eating behaviour. Statistically significant effects were observed in the linear relationships of time of year and TAR, STR and LBS and in the quadratic relationships between time of year and STR and BAC, indicating that these microflora populations do change significantly with respect to the time of year (p-values located in Table 1).

Significant linear and quadratic relationships were seen between observed leaf consumption behaviour and BAC and LBS, revealing that microflora do fluctuate significantly with respect to foraging behaviour of the giant panda (p-values located in Table 1). The linear fits for LBS and BAC displayed extremely significant p-values of 0.003 for both types, indicating that the level of leaf consumption has a highly significant effect on both types of microflora. The relationship between microflora types, LBS and BAC, and preference for leaf consumption can be seen in Fig. 3. It is interesting to note that when leaf consumption was at the lowest level (October 2009–April 2010), LBS were observed in higher values, whereas BAC were below the detection limit until 1 month prior to increasing leaf consumption. As the preference for leaf increased, BAC also increased and LBS declined and did not rise until the preference for leaf part decreased. The Pearson correlation procedure determined that LBS and BAC were not correlated, signifying that these two microbes' fluctuations were not dependent upon each other, but they were independently changing with respect to leaf consumption by giant pandas. Leaf consumption behaviour was chosen for comparison because that

Table 1 P-values for total anaerobes (TAN), total aerobes (TAR), streptococci (STR), total enterics (ENT), *E. coli* (EC), *Bacteroides* spp. (BAC), *Lactobacillus* spp. (LBS) and *Clostridium* spp. (CLO)

	Month		Leaf	
Bacterial groups	Linear	Quadratic	Linear	Quadratic
TAN	0.051	0.055	0.459	0.677
TAR	0.037*	0.106	0.746	0.641
STR	0.012*	0.038*	0.592	0.570
ENT	0.162	0.146	0.193	0.413
EC	0.050	0.077	0.159	0.308
BAC	0.241	0.013*	0.003*	0.012*
LBS	0.017*	0.059	0.003*	0.013*
CLO	0.264	0.359	0.229	0.444

P-values for linear and quadratic fits for month and observed leaf consumption behaviour effects on microflora for both male and female pandas at $\alpha = 0.05$. Significant values are indicated by *.

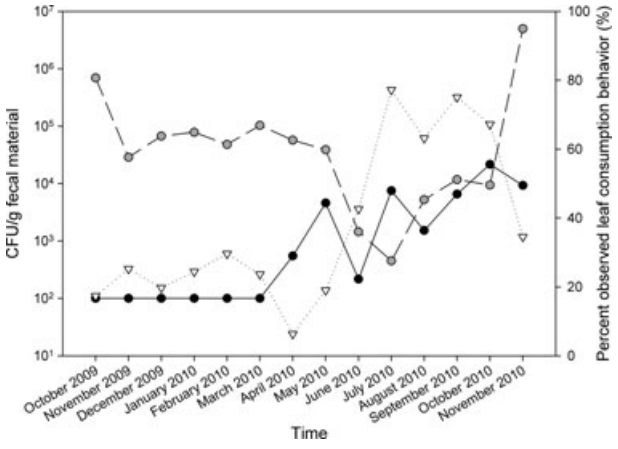

Fig. 3 Combined significant fluctuations of *Lactobacillus* spp. (LBS; —○—) and *Bacteroides* spp. (BAC; —●—) with respect to percentage of observed leaf consumption behaviour (···▽···) averaged for both male and female panda.

is the predominant preference throughout the year for the giant panda, and leaf and culm consumption behaviours on average are preferred approximately 97%, thus mirroring each other statistically. However, to be certain, the same statistical procedures were applied to the culm consumption behaviour, and there were no changes in levels of significance (data not shown).

Discussion

The present results demonstrate that some bacterial populations within the GIT of the giant panda fluctuate significantly with respect to time of year and panda foraging behaviour. In particular, TAR, STR, LBS and BAC populations all experienced marked changes due to the time of year, and LBS and BAC populations also displayed highly significant fluctuations with respect to the behaviour of bamboo part preference. This in-depth seasonal study relating panda foraging behaviours to microflora levels is the first study to correlate these factors and adds new information to the seminal studies on panda GIT microbes first conducted by Hirayama et al. (1989), Wei et al. (2007) and Zhu et al. (2011).

The study conducted by Hirayama et al. (1989) showed the population of the pathogenic *Clostridium perfringens* as high as 10^9, while other *Clostridium* spp. concentrations reached approximately 10^4–10^6. These values are higher than those seen in our study, but that could also be due to a difference in diet; pandas studied by Hirayama et al. (1989) were fed a higher protein gruel diet. *Lactobacillus* spp. were also quantified with levels ranging from 10^2 to 10^6 and 10^5 to 10^6 for the female and male bears respectively (Hirayama et al., 1989). Our study found similar levels of LBS for the female (10^3–10^5) and the male bear (10^2–10^7) located at the Memphis Zoo. In addition, Hirayama et al. (1989) found *Streptococcus* spp. at levels of 10^3–10^5 and 10^9 for the female and male bear, respectively, accounting for the highest population present in the faecal samples (Hirayama et al., 1989). These values were also similar in range with our study with *Streptococcus* spp. ranging from 10^7 to 10^9 in the female and from 10^5 to 10^9 in the male. Wei et al. (2007) also found *Clostridium* spp. and *Streptococcus* spp. in their analysis, but population sizes were not quantified; no *Lactobacillus* spp. were identified. The third study by Zhu et al. (2011) showed organisms from the class Clostridia comprising 60.8% of their total sequences, with *Clostridium* spp. being present in high numbers. Our current study reveals the importance of collecting samples more frequently and over a longer term to conduct analysis of GIT microbial populations and how their unique diet can impact bacterial groups.

GIT microbiota are always in a state of flux, and many factors, such as diet type, can affect the microbial flora present (Yokoyama and Johnson, 1988; Buddington and Sunvold, 1998; Collins and Gibson, 1999). After observing such dramatic shifts in observed feeding behaviour, one could assume that there may also be fluctuations within the microbial community inhabiting the gut. Multiple studies have been carried out on other animals utilizing controlled values of substrates allowing for better

correlation of dietary effects on GIT microflora (Varel et al., 1987; Varel and Yen, 1997; Zentek et al., 2003). However, working with an endangered species such as the giant panda offers several challenges. In particular, the limited ability to modify the animal's diet and their strict dependence on bamboo require a study design based on the animal's own foraging choices. Therefore, we took advantage of the shifts in observed behavioural feeding preferences as described by Hansen et al. (2010) to correlate their changing bamboo preference to the shift in microbial populations of the GIT. In this study, seasonal fluctuations in GIT microbes were observed, but few were significant. However, some linear and quadratic relationships between time of year and microflora were observed. These models showed that the time of year significantly affected TAR, STR, *Bacteroides* spp. and lactobacilli.

Only two linear and quadratic relationships displayed any significant change between values of observed leaf consumption and GIT microflora. These two groups, *Bacteroides* spp. and *Lactobacillus* spp., had highly significant linear relationships with respect to bamboo part preference. In swine, Varel and Pond (1985) studied the effects of high fibre diets on cellulolytic and hemicellulolytic microorganisms and determined that when higher fibre diets are consumed, there is an increase in fibrolytic microbes. *Bacteroides* species, previously unidentified in the GIT of the giant panda, most are known fibrolytic anaerobes; therefore, one would expect any change in microbial substrate (leaf proportion) to cause a shift in microflora populations within the GIT (Latham et al., 1978; Giuliano and Khan, 1984; Varel and Yen, 1997). However, if this is occurring, a shift in *Clostridium* species would also be expected because over half of the predictive genes involved in the production of enzymes for cellulose digestion in the giant panda have been shown to belong within the *Clostridium* genus (Zhu et al., 2011). Although levels of *Clostridium* spp. ranged between 10^2 and 10^6 in our study, no statistically significant changes were found when compared to leaf consumption. The genus level may not be sensitive enough to observe significance. Overall levels of *Clostridium* genera may not change, but species may fluctuate. A census at the species level may be required to observe significant relationships between dietary shifts and changes in microbial populations.

What was unexpected was the significant change in *Lactobacillus* spp. following a change in leaf proportion because lactobacilli are not fibrolytic, but there may be another stimulatory effect occurring. Pre-biotic studies indicate that dietary supplementation of fermentable fibres such as fructooligosaccharide and mannan oligosaccharide can cause an increase in populations of lactobacilli in the GIT (Kim et al., 2011). Bamboo composition studies have shown higher levels of non-digestible fibre in the culm portion when compared to the leaf portion (Edwards et al., 2006). As leaf consumption increases, levels of LBS decrease, and when levels of leaf consumption are low, levels of LBS are high. These fluctuations may be associated with the level of fibre present in their diet, and a better understanding of how fibres vary in bamboo plant part preference by giant pandas may expose the cause for the seasonal fluctuation of lactobacilli. Lactobacilli are considered beneficial organisms that will prevent or reduce colonization of pathogenic organisms in the gut, and any increase in lactobacilli populations can be seen as beneficial to host health (Buddington and Sunvold, 1998; Collins and Gibson, 1999; Gibson et al., 2004; Kim et al., 2011). Gastrointestinal disorders are the number one cause of death in captive pandas (Qiu and Mainka, 1993), and any information on improving overall gut health would be of the utmost importance.

No significant interactions between time of year and percentage of bamboo consumption were observed using mixed modelling. Studying an endangered species has many difficulties, one being there are so few to study. This study's small sample size ($n = 2$) may have made it difficult to collect enough data to glean any significant relationships. Additional animals may have strengthened the data set to understand whether a correlation exists between seasonal dietary changes and GIT microflora. Therefore, future studies with a larger sample size are imperative to correlate dietary shifts to changes in microflora. However, even with additional animals, no significant interactions may have been seen, primarily because the overall density of microflora does not alter following a dietary shift, but relative proportions within bacterial groups do change. Previous studies have found that while the overall levels of GIT microflora are static, within the microbiota, changes in substrate may cause a change in the relative proportion and the development of certain bacterial types (Yokoyama and Johnson, 1988; Buddington and Sunvold, 1998; Collins and Gibson, 1999). It is possible that the genus level microbial enumeration of this study is not specific enough to monitor fluctuations in microbial populations at the species level following a change in dietary intake. Quantitative tests at a more specific

level may be required to observe any clear correlation between the observed seasonal dietary shifts and fluctuations of the GIT microbiota of the giant panda.

More information on the relative bamboo nutritive levels may also be needed and would likely give better insight into the seasonal shift in the dietary preference affecting GIT microflora. Nutritive values related to total dietary fibre are changing with the season (Kouba, A. and Falcone, J., Memphis Zoo, unpublished data), and this could be the cause of the observed shift in preference of bamboo plant part. These seasonal changes in fibre consumption may impact specific bacterial types or species while not changing the overall genera of bacteria, such that total numbers of microflora remain unchanged. This study represents the first investigation into understanding how dietary shifts impact GIT microflora in giant pandas. Future work is needed to better understand the role nutrient levels influence the bacterial dynamics. With this information, a relationship between dietary shifts and GIT microbiota may become apparent.

Here, we describe for the first time the dynamic relationship between giant panda foraging behaviour, plant part preference and GIT microflora. Information from this study has provided a greater understanding of the ever-changing GIT microbiome within the giant panda in terms of dietary requirements and bamboo digestion. Moreover, we begin to have a clearer understanding of how important the symbiotic relationship between host and bacteria is to giant panda health. With the leading cause of mortality in captive giant pandas linked to GIT disorders, this study paves the way for continued investigations into how dietary modifications, and thus GIT bacteria flux, impact the way captive animals are managed. Lastly, these studies also lay the foundation for a comparative study to examine the natural bacterial fluctuations and nutritional ecology of wild giant pandas as they move spatially and temporally within the landscape, consuming different bamboo species and plant parts.

Acknowledgements

We thank the docents and the China Keepers (Kim Sopchak, Kathy Fay, Suzie Zaledzieski, Emily Macklin, Kendra Bell and Scott Lincoln) at the Memphis Zoo for their assistance in sample collections and Dr Dennis Rowe for statistical analysis. This study was supported by the Memphis Zoological Society.

References

Buddington, R. K.; Sunvold, G. D., 1998: Fermentable fiber and the gastrointestinal tract ecosystem. In: G. A. Reinhart, D. P. Carey (eds), *Recent Advances in Canine and Feline Nutrition*. Orange Frazer Press, Wilmington; OH. 1998 Iams Nutrition Symposium Proceedings, Vol. **2**, pp. 449–464.

Chesson, A.; Stewart, C. S.; Dalgarno, K.; King, T. P., 1986: Degradation of isolated grass mesophyll; epidermis and cell walls in the rumen and by cellulolytic rumen bacteria in axenic culture. *Journal of Applied Bacteriology* **60**, 327–336.

Christiansen, P., 2008: Feeding ecology and morphology of the upper canines in bears (Carnivora: Ursidae). *Journal of Morphology* **269**, 896–908.

Collins, M. D.; Gibson, G. R., 1999: Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *The American Journal of Clinical Nutrition* **69**, 1052–1057.

Dierenfeld, E. S.; Hintz, H. F.; Robertson, J. B.; Van Soest, P. J.; Oftedal, O. T., 1982: Utilization of bamboo by the giant panda. *The Journal of Nutrition* **112**, 636–641.

Edwards, M. S.; Zhang, G.; Wei, R.; Liu, X., 2006: *Giant Pandas: Biology, Veterinary Medicine and Management*. Cambridge University Press, Cambridge, UK.

Gibson, G. R.; Probert, H. M.; Loo, J. V.; Rastall, R. A.; Roberfroid, M. B., 2004: Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews* **17**, 259–275.

Gilbert, H. J.; Hazlewood, G. P., 1993: Bacterial cellulases and xylanases. *Journal of General Microbiology* **139**, 187–194.

Giuliano, C.; Khan, A. W., 1984: Cellulase and sugar formation by *Bacteroides cellulosolvens*; a newly isolated cellulolytic anaerobe. *Applied and Environmental Microbiology* **48**, 446–448.

Hansen, R. L.; Carr, M. M.; Apanavicius, C. J.; Jiang, P.; Bissell, H. A.; Gocinski, B. L.; Maury, F.; Himmelreich, M.; Beard, S.; Ouellette, J. R.; Kouba, A. J., 2010: Seasonal shifts in giant panda feeding behavior: relationships to bamboo plant part consumption. *Zoo Biology* **29**, 470–483.

Hirayama, K.; Kawamura, S.; Mitsuoka, T.; Tashiro, K., 1989: The faecal flora of the giant panda (*Ailuropoda melanoleuca*). *Journal of Applied Bacteriology* **67**, 411–415.

Hudson, M. J.; Marsh, P. D., 1995: *Human Colonic Bacteria*. CRC Press, Boca Raton, FL, USA.

Jousimies-Somer, H. R.; Summanen, P.; Citron, D. M.; Baron, E. J.; Wexler, H. M.; Finegold, S. M., 2002: *Anaerobic Bacteriology Manual*, 6th edn. Star Publishing Company, Belmont, CA, USA.

Kato, S.; Shin, H.; Zong, J. C.; Masaharu, I.; Yasuo, I., 2004: Effective cellulose degradation by a mixed-culture system composed of a cellulolytic *Clostridium*

and aerobic non-cellulolytic bacteria. *FEMS Microbiology Ecology* **51**, 133–142.

Kim, G. B.; Seo, Y. M.; Kim, C. H.; Paik, I. K., 2011: Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science* **90**, 75–82.

Latham, M. J.; Brooker, B. E.; Pettipher, G. L.; Harris, P. J., 1978: Adhesion of *Bacteroides succinogenes* in pure culture and in the presence of *Ruminococcus flavefaciens* to cell walls in leaves of perennial ryegrass (*Lolium perenne*). *Applied and Environmental Microbiology* **35**, 1166–1173.

Li, R.; Fan, W.; Tian, G.; Zhu, H.; He, L.; Cai, J. et al., 2010: The sequence and de novo assembly of the giant panda genome. *Nature* **263**, 311–317.

Loeffler, I. K.; Montali, R. J.; Rideout, B. A., 2006: *Giant Pandas: Biology; Veterinary Medicine and Management*. Cambridge University Press, Cambridge, UK.

O'Sullivan, C. A.; Burrell, P. C.; Clarke, W. P.; Blackall, L. L., 2004: Structure of a cellulose degrading bacterial community during anaerobic digestion. *Biotechnology and Bioengineering* **92**, 871–878.

Qiu, X.; Mainka, S. A., 1993: Review of mortality of the giant panda (*Ailuropoda melanoleuca*). *Journal of Zoo and Wildlife Medicine* **24**, 425–429.

Schaller, G. B.; Hu, J.; Pan, W.; Zhu, J., 1985: *The Giant Pandas of Wolong*. University of Chicago Press, Chicago, USA.

Tarou, L. R.; Williams, J.; Powell, D. M.; Tabet, R.; Allen, M., 2005: Behavioral preferences for bamboo in a pair of captive giant pandas (*Ailuropoda melanoleuca*). *Zoo Biology* **24**, 177–183.

Van Soest, P. J., 1996: Allometry and ecology and feeding behavior and digestive capacity in herbivores: a review. *Zoo Biology* **15**, 455–479.

Varel, V. H.; Pond, W. G., 1985: Enumeration and activity of cellulolytic bacteria from gestating swine fed various levels of dietary fiber. *Applied and Environmental Microbiology* **48**, 858–862.

Varel, V. H.; Yen, J. T., 1997: Microbial perspective of fiber utilization by swine. *Journal of Animal Science* **75**, 2715–2722.

Varel, V. H.; Robinson, I. M.; Jung, H. J., 1987: Influence of dietary fiber on xylanolytic and cellulolytic bacteria of adult pigs. *Applied and Environmental Microbiology* **53**, 22–26.

Wei, G.; Lu, H.; Zhou, Z.; Xie, H.; Wang, A.; Nelson, K.; Zhao, L., 2007: The microbial community in the feces of the giant panda (*Ailuropoda melanoleuca*) as determined by PCR-TGGE profiling and clone library analysis. *Microbial Ecology* **54**, 194–202.

Yokoyama, M. T.; Johnson, K. A., 1988: *Digestive Physiology and Nutrition*. Prentice Hall, Englewood Cliffs, NJ, USA.

Zentek, J.; Marquart, B.; Pietrzak, T.; Ballevre, O.; Rochat, F., 2003: Dietary effects on bifidobacteria and *Clostridium perfringens* in the canine intestinal tract. *Journal of Animal Physiology and Animal Nutrition* **87**, 397–407.

Zhu, L.; Wu, Q.; Dai, J.; Zhang, S.; Wei, F., 2011: Evidence of cellulose metabolism by the giant panda gut microbiome. *Proceedings of the National Academy of Science USA* **43**, 17714–17719.