SEASONAL VARIATIONS IN BAMBOO SELECTION AND UTILIZATION BY GIANT PANDAS

A Thesis

by

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ABSTRACT

Despite retaining a carnivore's characteristic simple gastrointestinal tract, giant pandas acquire the majority of their required nutrients from bamboo. The processes that allow giant pandas to meet their nutritional requirements from this high fiber diet are not yet fully understood due to this monogastric species' limited capability to digest plant structural components. Season dietary shifts in plant part selection have been observed in both wild and captive giant panda populations, and are believed to be an adaptive response to their specialized bamboo diet. We hypothesized that these dietary shifts are driven by temporal changes in the nutritive composition of bamboo and accompanied by changes in digestibility that maximize the absorption of bamboo nutrients in the giant panda. To examine changes in bamboo selection and potential responses in nutrient utilization, five feeding trials were conducted with two captive giant pandas at the Memphis Zoo (July 2014, January, March, May, October 2015).

Both giant pandas exhibited two distinctive culm-preference trials, one shoot-preference trial, and one leaf-preference trial. Despite higher nutritive quality of bamboo leaves, culm was the overall predominant plant part consumed by giant pandas during year. Maximum bamboo culm intake coincided with times of high starch concentration in culm. However, starch and hemicellulose content in culm was relatively low during October, when both giant pandas incorporated considerable amounts of leaves into their diets. Bamboo shoots were preferentially consumed when offered during May, likely due to their reduced proportion of plant part structural compounds (ADF and NDF) and increased CP content. Total dry matter digestibility of bamboo based diets by giant pandas is very low (averaging less than 30%), though giant pandas can apparently access and utilize non-fiber nutrients, such as starch, to a greater extent. When bamboo shoots were included in the diet, giant pandas achieved higher digestion rates for all nutrients. We propose that the basis of giant panda foraging strategy is maximization of digestible energy intake, often from carbohydrate sources, which has applications in the aforementioned areas of giant panda conservation, in addition to management of animals in captive scenarios.

ACKNOWLEDGEMENTS

Those of you who read this section probably know me well enough to know how poor of a job I do of vocally expressing my feelings. Fortunately, I have this opportunity to write them out; though if anyone ever references any of the sentiments found in the following pages to my face, I'll deny having ever thought such a thing and quickly change the subject. Nevertheless, I am truly overwhelmed with gratitude when I look back at the people and experiences I've been a part of in the last few years.

First and foremost, I'd like to thank my committee: Dr. Tryon Wickersham, Dr. Katrina Knott, Dr. Larry Redmon, Dr. Chad Paulk, and Dr. Josie Coverdale. All members not only contributed an area of expertise to that was fundamental to my Master's research, but have also guided me as I've realized the areas of study I am passionate about, and provided opportunities to help me further myself in those fields. To everyone in my committee, I truly appreciate your willingness to engage in my research, unconventional as it was.

In the most literal sense, Dr. Wickersham shaped my undergraduate and graduate experience from day one. During my New Student Conference prior to my freshman year, I was brought directly to Dr. Wickersham's office and I sat down for the first of what would be many (awkward) conversations in his office. Throughout the seven years I studied Animal Science at Texas A&M, Dr. Wickersham was present in my education, career, and my extracurricular activities. I can honestly say that outside of my family, no one has been more invested into my life and helped me to succeed, despite all of my resistance to his efforts. The crazy thing is, I know that I am not the only student who has been profoundly impacted by Dr. Wickersham, and I've seen him intentionally mentor many others over the time I've worked with him. He is driven by a genuine desire for his students to succeed academically, professionally, and personally, and I am truly grateful to have been one of them. I hope you never read this, but if you do, I hope that you are encouraged (and also that you shed at least one tear).

While I was still an undergraduate, Dr. Knott hired me for an internship at the Memphis Zoo that ultimately defined the direction in which my academic life would move, and allow me to conduct my Master's research. Interning with the Research and Conservation Department at the Memphis Zoo was an extremely influential and stimulating time for me, and much of this was thanks to Dr. Knott's mentorship. With her guidance, I was able to accomplish a major career dream: to research exotic animal nutrition. Additionally, Dr. Knott, as well as other staff at the Memphis Zoo, introduced me to ecology, conservation, and wildlife management, and helped me to integrate those fields with my knowledge of animal science. I am constantly inspired Dr. Knott's passion and curiosity for wildlife biology, as well as her empathy for the staff, visitors, and animals at the zoo. Katrina, thanks for all of the weeks you invested to help me sort through bamboo, keeping me sane during the trials, and encouraging me every step of the way.

Feeding trials involving giant pandas are an ordeal, and I am indebted to all of the research staff and zookeepers at the Memphis Zoo who made my project possible. I am especially grateful to Dr. Andy Kouba, who first approached me with the opportunity to research this incredibly unique species for my Master's. Additionally, I truly appreciate all of the research staff and zoo volunteers who spent hours disassembling bamboo with me; there's absolutely no way I would have been able to process my samples alone. A special thanks to the Memphis Zoo China Zookeepers, who provided me with all of my samples, and allowed my trials to interrupt their already hectic daily routine of caring for the many different animals in the China exhibit. And finally, a huge thank you to the true M.V.P.'s of my project, all of the student workers in College Station (Emily, Hope, Jordan, Natalie, Courtney) who helped me to grind dozens of unruly bamboo samples.

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Finally, thanks to my family for always being encouraging and taking an interest in my projects, despite my unwillingness to talk about them (it's not that I don't think y'all will understand it, but it's that most of the time, I don't understand it). My parents have always told me that my love for animals was a gift, and allowed me to pursue it from a young age, even though it mostly ended up as them keeping up with a variety of animals. Thank y'all for always being the ultimate examples: selfless, patient, level-headed, and strong. Mom, you were diagnosed, treated for, and overcame cancer in the couple of years I was in grad school, which is no doubt the greater accomplishment. Despite all you were going through, you never wavered in your compassion, support, and wit. Dad, thanks for being my constant source of encouragement, helping to carry my load when things went awry, keeping me updated on giant panda related news, and just being my on-call user manual for life in general. Finally, if it weren't for my parents, I wouldn't have been able to keep my cat, Jack, and horse, Cowboy, around. They may be expensive therapists, but they have given me so much joy and relief, and remind me every day why I study animals.

Many people and experiences shaped my time during graduate school, and provided for me a period to grow in intellect, maturity, and faith. Looking back at the way seemingly unrelated events came together and influenced my life, it is clear to me that there is a God who is in control and wants what is best for my life. Though there were many ups and downs, I'm incredibly blessed to have had this time in graduate school.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Despite its iconic status in popular culture, the giant panda (*Ailuropoda melanoleuca*) remains an enigmatic species. Reduced populations, as well as their elusive natural behaviors, predispose research of the giant panda to many challenges, and data regarding the species' unique biology is often inconclusive. Giant pandas are endemic to the mountainous bamboo forests of central China, where they have historically thrived on diets primarily composed of *Bashania, Fargesia, and Sinarundinaria* bamboos (Schaller *et al*. 1985; Carter 1999; Long *et al*. 2004). Though the nearly exclusive selection of bamboo by the giant panda is seemingly paradoxical, the species' history of inhabiting heavily vegetated bamboo forests may provide insight regarding this dietary choice. Schaller *et al*. described the lack of prey options available to the giant panda population in the Wolong Reserve, and hypothesized it to be energetically unrewarding for giant pandas to hunt (1985). Although some rodents, small ungulates, and red pandas populate the bamboo forests inhabited by giant pandas, the energy required to pursue and overcome these small prey would exceed that which the panda would consume (Schaller *et al*. 1985). On the other hand, bamboo was once an abundant and widespread resource in the giant panda's historical range, providing the quantity and flexibility of diet necessary to sustain a stable giant panda population (Lindburg and Baragona 2004). Fossil records indicate that giant pandas once occupied much of southeastern China in areas spanning from Northern Vietnam and Myanmar northward to Beijing; however, the current habitat is fragmented to five separate mountain ranges in central China due to due to anthropogenic encroachments, such as logging practices and urban development (Hu and Wei 2004; Loucks and Wang 2004). Each region hosts disparate giant panda populations, which inhabit elevations of 1,200 to 4,100 m, depending on specific mountain range and seasonal migrations (Hu and Wei 2004). The relatively recent and rapid decline in giant panda numbers is largely attributed to habitat reduction and separation. However, there have been significant efforts to preserve and restore areas of giant panda habitation, such as China's "Grain to Green" reforestation program and a commercial logging ban covering giant panda habitats (Lindburg and Baragona 2004; Swaisgood *et al.* 2011). Future conservation activities would undoubtedly benefit from a deeper knowledge regarding utilization of bamboo as a feed resource by giant pandas, and its implications for species' nutritional requirements, migration patterns, and habitat carrying capacity (Swaisgood *et al.* 2011).

Natural history of the giant panda

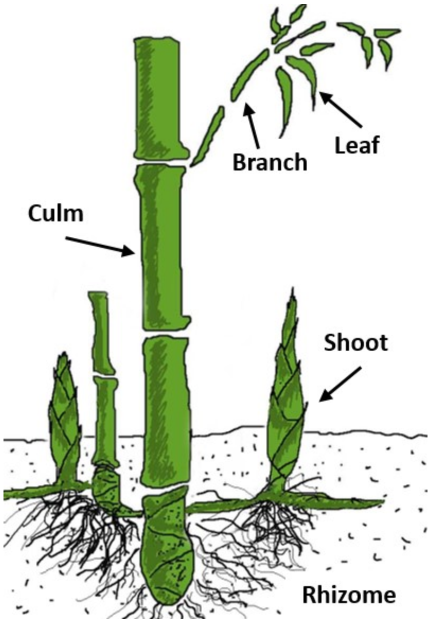
Over the course of the investigation of giant panda species history, there has been much speculation regarding the definitive phylogenetic relation between giant pandas and bears of the *Ursus* genus (Bininda-Emonds 2004; Garshelis 2004; Schaller *et al*. 1985). Most mammalian biologists, however, agree that the present-day giant panda most closely characterizes the ancestral *Ursidae* family, from which giant pandas and *Ursus* species developed (Bininda-Emonds 2004; Garshelis 2004; Spady *et al.* 2007). Mature giant panda body mass ranges 80 to 125 kg, and exhibit sexual dimorphism with males being approximately 10% - 20% larger than females (Schaller *et al*. 1985). Maximum life expectancy for giant pandas is 30 years with age of sexual maturity occurring between 5.5 to 6.5 years of age (Schaller *et al*. 1985) Schaller *et al*. further classifies giant panda age into three distinctive categories; infant (birth to 1.5 years, the approximate time of independence from the mother), subadults (1.5 to 5 years, or when the animal reaches sexual maturity), and adults (older than 5 years; 1985). Wild giant pandas generally travel alone and avoid other individuals when foraging for bamboo, with the exception of during breeding season or of a female and her cub (Schaller *et al*. 1985). Female pandas have a single estrous period, typically in the spring, during which male and female animals rely on chemical cues to locate one another (Snyder *et al*. 2004; Spady *et al.* 2007). Following a successful breeding, the female panda has a relatively short gestation (95-160 d), which exhibits “delayed implantation” of the embryo, or the embryo does not implant until late gestation such that the pregnancy can be terminated if environmental conditions are unsuitable for parturition (Schaller *et al*., 1985). Females may also exhibit pseudopregnancy, an ambiguous physiological state which mimics the hormonal and behavioral conditions of gestation (Kersey *et al*., 2010). Few observations of geriatric animals are recorded, and consequently little data exists regarding reproductive longevity.

The present condition of giant panda population is considered vulnerable and conservation-dependent; though it has only recently moved from the “endangered” classification by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (IUCN 2016). Current estimate of free-ranging individuals is nearly 1,900, not including young cubs still paired to their dams (Swaisgood *et al.* 2016). Over 300 giant pandas are currently kept in captivity worldwide, and many of the institutions housing these animals participate in breeding programs to promote *in situ* and *ex situ* population growth and genetic diversity (Xie *et al.* 2013). Though giant pandas have a viably reproducing wild population, it has proven difficult to recreate natural settings in captive situations. Factors affected by animal management decisions, such as diet, in addition to the species' single estrus period and potential for false pregnancies, result in low reproductive rates in captive facilities. Captive giant pandas are provided locally available bamboo species (i.e., *Phyllostachys, Pseudosasa*) and commercial supplements based on local availability (Sims *et al.* 2007; Hansen *et al.* 2010; Finley *et al.* 2011). Nutrient quality of bamboo and supplemental items (i.e., bread, eggs, fruit, gruel, canine kibble, etc.) therefore, vary appreciably among institutions (Dierenfeld *et al.* 1995). As proper nutrition is intimately associated with successful reproduction, it is critical that zoos and breeding centers offer a diet that best meets the giant panda's nutrient requirements. Efforts should be made to distinguish the captive giant panda's unique requirements, while maintaining a diet similar to that consumed by their wild counterparts. Gastrointestinal (GIT) discomfort and mucous stools have been observed from giant pandas consuming low quantities of fiber, further suggesting that their natural bamboo diet is key in maintaining animal health (Dierenfeld *et al*. 1995; Mainka et al. 1989; Qui and Mainka 1993). Greater comprehension regarding the nutritional composition of bamboo and its relation to animal health is needed, as GIT-related problems are the leading cause of giant panda mortality.

Bamboo physiology and nutritional potential

Bamboos are rhizomatous grasses characterized by tall, woody stalks, or culms, with recurring nodes and leafy branches (Figure 1.1). Over 1,500 bamboo species are reported to exist in both tropical and temperate climates worldwide, however the most diverse and concentrated populations of bamboo grow in Asia (Bystriakova *et al*. 2003; Ohrnberger 1999). A stand of bamboo grows from an underground rhizome complex which connects the aerial culms as a single plant, and reproduces annually in the form of juvenile bamboo shoots Although there are variations among species, bamboo stands are generally long-lived and evergreen; for instance, *Phyllostachys pubescens* reportedly flowers every 60-70 years, and sheds its leaves every two years during shooting season (Li *et al*. 1998). In the northern hemisphere, new bamboo shoots emerge in the spring and early summer months of April, May, and June. Shoots elongate at a rapid pace and reach full height, develop foliage, and complete lignification at approximately three months after emergence; however, a culm is not considered mature until its third year (Liese and Weiner 1996; Seki and Aoyama 1995). At the end of a bamboo plant's lifespan, all culms associated by a shared rhizome will bloom and expire. Due to this cycle, large expanses of bamboo will simultaneously die off, resulting in a significant loss of nutrients in the surrounding environment (Schaller *et al*. 1985; Scurlock *et al*. 2000).

Figure 1.1— Bamboo plant anatomy.



Plant cell wall constituents (e.g., cellulose, hemicellulose, lignin) provide the characteristic woody structure of bamboo culms, and allows certain species to grow to heights that dwarf other grasses. Not surprisingly, these structural compounds comprise a very large proportion of the mature bamboo plant. Similar to other grasses during growth from shoot to mature tiller, the composition of a new shoot transitions from high concentrations of water and soluble sugars to primarily cell wall constituents in the matured culm (Christian et al. 2015; Liese and Weiner 1996). Christian et al. observed a 14% increase in structural compounds in bamboo shoots of various species harvested at heights less than 60 cm to shoots harvested at greater than 180 cm (61% total dietary fiber on a DM basis to 75% DM, respectively [2015]). After the culm has reached full height (≈ three months after emergence), lignin is also incorporated into cell wall (Wallace et al. 1991; Wilson 1993). A mature culm is primarily comprised of long parenchyma and fiber cells featuring multi-layered cell walls (Liese 1992). At this point, subtle changes in chemical composition have been observed in bamboo culms. For instance, culm thickness increases with age, possibly in response to increased thickness of cell walls or stored starch content (Liese and Weiner 1996; Seki and Aoyama 1995).

For several species (i.e., giant panda, red panda, bamboo lemur, and bamboo rat) which inhabit areas heavily vegetated with bamboo stands, bamboo serves as an indispensable dietary component (Schaller et al. 1985; Wei et al. 2000). Not surprisingly, plant cell wall constituents contribute the majority of nutrients sourced from bamboo, in the form of dietary fiber. Due to the complex and stable structure of these compounds, dietary fiber is generally indigestible by mammalian gastrointestinal acids and enzymes. Additionally, high concentrations of dietary fiber preclude digestion and metabolism of intracellular nutrients, including starches, proteins, simple carbohydrates, fatty acids, vitamins, and minerals. This limited bioavailability of intracellular nutrients in high-fiber bamboo exacerbates their already reduced concentrations, such that they are considered to be of low-quality (Van Soest 1975; 1977; Schaller et al. 1985). Total dietary fiber content of mature bamboo culms range from 60-85% dry matter (DM) whereas crude protein (CP), another indicator of forage quality, has been reported in mature culms of various species to average 2-3% DM (Dierenfeld 1997; Mainka *et al*. 1989; Schaller *et al.*1985). Total cellulose and lignin (acid detergent fiber—ADF), the components of dietary fiber most resistant to degradation, comprise approximately 60% of culm DM (Table 1.1). In general, bamboo culms have been found to contain marginal proportions of fat (< 2% DM) and minerals (Xuanzhen et al. 2006; Wu et al. 2009). Bamboo leaves, which experience their own annual or biennial growth cycle, are generally less fibrous than culm, but still contain 60-70% CF (Table 1.1 [Schaller et al. 1985; Dierenfeld 1997; Xuanzhen *et al*. 2006]). However, leaves are 10-19% CP (DM basis), while indigestible ADF is only 30%-40% (Table 1.1). For bamboo consumers, the hemicellulose (HC) portion of dietary fiber is partially digestible, and concentrations range similarly between culm and leaves (Table 1.1 [Dierenfeld *et al.* 1982; Long *et al.* 2004]). Non-structural carbohydrates and minerals such as P, Ca, Mn, Zn, K, Mg, and Fe are found primarily within the plant cell (as opposed to cell wall), and are greater in bamboo leaves relative to culm (Li *et al*. 1998; Singh and Rai 2012). Bamboo shoots are considerably less fibrous than bamboo culms and leaves because the plant cell walls are not fully developed, and have equal or greater levels of digestible energy (DE) and CP than culms and leaves (Table 1.1 [Christian et al. 2015; Schaller et al. 1985]).

The giant panda exists as an obligate herbivore, and obtain the majority of their required nutrients from bamboo culms, leaves, and shoots. Plant part nutrient composition of bamboos offered to giant pandas in the available literature is summarized in Table 1.1. Wild individuals may opportunistically consume carrion or plants other than bamboo, however bamboo generally comprises over 99% of the giant panda diet (Long et al. 2004). Reported total bamboo dry matter digestibility in giant pandas is low, ranging from 6.9% - 39% (Table 1.2 [Dierenfeld et al. 1982; Finley et al. 2011; Mainka et al. 1989]). Thus, in order to meet their nutritional requirements, giant pandas must consume excessive amounts of bamboo (6-15% of body weight of fresh bamboo; 2-7% on DM basis), relying on morphological and behavioral adaptations developed to enhance digestibility of dietary fiber, accessibility of intracellular nutrients, and foraging efficiency (Dierenfeld 1997; Schaller et al. 1985).

Table 1.1.— Nutrient composition of bamboo species and plant parts consumed by giant pandas. *%Fat includes concentrations determined by ether extract and acid-hydrolysis methods

Species	Part	Age	Season	%DM	%OM	%Ash	%TDF	%NDF	%ADF	%HC	% ADL	%Silica	%CP	%Starch	%NSC	%Fat*	GE (kcal/kg)	Source
Phyllostachys mix**	culm	not specified	Winter		98.4	1.7	85.1		56.4	28.6	13.8		3.3	4.8		0.7		Knott *et al.* In review
P. aurea	culm	not specified	Early spring	46.1							3.8		5.0					Mainka *et al.* 1989
Phyllostachys mix**	culm	not specified	Early spring		98.5	1.5	80.5		51.9	28.6	12.8		3.6	8.8		0.6		Knott *et al.* In review
Phyllostachys mix**	culm	not specified	Late spring		98.1	2.0	88.1		62.4	25.7	14.4		4.5	0.9		0.7		Knott *et al.* In review
Phyllostachys mix**	culm	not specified	Summer		98.4	1.7	85.9		60.4	25.5	15.7		3.5	3.9		0.6		Knott *et al.* In review
P. bambusoides	culm	diameter < 5 mm	Fall	48.0	92.0	8.0		81.9	46.5	35.4			4.2		3.9	2.0		Senshu *et al.* 2007
P. bambusoides	culm	diameter 5-15 mm	Fall	60.2	96.5	3.5		88.1	57.0	31.1			2.4		4.5	1.5		Senshu *et al.* 2007
P. bambusoides	culm	diameter > 15 mm	Fall	59.0	97.4	2.6		91.4	72.5	18.9			1.7		3.2	1.0		Senshu *et al.* 2007
P. pubescens	culm	diameter < 15 mm	Fall	61.3	97.4	2.6		83.8	59.9	23.9	20.9	2.4	3.4		8.1	2.1		Senshu *et al.* 2007
P. pubescens	culm	diameter > 15 mm	Fall	73.8	98.4	1.6		87.5	64.3	23.2	21.1	0.4	2.0		8.1	0.8		Senshu *et al.* 2007
Phyllostachys mix**	culm	not specified	Fall		98.4	1.6	87.7		60.8	26.9	14.6		3.9	4.0		0.5		Knott *et al.* In review
F. spathacea	culm	2 yrs+	1 yr average		98.8	1.2		86.5	64.2	22.3	16.4	0.2	1.2					Schaller *et al.* 1985
Sinarundaria fangiana	culm	2 yrs+	1 yr average		98.0	2.0		85.0	61.9	23.1	16.2	0.3	2.4					Schaller *et al.* 1985
Sinarundaria fangiana	culm	old shoot	1 yr average		97.3	2.7		83.9	60.3	23.6	13.6	0.2	4.4.					Schaller *et al.* 1985
B. fargessii	culm	young	Not specified						45.9		14.7		1.6					Long *et al.* 2004
Not specified	culm	not specified	Not specified	62.4													4390.5	Liu *et al.* 2015
P. aureosulcata	culm	<5 mm diameter	Not specified	61.7	97.7	2.3		79.6	44.2	35.4	9.9		3.8				4600.0	Dierenfeld *et al.* 1982
P. aureosulcata	culm	5-15 mm diameter	Not specified	57.1	98.8	1.2		88.4	48.2	40.2	7.1		2.2				4600.0	Dierenfeld *et al.* 1982
Fargesia spathaceus	culm cover	not specified	Early spring	55.2							16.2		5.5					Mainka *et al.* 1989
P. aureosulcata	culm cover	not specified	Not specified	78.2	99.3	0.7		84.6	66.5	18.1	6.2		2.0					Dierenfeld *et al.* 1982
Fargesia spathaceus	culm pith	not specified	Early spring	48.2							1.7		8.3					Mainka *et al.* 1989
P. aureosulcata	culm pith	not specified	Not specified	65.6	99.1	0.9		82.7	60.2	22.5	7.2		1.9				4600.0	Dierenfeld *et al.* 1982
Phyllostachys mix**	leaf	not specified	Winter		88.6	11.4	51.2		30.9	20.4	5.3		19.0	2.1		2.0		Knott *et al.* In review
F. spathacea	leaf	not specified	Early spring						36.3		2.2		27.4					Mainka 1989
Fargesia spathaceus	leaf	not specified	Early spring	33.5							2.2		27.4					Mainka *et al.* 1989
P. aurea	leaf	not specified	Early spring	45.4							2.9		19.8					Mainka *et al.* 1989
Phyllostachys mix**	leaf	not specified	Early spring		87.4	12.6	52.0		32.1	19.9	6.0		18.3	2.5		1.7		Knott *et al.* In review
Phyllostachys mix**	leaf	not specified	Late spring		91.4	8.6	60.0		32.5	27.4	3.9		18.0	1.5		1.6		Knott *et al.* In review
Phyllostachys mix**	leaf	not specified	Summer		89.8	10.2	56.3		32.9	23.4	5.2		17.5	1.1		2.1		Knott *et al.* In review
P. bambusoides	leaf	not specified	Fall	43.7	86.0	14.0		69.5	34.2	35.3			13.1		0.0	3.4		Senshu *et al.* 2007
P. pubescens	leaf	not specified	Fall	44.8	91.1	8.9		66.9	32.9	34.0	8.6	5.2	17.6		2.4	4.3		Senshu *et al.* 2007
Phyllostachys mix**	leaf	not specified	Fall		88.0	12.0	56.0		35.6	20.4	5.8		18.5	1.1		1.9		Knott *et al.* In review
F. spathacea	leaf	all ages	1 yr average		91.2	8.8		71.2	38.2	33.0	9.7	2.3	14.1					Schaller *et al.* 1985
Sinarundaria fangiana	leaf	all ages	1 yr average		91.6	8.4		71.9	36.4	35.5	8.6	2.5	15.5					Schaller *et al.* 1985
B. *fargessii*	leaf	young	Not specified			3.5			33.7		10.0		9.6					Long *et al.* 2004
Not specified	leaf	not specified	Not specified	48.7													4366.7	Liu *et al.* 2015
P. aureosulcata	leaf	not specified	Not specified	52.0	92.2	7.8		65.6	27.7	37.9	6.2		13.4				4800.0	Dierenfeld *et al.* 1982
P. aurea	shoot	not specified	Early spring	23.9							2.7		21.3					Mainka *et al.* 1989
P. aurea	shoot	all ages	Late spring		93.4	6.6	69.0		36.7	32.4	3.0		15.9			3.3		Christian *et al*. 2015
P. aureosulcata	shoot	all ages	Late spring		93.0	7.0	63.9		28.5	35.4	2.1		19.8			3.4		Christian *et al*. 2015
P. bisettii	shoot	all ages	Late spring		93.1	6.9	64.0		32.5	31.5	2.7		18.6			3.7		Christian *et al.* 2015
P. glauca	shoot	all ages	Late spring		93.9	6.1	67.0		33.2	33.7	3.1		16.2			3.4		Christian *et al.* 2015
P. nuda	shoot	all ages	Late spring		92.7	7.3	61.0		29.3	31.7	1.7		21.2			3.6		Christian *et al.* 2015
P. rubromarginata	shoot	all ages	Late spring		93.5	6.5	69.1		34.8	34.4	2.6		14.5			3.1		Christian *et al.* 2015
Pseudosasa japonica	shoot	all ages	Late spring		93.6	6.4	73.6		42.4	31.3	5.9		8.6			2.7		Christian *et al.* 2015
Not specified	shoot	not specified	Not specified	8.2													4390.5	Liu *et al.* 2015

Gastrointestinal physiology of a monogastric species

Herbivores exhibit a spectrum of unique GIT adaptations, designed to accommodate high levels of dietary fiber intake. Most notably, the ruminant (e.g., cattle, sheep, giraffe) is highly adapted for utilization of a fibrous diet, having a voluminous multi-chambered stomach hosting fibrolytic microorganisms, and the ability to regurgitate and remasticate digesta (i.e., rumination; Van Soest 1994). The ruminal microbiome is responsible for enzymatic degradation of complex carbohydrates which would not otherwise be metabolized by mammalian enzymes of the host, whereas the act of rumination allows for extensive mechanical breakdown of these plant structural components. Ruminants further derive energy from volatile fatty acids (VFA), the product of microbial fermentation, and protein from microorganisms which continuously pass into and are digested by the small intestine (Van Soest 1994). While most monogastric species lack an initial site of microbial digestion of nutrients, and all lack the function of rumination, some species have a specialized hindgut (e.g., horse, rabbit, koala) which houses a unique microbiome capable of fiber degradation (Chivers and Hladik 1980; Hume 1989). Hind-gut fermenting species are characterized by their enlarged cecum attached to the large intestine, which functions similarly to the rumen as a site for microbial degradation of dietary fiber.

Fibrolytic capabilities of ruminal or hindgut microorganisms are further enhanced by increased length and surface area of the various gastrointestinal compartments, which slows movement of digesta and maximizes degradation and absorption of nutrients. The cecum of hind-gut fermenting species functions similarly. This phenomenon is present in other herbivorous species, which may feature a complexly folded stomach to slow digesta flow for extended acid-digestion of feeds (e.g., elephant, colobine monkey), or a multicompartmented stomach and complimentary microbial population (i.e., kangaroo, hippopotamus, though these are not considered ruminants as they lack the ability to regurgitate and remasticate digesta [Chivers and Hladik 1980; Hume 1989; Van Soest 1996]).

In spite of its nearly-exclusive bamboo diet, the giant panda's gastrointestinal morphology more closely resembles that of carnivorous species, and is little adapted for utilization of dietary fiber. Comprised of a simple stomach and relatively short intestinal tract lacking a cecum altogether, total gastrointestinal tract length is approximately one meter, and digesta passage rate is rapid, ranging from 5 to 16 hours, and averaging approximately 8 hours (Dierenfeld 1997; Mainka et al. 1989; Schaller et al. 1985). Mastication is an essential determinant of the giant panda's ability to utilize nutrients in bamboo, as well as being a key difference in the digestion processes distinguishing the species from other physiological carnivores. Giant pandas use their broad, flattened molars, an uncharacteristic feature for carnivorous species, to crush bamboo and weaken cell wall components prior to chemical digestion (Bleijenberg and Nijboer 1989; Schaller et al. 1985). Interestingly, the giant panda's enamel structure falls under the most complex and stress-resistant classification (zigzag HSB), a characteristic not shared by bears, but rather seen in animals known to consume or chew bones (e.g., canids [Stefen 2001]). The stomach serves as the initial site of protein degradation by means of acid digestion, and is sharply bent at the pylorus which may slow passage of digesta (Boisen and Eggum 1991; Raven et al. 1936). From the stomach, digesta passes into the small intestine, where enzymatic digestion and absorption of simple sugars, starches, proteins, and fatty acids occurs. As these nutrients are found primarily within the bamboo plant cell, they have limited bioavailability to the giant panda. Despite undergoing mechanical breakdown through mastication, high concentrations of plant structural components remain in the bamboo digesta, preventing full accessibility of nutrients that would independently be susceptible to enzymatic degradation. High digestibilities for CP and EE fat (avg. 68% and 75%, respectively) were reported for two captive giant pandas consuming primarily non-bamboo diets (bamboo = 23% - 40% of intake), indicating the potential for thorough protein and fat digestion as is seen in carnivorous species (Kametaka et al. 1988). Expressed as a ratio of total tract length to length of head and body, giant pandas have a comparatively lengthened GIT in respect to exclusively carnivorous felids, such as the jaguarondi and ocelot (643% compared to 389% and 394%, respectively), though still not quite as long as that of the omnivorous black bear (807% [Raven et al. 1936]). Furthermore, when compared on an index of body mass$^{0.75}$ to cellulose digestibility (being greatly influenced by GIT length), giant pandas fare nearly 40% less than other omnivorous and herbivorous species of the same metabolic body weight (i.e., humans, pigs, horses [Van Soest 1996]).

Several reports have identified fecal microflora unique to the giant panda that may aid in fiber degradation in the large intestine to an extent (Hirayama et al. 1989; Zhu et al. 2011; Fang et al. 2012; Williams et al. 2013). Despite the absence of a cecum, giant pandas are capable of partial hemicellulose digestion in the colon (10% to 56% across literature, avg. 23.5%), which was reported to account for 27.4% of the total daily energy requirement of wild individuals in the Qinling Mountain range (Table 1.2 [Dierenfeld et al. 1982; Kametaka et al. 1988; Long et al. 2004; Mainka et al. 1989; Schaller et al. 1984; Senshu et al. 2007; Sims et al. 2007]). Cellulose digestibility has been reported at levels as great as 46%, however it is improbable that the giant panda can utilize cellulose to a greater extent than hemicellulose; therefore, cellulose digestibility is likely within the more consistently observed range 0.5% to 11% (Table 1.2 [Dierenfeld et al. 1982; Kametaka et al. 1988; Long et al. 2004; Mainka et al. 1989; Senshu et al. 2007]). There are also reports of lignin disappearance (7% - 14.6%) in the giant panda gastrointestinal tract, which may be attributed to the recently identified lignolytic white-rot fungus (*Perenniporia medulla-panis*) and enzyme (laccase) present in the giant panda's fecal microbiome (Table 1.2 [Fang et al. 2012; Hirayama et al. 1989; Mainka et al. 1989; Senshu et al. 2007; Tun et al. 2014]). As colonic microorganisms digest structural carbohydrates, volatile fatty acids are produced and absorbed by the host to be used as metabolic substrates, namely energy sources for epithelial cells (Case et al. 2000; Van Soest 1996). While the giant panda gut microbiome has only recently begun to be investigated, it is very probable that gastrointestinal health is linked to this symbiotic relationship between microorganisms and host. In multiple reports, instances of mucoid feces and apparent discomfort occur in giant pandas consuming low levels of fiber, or experiencing an overall change in diet composition (Mainka et al. 1989; Williams et al. 2016). Williams et al. found that gastrointestinal microflora in the giant panda adapted to dietary changes caused by shifts between bamboo plant part consumption (Williams et al. 2013; Williams et al. 2016).

Table 1.2— Summary of giant panda feeding trial design and nutrient digestibilities from available literature. Nutrient digestibilities reported are for: dry matter (DM), organic matter (OM), ash, energy, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose (cell.), hemicellulose (HC), crude protein (CP), ether extract fat (EE fat), non-structural carbohydrates (NSC), and nitrogen-free extract (NFE).

												Apparent %Digestibility of Diet														
Source	Diet	Predominant diet component	Season	Location	Sampling duration (days)	Captive/Wild	Approx. subject age (years)	Subject gender	Subject body weight (kg)	DMI (% BW/day)	DMI (kg DM/day)	DM	OM	Ash	Energy	CF	NDF	ADF	ADL	Cell.	HC	CP	EE fat	NSC	NFE	Marker(s)	Notes
Dierenfeld et al., 1982	supp. 25% DMB	Leaf	not specified	National Zoo	7	Captive	9	M	123.0	4.20%	5.17	19.6%			23.0%				7.7%	8.0%	27.0%	90.0%				PEG and total fecal collection	Digestibility values were averaged between two subjects with three observations per subject
Dierenfeld et al., 1983	supp. 46% DMB	Supplement	not specified	National Zoo	7	Captive	9.5	F	114.0	1.60%	1.82	19.6%			23.0%				7.7%	8.0%	27.0%	90.0%				PEG and total fecal collection	Digestibility values were averaged between two subjects with three observations per subject
Finley et al., 2011	supp. 2.4% DMB	Mix	Late spring	Zoo Atlanta	7	Captive	8	M	133.6	2.47%	3.30				34.0%											Total fecal collection	
Finley et al., 2011	supp. 1.6% DMB	Mix	Winter	Zoo Atlanta	4	Captive	8	F	107.4	3.68%	3.95				25.0%											Total fecal collection	
Finley et al., 2011; Sims et al.,2007	supp. 3.8%	Leaf	Summer	Memphis Zoo	1	Captive	3	F	80.0	2.70%	2.16	26.7%	28.9%	3.5%	29.0%	27.3%	24.1%	18.4%			29.1%	36.5%	-16.3%			Total fecal collection, AIA, ADL, ADIN	Digestibility values for DM, OM, Ash, NDF, ADF, HC, CP, and EE were averaged across trials by subject
Finley et al., 2011; Sims et al.,2007	supp. 3.9%	Leaf	Fall	Memphis Zoo	1	Captive	3	F	82.2	3.20%	2.63	26.7%	28.9%	3.5%	38.9%	27.3%	24.1%	18.4%			29.1%	36.5%	-16.3%			Total fecal collection, AIA, ADL, ADIN	Digestibility values for DM, OM, Ash, NDF, ADF, HC, CP, and EE were averaged across trials by subject
Finley et al., 2011; Sims et al.,2007	supp. 1.9%	Mix	Winter	Memphis Zoo	2	Captive	3	F	85.1	3.90%	3.32	26.7%	28.9%	3.5%	26.7%	27.3%	24.1%	18.4%			29.1%	36.5%	-16.3%			Total fecal collection, AIA, ADL, ADIN	Digestibility values for DM, OM, Ash, NDF, ADF, HC, CP, and EE were averaged across trials by subject
Finley et al., 2011; Sims et al.,2007	supp. 1.6%	Leaf	Fall	Memphis Zoo	1	Captive	5	M	77.2	7.10%	5.48	11.5%	12.0%	5.9%	7.5%	14.3%	10.0%	13.0%			5.4%	30.2%	6.8%			Total fecal collection, AIA, ADL, ADIN	Digestibility values for DM, OM, Ash, NDF, ADF, HC, CP, and EE were averaged across trials by subject
Finley et al., 2011; Sims et al.,2007	unsupplemented	Leaf	Winter	Memphis Zoo	3	Captive	5	M	77.5	7.70%	5.97	11.5%	12.0%	5.9%	9.2%	14.3%	10.0%	13.0%			5.4%	30.2%	6.8%			Total fecal collection, AIA, ADL, ADIN	Digestibility values for DM, OM, Ash, NDF, ADF, HC, CP, and EE were averaged across trials by subject
Kametaka et al., 1988	supp. 77% fresh, 45% DMB	Supplement	Winter	Ueno Zoological Gardens	10	Captive	13	F	102.0	1.20%	1.22	57.5%				33.2%	37.2%	36.2%	46.9%	32.3%	38.9%	64.0%	71.2%		70.1%	Total fecal collection	Of bamboo, only leaves and branches were offered
Kametaka et al., 1988	supp. 61.9% fresh, 39% DMB	Supplement	Winter	Ueno Zoological Gardens	10	Captive	18	M	120.0	2.60%	3.12	62.2%				46.0%	51.8%	47.7%	53.2%	46.6%	56.2%	71.9%	79.1%		68.7%	Total fecal collection	Of bamboo, only leaves and branches were offered
Liu et al., 2015	87% shoots, 13% supp.	Shoot	Late spring	Anji Bamboo Expo Park (China)	7	Captive	4	M	102.4	2.75%	2.82	60.7%			65.9%		8.8%	4.9%			12.2%	87.9%	82.2%			Total fecal collection	
Liu et al., 2015	83% shoots, 17% supp.	Shoot	Late spring	Anji Bamboo Expo Park (China)	7	Captive	4	F	89.0	2.35%	2.09	61.0%			66.6%		11.1%	3.3%			17.9%	86.6%	76.7%			Total fecal collection	
Liu et al., 2015	84% shoots, 16% supp.	Shoot	Late spring	Anji Bamboo Expo Park (China)	7	Captive	9	M	93.6	2.39%	2.24	60.9%			64.9%		5.7%	6.2%			5.3%	86.4%	76.1%			Total fecal collection	Digestibility is for total, mixed diet
Liu et al., 2015	85% shoots, 15% supp.	Shoot	Late spring	Anji Bamboo Expo Park (China)	7	Captive	26	F	98.0	2.45%	2.40	53.0%			59.3%		-21.1%	-16.4%			-25.2%	84.0%	76.5%			Total fecal collection	
Mainka et al., 1989	supp. 37.7% DMB	Supplement	Early spring	Calgary Zoo	5	Captive	4.5	F	110.0	1.80%	2.00	17.2%								0.5%	17.5%					Total fecal collection	Digestibility and intake are for bamboo and sugar cane only, other feeds were accounted for
Mainka et al., 1989	supp. 44.7% DMB	Supplement	Early spring	Calgary Zoo	5	Captive	2.5	F	65.0	2.60%	1.61	15.9%								11.0%	22.4%					Total fecal collection	Digestibility and intake are for bamboo and sugar cane only, other feeds were accounted for
Senshu et al., 2007	supp. 19.1% DMB	Leaf	Fall	Adventure World (Japan)	7	Captive	6	M	116.0	2.50%	2.90	12.7%								7.2%	15.2%					Chromium dioxide	
Senshu et al., 2007	supp. 8.2% DMB	Leaf	Fall	Adventure World (Japan)	3	Captive	9	M	130.0	4.32%	5.61	15.2%							8.5%	28.0%	10.2%					AIA	
Long et al., 2004	unsupp. leaf only	Leaf	Early spring	Qinling Mountains	not specified	Wild	not specified	not specified	not specified	not specified	not specified	19.0%							6.7%	41.8%	23.4%					ADL	Intake measured by observations of rate of bamboo consumption multiplied by observed time spent eating
Long et al., 2004	unsupp. culm only	Culm	Early spring	Qinling Mountains	not specified	Wild	not specified	not specified	not specified	not specified	not specified	12.5%								0.0%	21.5%					ADL	Intake measured by observations of rate of bamboo consumption multiplied by observed time spent eating
Long et al., 2004	unsupp. culm only	Culm	Summer	Qinling Mountains	not specified	Wild	not specified	not specified	not specified	not specified	not specified	23.3%									26.0%					ADL	Intake measured by observations of rate of bamboo consumption multiplied by observed time spent eating
Schaller et al., 1985	unsupplemented	Culm	Late spring	Wolong Reserve	not specified	Wild	not specified	not specified	not specified	not specified	7.56	18.7%									18.2%					n/a	Schaller et al., calculated digestibilities for wild bears assuming 12.5 kg/d fresh consumption
Schaller et al., 1985	unsupplemented	Leaf	Fall	Wolong Reserve	not specified	Wild	not specified	not specified	not specified	not specified	4.95	40.0%														n/a	Schaller et al., calculated digestibilities for wild bears assuming 12.5 kg/d fresh consumption
Schaller et al., 1985	unsupplemented	Culm	Winter	Wolong Reserve	not specified	Wild	not specified	not specified	not specified	not specified	6.63	36.4%	37.8%		38.3%		24.3%	16.0%			33.1%		59.1%	82.1%		n/a	Schaller et al., calculated digestibilities for wild bears assuming 12.5 kg/d fresh consumption
Schaller et al., 1985	unsupp. shoots only	Shoot	Late spring	Wolong Reserve	not specified	Wild	not specified	not specified	not specified	not specified	3.83	21.6%	23.2%		22.7%		15.1%	14.4%	14.6%	7.8%	16.0%		30.2%	58.4%		n/a	Schaller et al., calculated digestibilities for wild bears assuming 12.5 kg/d fresh consumption

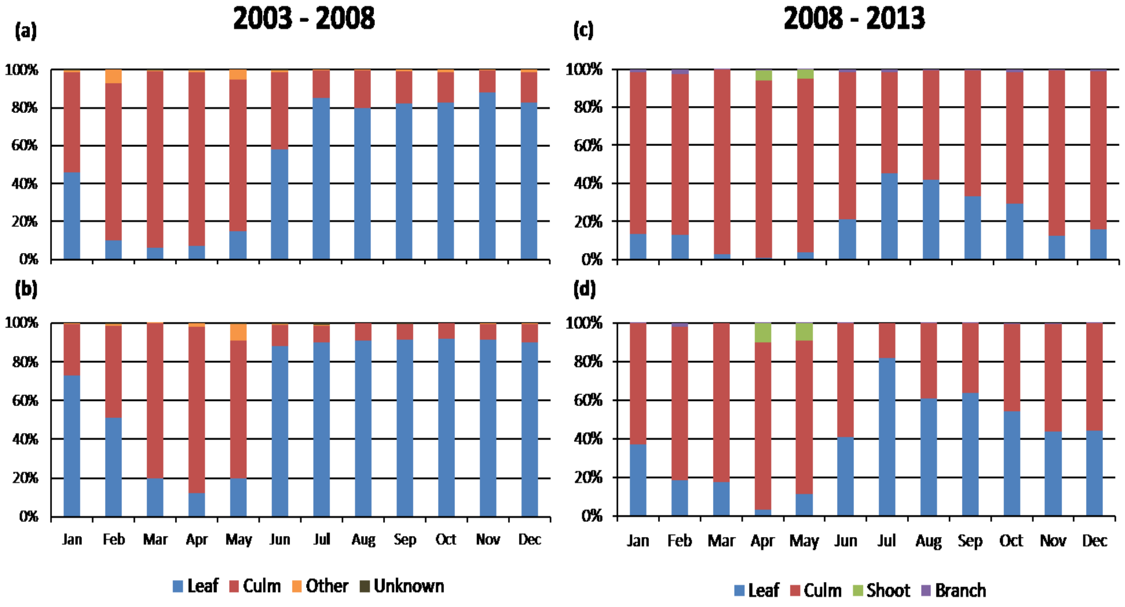
Foraging strategies exhibited by herbivores

All consumers display dietary preferences driven by desirable and adverse diet characteristics, for example digestibility and palatability versus the presence of indigestible or toxic compounds. Herbivores are presented with a nutritional challenge, as plants contain high levels of structural carbohydrates, change compositionally over time, and have developed defensive mechanisms to protect against predatory and environmental stressors. Thus, species often exhibit unique foraging behaviors, which work congruently with specialized GIT morphology, allowing the consumer to meet nutritional needs while consuming a high-fiber diet. For instance, smaller animals, with consequently smaller GIT size, generally experience a more rapid gut passage and consequently reduced digestive capacity, in addition to being limited by gut space for consumed feed items (Van Soest 1994). Not surprisingly, species constrained by GIT volume are predisposed to selective feeding behaviors, preferring more nutritious and digestible feed items to compensate for limited digestive ability. Optimal foraging theory proposes that foragers preferentially select foods to maximize energy intake, while engaging in foraging behaviors to reduce energy expenditure (Sih and Christensen 2001). For example, smaller ruminants, such as the blue duiker and white-tail deer, browse selectively for energy-dense leaves, fruits, and seeds (Van Soest 1994). Selective feeding is also present in generalist grazers (consumers of leaves and culms of grasses and legumes; e.g., cattle, horses, sheep), where “patch-grazing” refers to predation on favored plant species and immature, succulent plant growth, which promotes development of undesirable plants and results in disparate patches of vegetation types (Chapman et al. 2006; Teague et al. 2004; Vries and Daleboudt 1994; Wilmshurst et al. 1995). In some scenarios, the avoidance of certain plants due to the presence of anti-nutritional compounds, which often indicate unpalatability or toxicity on behalf of the consumer, drives herbivore diet selectivity. Such compounds are believed to function as defensive agents in plants, and include silica, endophytes, and secondary metabolites (Massey et al. 2009; Huitu et al. 2014).

Compared to other herbivores across all varieties of GIT morphology, giant pandas are especially disadvantaged. It is reasonable to conclude that giant pandas consume a diet of lesser quality, while possessing a digestive system least adapted for fiber utilization, than any other species. In light of this inefficient combination of diet selection and gut morphology, foraging strategy is key to giant panda survival, as it is the primary adaptation enabling them to meet their nutritional requirements. As previously discussed, bulk consumption of bamboo can provide sufficient nutrients to meet giant panda dietary needs. However, a degree of bamboo selectivity has been observed in both wild and captive settings, and may also contribute to fulfilling nutritional requirements (Schaller et al. 1985; Long et al. 2004; Hansen et al. 2010). Theoretically, bamboo selection balances the energy expenditure of constant foraging activity with intake of the most nutritionally-rewarding bamboo (Long et al. 2004). Giant pandas are notoriously picky consumers; thoroughly investigating bamboo by smell before they consume it, and discarding what they deem unacceptable (Parsons 2013; Schaller et al. 1985; personal observation). Unlike other carnivorous species, such as the cat and bottlenose dolphin, giant pandas have maintained the ability to perceive sweet tastes (Jiang et al. 2012). This proposed “sweet tooth” may provide a means to distinguish energy-dense foods, as sweetness is indicative of digestible simple sugar content, and impact food preference (Jiang et al. 2014). Furthermore, analysis of the giant panda genome revealed a mutation replacing the sequence for umami perception with a pseudogene, and as such, protein may not be a driving factor in diet selection (Li et al. 2010). The selection of bamboo for palatability incentives is further supported by descriptions of bamboo rejected by giant pandas in captive feeding trials. Dierenfeld et al. reported that rejected bamboo had lesser concentrations of both gross energy (GE) and protein, and greater concentrations of dietary fiber, than the average concentrations of respective nutrients in bamboo offered, indicating that the subjects selected for bamboo with greater nutritional potential (1982). During periods of culm intake, giant pandas further the process of diet selection by removing the surface layer, which is hypothesized to primarily be composed of fiber and waxes, and consume only the pith, or interior portion, of bamboo (Dierenfeld et al. 2010; Hansen et al. 2010; Long et al. 2004).

Giant pandas exhibit distinctive, seasonal patterns of dietary shifts defined by the part of bamboo which is selected for consumption. Dietary shifts in plant part consumption have been reported in both wild and captive giant panda populations, however the timing is known to vary among regions and institutions. In general, giant pandas prefer bamboo culm in the late winter and spring, young shoots when available in the late spring to early summer, and leaves in late summer through winter (Hansen et al. 2007; Long et al. 2004; Schaller et al. 1985; Williams et al. 2012). Giant pandas inhabiting the Wolong Reserve of China were reported to prefer leaves from July to October, and culm from November to March (Schaller et al. 1985). Seasonal dietary shifts exhibited by giant pandas in the Qinling Mountain region were correlated to the animals' annual migration, as different bamboo species grow in different altitudes (*Bashania* grows on lower slopes, *Fargesia* grows on higher). Giant pandas consumed leaves of *Bashania* bamboo growing in the lower altitudinal region from September to February, when leaves of those bamboos at their lowest dietary fiber and greatest CP concentrations. The populations shifted to consumption of new *Bashania* shoots as they became available in April and May, then climbed to higher altitudes in late May and June when *Fargesia* bamboo shoots emerged. Giant pandas remained in higher regions consuming *Fargesia* until late September, at which time they returned to the lower levels and fed on *Bashania* leaves (Long et al. 2004). Thus, wild giant pandas exclusively forage on newly emerged bamboo shoots when they are accessible, and as such, bamboo shoots have been hypothesized to provide a vital nutritive opportunity to improve body condition and reproductive fitness (Long et al. 2004; Schaller et al. 1985). However, dietary shifts of captive giant pandas are less defined by availability of bamboo shoots, as fresh shoots are difficult to provide *ad libitum* in captivity. Plant part selection by two giant pandas has been an ongoing study at the Memphis Zoo since the animals' arrival in 2003, and has been previously described by Hansen et al. (2010) and Williams et al. (2013). During 2003 through 2008, giant panda foraging behavior indicated leaf preference from June through December (Figure 1.2 [Hansen et al. 2010]). From 2008 to 2013, average leaf intake peaked during July (45% and 82% for the male and female, respectively) and remained relatively elevated through October (Figure 1.2 [Williams et al. 2012; Memphis Zoo Records—Personal communication]).

Figure 1.2— Bamboo plant part selection by two giant pandas at the Memphis Zoo. Months presented as averages within the duration of the observation periods (2003 – 2008 or 2008 – 2013). (A) Bamboo consumption for male 2003-2008. (B) Bamboo consumption for female 2003-2008. (C) Bamboo consumption for male 2008-2013. (D) Bamboo consumption for female 2008-2013. Plant part consumption was determined from daytime behavioral observations. The change in key between figures A, B and C, D denotes difference in ethogram data collection between two reports (A, B: Hansen et al., 2010; C,D: Williams et al. 2012; Memphis Zoo Records—Personal communication).



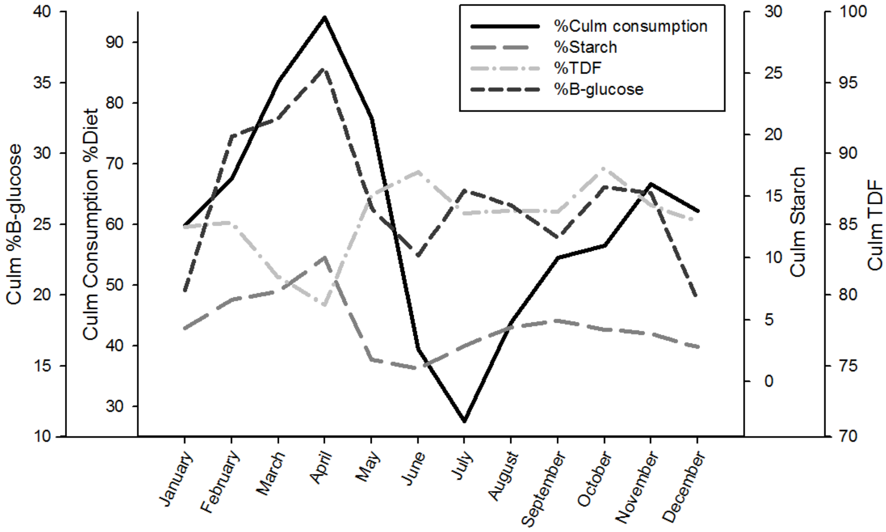
Seasonal diets have also been observed in other obligate and natural herbivores, such as the red panda, black bear, elephant, and impala, and appear to be dependent on the most nutritious and readily available food source at that time of year (Eagle and Pelton 1983; Hellgreen *et* al. 1989; Kos et al. 2010; Wei et al. 2000). Furthermore, seasonal dietary shifts are the chief response by which other herbivorous and omnivorous bear species subsist on low-quality, vegetarian diets. In the habitat of the wild giant panda, however, bamboo culms and leaves are available year-round in relatively constant amounts, except in the rare occurrence of bamboo flowering and die-off. As such, their seasonal dietary transitions are speculated to be a response to changes in nutrient content of different bamboo parts throughout the year, though it is unclear which nutrients, or combination of nutrients, most impact plant part selection. Of bamboo culms offered to giant pandas at the Memphis Zoo, monthly nutritional data revealed increases in starch, and bound glucose, and a decrease in fiber during the spring, while protein and fat remained relatively constant year-round (Figure 1.1 [Katrina Knott, Memphis Zoo—personal correspondence]). This is consistent with the description of foraging patterns of the two giant pandas at the Memphis Zoo, taken over a period of five years, which reported that the bears consumed primarily culm from February to May (Hansen et al. 2007). Seki and Aoyama report that starch and free sugars both peak in culms prior to shooting season and decline while shoots emerge and grow, and hypothesize that excess sugars are being mobilized to promote shoot growth (1995). Leaves, on the other hand, are known to reach peak concentrations of silica, an indigestible compound functioning as a plant defense mechanism, at this time of the year (Lux et al. 2003; Schaller et al. 1985; Tabet et al. 2004). Bamboo shoots sampled from the Memphis Zoo Bamboo Farm (Shelby Farms, Memphis, TN) had greater protein and fat, and lower fiber contents than culm and leaves (Christian et al. 2015).

It is also possible that the female giant panda’s unique reproductive cycle, characterized by an annual monoestrous and delayed embryo implantation, influences nutrient needs, and consequently foraging behavior throughout the year. Interestingly, shooting season of bamboos consumed by giant pandas often coincides with their annual estrus and early gestational periods in the spring (Schaller *et* al. 1985; Swaisgood et al. 2011).

Digestibility study design and internal dietary markers

Evaluation of diet or feed digestibility, in relation to the subject species, is ascertained through controlled and replicated feeding trials. In such experiments, feed offered, feed rejected, and fecal output can be observed, sampled, and analyzed to determine diet selection, nutritional composition of the consumed diet, and digestibility of nutrients consumed. Complete collection of the subject's feces throughout the trial is the classical method used to determine a nutrient digestibility coefficient, as digestibility is roughly the ratio of nutrient consumed to nutrient excreted. However, total fecal collection is not feasible for free-ranging animals, and can be laborious in confined studies. Dietary markers provide a calculable reference of nutrient disappearance in digesta and feces, and allow the examiner to estimate total fecal production. An internal dietary marker is an indigestible, naturally-occurring compound within the feedstuff, such as plant structural components, that can be reliably quantified in the feed and feces (Cochran et al. 1986; Titgemeyer 1997). Internal markers are especially useful for experiments which involve herbivorous species consuming high-fiber forages, as these feedstuffs contain high levels of indigestible compounds. From a management perspective, internal markers may also be ideal when studying intake and digestibility of free-grazing animals because they do not need to be added to the subject's diet and they allow for determination of fecal output without total fecal collection. This could be particularly valuable in nutritional studies involving wild giant pandas, though a suitable internal marker must first be identified to accurately predict fecal output and nutrient digestibility. In previous trials with giant pandas, acid detergent lignin (ADL) and acid

Figure 1.3— Annual changes in carbohydrates of bamboo culm relative to culm consumption by giant pandas at the Memphis Zoo. Observed constituents include starch, total dietary fiber (TDF), and bound glucose (B-glucose) concentrations in bamboo culms offered from 2008 – 2010 (Katrina Knott, Memphis Zoo—personal communication).



insoluble ash (AIA) have been used as internal dietary markers alongside total fecal collection to measure nutrient disappearance from consumed bamboo (Long *et* al. 2004; Sims et al. 2007). As previously mentioned, however, recent studies have reported lignin disappearance in the giant panda, proving it to be an unreliable dietary marker (Fang et al. 2012; Hirayama et al. 1989; Senshu et al. 2007; Tun et al. 2014). In one study, AIA was found to accurately predict fecal output when compared to the values obtained through total fecal collection, whereas ADL and acid detergent insoluble nitrogen (ADIN) could not (AIA fecal output $r = 0.99$ vs. 0.84 and 0.85 for ADL and ADIN, respectively [Sims et al. 2007]). Acid detergent insoluble ash (ADIA), the inorganic residue of ADF, has not been reviewed as a dietary marker for bamboo, though it may be another suitable option for digestibility studies with giant pandas, as it is relatively simple to quantify and has been used successfully in ruminant studies (Bodine et al. 2002; Kanani et al. 2014). A feed additive, polyethylene glycol, has also been used to estimate nutrient digestibility in giant pandas, although it is liquid-phase marker, and may not be suitable for a highly indigestible diet in which digesta is primarily solid (Dierenfeld et al. 1982; Macrae 1974; Manners and Kidder 1968).

Review of available nutritional data regarding the giant panda

The fragile status of the giant panda population necessitates the use of utmost caution when studying these animals in both captive and wild scenarios. Unfortunately, these circumstances have allowed only a few opportunities to discern giant panda nutrient requirements. Data from giant panda nutritional studies is not only scarce, but further complicated by variations amongst trials in bear age, time of year, reproductive status, and most especially, diet variations amongst institutions. A current issue in the management of captive giant pandas in zoos is a lack of consistency concerning diet composition and bamboo provision in diets of different institutions. Reported diets provided to giant pandas in five Chinese zoos during the 1990s consisted of 17 to 82% bamboo with additional fruit and vegetables, grain products, occasional meat products, and vitamin and mineral supplements formulated for canines (Dierenfeld et al. 1995). More recently, giant pandas in four U.S. zoos were reported to consume diets of 87.3 to 96.3% bamboo, with the remaining diet portion being various fruits or fiber biscuits for training purposes (Wiedower et al. 2012). Future research in the area of giant panda nutrition should be directed at establishing dietary recommendations based on wild and captive pandas, and implementing in zoos and reserves a diet that both meets these regulations and accurately replicates the diet of the wild panda.

In a review of the available literature, Dierenfeld estimated that the nutrient content of bamboo consumed by the giant panda diet to be approximately 9% CP, 73% fiber, 13% soluble sugars, and 1000 kcal kg^{-1} on a dry matter basis. When consumed at high volumes, these levels are expected to meet the approximated panda's nutrient requirements (Dierenfeld 1997). Other feeding guidelines for the captive giant panda are largely derived from known canine dietary requirements, given the similarity of their digestive tracts. However, some requirements have been approximated through observation of feeding habits of captive and wild giant pandas. Energy needs of the giant panda may be the most comprehensively studied of nutrient requirements, however the data available is nevertheless widely variable. Energy-bearing carbohydrates, protein, and fatty acids are present in bamboo; however, fat content is marginal and protein concentrations are low in this plant-based diet. It is likely that giant panda meet most of their energetic needs through partially digestible carbohydrates, as bamboo is almost completely constituted of carbohydrate polymers. For free-ranging individuals in the Wolong Reserve, Schaller et al. estimated the energy expenditure of a 100 kg, non-reproductive giant panda to be 3,132 kcal/day, with the average daily digestible energy (DE) intake being 4,300 to 5,500 kcal (1985). On the other hand, a daily energy expenditure averaged 1,482 kcal and a 6,405 kcal daily DE requirement were observed (in separate studies) for wild giant pandas in the Qinling mountain range (Long et al. 2004; Nie et al, 2015). From data reported by Finley et al., gross energy (GE) and digestible energy (DE) intake can be calculated for captive giant pandas, ranging from 11,908 to 28,130 and 2,009 to 6,245, respectively (Table 1.3 [2011]). These values indicate captive giant pandas consume sufficient DE to meet the daily energy expenditure of captive giant pandas estimated at 1,099 kcal/day by Nie et al. (2015). Additionally, individuals with lower GE digestibilities (7.5%, 9.2%) consumed greater quantities of GE per day than those displaying more efficient GE utilization (25% - 39% [Finley et al. 2011]).

Table 1.3— Intake of gross energy (GE) and digestible energy (DE) by captive giant pandas in U.S. zoos. Adapted from Finley *et al.* (2011).

Animal ID	Location	Month	GE Intake (kcal/day)	DE intake (kcal/day)	Apparent energy digestibility (%)
507	Memphis	February	19,682	5,255	26.7%
461	Atlanta	June	15,713	5,342	34.0%
507	Memphis	August	11,908	3,453	29.0%
466	Memphis	October	26,785	2,009	7.5%
507	Memphis	November	16,054	6,245	38.9%
466	Memphis	December	28,130	2,588	9.2%
452	Atlanta	December	19,228	4,807	25.0%

Large quantities of dietary fiber are suggested to be essential to giant panda health as gastrointestinal tract (GIT) related problems in the mid-1990s' accounted for 28% of captive giant panda deaths, and mucous stools have been observed from giant pandas consuming low fiber diets (Qui and Mainka 1993; Dierenfeld et al. 1995). Mainka et al. reported frequent incidences of mucous stools in captive bears on a diet consisting of 56-63% bamboo, indicating a dependence on fiber for gut health (1989). Additionally, diets high in soluble fibers, such as pectin, increase digesta viscosity and slows rate of passage through the gut, potentially improving digestibility (Van Soest 1996).

In addition to being a concentrated source of energy, fats are also essential to body heat regulation, cellular structure, and are precursors to hormones and bile salts (among other necessary bodily compounds [Case et al. 2000]). Fatty acid requirement has not been reviewed for giant pandas, possibly due to the difficulty of quantifying fat disappearance in a diet with minimal fat concentrations (less than 3.5% in leaves, 1% in culm [Katrina Knott, Memphis Zoo—Personal communication; Schaller et al. 1985]). Protein is an additional energy-bearing nutrient; however, it is primarily utilized as a source of nitrogen for the body to synthesize essential amino acids and nitrogen-containing compounds (Case et al. 2000). As suggested by Dierenfeld, the NRC crude fat requirement for the dog (5% DM of diet) may satisfy the giant panda's dietary needs (1997). Protein requirements have been examined in few feeding studies, and are estimated to be 100 g/d (Dierenfeld et al. 1995; Dierenfeld 1997; Schaller et al. 1985). Liu et al. observed protein intake in captive, subadult (aged 1.5 to 4.7 years) giant pandas consuming a mixed diet of concentrate supplements and bamboo, and reported CP intake ranging from 9.95 g per kg metabolic body weight (MBW: animal weight$^{0.75}$) for the youngest animals to 13.54 g CP/kg MBW by the older animals (2002). The subadult individuals in the previous study were still considered to be growing, no direct relationship was found between CP intake and weight gain (Liu et al. 2002). Methionine is the limiting amino acid in bamboo; however, there is little research on essential amino acid balance in pandas (Schaller et al., 1985). In general, though there are low concentrations of fat and protein in bamboo, deficiencies of these nutrients are apparently of little concern to giant pandas consuming bamboo *ad libitum*. In prior reports available on vitamin and mineral levels of giant pandas, there have been no reported incidences of serious deficiencies in captive or wild bears (Dierenfeld 1997; Mainka et al. 1991). However, a recent study suggests that low soil iodine levels in the pandas' mountain range habitats may be a factor in poor reproductive rates (Milewski and Dierenfeld 2012). Nie et al. proposed calcium, phosphorous, and nitrogen imbalances in bamboo culm may interfere with mineral absorption in the giant panda (2014).

Digestibility trials observing giant pandas have been conducted, however, given the complicated nature of studying this species, studies are few and highly varied in methods, determination of digestibility, and description of data (summarized in Table 1.2). For instance, levels of supplementation in the subject diet range from unsupplemented to 77% of intake comprised of non-bamboo feeds (on a fresh basis; Table 1.2 [Kametaka et al. 1988; Schaller et al. 1985]). Additionally, the variety and potential inaccuracy of digestibility markers used in previous trials already been acknowledged in this review undoubtedly introduces error when comparing results of available literature. The earliest reported digestibility study was a series of three traditional digestibility trials carried out by Dierenfeld et al. (1982). The trials were carried with two captive giant pandas, which allowed investigators to closely observe diet intake, administer a known amount of a dietary marker, and measure fecal production (Dierenfeld et al. 1982). Digestibility of nutrients in bamboo (Table 1.2) were reported as the average between the two bears across all trials, and were calculated by subtracting the contribution of digestible nutrients in supplemental diet items (Dierenfeld et al. 1982). In contrast, Long et al. opportunistically collected video observations of wild giant pandas in the Qinling Mountains, and used video data to estimate time spent feeding per day, bamboo plant part selection and feeding rate, which then allowed researchers to calculate daily bamboo intake, assuming bamboo. From these estimations of intake and analysis of acid detergent lignin (ADL) in fecal samples to approximate fecal output, dry matter, cellulose, and hemicellulose digestibilities were determined according to plant part and season (Table 1.2 [Long et al. 2004]).

Five feeding trials were conducted from 2003-2004 at the Memphis Zoo with a female (ID: 507) and male (ID: 466) giant panda, aged 3 and 5, respectively. Subjects were offered bamboo-based, mixed diets (supplements comprised 1.6% to 3.9%) in four trials (Finley et al. 2011; Sims et al. 2007). The fifth trial examined nutrient digestibilities from a total bamboo diet in both bears; however, Finley et al. excluded data from the female bear, as she had a mucus stool during the study (2011). These feeding trials varied in length from 1 to 3 days, and occurred from August through the following February. Sims *et al*. reported average digestibility coefficients for DM, ash, OM, CP, CF, NDF, ADF, hemicellulose, and EE (Table 1.2 [2007]). Significant differences in digestibility between bears were observed for only hemicellulose ($P = 0.014$ [Sims et al. 2007]). In a separate manuscript, Finley *et al*. reported energy digestibilities from the same trials (Table 1.2 [2011]). In these trials, supplements contributed 7.6% to 35.6% of the energy consumed. While the study was not designed to observe seasonal changes in nutrient digestibility, they did not report evidence to suggest giant pandas made foraging selections to increase energy assimilation (Finley et al. 2011). Thus far, there have been no repeated studies on the same individuals to detect age effects on digestibility; however, differences in bacterial and fungal populations in the giant panda fecal microbiome have been observed between adult and geriatric animals (exact ages not given), indicating potential changes in carbohydrate digestion (Tun et al. 2014). Additionally, Liu et al. observed reduced digestibilities for several nutrients in an older individual (aged 26 years vs. 4 and 9 years), most conspicuously for NDF, ADF, and hemicellulose (Table 1.2 [2015]).

Previously at the Memphis Zoo, bamboo shoots were offered to the giant pandas as treats; though in recent years they have been more heavily integrated into the diet during shooting season due to their nutritional value and enthusiastic consumption by the bears (Memphis Zoo Zookeepers—personal communication). The effects of the inclusion of bamboo shoots as a major component in captive diets during shooting season have not been reviewed, although shoot digestibility in giant panda is recorded to be 40% DM (Schaller et al. 1985). Moreover, Liu et al., concluded that bamboo shoot-based diet provided ad libitum for captive giant pandas (83% to 87% *Phyllostachys vivax* shoot, supplemented with concentrate) met subject energy and protein requirements (2015).

Research objectives and project overview

Although data regarding giant panda nutrition is available, it is not sufficient to draw reliable and universal conclusions regarding the species as a whole, and necessitates repeated trials for authentication. Furthermore, gaps in knowledge accentuate the need to also perform unique studies that elucidate unknown aspects of giant panda feeding behavior and nutrient requirements. The situation of captive animals provides a unique opportunity to carry out nutritional studies, as their diet is relatively controlled, fecal output is observed, and behavior is monitored on a daily basis. We conducted an investigation to examine seasonal variances of macronutrient selection and digestibility, with an emphasis on energy, for captive giant pandas consuming a bamboo-based diet (>95%). This study supplements previous data concerning bamboo digestibility in the giant panda, while also examining whether varying nutrients in bamboo culms, leaves, and shoots impact dietary shifting behaviors and digestibility patterns. Additionally, results from this study will compliment previous digestibility studies performed on the same individuals ten years prior when the subjects had not yet reached maturity.

We hypothesized that dietary shifts in bamboo plant part selection are driven by temporal changes in the nutritive composition of bamboo and accompanied by changes digestibility that maximize the absorption of bamboo nutrients in the giant panda. To examine changes in bamboo selection and potential responses in nutrient utilization, five feeding trials were conducted with two captive giant pandas at the Memphis Zoo (July 2014, January, March, May, October 2015). During each trial, bamboo offered, bamboo rejected, and fecal output were measure during each trial as to determine bamboo selection, intake, fecal output, and digestibilities of macronutrients. The objectives of this project were to: (1) determine an accurate representation of bamboo dry matter intake and digestibility in giant pandas; (2) establish an accurate measure of bamboo plant part selection; (3) evaluate diet selection by giant pandas in relation to temporal variations in bamboo plant part and nutrient composition; and (4) evaluate utilization of nutrients in bamboo-based diets by the giant panda.

CHAPTER II

DETERMINATION OF TOTAL DRY MATTER INTAKE AND DRY MATTER DIGESTIBILITY OF THE BAMBOO-BASED DIET CONSUMED BY THE GIANT PANDA (*AILUROPODA MELANOLEUCA*)

Summary

Despite retaining a carnivore's characteristic simple gastrointestinal tract, giant pandas acquire the majority of their required nutrients from bamboo. The processes that allow giant pandas to meet their nutritional requirements from this high fiber diet are not yet fully understood due to this monogastric species' limited capability to digest plant structural components. Nutritional observations of captive individuals undoubtedly benefit their free-ranging counterparts by providing insights regarding habitat utilization and requirements. We evaluated methods to determine daily dry matter intake (DMI), fecal output (DFO), and dry matter digestibility (DMD) in captive giant pandas consuming a bamboo-based diet (> 95% bamboo on a DM basis). In giant pandas, day-to-day DMI varies broadly while fecal output remains relatively consistent, and a period longer than three days was necessary to evaluate bamboo intake and digestibility in giant pandas. We determined that mean DMI taken over a 17-day period best represented bamboo intake, and predicted reliable DMD values, as compared to median DMI and trial DMI estimations. The 17-d mean for DMI also exhibited a strong quadratic relationship with daytime bamboo consumption behavior observed through visual observation ($P < 0.01$; $R^2 = 0.89$). The internal dietary markers ADL, AIA, and ADIA could not accurately calculate DMI and DMD in the two giant pandas, likely due to difficulty quantifying intake of each marker in a bamboo diet.

Introduction

Giant pandas (*Ailuropoda melanoleuca*) historically thrived on diets primarily comprised of bamboo, a grass characterized by its tall, woody structure and evergreen foliage (Schaller et al. 1985; Carter 1999; Long et al. 2004). Among specialist herbivores, giant pandas are unique in their retention of a carnivorous gastrointestinal tract (GIT), lacking specialized gastrointestinal compartments to facilitate degradation of plant structural components (dietary fiber [Van Soest 1994]). Consequently, reported total bamboo dry matter digestibility in giant pandas is low, ranging from 6.9% - 39% (Dierenfeld et al. 1982; Finley *et al*. 2011; Mainka et al. 1989). Although near-exclusive selection of bamboo by the giant panda is paradoxical, the species' history of inhabiting heavily vegetated forests of central China suggests that bamboo abundance drove their dependence on this feed source (Schaller et al. 1985).

Presently, the giant panda population is considered vulnerable and conservation-dependent; though the species was only recently moved from the "endangered" classification by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (Swaisgood et al. 2016). Most recent surveys estimate nearly 1,900 free-ranging giant pandas, not including young cubs still paired to their dams (Swaisgood et al. 2016). Over 300 giant pandas are currently kept in captivity worldwide, and many of the institutions housing these animals participate in breeding programs to promote *in situ* and *ex situ* population growth and genetic diversity (Xie et al. 2013). Though giant pandas have a viably reproducing wild population, it has proven difficult to recreate natural settings in captive situations. Captive giant pandas are provided locally grown bamboo species (i.e., *Phyllostachys, Pseudosasa*) and commercial supplements based on availability (Finley et al. 2011; Hansen et al. 2007; Sims et al. 2007). Nutrient quality of bamboo and supplemental items, (i.e., bread, eggs, fruit, gruel, canine kibble, etc.) therefore, vary appreciably among institutions (Dierenfeld et al. 1995). As proper nutrition is intimately associated with overall animal health and successful reproduction, it is critical that zoos, reserves, and breeding centers offer a diet that meets the giant panda's unique dietary requirements.

Diet intake and digestion is evaluated through controlled and replicated feeding trials. In such experiments, feed offered, feed rejected, and fecal output are observed, sampled, and analyzed to determine diet selection, total intake, and diet digestibility. Complete collection of the subject's feces throughout the trial is the classical method used to determine diet digestibility, as it is roughly the ratio of nutrient consumed to nutrient excreted. However, total fecal collection is not feasible for free-ranging animals, and can be laborious in confined studies. Dietary markers provide a calculable reference of nutrient disappearance in digesta and feces, and allow the examiner to estimate total fecal production (Cochran et al. 1986). An internal dietary marker is an indigestible, naturally-occurring compound within the feedstuff, such as plant structural components, that can be reliably quantified in the feed and feces (Cochran et al. 1986; Titgemeyer 1997). Internal markers are especially useful when examining herbivorous species that consume high-fiber forages, as these feedstuffs contain high levels of indigestible compounds. From a management perspective, internal markers may also be ideal when studying intake and digestibility of free-grazing animals because they do not need to be added to the subject's diet and they allow for determination of fecal output without total fecal collection. This could be particularly valuable in nutritional studies involving wild giant pandas, though a suitable internal marker must first be identified to accurately predict fecal output and diet digestibility.

In previous trials with giant pandas, acid detergent lignin (ADL) and acid insoluble ash (AIA) have been used as internal dietary markers alongside total fecal collection to measure nutrient disappearance from consumed bamboo (Long et al. 2004; Sims et al. 2007). However, recent studies have reported lignin disappearance (7% - 15%) in the giant panda, proving it to be an unreliable dietary marker (Dierenfeld et al. 1982; Fang et al. 2012; Hirayama et al. 1989; Mainka et al. 1989; Senshu et al. 2007; Tun et al. 2014). Sims et al. suggested that AIA was a more appropriate dietary marker in giant pandas, as accurately predicted fecal output when compared to the values obtained through total fecal collection. Acid detergent insoluble ash (ADIA), the inorganic residue of ADF, has not been reviewed as a dietary marker for bamboo, though it may be another suitable option for digestibility studies with giant pandas, as it is relatively simple to quantify and has been used successfully in ruminant studies (Bodine et al. 2002; Kanani et al. 2014).

Digestibility trials observing bamboo utilization by the giant panda are complicated by dissimilar subjects, variations in experimental design, as well as general characteristics of the species' foraging behavior (summarized in Table 2.1). For instance, levels of supplementation in observed diets range from unsupplemented to 77% of intake comprised of non-bamboo feeds (on a fresh basis [Kametaka et al. 1988; Schaller et al. 1985]). Additionally, giant pandas are known to exhibit changes in foraging behavior at different times of the year, relating to both plant part selection and volume of total bamboo intake (Long et al. 2004; Mainka and Zhang 2004; Schaller et al. 1985; Hansen et al. 2010). In general, giant pandas prefer bamboo culm in the late winter and spring, shoots when available in the late spring to early summer, and leaves in late summer through winter (Hansen et al. 2007; Long et al. 2004; Schaller et al. 1985; Williams et al. 2012). Reported bamboo DMI in giant pandas ranges from 2.5% BW/day to 7.7% BW/day, and foraging activity has been observed to decrease in the late summer (July – August) in both captive and wild animals (Table 2.1 [Dierenfeld et al. 1982; Liu et al. 2015; Mainka and Zhang 2004; Schaller et al. 1985; Sims et al. 2007; Senshu et al. 2007]).

Despite inherent complexities that accompany research with this species, the situation of captive giant pandas provides a unique opportunity to carry out nutritional studies. Furthermore, nutritional observations of captive individuals undoubtedly benefit their free-ranging counterparts by providing insights regarding bamboo utilization and thus, habitat requirements (Swaisgood et al. 2011). We conducted an investigation to evaluate methods of daily dry matter intake (DMI), daily fecal output (DFO), and dry matter digestibility (DMD) determination in captive giant pandas consuming a bamboo-based diet (> 95%). Five feeding trials were timed throughout the year to capture day-to-day and seasonal variations in bamboo intake and digestibility.

Table 2.1— Summary of giant panda intake and dry matter digestibility (DMD) trials in available literature. Trials were characterized by giant panda diet composition, where a bamboo plant part (“culm”, “leaf”, “shoot”) was considered predominant if it represented greater than 65% of dry matter intake (DMI). A “mixed” diet had multiple plant parts being consumed at 40% - 60% of DMI, and “supplement” represented diets where greater than 30% of DMI were non-bamboo items. Trials were also classified into seasons, with “winter” representing December - February, “early spring” representing March - April, “late spring” representing May - June, “summer” representing July - September, and “fall” representing October - November.

Source	Predominant diet component	Season	Sampling duration (days)	Captive/Wild	DMI (% BW/day)	DMD (%)	Determination of DFO	Notes
Dierenfeld et al. 1982	Leaf	not specified	7	Captive	4.2%	19.6%	PEG and total fecal collection	DMD values were averaged between two subjects with three observations per subject
Dierenfeld et al. 1982	Supplement	not specified	7	Captive	1.6%	19.6%	PEG and total fecal collection	DMD values were averaged between two subjects with three observations per subject
Finley et al. 2011; Sims et al. 2007	Leaf	Summer	1	Captive	2.7%	26.7%	Total fecal collection, AIA, ADL, ADIN	DMD values were averaged across trials by subject
Finley et al. 2011; Sims et al. 2007	Leaf	Fall	1	Captive	3.2%	26.7%	Total fecal collection, AIA, ADL, ADIN	DMD values were averaged across trials by subject
Finley et al. 2011; Sims et al. 2007	Mix	Winter	2	Captive	3.9%	26.7%	Total fecal collection, AIA, ADL, ADIN	DMD values were averaged across trials by subject
Finley et al. 2011; Sims et al. 2007	Leaf	Fall	1	Captive	7.1%	11.5%	Total fecal collection, AIA, ADL, ADIN	DMD values were averaged across trials by subject
Finley et al. 2011; Sims et al. 2007	Leaf	Winter	3	Captive	7.7%	11.5%	Total fecal collection, AIA, ADL, ADIN	DMD values were averaged across trials by subject
Kametaka et al. 1988	Supplement	Winter	10	Captive	1.2%	57.5%	Total fecal collection	Of bamboo, only leaves and branches were offered
Kametaka et al. 1988	Supplement	Winter	10	Captive	2.6%	62.2%	Total fecal collection	Of bamboo, only leaves and branches were offered
Liu et al. 2015	Shoot	Late spring	7	Captive	2.8%	60.7%	Total fecal collection	DMD is for total, mixed diet
Liu et al. 2015	Shoot	Late spring	7	Captive	2.4%	61.0%	Total fecal collection	DMD is for total, mixed diet
Liu et al. 2015	Shoot	Late spring	7	Captive	2.5%	53.0%	Total fecal collection	DMD is for total, mixed diet
Mainka et al. 1989	Supplement	Early spring	5	Captive	1.8%	15.2%	Total fecal collection	DMI and DMD are for bamboo and sugar cane only, other feed items were accounted for
Mainka et al. 1989	Supplement	Early spring	5	Captive	2.6%	19.0%	Total fecal collection	DMI and DMD are for bamboo and sugar cane only, other feed items were accounted for
Senshu et al. 2007	Leaf	Fall	7	Captive	2.5%	24.7%	Chromium dioxide	Bamboo leaf DMD calculated separately from other feed items
Senshu et al. 2007	Leaf	Fall	3	Captive	4.3%	15.8%	AIA	Bamboo leaf DMD calculated separately from other feed items
Long et al. 2004	Leaf	Early spring	not specified	Wild	not specified	17.2%	ADL	DMI estimated by rate of bamboo consumption multiplied by observed time spent eating
Long et al. 2004	Culm	Early spring	not specified	Wild	not specified	15.9%	ADL	DMI estimated by rate of bamboo consumption multiplied by observed time spent eating
Long et al. 2004	Culm	Summer	not specified	Wild	not specified	12.7%	ADL	DMI estimated by rate of bamboo consumption multiplied by observed time spent eating
Schaller et al. 1985	Culm	Late spring	not specified	Wild	not specified	12.5%	Fecal collection	Assumed 12.5 kg/d fresh bamboo intake
Schaller et al. 1985	Leaf	Fall	not specified	Wild	not specified	23.3%	Fecal collection	Assumed 12.5 kg/d fresh bamboo intake
Schaller et al. 1985	Culm	Winter	not specified	Wild	not specified	18.7%	Fecal collection	Assumed 12.5 kg/d fresh bamboo intake
Schaller et al. 1985	Shoot	Late spring	not specified	Wild	not specified	40.0%	Fecal collection	Assumed 12.5 kg/d fresh bamboo intake

Materials and methods

Feeding trials

Five feeding trials were completed with two captive giant pandas housed at the Memphis Zoo (Memphis, TN). Two trials were timed to correspond with the period of maximum culm consumption by the giant pandas (January 3-5, 2015; March 23-25, 2015), two trials with leaf consumption (July 21-23, 2014; October 27-30, 2015) and one trial with bamboo shoot consumption (May 21-23, 2015), with predicted plant part selection based on previous foraging data (Hansen et al. 2010; Williams et al. 2012). During the trials, a male (466, aged 16) and female (507, aged 14), were housed in separate indoor, air-conditioned habitats during the day and moved to a separate enclosure overnight. Access to an outdoor exhibit was offered in cooler weather.

Feeding trials were designed to be minimally invasive and not alter the giant pandas' regular diets and daily routines. Consequently, bamboo feeding frequency and sample collections were contingent on the zookeepers' schedules. Bamboo was harvested locally prior to feeding (Shelby County, TN), bundled by species, and stored at 16° C under misters until fed. Bamboo was available *ad libitum* at all times, and fresh bamboo was provided several times per day. All trials except for the one in October occurred over the course of three days, with sample collection lasting approximately 48 hours, or approximately 4× the maximum passage rate of the giant panda (Dierenfeld 1997). Fecal and ort sample collection began approximately 12 hours after the first diet sampling and ended 12 hours after the final diet sampling to ensure ort and feces corresponded to diet sampled. The October trial included an additional day, resulting in approximately 72 hours of sampling. At the start of the October trial, 30 corn kernels were fed to the male bear to mark the first sampled diet and to determine GIT transit time, and ort and feces were sampled following excretion of the kernels. Uncooked corn kernels were fed to the male bear again at the last diet sampling, and ort and feces collection ended when kernels were defecated. An additional GIT passage trial using corn kernels was carried out separately at a later time (December 2015) with the female giant panda.

Fresh bamboo samples (approximately 2 kg) from bamboo bundles were randomly drawn and weighed by zookeepers, and the remaining bamboo was fed to the giant pandas. Rejected bamboo culms, leaves, branches, and the culm coverings (fragments of the culm exterior layer peeled away by the giant panda), were collected throughout the day when the animals' enclosures were cleaned. After removal from the animal enclosure, total rejected bamboo was weighed, and culm exterior fragments were sorted and weighed separately from whole bamboo. Approximately 2 kg of the whole bamboo portion and 10% of the culm coverings were randomly sampled. Bamboo offered and rejected samples were separated by hand into culm, culm covering (for orts), leaf, and branch fraction to estimate plant part proportions of the bamboo offered and rejected. All feces were also removed from the enclosure during cleaning, and subsequently weighed, hand-mixed, and a sample (10%) of feces was immediately frozen until the end of the trial. At that time, all fecal samples collected respective to each giant panda were thawed and composited to represent fecal output from that individual over the course of the trial. Bamboo plant part and fecal samples were dried in a forced-air oven at 60° C until a reaching a constant partial dry matter (PDM) weight (leaves, branches, culm coverings: 24 hours; culm: 7 days; feces: 72 hours). Offered and rejected bamboo samples were composited by plant part, so that there were three samples corresponding to a species of offered bamboo (culm, leaf, branch) and four samples corresponding to the rejected bamboo of each animal (culm, leaf, branch, culm cover) within a trial. Bamboo and fecal samples were further homogenized through a Wiley Mill (Model 4) to pass a 1 mm screen, and dried at 100º C for 24 hours to determine laboratory DM. Species' whole bamboo (prior to disassembly) DM coefficient was determined for each trial, and calculated with plant part DM values weighted proportionally to the plant part composition of the respective species at that time. Feces DM coefficient was the product of PDM and laboratory DM.

Total bamboo offered, rejected, and total fecal output during the trial were calculated for each animal on a DM basis. Total bamboo DM consumed was the difference between bamboo offered and bamboo rejected. Daily dry matter intake (DMI) was the total bamboo consumed (kg DM) divided by the length of the trial (days). Likewise, total fecal output (kg DM) was divided by the length of the trial to determine daily fecal output (DFO).

Daytime activity budget

During the trial period, daily giant panda activity and bamboo consumption was performed by video surveillance. Daytime behavioral data was collected according to an ethogram described in detail by Hansen et al. (2010). In brief, giant panda daytime activities were reported every 30 minutes over 12 hours (6:00 AM – 6:00 PM), so that a total of 24 observations per day were recorded. For the present study, the percentage of daytime spent eating was based on behavioral observations spanning 17 days, with the observation period beginning one week before the start of the feeding trial, and ending a week after the trial's conclusion.

Daily husbandry records

Zookeepers maintained daily husbandry reports on each giant panda, which includes dietary records of the species and quantity of fresh bamboo offered and rejected by each animal, during the respective day. For each feeding trial, DMI was estimated using these records over 17 days, with the observation period beginning one week before the start of the trial and ending a week after the trial's conclusion, allowing three days to represent the trial period. Daily DMI values were estimated from the daily husbandry reports of bamboo offered and rejected, which were converted to DM basis using coefficients for bamboo species and orts determined during the corresponding feeding trial. Days when giant pandas were offered bamboo species that were not sampled during the corresponding short-term feeding trial were excluded, as we did not have a DM coefficient to represent that species. Daily fecal output (kg) was also recorded by the zookeepers, and multiplied by the corresponding feeding trial fecal DM coefficient to estimate fecal output on a DM basis over the same 17 days. For each giant panda and feeding trial, 17-d mean and 17-d median DMI and DFO values were estimated using data from this observation period.

Calculation of dry matter digestibility

Three estimates of dry matter digestibility were calculated based on DMI and DFO values determined during the short-term feeding trials, and the 17-d mean and 17-d median DMI and DMO values from 17-day husbandry records for each giant panda. Percent dry matter digestibility (DMD) was calculated as:

$$DMD\ (\%) = 100 \times \frac{DMI - DFO}{DMI}$$

Evaluation of internal dietary markers: ADL, ADIA, and AIA

Offered bamboo (classified by species and plant part), rejected bamboo (classified by animal and plant part), and fecal samples were analyzed for three internal dietary markers: ADL, ADIA, and AIA. Acid detergent lignin (ADL) was determined by Van Soest's method (1963) modified according to the recommendations of Ankom, where ADF was performed with an Ankom Fiber Analyzer and followed by a three-hour digestion in sulfuric acid using a Daisy II Incubator (rotation only; Ankom Technology Corp., Macedon, NY). Acid detergent insoluble ash (ADIA) was the inorganic content remaining after ADF residue was combusted at 450º for six hours. Acid insoluble ash (AIA) was determined according to Van Keulen and Young (1977) acid digestion of subsample ash, followed by vacuum filtration to isolate insoluble residue (DigiPrep SCP Science, D'Urfé, Quebec). Plant part proportions of bamboo samples collected by species represented the total amount of the same species offered, and total dietary marker offered (kg/day) was the sum of the marker (kg) contributed by each plant part, specific to species, offered.

$$\begin{aligned} &Total\ marker\ offered\ (kg) \\ &\quad = \big\{[marker]_{leaf} \times leaf\ offered\ (kg\ DM) + [marker]_{culm} \times culm\ offered\ (kg\ DM) \\ &\qquad\qquad + [marker]_{branch} \times branch\ offered\ (kg\ DM)\big\}_{sp.A} + \{\cdots\}_{sp.B} + \{\cdots\}_{sp.C} + \{\cdots\}_{sp.D} \end{aligned}$$

Total dietary marker (kg) in rejected bamboo was calculated similarly, with plant part proportions of rejected bamboo samples representing the quantity of plant parts rejected.

$$Total\ marker\ rejected\ (kg) = \{[marker]_{leaf} \times leaf\ rejected\ (kg\ DM) + [marker]_{culm} \times culm\ rejected\ (kg\ DM) + [marker]_{branch} \times branch\ rejected\ (kg\ DM) + [marker]_{culm\ cover} \times culm\ cover\ rejected\ (kg\ DM)\}_{sp.A} + \{\cdots\}_{sp.B} + \{\cdots\}_{sp.C} + \{\cdots\}_{sp.D}$$

Consumption of the marker by the giant pandas was determined as the difference of the quantity of the marker in offered and rejected bamboos, and the concentration of the marker in the diet consumed was calculated in respect to total bamboo daily intake, as estimated by 17-d mean DMI.

$$\%marker\ in\ diet = \frac{marker\ offered\ (kg) - maker\ rejected\ (kg)}{Total\ bamboo\ DMI\ (kg\ DM)}$$

Daily DMI was calculated using each dietary marker as:

$$DMI\ (kg) = \frac{fecal\ output\ (kg) \times \%marker\ in\ feces}{\%marker\ in\ diet}$$

Dry matter digestibility was estimated by the previously described formula, with DMI represented by dietary marker calculation and 17-d mean DFO.

Statistics and data evaluation

Statistical analyses were performed with SAS CORR and REG procedures to model linear and quadratic relationships between daytime eating behavior and each of the trial, 17-d mean, and 17-d median DMI values (SAS 9.3, SAS Institute, Cary, NC). Giant panda feeding trial data collected during the July trial was excluded from statistical analyses due to abnormal feeding behaviors exhibited by the female giant panda, which involved increased time spent resting and reduced bamboo intake. A separate endocrine analysis indicated substantial changes in the female giant panda's hormone concentrations during the time of the feeding trial, which likely caused decreased foraging behavior and bamboo intake (Beth Roberts [Memphis Zoological Society], personal communication [2014]).

Results

Bamboo dry matter intake (DMI) and daily fecal output (DFO), and daytime activity budget

Across all trials, estimated trial DMI ranged from approximately 2.1 – 8.1 kg DM of bamboo per day (Figure 2.1). Trial DMI did not remain consistent among trials; rather both giant pandas exhibited alternating decreasing and increasing intake throughout the year. Trends in trial DFO reflected those observed in trial DMI, and ranged from approximately 1.5 - 5 kg DM (2.4% - 9.9% BW; Figure 2.1). Trial DMI and trial DFO respective to the individual giant panda during each trial is presented in Table 2.3. Gut passage rate during the fall of 2015 was 6 hours for the male giant panda, though it ranged from 5 to 6 hours in the female.

Based on zookeeper husbandry records, day-to-day DMI ranged widely during the 17-day observation periods for both giant pandas. Dry matter intake and DFO ranges and standard deviations (*SD*) by individual animal and observation period are reported in Table 2.2, and daily variance in DMI and DFO is illustrated by daily husbandry data in Figure 2.1. Greatest variability occurred during March and May, with ranges in 17-dat DMI exceeding 23 kg for the male (*SD* = 7.5 and 6.3 kg/day for March and May, respectively) and 12 kg for the female (*SD* = 3.9 kg/day for both trials; Table 2.2; Figure 2.1). For all other trials, ranges in day-to-day DMI was less than 8 kg for both animals (excluding outliers; Figure 2.2). When comparing 17-d mean DMI of both animals during all trials, bamboo intake ranged from 0.8 - 7.4 kg DM (0.8% - 8.3% BW). Similarly, 17-d median DMI ranged from 0.8– 8.6 kg DM (0.8% - 9.6% BW). As was observed with trial DMI, 17-d mean and 17-d median DMI varied among trials, and direction of change (increase vs. decrease) was consistent between the three calculation methods. Despite this variability of intake values, DFO was more precise within each 17-day period ($SD \leq .82$ for both animals and all trials; Figure 2.1), as well as among the three calculation methods (Table 2.3). Changes in DFO between trials roughly reflected the trends of 17-d mean and 17-d median DMI (Figure 2.1). All 17-d mean and 17-d median DMI and DFO values for both giant pandas during each observation period are presented in Table 2.4.

The male giant panda spent approximately 45% of daytime activity engaging in bamboo eating behaviors during all trials, except July, when eating behavior was observed at 28.4% (Figure 2.2.a). Daytime eating behavior by the female dropped similarly from the May to the July trial; however, in January her bamboo consumption behaviors further decreased to 20% of total daytime activity budget (Figure 2.2.b). For both animals, trial DMI and 17-d mean DMI(kg/day) related linearly to the daytime bamboo consumption behavior, while 17-d median DMI data did not ($P = 0.03$ and 0.02 vs. $P = 0.20$ for trial DMI and 17-d mean DMI vs. 17-d median DMI, respectively). On the other hand, 17-d mean DMI exhibited a strong quadratic relationship with daytime bamboo consumption behavior ($P < 0.01$; $R^2 = 0.89$; Figure 2.3).

Table 2.2— Minimum, maximum, and standard deviation (*SD*) values for daily dry matter intake (DMI) and daily fecal output (DFO) by two giant pandas at the Memphis Zoo. Observations recorded over five 17-day periods, according to daily zookeeper husbandry reports.

Trial	Animal ID	DMI (kg/day)			DFO (kg/day)		
		Min	Max	*SD*	Min	Max	*SD*
January	466	0.58	9.73	2.20	3.77	5.22	0.36
	507	-2.46	9.85	2.41	0.12	2.51	0.51
March	466	-3.35	20.47	7.58	3.25	6.23	0.62
	507	0.83	13.80	3.92	2.48	4.29	0.47
May	466	-1.97	24.05	6.30	2.98	5.77	0.67
	507	0.06	12.32	3.94	1.82	4.02	0.48
July	466	0.20	5.28	1.44	0.40	3.43	0.82
	507	-2.87	10.02	2.78	1.36	3.00	0.55
October	466	1.80	9.71	2.32	2.79	4.47	0.48
	507	3.35	9.78	1.75	3.24	4.86	0.45

Figure 2.1— Daily dry matter intake (DMI) and daily fecal output (DFO) for giant pandas at the Memphis Zoo during five months. Individual animal observations for the male (466; 2.1.a) and female (507; 2.1.b) giant pandas (*Ailuropoda melanoleuca*) at the Memphis Zoo were recorded in January, March, May, July, and October. Samples of offered bamboo, rejected bamboo, and feces were collected during short-term feeding trials corresponding to each time period. Dry matter intake and DFO values were estimated from daily zookeeper husbandry reports over 17 days, with the observation period beginning one week before the start of the feeding trial and ending a week after the trial’s conclusion, allowing three days for the trial.

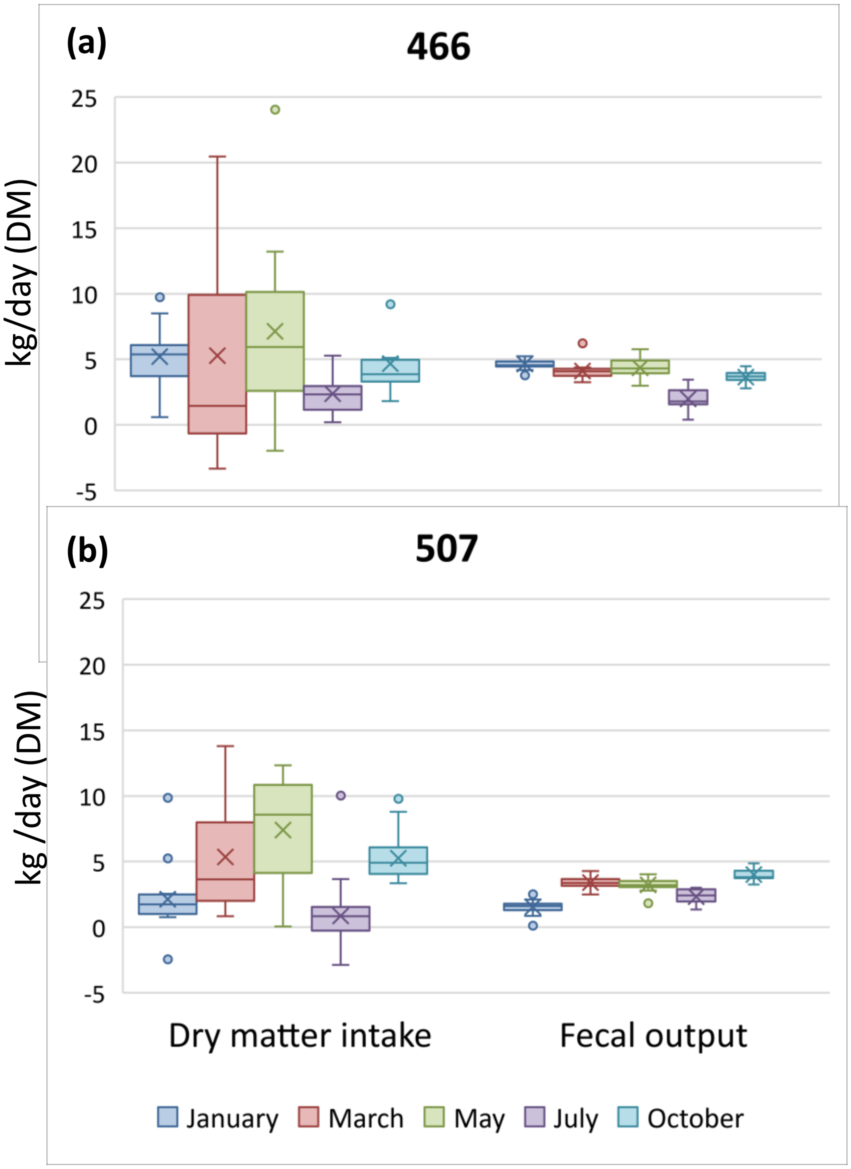


Figure 2.2— Daytime feeding behavior by giant pandas at the Memphis Zoo during five feeding trials. Feeding behavior was reported as time spent eating of total daytime activity budget, for the male (466; 2.2.a) and female (507; 2.2.b) giant pandas (*Ailuropoda melanoleuca*) at the Memphis Zoo during January, March, May, July, and October. Data corresponding to each trial was observed over 17 days, with the observation period beginning one week before the start of a short-term feeding trial and ending a week after that trial's conclusion, allowing three days for the trial.

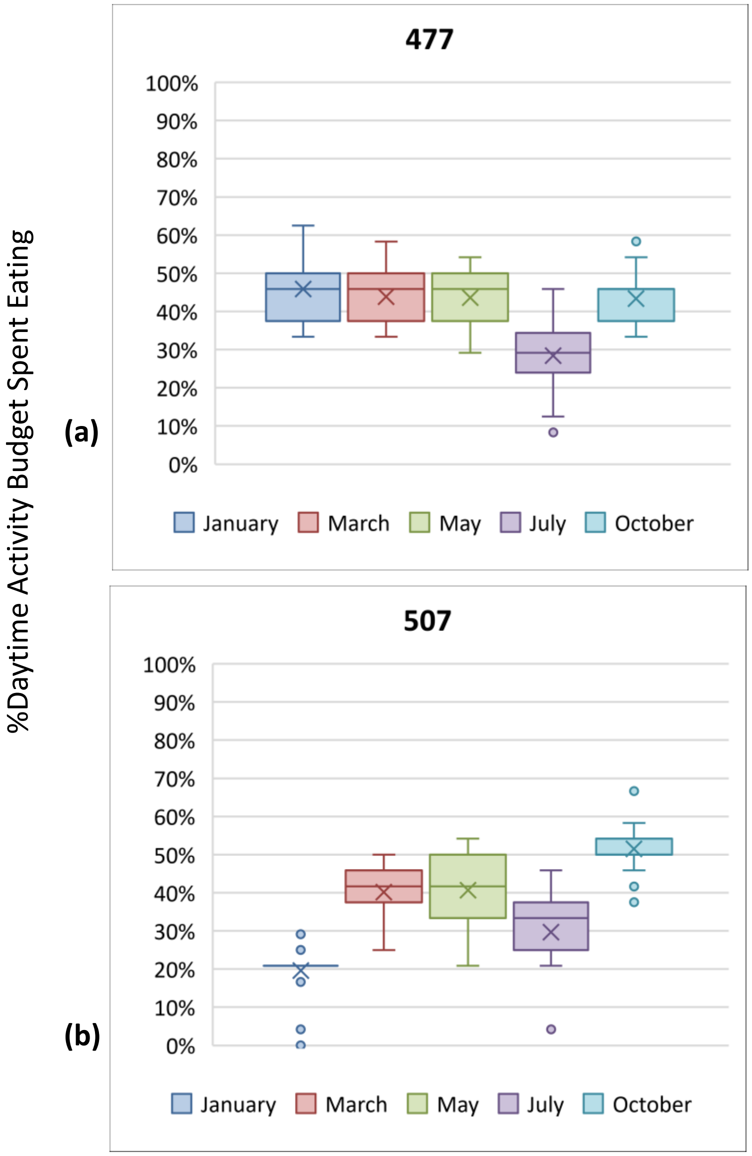


Table 2.3— Daily intake, fecal output, and DMD values estimated by trial, mean, and median calculation methods. Individual body weight (BW), fresh intake, dry matter intake (DMI), daily fecal output (DFO), and dry matter digestibility (DMD) for the male (466) and female (507) giant pandas (*Ailuropoda melanoleuca*) at the Memphis Zoo were observed during five observation periods (January, March, May, July, October). "Trial" values refer to data collected during a short-term feeding trial, while "17-d mean" and "17-d median" values are sourced from zookeeper husbandry reports over 17 days, with the observation period beginning one week before the start of the corresponding short-term feeding trial and ending a week after that trial's conclusion, allowing three days for the trial.

Animal	**Trial**	**Animal BW (kg)**	**Fresh bamboo intake (kg/day)**			**DMI (kg DM/day)**			**Fecal output (kg DM/day)**			**DMD**		
			Trial	17-d Mean	17-d Median	Trial	17-d Mean	17-d Median	Trial	17-d Mean	17-d Median	Trial	17-d Mean	17-d Median
Male (466)	January	104.3	17.57	16.87	16.98	5.36	5.65	5.19	4.53	4.64	4.55	21.21%	10.68%	15.25%
	March	109.6	14.19	28.18	23.55	2.58	5.27	1.45	4.16	4.10	4.08	-61.07%	22.25%	-182.13%
	May	112.7	27.83	32.29	30.62	5.56	7.14	5.93	4.92	4.35	4.30	11.52%	39.03%	27.46%
	July	107.6	11.83	10.87	10.24	2.82	2.38	2.33	1.59	1.97	1.78	43.66%	17.12%	23.74%
	October	99.4	23.46	15.63	13.82	7.85	4.67	3.87	3.73	3.64	3.70	52.56%	21.98%	4.26%
Female (507)	January	84.0	7.08	8.63	8.10	2.06	2.13	1.75	1.37	1.54	1.61	33.18%	27.74%	7.55%
	March	86.1	21.74	20.53	18.90	6.68	5.35	3.65	3.65	3.39	3.38	45.39%	36.75%	7.37%
	May	89.7	19.33	22.36	24.43	5.46	7.41	8.57	3.19	3.23	3.20	41.63%	56.40%	62.67%
	July	104.0	11.50	11.44	10.13	2.58	0.85	0.84	2.54	2.32	2.41	1.33%	-174.02%	-186.13%
	October	81.3	24.42	16.71	15.04	8.06	5.25	4.91	3.79	4.00	3.82	52.94%	23.86%	22.13%

Dry matter digestibility (DMD) of bamboo: trial vs. 17-d mean vs. 17-d median data

Subtle differences in DMI and DFO can dramatically affect DMD, and as such, bamboo DMD varied across the different calculation method, between animals, and between trials (Table 2.3). Dry matter digestibility ranged widely within each calculation method (Trial: -61.1% to 52.9%; 17-d mean: -174.0% to 56.4%; 17-d median: -186.1% to 62.8%), and data from the female giant panda displayed more extreme values (Table 2.3). Within DMD values relating to the individual animal, 17-d median intake and fecal output data estimated the largest range of digestibility values for both giant pandas. Between both animals, all three calculation methods yielded at least one negative DMD value. For the male giant panda, trial and 17-d median DMD values were negative during March, while the 17-d mean and 17-d median DMD values were negative for the female during the July trial.

Evaluation of internal dietary markers: ADL, ADIA, and AIA

Acid detergent lignin, ADIA, and AIA concentrations in the diet and feces failed to predict realistic values for DMI and subsequent DMD (Table 2.4). Although, ADL was present in relatively considerable amounts in all plant parts consumed (13% - 18% for culm; 7% -12% for leaves), except for shoots (less than 2% of DM), it either underestimated, overestimated, or produced negative DMI values (relative to 17-d mean DMI; Table 2.4). Lignin content of consumed bamboo ranged from approximately 5% - 35% across both individuals and all trials, however ADL disappearance was observed in most trials. Acid detergent insoluble ash (ADIA) and AIA tended to underestimate DMI or produce negative DMI values, which resulted in negative DMD values, or DMD values which exceeded 100% (Table 2.4). Throughout all trials, ADIA and AIA were present at very low (2% or less) concentrations in the culm of offered and rejected bamboos, while ADIA and AIA contents in offered and rejected leaves were more substantial (ADIA: 2.0% - 7.7%; AIA: 4.8% - 10.8%). However, leaves comprised a minor proportion of total bamboo dry matter, and consequently ADIA and AIA concentrations of the diet offered was less than 5% for both bears during most trials. Acid insoluble ash content in bamboo consumed during the October trial is slightly higher than in other trials (5.5% and 7% for the male and female, respectively), which likely reflects an increase in leaf consumption in the diet. Both ADIA and AIA content in feces were less than 2.2% DM across all trials. These low concentrations of ADIA and AIA in diet and feces likely introduced error when quantifying the total amount of these markers consumed and recovered, which in turn precluded us from accurately predicting intake and digestibility.

Figure 2.3— Correlations between 17-d mean dry matter intake (DMI) and daytime feeding behavior for two giant pandas at the Memphis Zoo. Correlation between 17-d mean bamboo DMI and daytime feeding behavior were reported as time spent eating of total daytime activity budget, for two giant pandas over five feeding trials. Nine observations (five from the male and four from the female) were used to determine a quadratic model between each of the bamboo DMI estimates and daytime feeding behavior.

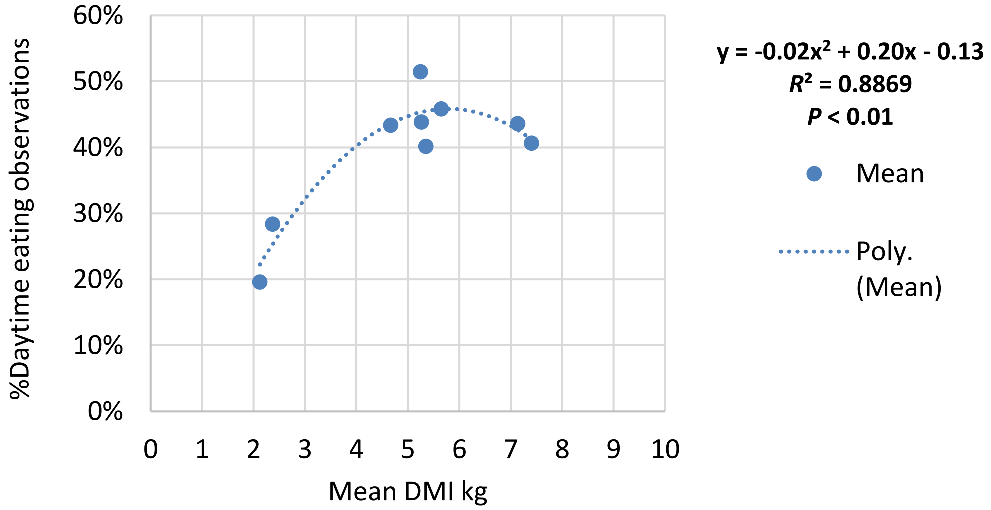


Table 2.4— Daily marker intake**,** Dry matter intake (DMI) and dry matter digestibility (DMD) estimated by three internal dietary markers (ADL, ADIA, and AIA). Dry matter intake and DMD are compared to the observed (mean) DMI and DMD for two giant pandas (*Ailuropoda melanoleuca)* at the Memphis Zoo, over five trials (January, March, May, July, October). Mean DMI and DMD were determined from daily DMI and DFO observations over a 17-day period corresponding to the respective trial.

Animal	**Trial**	**Marker Intake, kg/day**			**DMI, kg/day**				**DMD, %**			
		ADL	ADIA	AIA	Mean	ADL	ADIA	AIA	Mean	ADL	ADIA	AIA
Male (466)	January	1.85	-0.16	0.13	5.36	1.83	-1.30	2.71	10.68%	-148.15%	449.09%	-67.26%
	March	0.38	-0.33	0.76	3.08	7.11	-0.41	0.12	22.25%	41.51%	1125.99%	-3240.44%
	May	0.72	-0.26	0.01	5.56	4.43	-1.56	12.92	39.03%	-11.23%	415.94%	61.90%
	July	0.06	0.01	-0.19	2.82	7.45	4.48	-0.27	17.12%	78.65%	64.48%	681.32%
	October	0.57	-0.28	0.25	7.85	3.25	-0.54	0.27	21.98%	-14.68%	796.06%	-1278.68%
Female (507)	January	0.62	-0.04	0.07	2.13	0.75	-0.96	0.94	27.74%	-104.50%	259.75%	-62.83%
	March	0.64	-0.19	0.01	5.35	3.30	-0.94	25.54	36.75%	-2.46%	459.08%	86.75%
	May	1.17	-0.24	-0.04	7.41	1.95	-0.70	-2.32	56.40%	-65.54%	560.05%	239.40%
	July	-0.20	-0.01	-0.04	0.85	-1.29	-0.96	-0.13	-174.02%	279.45%	342.64%	1910.28%
	October	0.48	-0.19	0.37	5.25	4.40	-2.14	1.23	23.86%	9.22%	286.94%	-224.75%

Discussion

Giant pandas exhibit wide variation in day-to-day bamboo DMI, while DFO is relatively consistent. Some variation in daily intake is to be expected by this species, as they are known to spend large bouts of time consuming bamboo en-masse, followed by a long period of rest (Schaller et al. 1985). These alternating, extended stretches of eating and resting may not follow a distinct 24-hour period; thus, daily intake calculations are constrained by the beginning and end points of the 24 hours under observation. Nevertheless, some DMI values from daily husbandry records were unrealistic (e.g. negative values or values exceeding 20% of BW), and our challenge was to balance the natural inconsistencies of giant panda feeding behavior with the seemingly improbable intake values observed measuring the offered and rejected bamboo. Unrealistic DMI values may be attributed to the highly variable moisture content of fresh bamboo that was rinsed by a sprinkler system prior to weighing and feeding, as well as general record-keeping inconsistencies among individual zookeepers. Another possible source of day-to-day DMI variation could be attributed to plant part being consumed. Daily DMI was most variable during March, when maximum culm consumption was observed for both animals (100% and 96.7% of DMI for the male and female, respectively). Culm is bulky and dense, compared to leaves, and constituted the majority of bamboo DM offered to both giant pandas. When calculating intake from the large amounts of bamboo offered and refused, a missing or unreported culm fragment could substantially impact the measure of total bamboo consumption. As a rule, we elected that DMI values which resulted in DMD values less than 5%, including negative values, were likely to be inaccurate. Likewise, DMI values that yielded digestibilities greater than 40% were also considered unlikely, with the exception of the May trial during which both giant pandas were consuming highly digestible bamboo shoots (Table 2.1 [Dierenfeld et al. 1982; Long et al. 2004; Senshu et al. 2007; Sims et al. 2007; Schaller et al. 1985]). At this time, we expected DMD to be roughly between 30% - 60%, based on previous reports of bamboo shoot digestibility in giant pandas (Table 2.1 [Liu et al. 2015; Schaller et al. 1985]). When examining our data by individual bear and trial, each calculation method for DMI (trial, 17-d mean, 17-d median) yielded what we considered to be unrealistic DMI values (trial = 6 unrealistic values; mean = 1 unrealistic value; median = 3 unrealistic values). Seventeen-day mean DMI estimations resulted in only one unrealistic DMI value, which corresponded to the observation of unusual foraging behavior and hormone activity in the female giant panda (507) during July.

Additionally, we anticipated variations in DMI and DMD between trials due to differences in diet composition and animal physiology. Because the goal of this study was to determine an accurate representation of DMI and DMD, we did not evaluate the changes in intake observed between trials. Rather, we used those temporal patterns to support reasonable DMI values by comparing them to historical data. Daytime activity budgets recorded with the same giant pandas in previous years revealed a recurring pattern in foraging behavior, where time spent foraging bamboo peaked in winter months (45% - 60% of daytime activity), but was nearly halved during the summer (Barbara Gocinski [Memphis Zoological Society], personal communication [2015]). According to daytime activity budgets taken over the course of our study, both animals continued to exhibit a similar seasonal cadence in foraging behavior, with the exception of the female during January, during which she spent less time foraging than in any other trial (Figure 2.2). Although activity budget data is constrained to daytime observations and rate of fresh bamboo intake, we expected that an accurate DMI value would reflect time spent foraging. Similar to the activity budgets, DMI displayed a decrease in bamboo intake between May and July in the three calculation methods, for both animals. However, trial and 17-d median DMI values were lowest for the male (466) during March; a change in intake not observed in daytime foraging behavior. Of the three DMI calculation methods, 17-d mean DMI values were most likely to predict time spent foraging ($R^2 = 0.89$; $P < 0.01$; Figure 2.3). Stronger linear relationships to foraging activity, using any of the trial, 17-d mean, and 17-d median DMI values, were likely inhibited by the small number of observations, as well as the disparity between fresh bamboo intake and actual DM content of that bamboo consumed. For instance, moisture content is greater in bamboo leaves than in culm, such that for a given quantity of fresh culm consumed, the giant panda must consume a greater amount of fresh leaves to match culm intake on a DM basis. Rate of bamboo intake may also affect the application of time spent foraging as a direct reflection of DMI. An investigation of intake rates of different plant parts in relationship to DMI of those plant parts may prove useful and non-invasive method for monitoring DMI by giant pandas. This could be especially applicable for studying intake habits of giant pandas in the wild, as feeding trials require a controlled environment and labor-intensive sampling protocol. In one report, Long et al. utilized bamboo feeding rates to predict intake in free-ranging giant pandas, however they did not discuss differences in intake rates between plant parts (2004).

Based on the results of the present study and supported by available data from the literature, we concluded that 17-d mean DMI data is the most accurate representation of bamboo intake, and resulting bamboo DMD. Averaging DMI over the 17-day observation period diffused day-to-day variation in bamboo consumption, which could not be accounted for in the feeding trials lasting only three to four days. Additionally, 17-d mean DMI apparently distributed error caused by wide-ranging and extreme daily DMI values to a greater extent than trial and 17-d median DMI of the same observation period. For instance, the March 17-day observation of the male giant panda (466) displayed a considerably broad range in daily bamboo DMI (-3.3 to 20.5 kg/day, no outliers; Table 2.3; Figure 2.1), and only the mean 17-d DMI value provided a realistic estimation of bamboo intake (Table 2.4).

Daily fecal output was remarkably consistent for both giant pandas within each 17-day observation period (Table 2.3; Figure 2.1), suggesting a flexible gastrointestinal passage rate may accommodate for sporadic and excessive consumption of bamboo. Passage of corn kernels by the two giant pandas in our study ranged from 5 – 6 hours, which is consistent with previously reported mean retention times (MRT) in the same animals (4 – 6 hours; Sims et al. 2007). Giant panda MRT values from other studies range from 4 to 13 hours (Dierenfeld 1997; Schaller et al. 1985). Giant pandas consuming bamboo exhibit MRT that are not only rapid compared to herbivores with more complex gastrointestinal physiology (e.g., ruminants and hind-gut fermenters), but are also notably shorter than physiologically similar bear species consuming meat-based diets. Mean retention times in grizzly (*Ursus arctos*) and polar bears (*Ursus maritimus*) have been reported between 13 and 19 hours (Best 1985; Pritchard and Robbins 1989). Although the giant panda shares its simple gastrointestinal physiology with the grizzly and polar bear, its shorter MRT is likely a consequence of a diet concentrated in slow-degrading and non-degradable fiber. In simple-stomached species which lack the capability of foregut fermentation, such as humans and chimpanzees, increased dietary fiber intake has been shown to reduce gut transit time as a response to increased digesta bulk and water absorption (Hillemeier 1995; Milton and

Dimment 1988). While this constrains mechanical and enzymatic digestion, it is believed to be advantageous for bulk consumers, including the giant panda, which require frequent gastrointestinal movement to provide capacity for extensive foraging sessions (Dierenfeld et al. 1982; Pritchard and Robbins 1989). Although we only measured gut transit at one time point, seasonal shifts in plant part selection and overall volume of bamboo consumption likely impact giant panda MRT, as plant parts differ in dietary fiber composition (Christian et al. 2015; Mainka et al. 1989; Schaller et al. 1985).

Although it is probable that the three internal dietary markers observed (ADL, ADIA, and AIA) are highly, if not completely, indigestible, they could not accurately predict DMI and DMD according to giant panda fecal output. The failure of ADL, ADIA, and AIA to provide reasonable estimates of DMI is more likely the result of incorrect measurement and allocation of bamboo species and plant parts within total bamboo offered, as marker concentrations were first determined according to species and plant part. Quantification of ADIA and AIA were further complicated due to minimal concentrations of those constituents in bamboo culm (ADIA or AIA < 2% DMB). Because culm was the predominant plant part consumed, ADIA and AIA concentrations were also minimal in giant panda feces. However, ADIA and AIA may be suitable dietary markers for giant pandas consuming leaf-only diets.

During two of our feeding trials, the female giant panda exhibited considerably low bamboo daily intake levels, relative to other intake values from our study as well has those reported for giant pandas in the literature (DMI < 3% BW). Interestingly, these events of decreased bamboo consumption coincided with abrupt hormonal shifts occurring in the female's reproductive cycle. In general, female giant pandas experience one reproductive cycle per year, which in turn limits breeding opportunities to a single, brief estrous period in the spring. However, there have been few reported instances of a second fall ovulation, if the female failed to conceive or implant earlier in the year (Spady et al. 2007; Beth Roberts [Memphis Zoological Society], personal communication [2016]). Our observations suggest an association between abnormal hormone activity and foraging behavior. This is unsurprising, as diet selection and foraging activity of similar bear species, such as the black and grizzly bears, are intimately associated with their characteristic reproductive cycle seasonality. Further, it is hypothesized that the evolution of reproduction seasonality in bear species was in part driven by diet availability coinciding with seasonal climate patterns (Spady et al. 2007). Further understanding feeding behavior of female giant pandas during estrous, pregnancy, and parturition would undoubtedly have beneficial applications for management and propagation of this threatened species.

Available information regarding giant panda bamboo intake and digestibility is wide-ranging in resultant data, as well as in methodology (Table 2.1). In order to achieve more consistent measurements of DMI and DMD, observation periods must be of a sufficient length (> 3 days) to account for daily variation in DMI, however not so long as to interact with deliberate temporal shifts in foraging behavior exhibited by giant pandas. Visual assessment of bamboo intake behaviors relative to total activity is an insightful tool for giant panda management and research; however, it may not directly relate to the dry quantity of bamboo consumed. In scenarios where precise DMI measurements are essential (for instance, digestibility determination or habitat carrying capacity assessments), a feeding trial of sufficient length would ensure the most accurate representation of daily bamboo intake.

CHAPTER III

ALTERNATIVE METHODS TO EVALUATE PLANT PART SELECTION BY THE GIANT PANDA

Summary

Giant pandas historically thrived on diets primarily comprised of bamboo, a grass characterized by its tall, woody structure and evergreen foliage (Carter 1999; Long et al. 2004; Schaller *et al*. 1985). Earliest reports of giant panda ecology described seasonal transitions in bamboo plant part selection, which have since been confirmed in numerous groups of free-ranging and captive giant pandas. Describing plant part selection by giant pandas is difficult, as differences in water content and bamboo intake rate between bamboo plant parts preclude behavioral observations from accurately predicting actual dry matter consumed. We concluded that sampling of offered and rejected bamboo provided a more realistic assessment of plant part intake by giant pandas. Image analysis of giant panda feces at different levels of leaf intake revealed that feces greenness was strongly correlated to leaf dry matter intake for two giant pandas ($P < 0.01$; $r^2 = 0.91$; n = 9), and has potential field applications as a non-invasive, simple procedure to evaluate giant panda feeding behavior.

Introduction

All plant consumers display dietary preferences driven by desirable and adverse diet characteristics, for example digestibility and palatability versus the presence of indigestible or toxic compounds. Herbivores are presented with a nutritional challenge, as plants contain high levels of structural carbohydrates (dietary fiber), change compositionally over time, and have developed defensive mechanisms to protect against predatory and environmental stressors. Compared to other herbivores, giant pandas are especially disadvantaged, as they possess a digestive system little adapted for utilization of dietary fiber. Foraging strategy is key to giant panda survival, and is the primary adaptation enabling them to fulfill their nutritional requirements. Nutritional studies have suggested that bulk consumption of bamboo can provide sufficient nutrients to meet these dietary needs (Schaller et al. 1985; Long et al. 2004; Hansen et al. 2010). Additionally, it is well-documented that giant pandas exhibit distinctive seasonal dietary shifts relative to the part of bamboo which is selected for consumption (Hansen et al. 2007; Long et al. 2004; Schaller et al. 1985). In general, giant pandas prefer bamboo culm in the late winter and spring, young shoots when available in the late spring to early summer, and leaves in late summer through winter (Hansen et al. 2007; Long et al. 2004; Schaller et al. 1985; Williams et al. 2012). Dietary shifts of captive giant pandas are less defined by availability of bamboo shoots than those of wild populations, as fresh shoots are difficult to provide *ad libitum* in captivity. Nevertheless, the seasonal nature of plant part selection is a key aspect of giant panda nutritional ecology. A greater understanding of giant panda foraging behavior has implications for managing individuals in captivity, as well as for recognizing free-ranging giant panda habitat needs.

Plant part selection by giant pandas has been an ongoing study at the Memphis Zoo since the animals' arrival in 2003, and has been previously described by Hansen et al. (2007) and Williams et al. (2012). Bamboo culm or leaf intake is monitored by daytime visual observation, and distinctive shifts in diet selection are distinguishable based on time spent by the giant panda consuming specific plant parts. However, plant part dry matter intake (DMI) has yet to be related to this visible assessment of foraging behavior. We describe and compare these two direct measurements of plant part intake, as well as introduce a new technique to estimate leaf intake based on feces image analysis.

Materials and methods

Feeding trials

Five feeding trials were completed with two captive giant pandas housed at the Memphis Zoo (Memphis, TN). Two trials were timed to correspond with the period of maximum culm consumption by the giant pandas (January 3-5, 2015; March 23-25, 2015), two trials with leaf consumption (July 21-23, 2014; October 27-30, 2015) and one trial with bamboo shoot consumption (May 21-23, 2015), with predicted plant part selection based on previous foraging data (Hansen et al. 2010; Williams et al. 2012; Barbara Gocinski [Memphis Zoological Society], personal communication [2015]). During the trials, a male (466, aged 16) and female (507, aged 14), were housed in separate indoor, air-conditioned habitats during the day and moved to a separate enclosure overnight. Access to an outdoor exhibit was offered in cooler weather. Bamboo was available *ad libitum* at all times, and new bamboo was provided several times per day. The feeding trials were designed to be minimally invasive and not alter the giant pandas' regular diets and daily routines. Consequently, bamboo feeding frequency and sample collections were contingent on the zookeepers' schedules. Bamboo was harvested locally prior to feeding, bundled by species, and stored at 16° C under misters.

All trials except for the one in October occurred over the course of three days, with sample collection lasting approximately 48 hours, or approximately 4× the maximum passage rate of the giant panda (Dierenfeld 1997). Fecal and ort sample collection began approximately 12 hours after the first diet sampling and ended 12 hours after the final diet sampling to ensure ort and feces corresponded to diet sampled. The October trial included an additional day, resulting in approximately 72 hours of sampling.

Fresh bamboo samples (approximately 2 kg) from bamboo bundles were randomly drawn and weighed by zookeepers, and the remaining bamboo was fed to the giant pandas. Rejected bamboo culms, leaves, branches, and the culm coverings (fragments of the culm exterior layer peeled away by the giant panda), were collected throughout the day when the animals' enclosures were cleaned. After removal from the animal enclosure, total rejected bamboo was weighed, and culm exterior fragments were sorted and weighed separately from whole bamboo. Approximately 2 kg of the whole bamboo portion and 10% of the culm coverings were randomly sampled. Bamboo offered and rejected samples were separated by hand into culm, culm covering (for orts), leaf, and branch fraction to estimate plant part proportions of the bamboo offered and rejected. All feces were also removed from the enclosure during cleaning, and subsequently weighed, hand-mixed, and a sample (10%) of feces was immediately frozen until the end of the trial. At this time, all fecal samples taken from one animal were thawed and composited to represent fecal output from that individual over the course of the trial. Bamboo plant part and fecal samples were dried in a forced-air oven at 60° C until a reaching a constant partial dry matter (PDM) weight (leaves, branches, culm coverings: 24 hours; culm: one week; feces: 72 hours). Offered and rejected bamboo samples were composited by plant part, so that there were three samples corresponding to a species of offered bamboo (culm, leaf, branch) and four samples corresponding to the rejected bamboo of each animal (culm, leaf, branch, culm cover) within a trial. Bamboo and fecal samples were further homogenized through a Wiley Mill (Model 4) to pass a 1 mm screen, and dried at 100º C for 24 hours to determine laboratory DM. Species' whole bamboo (prior to disassembly) DM coefficient was determined for each trial, and calculated with plant part DM values weighted proportionally to the plant part composition of the respective species at that time. Feces DM coefficient was the product of PDM and laboratory DM.

For each trial, proportions of culm, leaves, and branches in offered and rejected bamboo were calculated on a dry matter basis according to species, and used to estimate the quantity (kg DM) of each plant part, specific to bamboo species, offered. Bamboo shoots were measured separately from mature bamboo when offered during May. Similarly, we used dry proportions of culm, leaves, branches, and culm cover in rejected bamboo samples to estimate plant part composition of total reject bamboo. Plant part intake was the difference between total offered and rejected plant parts:

$$\begin{aligned} &\textit{Plant part intake (kg DM)} \\ &\qquad = \left(\textit{plant part}_{\,sp.A} + \textit{plant part}_{\,sp.B} + \textit{plant part}_{\,sp.C} + \textit{plant part}_{\,sp.D}\right) \\ &\qquad - \textit{plant part}_{\,rejected} \end{aligned}$$

We corrected for any negative plant part intake values by assuming that the giant panda did not consume that plant part during the trial, and rejected quantity of that plant part was substituted as the offered quantity so that total plant part intake was zero kg DM.

Giant panda feeding behavior

Daily giant panda activity and bamboo consumption is under continuous video surveillance at the Memphis Zoo, and behavioral data is collected according to an ethogram specific to giant panda foraging behaviors, described in detail in Hansen *et al*., 2010.

Bamboo plant part consumption was recorded throughout the previously described feeding trials, and data was collected in 20-min blocks with observations occurring at 30-second intervals. Consumption of bamboo plant parts were recorded as “culm-eat,” “leaf-eat,” and “other/unknown-eat” for consumption of bamboo plant parts that could not be distinguished due to animal and camera positions (Hansen *et al*. 2010).

Fecal image analysis

Giant panda feces were thawed, composited, and thoroughly mixed according to trial and individual animal. Photographs of each fecal composite were analyzed to determine green pixel content of the image as a potential quantitative indicator of dietary leaf composition. Three photos were taken of each fecal sample, with feces being mixed between photos. Photo analysis for %green pixels was carried out by *ImageJ* image processing software, and the average %green pixels for each sample was recorded (Schneider et al. 2012).

Statistics and data evaluation

Statistical analyses were performed with SAS CORR and REG procedures to model linear relationships between daytime leaf-eating behavior observations, leaf DMI calculated from the feeding trial, %green pixilation in feces, and %AIA in feces (SAS 9.3—SAS Institute Inc.). Giant panda feeding trial data collected during the July trial for the female giant panda was excluded from statistical analyses due to abnormal feeding behaviors, which involved increased time spent resting and reduced bamboo intake.

Results

Feeding trials

Bamboo culm was consumed by both giant pandas during all trials, and was the predominant plant part selected during January and March (culm intake > 80% of total DMI). Culm remained the primary bamboo part consumed by the male during July and October, while the female consumed an approximately 50:50 mixture of culm and leaves during October. Both giant pandas selected for bamboo shoots when they were offered in May, and shoots comprised approximately 50% of their intake on a dry matter basis (55.6% and 47.4% of DMI for male and female, respectively; Figure 3.1.a, 3.1.b). In general, the female giant panda consumed more leaves than the male, with the exception of the May trial, when no leaves were selected by either animal.

Giant panda feeding behavior

Culm-eat was the predominant feeding behavior observed for both animals during all trials (greater than 60%), and was highest during January and March (culm-eat > 90% total DMI for male, > 79% total DMI for female; Figure 3.1.c, 3.1.d). Visual estimates of leaf intake were generally greater than feeding trial leaf intake values, with the exception of October, when leaf-eating behaviors were less than 21% of total feeding behaviors for the female, and less than 4% for the male. Instances of other/unknown-eat behaviors were more common in May than all other trials (approximately 25% of total feeding behaviors), likely reflecting bamboo shoot intake during this time. Additionally, large bamboo shoots may have been indistinguishable from bamboo culm, and categorized with culm-eat behaviors.

Fecal image analysis

Percent of green pixilation in fecal images ranged from 0% to 61%. Figure 3.2 is provided as visual reference for feces appearance at different levels of leaf intake. In general, greenness of feces followed the same trend as feeding trial leaf DMI and observed leaf eat-behavior for both animals, with maximum % green occurring in October, and minimum during March and May. However, feces greenness was more strongly related to leaf intake values estimated through the feeding trial than leaf-eat behavior trends (r^2 = 0.91 vs. r^2 = 0.27 for %leaf DMI and %leaf-eat, respectively; Figure 3.2).

Figure 3.1— Plant part selection by two giant pandas at the Memphis Zoo across five feeding trials as determined by DMI and daytime feeding behavior. Plant part selection by the male (466) and female (507) giant panda (*Ailuropoda melanoleuca*) was reported during January, March, May, July, and October, as determined by feeding trial dry matter intake (DMI; 3.1.a, 3.1.b) and feeding behavior observations over a 17-day period (3.1.c, 3.1.d). Data from the July trial was excluded for 507 due to abnormal feeding behavior occurring at that time.

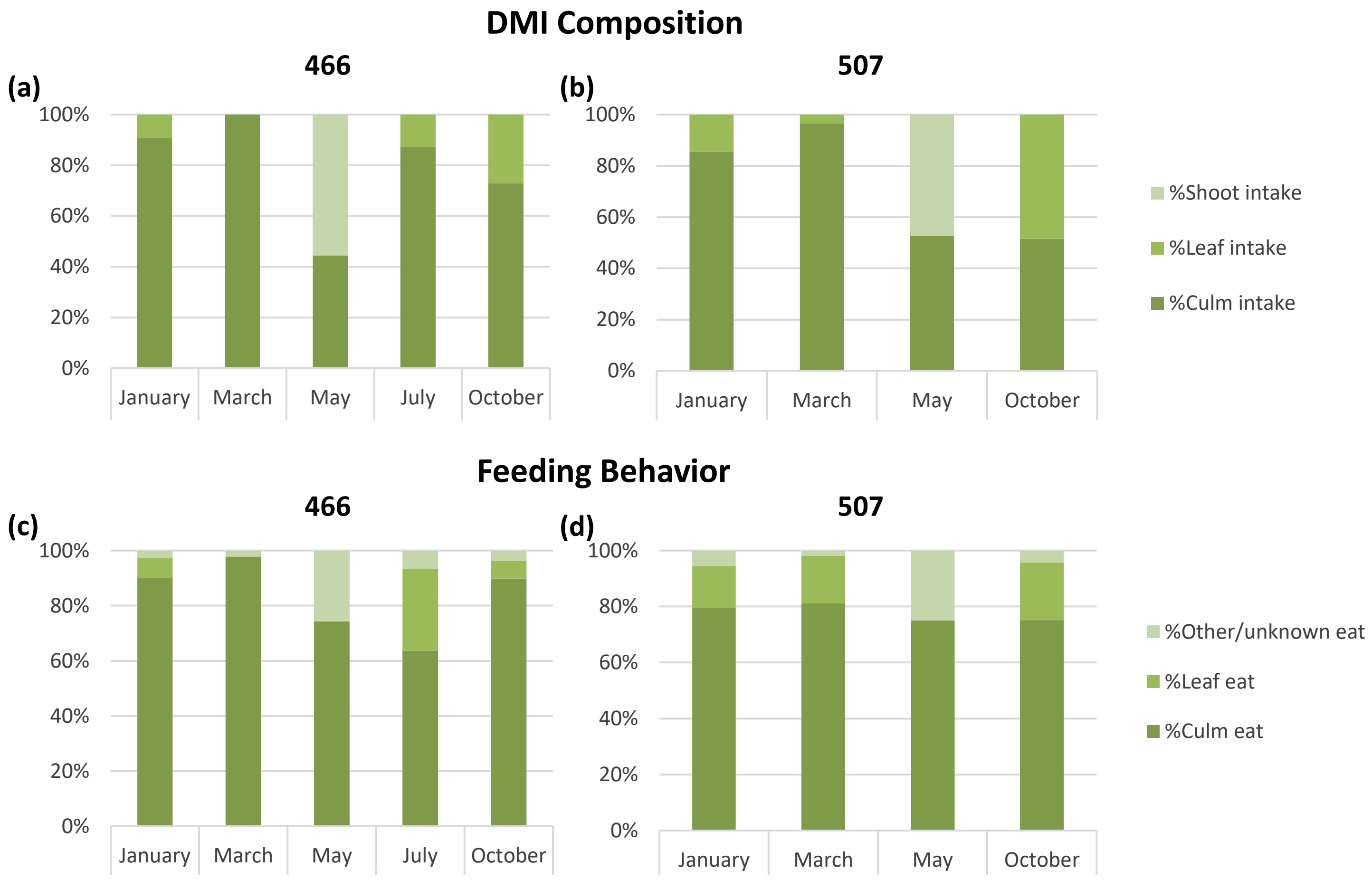


Figure 3.2— Images of composited giant panda feces analyzed for %green pixilation. Leaf composition of diet consumed (% of total DMI) was 0%, 14.6%, and 61.4% for images 3.2.a, 3.2.b, 3.2.c, respectively. Green pixilation was 1.3%, 16.0%, and 61.4% for images 3.2.a, 3.2.b, 3.2.c, respectively.

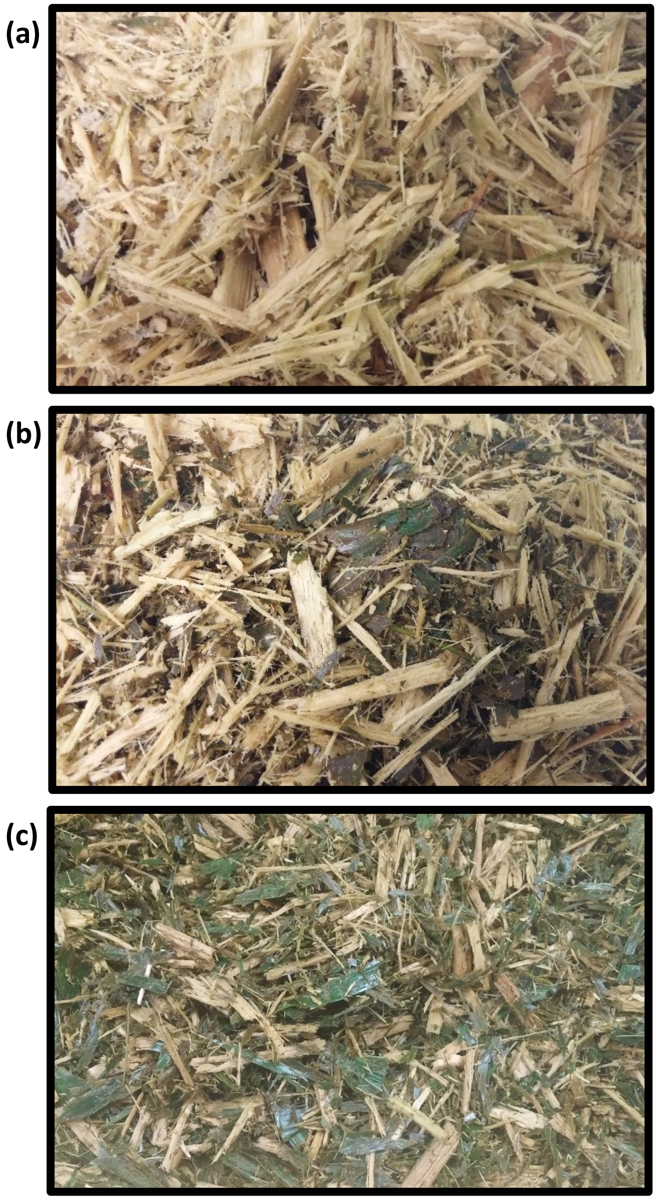
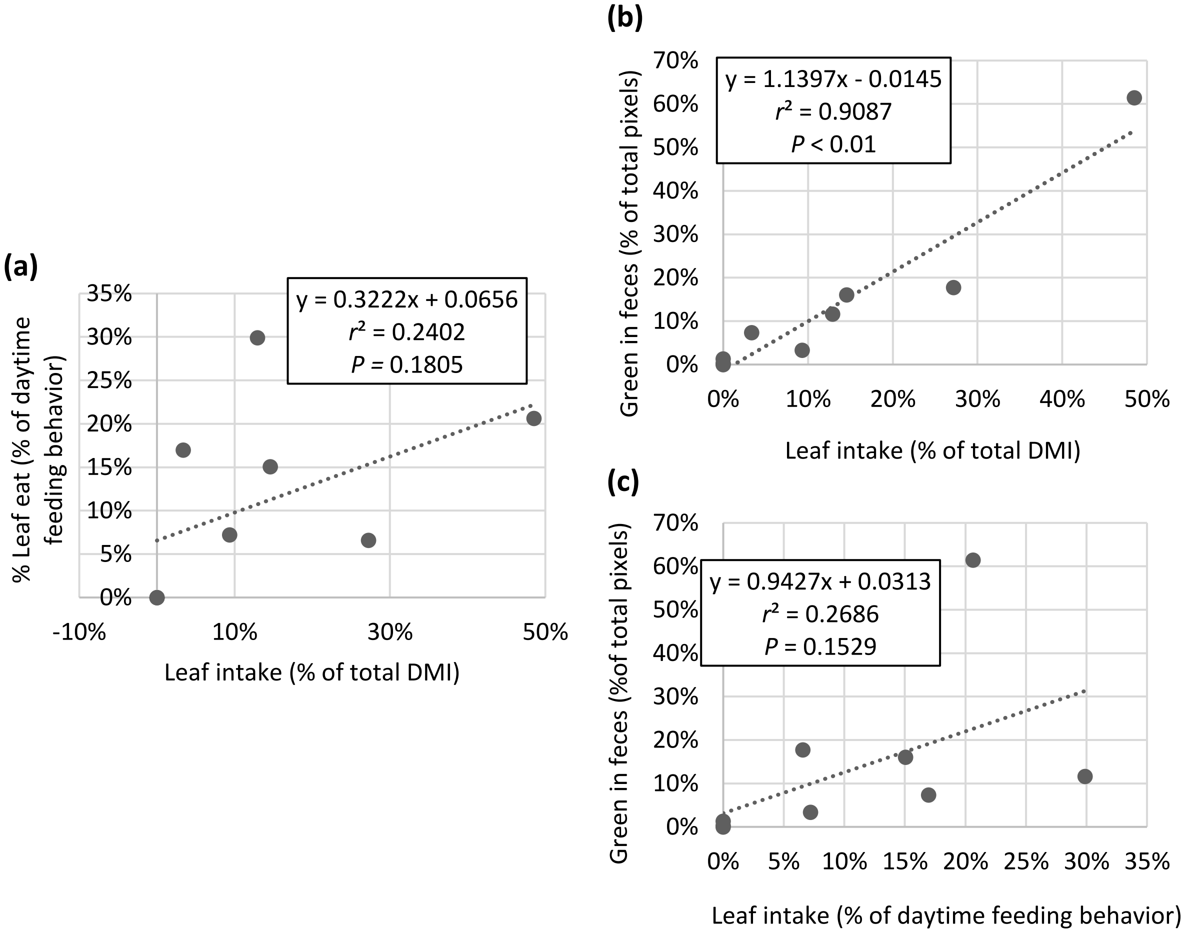


Figure 3.3— Linear relationships between leaf consumption by two giant pandas (*Ailuropoda melanoleuca)* at the Memphis Zoo: leaf intake (as % of total DMI) and leaf-eat behavior (as % of total daytime eating behaviors) observations (3.3.a); leaf consumption (as estimated by leaf intake and leaf-eat behaviors) and %green pixilation in feces (3.3.b, 3.3.c); leaf consumption (as estimated by leaf intake and leaf-eat behaviors) Total observations = 9, as the observation for the female giant panda during July was excluded do to abnormal feeding behavior and hormone activity.



Discussion

Temporal shifting of plant part selection by giant pandas is a key adaptation allowing these physiological carnivores to consume a near-exclusive bamboo diet, as well as being a noteworthy attribute of the species' ecology. Unlike some closely-related *Ursid* species which seasonally change forage or prey types based on food availability and preparation for denning, giant pandas do not hibernate, and bamboo is an available food source year-round. Additionally, studies involving both captive and wild giant pandas report patterns in plant part selection that are often unique to the population of animals at certain locations, suggesting further specificity to the level of diet selection exhibited by giant pandas (Long et al. 2004; Hansen et al. 2007; Schaller et al. 1985). The question remains as to why giant pandas exhibit changes in bamboo plant part preference, and how this relates to the species' unique biology. Similar to other ursids, giant pandas exhibit season-dependent reproductive cycling, generally undergoing a single, brief estrous during the spring and delayed embryo implantation in the late summer (assuming favorable gestation conditions; Spady et al. 2007; Schaller et al. 1985). However, seasonal mono-estrous is unique to giant pandas, and the period of embryonic development following implantation is shortest (approx. 40 – 45 days) among ursids (Spady et al. 2007). Additionally, the giant panda's gastrointestinal tract is known to respond to shifts in plant part intake, specifically with changes in gut microbiota according to the plant part being consumed (Williams et al. 2013; Williams et al. 2016.) Further, abrupt shifts in gut microbiota are linked to gastrointestinal discomfort, and gastrointestinal disease were found to the most common cause of mortality in giant pandas (Qui and Mainka 1993; Hansen et al. 2016). In light of the species' highly specific reproductive cycle and sensitivity to gastrointestinal stressors, the seasonal nature of plant part selection is an integral facet of giant panda biology. Monitoring foraging behavior has implications for managing individuals in captivity, as well as the understanding of habitat needs for free-ranging populations.

Visual observation of giant panda foraging behavior at the Memphis Zoo began in 2003, and has since provided valuable information regarding plant part selection patterns by the two individuals observed in the present study (Hansen et al. 2010; Williams et al. 2013). Previous feeding trials at the Memphis Zoo have also been used to quantify plant part intake; however there have not yet been any attempts to link the two methods (Finley et al. 2011). We compared plant part intake data resulting from five feeding trials to visual assessments of foraging behavior from the same time. Both methods yielded similar general trends in plant part selection by each animal, however inconsistencies between the two methods were apparent in the magnitude and direction of intake changes among trials. With the exception of the October trial, foraging behavior observations suggested higher levels of leaf intake, relative to feeding trial data, by both giant pandas. Additionally, behavioral observations did not specifically account for bamboo shoots consumed during May, however the proportion of “other/unknown-eat” behavior likely reflected shoot consumption (Figure 3.1). The greatest discrepancy of consequence are the leaf intake measurements for both giant pandas during October, as this was the expected period for greatest leaf intake, though behavioral observations indicating leaf foraging behavior was similar to January and March (Figure 3.1).

Both feeding trial and foraging behavior measurements of plant intake are inherently limited their respective methodologies, which likely explains disparity between their estimates of plant part selection. In the previous chapter, we described the difficulties involved with determining total dry matter intake (DMI), in short due to inconsistent day-to-day bamboo intake by the giant pandas and highly variable moisture content in fresh bamboo. Assuming a reasonable value for total bamboo DMI, plant part composition should be proportionally well-represented as those were determined in offered and rejected bamboos on a DM basis. However, bamboo plant part composition itself may contribute error when determining actual quantities of plant part intake. Bamboo pieces offered to the giant pandas are large and highly variant in moisture and plant part composition, and obtaining a representative sample is constrained by the time-sensitivity of water loss from the fresh bamboo. For instance, for bamboo offered to both the male and female giant pandas, culm represented the majority of bamboo biomass on a DM basis during all trials. Considering the low DMI value relative the large amounts of bamboo offered and refused, a missing or unreported culm fragment could result in an unrepresentative sample, substantially impacting the representation of plant parts in bamboo consumed. Conversely, leaves contributed the least to offered and rejected bamboo biomass (leaf composition < 20% total plant biomass, DM basis), which could impede the detection of subtle fluctuations in leaf selection. Nevertheless, we were able to distinguish relatively substantial changes in leaf composition of offered bamboo between October (16% – 17% total plant biomass, DM basis) and all other trials (2% - 8% total plant biomass, DM basis), which is further discussed in the following chapter. On the other hand, visual observation of foraging behavior may not directly represent plant part DMI due to differences in moisture content and consumption rates between different plant parts. For instance, the observed heightened levels of leaf intake in foraging behavior data, as compared to feeding trial DMI data, suggests that giant pandas consumed leaves at a slower rate than culm. Foraging behavior observations were also limited to daytime activity, while feeding trial data accounted for overnight bamboo intake.

An investigation of intake rates of different plant parts in relationship to dry matter intake of those plant parts may prove useful and non-invasive method for monitoring DMI by giant pandas. This could be especially applicable for studying intake habits of giant pandas in wild and captive setting, as feeding trials require a controlled environment and labor-intensive sampling protocol. In one report, Long et al. utilized bamboo feeding rates to predict intake in free-ranging giant pandas, however they did not discuss differences in intake rates between plant parts (2004).

To address the discrepancies between measurement of plant part intake by feeding trial and foraging behavior observations, we proposed image analysis of fecal samples to evaluate leaf consumption by giant pandas. Examination of animal feces has have long provided key insights regarding wildlife diet selection, and is especially relevant for the giant panda, whose low bamboo digestibility (DMD < 30%) allows feces to retain many of the compositional characteristics and appearance of the bamboo consumed (Eagle and Pelton 1983; Holechek et al. 1982; Sims et al. 2007; Schaller et al. 1985). As such, the color of giant panda feces reflects bamboo plant parts in the diet, with dark green and yellow indicating leaf and culm consumption, respectively. While it is possible that leaves, being lesser in fiber content, would be more digestible than culm, and thus alter the culm:leaf ratios between what was consumed and what was detected in feces, our digestibility data (described in CH. V) did not distinguish changes in DMD between the leaf-dominated trial (October) and culm-dominated trials (January, March). Image analysis of giant panda fecal samples provided a quantity for feces greenness, representing the concentration of leaf-sourced material, and made it possible to pair feces coloration to our direct observations of plant part intake. We found feces greenness to be much more strongly correlated with leaf DMI resulting from feeding trial data than leaf-eat foraging behavior (Figure 3.3). Although our number of observations was low (n = 9), feces greenness ranged from 0% to 61%, and predicted similar increases in leaf DMI during the feeding trials. As a rule, green content in feces unquestionably indicated leaf intake by the giant panda. Weaknesses in the model for feces greenness and leaf-eat foraging behavior were evident in our data pairs, as low levels of leaf foraging corresponded to relatively high greenness content in feces (e.g. 61.4% feces greenness and 20.6% foraging activity spent leaf-eating), with pairings of the opposite scenario also occurring (e.g. 11.3% feces greenness and 29.9% foraging activity spent leaf-eating).

Although visual observation of giant panda behavior remains a versatile tool for studying this species, we concluded that sampling of offered and rejected bamboo provided a more realistic assessment of plant part intake by giant pandas. Studies requiring accurate values of leaf consumption, such as nutrient intake and digestibility studies, may call for this more precise method. Additionally, leaf DMI measured in feeding trials closely reflected presence of leaf-sourced material in feces, as determined by feces greenness. With further development, image analysis of fecal samples could predict leaf composition of giant panda diets. Such a tool would be extremely useful when studying the nutrition habits of free-ranging animals, as it is non-invasive toward the subject, and sampling diet and rejected bamboo or frequent behavior assessments is impractical.

CHAPTER IV

NUTRITIVE COMPOSITION OF BAMBOO LEAF, CULM, AND SHOOTS SELECTED BY TWO CAPTIVE GIANT PANDAS

Summary

Giant pandas face a unique nutritional challenge, as they retain a carnivorous gastrointestinal system, but consume a bamboo-based diet containing high levels of structural carbohydrates (dietary fiber). Seasonal patterns of dietary shifts, defined by the part of bamboo which is selected for consumption, may be a key adaptation allowing giant pandas to survive on this poorly-digested feed sourced. We evaluated bamboo selection by two captive giant pandas in relation to temporal variations in plant part and nutrient composition over the course of four feeding trials. Despite apparent higher nutritive value (leaf CP >12% DM, ADF <40% DM) culm was the predominant plant part consumed by giant pandas during the majority of trials. Maximum bamboo culm intake coincided with times of culm starch > 6% DM and culm hemicellulose >25% DM. However, starch and hemicellulose concentrations in culm were relatively low during May and October, when both giant pandas incorporated the highest observed levels of shoots or leaves into their diets. Bamboo shoots were preferentially consumed when offered during May, likely due to their reduced proportion of plant part structural compounds (ADF and NDF) and increased CP content. As opposed to selecting a diet that is nutritionally consistent year-round, giant pandas apparently forage for digestible carbohydrates as energy sources, which results in shifting plant part preferences throughout the year in response to nutrient fluctuations in available bamboo.

Introduction

Despite its iconic status in popular culture, the giant panda (*Ailuropoda melanoleuca*) remains an enigmatic species. Reduced animal numbers, as well as their naturally elusive nature, cause available data regarding the species' unique biology is often inconsistent and inconclusive. Giant pandas are endemic to the mountainous bamboo forests of central China, where they historically thrived on bamboo diets; bamboo generally being an abundant and readily available resource throughout the year (Schaller et al. 1985; Carter 1999; Long et al. 2004). In spite of its vegetarian diet, the giant panda's gastrointestinal tract (GIT) morphology more closely resembles that of carnivorous species, and is little adapted for degradation of plant structural components (dietary fiber [Raven et al. 1936; Van Soest 1994]). Consequently, reported total bamboo DMD in giant pandas is low, ranging from 6.9% - 39% (Dierenfeld et al. 1982; Finley et al. 2011; Mainka et al. 1989). Thus, to meet their nutritional requirements, giant pandas must consume excessive amounts of bamboo (6-15% of body weight of fresh bamboo), relying on morphological and behavioral adaptations developed to enhance digestibility of dietary fiber, accessibility of intracellular nutrients, and foraging efficiency (Dierenfeld 1997; Schaller et al. 1985).

All consumers display dietary preferences driven by desirable and adverse diet characteristics, for example digestibility and palatability versus the presence of indegradable or toxic compounds. Herbivores are presented with a nutritional challenge, as plants contain high levels of structural carbohydrates, change compositionally over time, and have developed defensive mechanisms to protect against predatory and environmental stressors. Compared to other herbivores, giant pandas are especially disadvantaged due to the combined characteristics of their simple GIT and fibrous bamboo diet. Consequently, foraging strategy is key to giant panda survival, as it is the primary adaptation enabling them to meet their nutritional requirements. Bulk consumption of bamboo can provide sufficient nutrients to meet giant panda dietary needs; however, a degree of bamboo selectivity has been observed in both wild and captive settings (Schaller et al. 1985; Long et al. 2004; Hansen et al. 2010). Theoretically, diet selection balances the energy expenditure of constant foraging activity with intake of the most nutritionally-rewarding bamboo (Long et al. 2004). One foraging strategy which has been well-documented is a distinctive, seasonal pattern of dietary shifts defined by the part of bamboo which is selected for consumption (Hansen et al. 2007; Long et al. 2004; Schaller et al. 1985). In general, giant pandas prefer bamboo culm in the late winter and spring, young shoots when available in the late spring to early summer, and leaves in late summer through winter (Hansen et al. 2007; Long et al. 2004; Schaller et al. 1985; Williams et al. 2013). Seasonal diets have also been observed in other obligate herbivorous and omnivorous species (e.g. red panda, black bear, and brown bear), and appear to be dependent on the most nutritious and readily available food sources at different times of year (Eagle and Pelton 1983; Hellgreen et al. 1989; Wei et al. 2000). In the habitat of the wild giant panda, however, bamboo culms and leaves are available year-round in relatively constant amounts, except in the rare occurrence of bamboo flowering and die-off. As such, their seasonal dietary transitions are speculated to be a response to changes in nutrient content of different bamboo parts throughout the year, though it is unclear which nutrients, or combination of nutrients, most impact plant part selection.

Fossil records indicate that giant pandas once occupied much of eastern China, Northern Vietnam, and Myanmar. However, the current habitat is fragmented to five separate mountain ranges in central China due to due to anthropogenic encroachments, such as logging practices and urban development (Hu and Wei 2004; Loucks and Wang 2004). Limited bamboo availability is undeniably linked to the current vulnerable population of free-ranging giant pandas. Additionally, maintaining giant pandas in captivity is beleaguered by the species' reliance on bamboo quantity and variety. Our aim was to examine plant part consumption shifts by giant pandas in relation to changing nutrient status in the bamboo offered to two animals housed at the Memphis Zoo, so to further understand the unique nutritional ecology of this enigmatic species. We predicted that giant pandas would select for energy maximizing nutrients: hemicellulose (HC), starch, and protein; and select against indigestible components of dietary fiber: cellulose and lignin.

Materials and methods

Feeding trials

Five feeding trials were completed with two captive giant pandas housed at the Memphis Zoo (Memphis, TN). Two trials were timed to correspond with the period of maximum culm consumption by the giant pandas (January 3-5, 2015; March 23-25, 2015), two trials with consumption (July 21-23, 2014; October 27-30, 2015) and one trial with bamboo shoot consumption (May 21-23, 2015), with predicted plant part selection based on previous foraging data (Hansen et al. 2010; Williams et al. 2012). However, data collected during the July trial was excluded from evaluation due to abnormal feeding behaviors by the female giant panda, which involved increased time spent resting and low bamboo intake caused by a significant shift in hormone concentrations at that time. During feeding trials, one male (466, aged 16) and one female (507, aged 14) giant panda were housed in separate indoor, air-conditioned habitats during the day and moved to a separate enclosure overnight. Access to an outdoor exhibit was offered in cooler weather. Bamboo was provided *ad libitum* at all times, and new bamboo was offered several times per day. The feeding trials were designed to be minimally invasive and not alter the giant pandas' regular diets and daily routines. Consequently, bamboo feeding frequency and sample collections were contingent on the zookeepers' schedules. Bamboo was harvested locally prior to feeding, bundled by species, and stored at 16° C under misters. Across all five trials, bamboo species offered were: *Phyllostachys* (*P.*) *aureosulcata, P. bissetii, P. nuda,* and *Pseudosasa japonica*.

All trials except for the one in October occurred over the course of three days, with sample collection lasting approximately 48 hours, or approximately 4× the maximum passage rate of the giant panda (Dierenfeld 1997). The October trial included an additional day, resulting in approximately 72 hours of diet and ort sampling. Fresh bamboo samples (approximately 2 kg) from bamboo bundles were randomly drawn and weighed by zookeepers, and the remaining bamboo fed to the giant pandas. Rejected bamboo culms, leaves, branches, and the culm coverings, which were pieces of the culm exterior layer peeled away by the giant panda to consume the pith, were considered orts. After removal from the animal enclosure, total orts were weighed, and whole bamboo orts and culm exterior fragments were sorted and weighed separately. Approximately 2 kg of the whole bamboo portion and 10% of the culm coverings were randomly sampled. Bamboo offered and rejected samples were separated by hand into culm, culm covering (for orts), leaf, and branch fraction to estimate plant part proportions of the bamboo offered and rejected. Bamboo plant part samples were weighed, and dried in a forced-air oven at 60° C until a reaching a constant partial dry matter (PDM) weight (leaves, branches, culm coverings: 24 hours; culm: one week).

Bamboo sample analysis

Offered and rejected bamboo plant part samples were ground through a Wiley Mill (Model 4) to pass a 1 mm screen. Subsamples were weighed dried at 100º C for 24 hours to determine DM, and the following nutrient concentrations were determined in respect to DM. Organic matter was considered as the content lost during combustion at 450º C for six hours. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were extracted according to Van Soest's method (1963) and performed with an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Hemicellulose (HC) was considered the difference between NDF and ADF. Acid detergent lignin (ADL) was also determined by Van Soest's method (1963) with Ankom standard procedures, where ADF was performed with an Ankom Fiber Analyzer and followed by a three-hour digestion in sulfuric acid using a Daisy II Incubator (rotation only; Ankom Technology Corp., Macedon, NY). Acid insoluble ash (AIA), as a representation of silica content, was determined according to Van Keulen and Young (1977) acid digestion of subsample ash, followed by vacuum filtration to isolate insoluble residue (DigiPrep SCP Science, D'Urfé, Quebec). Crude protein (CP = %total nitrogen × 6.25) was assessed by a Leco Nitrogen Analyzer (model FP-2000, Leco Corporation, St. Joseph, MI; AOAC, 2000). Total starch content was determined by two-stage α-amylase, glucoamylase digestion of subsample and subsequent recording of absorbance at 510 nm (Hall 2009; Thivend 1972). Nutrient analyses were performed in duplicate, and averages of less than 0.05 error were reported.

Diet selection and intake

Offered and rejected bamboo plant parts were used to characterize the nutrient and plant part composition of the individual animal's selected diet. Plant part (culm, leaf, branch) proportions of bamboo samples collected by species represented the total amount of plant part respective to species offered, and total nutrient offered (kg/day) was the sum of the nutrient (kg) contributed by each plant part, specific to species, offered. Quantity of a nutrient or plant part offered (on a DM basis) and composition of the diet was calculated for each animal and trial using the following formulae:

$$
\begin{aligned}
&Total\ plant\ part\ offered\ (kg\ DM)\\
&\qquad = \{sp.A\ offered\ (kg\ DM) \times \%plant\ part\ _{sp.A}\} + \{\cdots\}_{sp.B} + \{\cdots\}_{sp.C} + \{\cdots\}_{sp.D}
\end{aligned}
$$

$$
\begin{aligned}
&Total\ nutrient\ offered\ (kg)\\
&= \big\{\%nutrient_{leaf} \times leaf\ offered\ (kg\ DM) + \%nutrient_{culm} \times culm\ offered\ (kg\ DM)\\
&\qquad + \%nutrient_{branch} \times branch\ offered\ (kg\ DM)\big\}_{sp.A} + \{\cdots\}_{sp.B} + \{\cdots\}_{sp.C} + \{\cdots\}_{sp.D}
\end{aligned}
$$

Plant parts and nutrients (kg DM) in rejected bamboo were calculated similarly, with plant part proportions of rejected bamboo samples representing the quantity of plant parts or nutrients rejected. We corrected for any negative plant part intake values by assuming that the giant panda did not consume that plant part during the trial, and rejected quantity of that plant part was substituted as the offered quantity so that total plant part intake was zero kg DM.

$$Total\ plant\ part\ rejected\ (kg\ DM) = total\ bamboo\ rejected\ (kg\ DM) \times \%plant\ part_{rejected}$$

$$\begin{aligned} Total\ nutrient\ rejected\ (kg) = & \big\{\%nutrient_{leaf} \times leaf\ rejected\ (kg\ DM) \\ & + \%nutrient_{culm} \times culm\ rejected\ (kg\ DM) \\ & + \%nutrient_{branch} \times branch\ rejected\ (kg\ DM) \\ & + \ \%nutrient_{culm\ cover} \\ & \times culm\ cover\ rejected\ (kg\ DM)\big\}_{sp.A} \\ & + \ \{\cdots\}_{sp.B} + \ \{\cdots\}_{sp.C} + \ \{\cdots\}_{sp.D} \end{aligned}$$

Consumption of the nutrient or plant part by the giant pandas was determined as the difference of the quantity in offered and rejected bamboos, and the concentration in the diet consumed was calculated in respect to total bamboo daily intake, as estimated by mean DMI.

$$\%nutrient\ in\ diet = \frac{nutrient\ offered\ (kg) - nutrient\ rejected\ (kg)}{Total\ bamboo\ DMI\ (kg\ DM)}$$

To measure degree nutrient selection between trials, a relative preference index (RPI) was calculated as the ratio of the nutrient in bamboo offered to the bamboo consumed, such that a RPI of 1 indicated no selection, RPI < 1 indicated selection against, and RPI > 1 indicated selection for a nutrient (Westoby 1974):

$$\text{RPI} = \frac{\text{Nutrient consumed (kg)}}{\text{Nutrient offered (kg)}}$$

Statistics and data evaluation

Relative preference index least-squares means (LSM) and between-trial differences were determined for NDF, ADF, HC, ADL, starch, CP, using SAS Mixed Procedure (SAS 9.3, SAS Institute, Cary, NC). There were four observation periods (January, March, May, October) with two giant pandas being replicates within each trial. Tables and figures for nutrient and plant part selection, as well as calculations for total diet composition, were prepared in Microsoft Excel (Microsoft Office 2016, Microsoft Corporation, Redmond, WA). Triangle diagrams comparing culm, leaf, and consumed diet concentrations of starch, HC, and ADF were plot in Excel using *Tri-plot* model, developed by Graham and Midgely (2000). Offered culm and leaf nutrient values were included as individual species, to indicate the range of selection that was available to the giant pandas during a feeding trial. Percentage of nutrient in the triangle diagrams is represented proportionally to total starch, HC, and ADF content, rather than total DM.

Results

Bamboo plant part selection

Throughout all months, culm represented the majority of plant biomass on a dry matter basis in bamboo diets offered to both the male and female giant pandas. However, leaf comprised 16% to 17% of offered bamboo DM offered during October, as compared to 2.5% to 7.8% of DM in the other months (Figure 4.1).

Bamboo culm was consumed by both giant pandas during all months, and was the predominant plant part selected in January and March (culm intake > 80% of total DMI). Culm remained the primary bamboo part consumed by the male during October, while the female consumed an approximately 50:50 mixture of culm and leaves. Both giant pandas selected for bamboo shoots when they were offered in May, and shoots comprised approximately 50% of their intake on a DM basis (55.6% and 47.4% of DMI for male and female, respectively; Figure 4.2). In general, the female giant panda consumed more leaves in proportion to culm than the male, with the exception of May, when no leaves were selected by either animal.

Figure 4.1— Plant part composition of bamboo offered to two giant pandas at the Memphis Zoo. Plant part composition of bamboo offered to the male (466; 2.a) and female (507; 2.b) giant pandas at the Memphis Zoo during January, March, May, and October, expressed as a percent of total bamboo dry matter (DM).

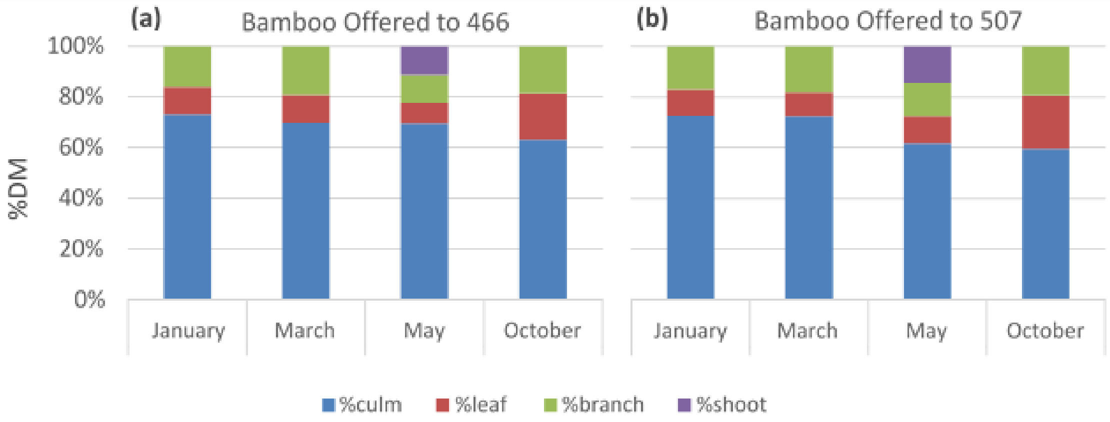
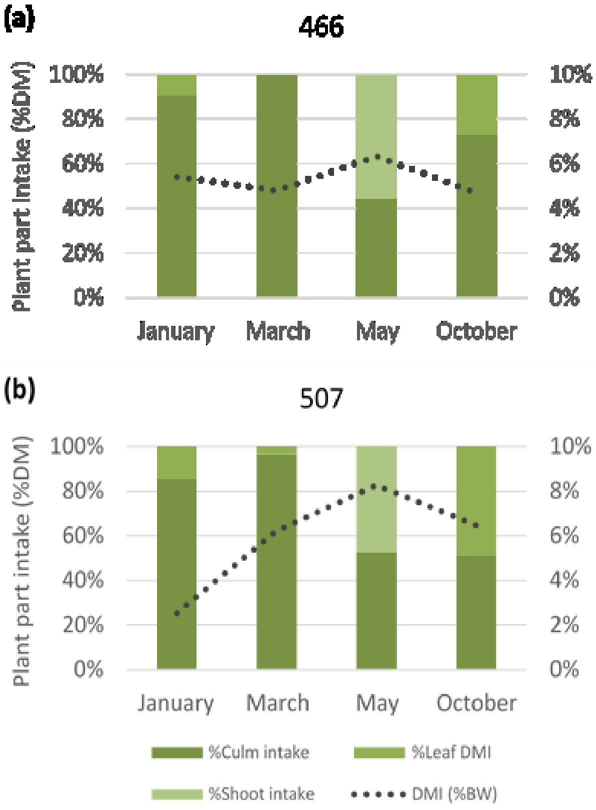


Figure 4.2— Plant part selection and dry matter intake (DMI) by two giant pandas at the Memphis Zoo. Plant part selection and DMI are expressed individually for the male (466) and female (507) giant pandas (*Ailuropoda melanoleuca*), during four months (January, March, May, October). Plant part intake is expressed as percent of total DMI (primary Y-axis), and DMI is the mean daily bamboo dry matter intake over a 17-d period, as a percentage of the animal's total body weight at the time of the trial (secondary Y-axis).



Nutrient selection in consumed bamboo

Based on the relative preference index (RPI), both giant pandas selected for hemicellulose and starch, and against ADF in bamboo, however degree of selection differed by month (Figure 4.3). Relative preference indices that differed from baseline (1 = no preference) were observed in ADL and CP, however observations were highly variable between animals and overall concentrations of these constituents in the diet were low, potentially causing a high degree of error to be associated with these RPI values (SE = 0.26 and 0.38 for ADL and CP, respectively; Table 4.1). No selection for or against NDF was observed across all months (Figure 4.1).

Between both animals, selection for HC and starch was highest during January (RPI = 1.96 and 2.85 for HC and starch, respectively), with RPI for HC being significantly greater than all other months ($p < 0.05$; Figure 4.3). Conversely, selection against ADF was significantly stronger in January than all other months (RPI = 0.55, $p < 0.05$; Figure 4.3).

Triangle plots comparing starch, HC, and ADF did not indicate selection for a consistent nutrient balance in bamboo consumed across all months (Figure 4.4). However, concentrations of starch and hemicellulose were similar in the selected diets during January and March (Figure 4.4.a, 4.4.b). Both giant pandas did not select diets of the lowest attainable ADF concentrations, with the exception of during January, despite the availability of bamboo leaves (corresponding in the plots to the feed items containing the smallest proportion of ADF). However, ADF in consumed bamboo was consistently lower than ADF in offered culm. Across months, offered bamboo culm varied in starch, HC, and ADF balance, due to temporal changes in nutrient concentrations and compositional differences between bamboo species. Leaves were more consistent across months and species, with all samples having near 1:1 proportions of HC and ADF, while starch was virtually non-existent.

Figure 4.3— Mean relative preference indices (RPI) for various nutrients by two giant pandas at the Memphis Zoo. Mean RPI values for neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HC), crude protein (CP), and starch in bamboo by two giant pandas (*Ailuropoda melanoleuca*) were calculated during four months (Jan, Mar, May, Oct). Relative preference index > 1 indicates selection for a nutrient and RPI < 1 indicates selection against a nutrient. Data columns marked by an asterisk represent nutrient selection during a month that significantly differed from other months ($p < 0.05$).

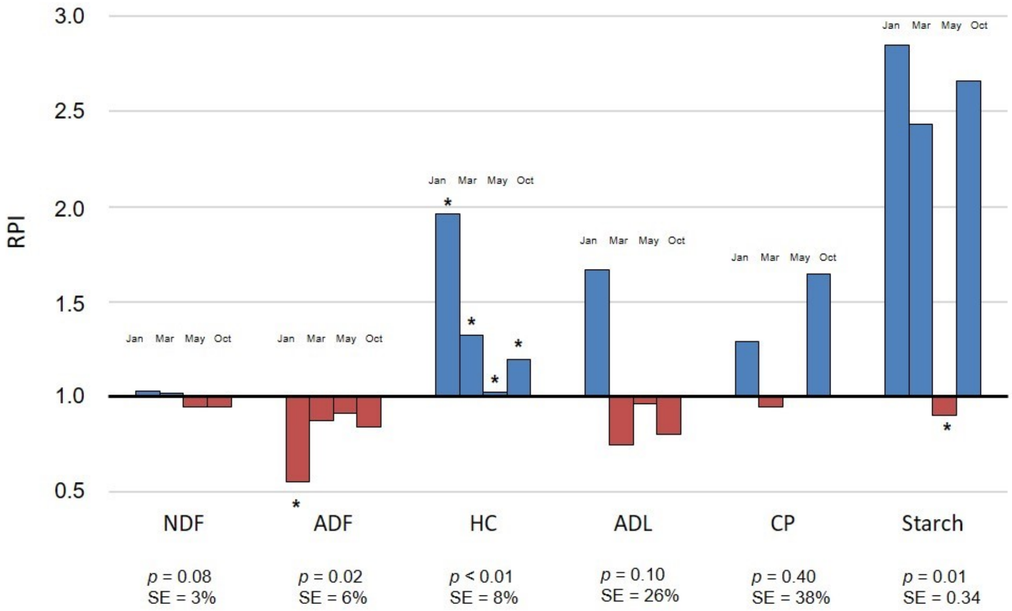
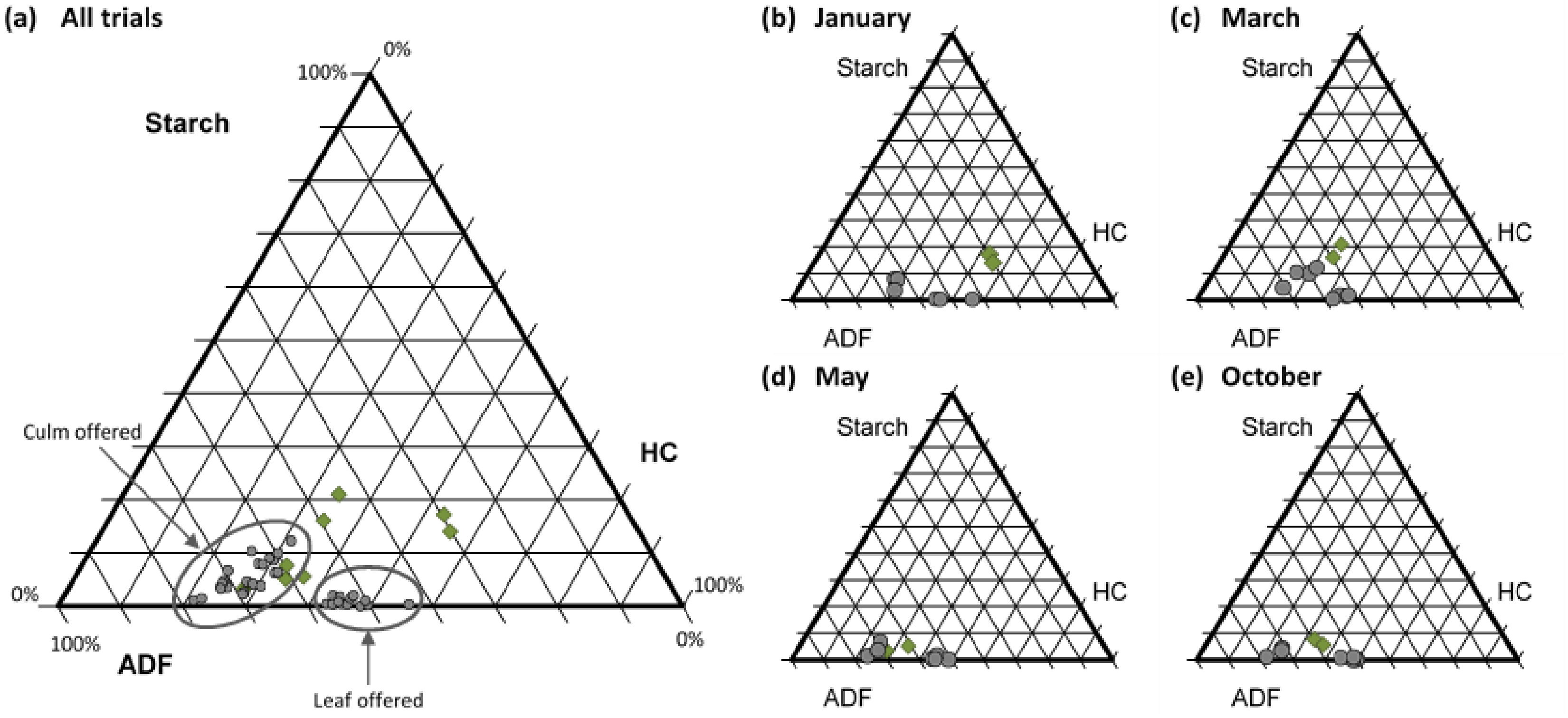


Figure 4.4— Triangle plots displaying starch, hemicellulose (HC), and acid detergent fiber (ADF) availability and selection by two giant pandas at the Memphis Zoo. Concentrations of starch, hemicellulose (HC), and acid detergent fiber (ADF) in bamboo culm and leaves offered to two giant pandas at the Memphis Zoo are represented by gray circles, and the diet consumed by the giant pandas is represented by green diamonds, with observations taken over four months (January, March, May, October). Percentage of nutrient in the triangle diagrams is represented proportionally within total starch, HC, and ADF content, rather than as a proportion of total dry matter



Bamboo plant part nutritive values in relation to plant part selection

We did not evaluate bamboo species preferences by the giant pandas during each month. Therefore, we expressed nutrient values plant parts (culm, leaf, shoot, culm cover) as the average of all species offered over the course of the feeding trial corresponding to that month (Table 4.1). Throughout all months, culm was more concentrated in NDF, ADF and starch concentrations than leaves. Culm cover, which was always peeled away prior to culm consumption, was marginally higher in ADF, ADL, and lower in CP compared to culm as a whole. On the other hand, leaves were consistently more concentrated in ash, HC, AIA, and CP than culm.

Both giant pandas consumed primarily bamboo culm during January and March, therefore these two months were considered to be part of "culm consumption season." In January, maximum concentrations HC were observed in both culm and leaf in January (HC = 27.2% and 33.8% for culm and leaf, respectively; Table 4.1), while CP content of leaves also peaked at this time (CP = 19.6%; Table 4.1 However, leaf CP dropped to its lowest concentration during March (CP = 15.2%; Table 4.1). At the same time, %ADL in culm decreased by nearly 5% (ADL = 13.3%) and culm starch increased to its peak concentration at 8.9% (Table 4.1).

Although bamboo shoots were offered in limited quantities during May, both giant pandas displayed a strong preference for this plant part. Thus, May was defined as "shoot consumption season." Bamboo shoots were more concentrated in HC than both leaves and culm (HC = 33%; Table 4.1), while NDF, ADF, and CP concentrations were intermediate to leaf and culm (NDF = 74.6%; ADF = 66.4%; CP = 10.5%; Table 4.1), and quantities of ADL, AIA, and starch were minimal (< 2%; Table 4.1).

Of the four observation periods in this study, leaves were most prominent in the diets of both giant pandas during October. Based on our data, as well as historical foraging behavior records of the same animals, this month was considered to represent “leaf consumption season.” Previously, culm ADF concentration increased by 5% from March to May, and in October it remained at this higher concentration (ADF = 66% - 67%; Table 4.1), whereas leaf ADF remained relatively constant at lower concentrations during all months (ADF = 35% - 37%). Culm HC and starch concentrations dropped from March to May, and were also found at similar concentrations during October (%HC = 22%; %starch < 4%; Table 4.1). Crude protein concentration in leaves during October was approximately 3% higher than in March and May (October leaf CP = 18.3%), while culm CP was between 3% and 4.5% across all months (Table 4.1).

Table 4.1— Nutrients in bamboo culm, leaves, culm cover, and shoots offered to two giant pandas (*Ailuropoda melanoleuca*) at the Memphis Zoo over four months (January, March, May, October). Based on feeding trial plant part preferences, January and March were classified as occurring during "culm consumption season," May as "shoot consumption season," and October as "leaf consumption season." Nutrient values are the average of bamboo species offered during the corresponding trial, expressed as percentage of plant part total dry matter (DM).

Month	Plant part	OM	NDF	ADF	HC	ADL	CP	Starch
January	culm	98.33%	87.26%	60.07%	27.19%	18.07%	4.67%	6.13%
	leaf	89.99%	68.88%	35.06%	33.82%	9.85%	19.63%	0.23%
	cover	98.77%	85.70%	66.88%	18.82%	19.10%	3.10%	6.50%
March	culm	98.14%	85.95%	59.46%	26.49%	13.29%	4.67%	8.90%
	leaf	87.30%	64.46%	35.31%	29.15%	7.32%	15.18%	0.98%
	cover	98.70%	86.43%	65.29%	21.15%	16.05%	3.85%	7.60%
May	culm	98.20%	88.43%	66.44%	21.99%	16.17%	3.03%	3.60%
	leaf	86.94%	68.24%	36.77%	31.47%	8.59%	15.33%	0.57%
	cover	97.76%	87.69%	65.23%	22.46%	14.60%	3.35%	4.40%
	shoot	93.69%	74.56%	41.57%	32.99%	1.25%	10.50%	0.30%
October	culm	98.19%	88.66%	67.10%	21.56%	14.73%	4.28%	2.88%
	leaf	87.47%	68.27%	35.53%	32.74%	7.11%	18.35%	0.35%
	cover	98.40%	88.85%	70.19%	18.67%	17.80%	3.35%	2.75%

Discussion

In the nutritional ecology of many wildlife species, it is often found that animals forage in a way to optimize intake of critical nutrients, and avoid ingesting compounds that are potentially harmful or inhibit digestion and absorption (Chapman and Chapman 2002; Duncan et al. 2006; Owen-Smith 1994; Sih and Christensen 2001; Westoby 1974). For species consuming primarily plant-sourced foods, forage selection often manifests itself in diets that are relatively higher in energy-bearing nutrients (e.g. protein, simple sugars, and fat) and low in indigestible plant cell wall constituents (dietary fiber), relative to other available forage sources (Doucet and Fryxell 1993; Zweifel-Schielly et al. 2012). Diet selection prioritizing digestible, energy-dense foods is especially pronounced in monogastric animals lacking capabilities for extensive plant cell wall degradation, such as brown bears, black bears, gorilla, and baboons (Doran-Sheehy et al. 2009; Erlenbach et al. 2014; Kimball et al. 1998; Rothman et al. 2011; Whiten et al. 1991). Previous reports of nutrient selection by giant pandas indicated selection for dietary protein, which was achieved by preferential consumption of bamboo leaves rather than culm (Dierenfeld et al. 1982; Wei et al. 2015). Nevertheless, captive giant pandas in our study consumed predominantly bamboo culm during the majority of our feeding trials despite apparent higher nutritive quality of bamboo leaves, as indicated by relatively high CP and low ADF concentrations of leaves compared to culm. While this is unexpected for an animal with such limited ability to digest dietary fiber, nutrient selection indices and seasonal fluctuations in bamboo plant part nutrients may explain this contradictory foraging strategy.

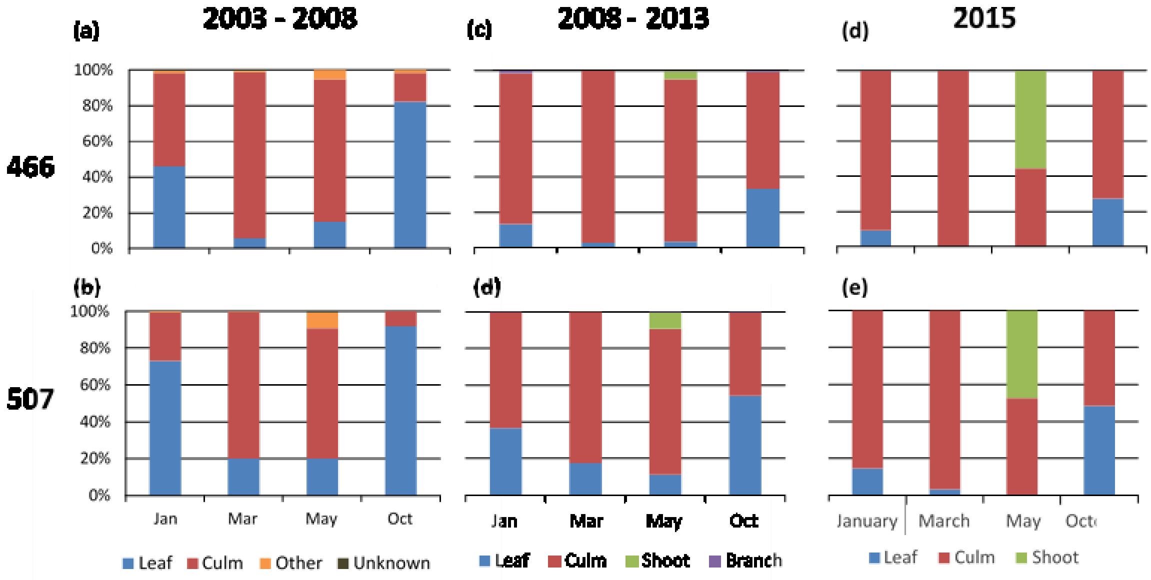
Nutrient RPI indicate that both giant pandas selected for increased starch and hemicellulose rather than protein concentrations across all but the shoot-consumption season trial (May). Starch, functioning in the plant to store surplus energy, is a carbohydrate polymer known to be digested by mammalian enzymes. Provided that bioavailability is not impeded by high concentrations of dietary fiber, starch would be a valuable source of digestible energy for giant pandas. Year-round, starch was present in bamboo culm in considerable amounts (culm starch = 3% - 9%) and peaked during culm-consumption season (January and March), while virtually nonexistent in bamboo leaves (leaf starch < 1%). From January to March, culm ADL also decreased from 18% to 13%, which would likely result in improved bioavailability of starch. These nutrient fluxes of increased starch and decreased dietary fiber during culm-consumption season were consistent with previous nutritional analyses of bamboo culm, including of those previously conducted at the Memphis Zoo (Knott et al. 2016—personal communication; Okahisa et al. 2006). In all trials of the present study, giant pandas selected to reduce cellulose and lignin (represented by ADF) intake, though not to the extent that would have been observed in leaf-dominated diets. Nevertheless, selection against cellulose and lignin is consistent with previous reports of diet selectivity in giant pandas and other specialist herbivores (Dierenfeld et al. 1982; Doran-Sheehy et al. 2009; Van Soest 1996; Zweifel-Schielly et al. 2012). One mechanism which allows giant pandas to decrease indigestible fiber concentrations when consuming culm is to strip the outer layer and consume only the interior pith (Hansen et al. 2010; Schaller et al. 1985). Nutritional analyses of culm coverings left after the pith was consumed confirmed higher concentrations of cellulose and lignin in these fragments than culm as a whole (Table 4.1). Hemicellulose, on the other hand, is a fraction of dietary fiber shown to be somewhat digestible by giant pandas (HC digestibility in bamboo culm ≈ 20%; Long et al. 2004; Schaller et al. 1985). Comprised of relatively stable polysaccharides and gel-like pectin, hemicellulose is believed to be broken down in the large intestine by the giant panda's unique microbial community (Long et al. 2004; Zhu et al. 2011). Though HC was consistently more concentrated in bamboo leaves than culm, culm HC increased, similar to starch, during the culm-consumption season trials. Culm coverings were also generally less concentrated in HC than culm as a whole Table 4.1.

It is unclear as to why giant pandas may prefer starch as an energy source to protein, and therefore select culm rather than leaves; however, starch selection may serve as a trade-off for anti-nutritional compounds prevalent in bamboo leaves. As expected, silica (represented by AIA) was considerably present in leaves (AIA = 7% - 8%), while nearly undetectable in culm (AIA < 1%). Silica in bamboo leaves has also been reported to increase during late spring, the season of culm consumption characteristically shared by most giant pandas, however our data did not suggest any notable fluxes in leaf silica between trials (Lux et al. 2003; Schaller et al. 1985; Tabet et al. 2004). Selection for carbohydrate sources of energy rather than protein is also accordant to the giant panda's ability to perceive sweet tastes, while they lack the taste receptor responsible for detecting savory flavors associated with proteinaceous foods (Jiang et al. 2014; Jin et al. 2011). Perception of sweet flavors is a function lost to strictly carnivorous species, and its preservation by giant pandas suggests that carbohydrate recognition in bamboo may impact diet preference. It is worth noting, that protein selection may have been underestimated due to the high degree of error associated with such low overall diet protein concentrations in bamboo. However, we detected a relatively high (RPI = 1.6) for CP by giant pandas during October, likely influenced by selection for leaves at that time (Figures 4.1; 4.3).

When bamboo shoots were offered and highly favored by the giant pandas in May, starch and hemicellulose selection was markedly lower than the other three trials. It's likely that shoots differed much more from culm and leaves in nutrients not examined, such as simple sugars, vitamins, and minerals (Christian et al. 2015). Bamboo shoots also had higher concentrations of CP than culm and lesser concentration of AIA than leaves, which would provide an alternate source of energy during this time when little starch was consumed, without the antinutritive influence of silica. We note that although our nutrient values for bamboo shoots are representative of all shoots offered to the giant pandas during the time of the trial, individual shoots are variable in composition. Nutrient concentrations rapidly change, and particularly dietary fiber fractions increase, as the shoot rapidly grows and matures into culm (Christian et al. 2015). Bamboo shoots of various stages of growth were offered to both giant pandas, though all shoots were nearly completely consumed. Interestingly, emergence of bamboo shoots, which occurs in the late spring, occurs shortly after female giant pandas undergo their single, annual estrous. Similar to brown bears, black bears, and polar bears, which accumulate energy reserves prior to denning and parturition, giant pandas may capitalize on bamboo shoot availability to lay a nutritional foundation to support gestation (Brody and Pelton 1988; Elowe and Dodge 1989; Lopez-Alfaro et al. 2013; Nelson et al. 1983). In free-ranging population of the Qinling Mountains, giant pandas exhibited elevational migrations to consume shoots of multiple bamboo species emerging at different times, such that they were able to exclusively consume bamboo shoots throughout the summer and leading to parturition in early fall (Long et al. 2004).

Plant part selection across months was similar to records of plant part consumption by the same animals taken from 2008 - 2013 (determined by visual observation of foraging behavior), where both giant pandas spent more of daytime foraging behavior consuming culm than leaves throughout the majority of the year (Williams et al. 2012). During this period (2008 – 2013) average leaf intake peaked during July (45% and 82% for the male and female, respectively) and remained relatively elevated through October, and we observed greatest leaf intake in our October trial (Williams et al. 2012). Interestingly, foraging behavior of the same animals from 2003 – 2008 indicated a much longer season of leaf preference, such that leaves were the predominant plant part consumed from June through December (Hansen et al. 2010). The female giant panda appeared to consume leaves to a greater extent than the male, year-round, in both the historical foraging data (2003 – 2013) and the present study. Our feeding trials also depict increased consumption of bamboo shoots compared to previous years, however bamboo shoots were historically offered at a lower level in the giant pandas' diets at the Memphis Zoo prior to the years of our study (Memphis Zoo Zookeepers—Personal communication). Data resulting from this study indicates a distinctive leaf-consumption season for the Memphis Zoo giant pandas; however, it also suggests that a nearly year-round preference for bamboo culm by both animals developed over time (2003 – 2015; Figure 4.5). There are very few reports on changes in diet selection or nutritional requirements with age in giant pandas; however, this shift toward culm preferences could also be a function of changes in bamboo nutrients or an adaptation to captivity. All of the bamboo provided to the giant pandas was locally-sourced, with the majority harvested from one site in Shelby County, TN. Bamboo stands at this location were established upon the arrival of the giant pandas to the Memphis Zoo in 2003, and therefore were approximately 10+ years old at the time of the study. In the year following the study, zoo staff determined that productivity of these stands was unsatisfactory, and stands were either clear-cut or extensively pruned (Memphis Zoo Zookeepers—Personal communication). Though chemical composition in individual mature culms does not appear to considerably change with age, it is possible that stand quality had been in decline and caused shifts in diet preferences of giant pandas (Liese and Weiner 1996). Additionally, at the time of our trial both giant pandas had lived under captive conditions their entire lives, and been at the Memphis Zoo for the majority of that time. The two giant pandas may have learned to exhibit diet preferences that would not be ecologically favorable for wild individuals, but would be accommodated by the *ad-libitum* supply of fresh bamboo, provision of supplemental diet items, and reduced energy expenditure typical of captivity scenarios.

Figure 4.5— Bamboo plant part selection by two giant pandas (466, male; 506, female) at the Memphis Zoo during January, March, May, and October from 2003 – 2015. Data from 2003 – 2008 was adapted from Hansen et al. (2010). Data from 2008 - 2013 was adapted from Williams et al. and zookeeper historical records (2012). Plant part intake in the present study is 2015 data.



Nutrients not evaluated in this study have also been proposed to drive foraging behavior in free-ranging animals. Seasonal shifts in bamboo selection by giant pandas in the Foping Reserve of the Qinling Mountains were attributed to availability of minerals in these plant parts (Nie et al. 2014). Nie et al. suggested giant pandas sought specific species or plants to balance or maximize intake of calcium (Ca), phosphorus (P), and nitrogen (N), though nitrogen concentrations likely represented protein content (2014). Additionally, mineral concentrations in old shoots (first-year culms) sampled from the Wolong Natural Reserve plummeted in September, fell within the leaf-consumption season of giant pandas in that area (Schaller *et al.* 1985). Fat, though more calorically-dense than carbohydrates and protein, was not examined in the present study, as concentrations in all bamboo plant parts are generally less than 3% (Memphis Zoo, Katrina Knott—Personal communication; Senshu et al. 2007).

Optimizing intake of key nutrients, described by nutritional geometry, is gaining attention as tool to explain foraging behaviors and diet selection in many wildlife species, including giant pandas (Nie et al. 2014; Raubenheimer et al. 2015). Nie et al. examined balance of mineral intake in free-ranging giant pandas, while we focused on energy-providing or -inhibiting macronutrients that have been observed to influence diet selection of other species (Coogan et al. 2014; Rothman et al. 2011). In our trials, giant pandas appeared to exhibit a strong selective response to starch, ADF (representative of cellulose and lignin), and hemicellulose. When balanced on a triangle plot, giant pandas did not select diets of consistent starch : HC : ADF ratios throughout the different feeding seasons, though all showed increased HC and decreased ADF concentrations in the diet as compared to offered culm. Rather, when offered bamboo culms had starch concentrations greater than 5% (January and March), giant pandas were able to select to increase dietary starch concentrations in consumed diet. As opposed to selecting a diet that is nutritionally consistent year-round, giant pandas apparently forage for digestible carbohydrates as energy sources, which results in shifting plant part preferences throughout the year in response to nutrient fluctuations in available bamboo. Nevertheless, a diet balanced in all essential macro- and micro-nutrients is foundational for giant panda health and reproductive success in captive and wild scenarios. Further observations of nutrient maximization versus optimization by the giant panda will provide a basis of understanding for the species' unique foraging decisions and nutrient requirements, which have so far remained in relative obscurity. These insights would have implications for habitat preservation and restoration for free-ranging populations, as well as the propagation of individuals in captivity.

CHAPTER V

SEASONAL NUTRIENT UTILIZATION BY TWO CAPTIVE GIANT PANDAS CONSUMING A BAMBOO-BASED DIET

Summary

Giant pandas exhibit distinctive seasonal preferences in bamboo plant part selection as a foraging strategy to meet nutrient requirements while consuming a poorly-utilized diet. We examined digestibility of macronutrients in two captive giant pandas consuming bamboo-based diets across four months (January, March, May, October), which encompassed culm-, shoot-, and leaf-consumption seasons. Giant pandas have a very limited ability to utilize diets primarily comprised of mature bamboo culm and leaves, with total dry matter digestibility being less than 30%. Though the majority of bamboo dry biomass are plant-structural compounds (dietary fiber), giant pandas can apparently access and utilize some fiber and non-fiber nutrients, such as hemicellulose and starch, to a greater extent. When bamboo shoots were included in the diet (May), giant pandas achieved higher digestion rates for DM, OM, NDF, ADF, HC, and GE. We proposed that the basis of giant panda foraging strategy is maximization of digestible energy intake, often from carbohydrate sources, which has applications in areas of giant panda conservation and management of animals in captive scenarios.

Introduction

Giant pandas historically thrived on diets primarily comprised of bamboo, a grass characterized by its woody structure and evergreen foliage (Schaller et al. 1985; Carter 1999; Long et al. 2004). Among specialist herbivores, giant pandas are unique in their retention of a simple, monogastric digestive system analogous to those of carnivorous species. Though near-exclusive selection of bamboo by the giant panda is paradoxical, the species' history of inhabiting heavily vegetated forests of central China suggests that bamboo abundance drove their dependence on this feed source (Schaller et al. 1985). Nevertheless, giant pandas lack specialized gastrointestinal compartments, such as those seen in ruminant and hindgut-fermenting herbivorous species, to facilitate degradation of plant structural components (dietary fiber [Van Soest 1996]). Consequently, reported total bamboo dry matter digestibility in giant pandas is low, ranging from 6.9% - 39% (Dierenfeld et al. 1982; Finley et al. 2011; Mainka et al. 1989). In order to meet their nutritional requirements, giant pandas must consume excessive amounts of bamboo (6-15% of body weight of fresh bamboo; 2-7% on DM basis), relying on morphological and behavioral adaptations developed to enhance digestibility of dietary fiber, accessibility of intracellular nutrients, and foraging efficiency (Dierenfeld 1997; Schaller et al. 1985).

Bamboo selectivity by giant pandas has been observed in both wild and captive settings, and likely contributes to fulfilling nutritional requirements while consuming an ineffectively-utilized diet (Schaller et al. 1985; Long et al. 2004; Hansen et al. 2010). Theoretically, bamboo selection balances the energy expenditure associated with frequent foraging activity to intake of the most nutritionally-rewarding bamboo (Long et al. 2004). Seasonal patterns in diet selection, defined by the part of bamboo consumed, is a well-documented but poorly-understood component of giant panda foraging strategy. In general, giant pandas prefer bamboo culm (stem) in the late winter and spring, young shoots when available in the late spring to early summer, and leaves in late summer through winter (Hansen et al. 2010; Long et al. 2004; Schaller et al. 1985; Williams et al. 2013). Giant pandas inhabiting the Wolong Reserve of China were reported to prefer leaves from July to October, and culm from November to March (Schaller et al. 1985). Seasonal dietary shifts exhibited by giant pandas in the Qinling Mountain region were correlated to the animals' migratory habits, as different bamboo species grow in different elevations, and shoots of these species emerge at different times. Wild giant pandas exclusively forage on newly emerged bamboo shoots when they are accessible, and as such, bamboo shoots have been hypothesized to provide a vital nutritive opportunity to improve body condition and reproductive fitness (Long et al. 2004; Schaller et al. 1985). However, dietary shifts of captive giant pandas are less defined by availability of bamboo shoots, as fresh shoots are difficult to provide *ad libitum* in captivity. At the Memphis Zoo, giant pandas historically consumed bamboo leaves throughout the year, excepting a springtime period of culm consumption, with shoots offered as treats during the late spring (Hansen et al. 2007). More recent reports, however, have indicated a stronger preference for culm beginning in the early winter and continuing into summer (Ch. IV).

Seasonal diets are reported of other obligate and natural herbivores (e.g., red panda, black bear, elephant, and impala), and appear to be dependent on the most nutritious and readily available food source at that time of year (Eagle and Pelton 1983; Hellgren et al. 1989; Kos et al. 2010; Wei et al. 2000). Furthermore, seasonal dietary shifts are the chief response by which other bear species subsist on low-quality, omnivorous diets. In the habitat of the wild giant panda, however, bamboo culms and leaves are available year-round in relatively constant amounts, except in the rare occurrence of bamboo flowering and die-off. As such, their seasonal dietary shifts are speculated to be a response to changes in nutrient content of different bamboo parts throughout the year; though it is unclear which nutrients, or combination of nutrients, most impact plant part selection. Of bamboo culms offered to giant pandas at the Memphis Zoo, monthly nutritional data revealed increases in digestible carbohydrates (starch, free fructose and glucose), and a decrease in fiber during the spring, while protein and fat remained relatively constant year-round (Katrina Knott, Memphis Zoo —personal communication). This is consistent with the description of foraging patterns of the two giant pandas at that time, which reported that the bears consumed primarily culm from February to May (Hansen et al. 2010). In the previous chapter (Ch. IV), we found that the giant pandas observed in our trials foraged selectively for energy-bearing nutrients, notably starch and hemicellulose, which resulted in plant part preferences that coincided with seasonal nutrient fluctuations in offered bamboos. Additionally, both animals selected to reduce indigestible plant structural components cellulose and lignin (represented by ADF) concentrations in consumed bamboo (versus offered bamboo), and highly favored young bamboo shoots when offered in May (shoots ≈ 50% DMI). These foraging tendencies suggest that giant pandas select bamboo in such a manner as to maximize caloric intake through digestible carbohydrates.

A small number of feeding trials have been conducted observing diet digestibility by the giant panda; however, given the complicated nature of studying this species, studies are highly varied in methods, determination of digestibility, and description of data (summarized in Table 1.2). For instance, levels of supplementation in the subject diet range from unsupplemented to 77% of intake comprised of non-bamboo feeds (on a fresh basis; Kametaka et al. 1988; Schaller et al. 1985). Additionally, the variety and potential inaccuracy of digestibility markers used in previous trials already been acknowledged (Ch. II), and likely introduces error when comparing results of available literature. Five feeding trials were conducted between 2003 – 2004 with giant pandas housed at the Memphis Zoo. Subjects were offered bamboo-based diets (supplements comprised 1.6% to 3.9%) in four trials, while the fifth trial examined nutrient digestibilities from a total bamboo diet (Finley et al. 2011; Sims et al. 2007). Sims et al. reported average digestibility coefficients for DM, ash, OM, CP, CF, NDF, ADF, hemicellulose, and EE (Table 1.2 [2007]). Significant differences in digestibility between bears were observed for only hemicellulose (Sims et al. 2007). In a separate manuscript, Finley et al. reported energy digestibilities from the same trials (Table 1.2 [2011]). In these trials, supplements contributed 7.6% to 35.6% of the energy consumed. While the study was not designed to observe seasonal changes in nutrient digestibility, they did not report evidence to suggest giant pandas made foraging selections to increase energy assimilation (Finley et al. 2011). Thus far, there have been no repeated studies on the same individuals to detect age effects on digestibility; however, differences in bacterial and fungal populations in the giant panda fecal microbiome have been observed between adult and geriatric animals (exact ages not given), indicating potential changes in carbohydrate digestion (Tun et al. 2014). Additionally, Liu et al. observed reduced digestibilities for several nutrients in an older individual (aged 26 years vs. 4 and 9 years), most conspicuously for NDF, ADF, and hemicellulose (Table 1.2 [2015]).

The fragile status of the giant panda population necessitates the use of caution when studying these animals in both captive and wild scenarios. Unfortunately, these circumstances cause giant panda nutritional data to not only be scarce, but also variant amongst studies in animal age, time of year, reproductive status, and diet variation amongst institutions. Digestibility trials observing bamboo utilization by the giant panda are further complicated by dissimilar methods and description of data (summarized in Table 1.2). Nevertheless, the situation of captive giant pandas provides opportunities to gain valuable insights regarding bamboo selection and utilization. Seasonal plant part selection is clearly a significant element of giant panda ecology, though the circumstances driving the foraging decisions remain poorly-understood. We propose a study of nutrient digestibility by the giant panda, which will supplement available data regarding bamboo utilization, as well as be paired with seasonal foraging behaviors and evaluated for temporal responses to changes in plant part selection.

Materials and methods

Feeding trials

Diet selection and digestibility by two captive giant pandas housed at the Memphis Zoo (Memphis, TN) was measured during five months: July, January, March, May, and October). The January and March trial were timed to correspond with the period of maximum culm consumption by the giant pandas (January 3-5, 2015; March 23-25, 2015), the July and October with leaf consumption (July 21-23, 2014; October 27-30, 2015) and the May trial with bamboo shoot consumption (May 21-23, 2015). Plant part selection was predicted based on historical foraging data of the same subjects (Hansen et al. 2010; Williams et al. 2012). However, data collected during July 2014 was excluded from evaluation due to abnormal feeding behaviors by the female giant panda, which involved increased time spent resting and low bamboo intake caused by a significant shift in hormone concentrations at that time. During feeding trials, one male (466, aged 16) and one female (507, aged 14) giant panda were housed in separate indoor, air-conditioned habitats during the day and moved to a separate enclosure overnight. Access to an outdoor exhibit was offered in cooler weather. Bamboo was provided *ad libitum* at all times, and new bamboo was offered several times per day. The feeding trials were designed to be minimally invasive and not alter the giant pandas' regular diets and daily routines. Consequently, bamboo feeding frequency and sample collections were contingent on the zookeepers' schedules. Bamboo was harvested locally prior to feeding, bundled by species, and stored at 16° C under misters. Across all five trials, bamboo species offered were: *Phyllostachys* (*P.*) *aureosulcata, P. bissetii, P. nuda,* and *Pseudosasa japonica*.

All trials except October occurred over the course of three days, with sample collection lasting approximately 48 hours, or approximately 4× the maximum passage rate of the giant panda (Dierenfeld 1997). The October trial included an additional day, resulting in approximately 72 hours of diet and ort sampling. Fresh bamboo samples (approximately 2 kg) from bamboo bundles were randomly drawn and weighed by zookeepers, and the remaining bamboo fed to the giant pandas. Rejected bamboo culms, leaves, branches, and the culm coverings, which were pieces of the culm exterior layer peeled away by the giant panda to consume the pith, were considered orts. After removal from the animal enclosure, total orts were weighed, and whole bamboo orts and culm exterior fragments were sorted and weighed separately. Approximately 2 kg of the whole bamboo portion and 10% of the culm coverings were randomly sampled. Bamboo offered and rejected samples were separated by hand into culm, culm covering (for orts), leaf, and branch fraction to estimate plant part proportions of the bamboo offered and rejected. Bamboo plant part samples were weighed, and dried in a forced-air oven at 60° C until a reaching a constant partial dry matter (PDM) weight (leaves, branches, culm coverings: 24 hours; culm: one week).

Bamboo and feces nutrient analysis

Offered and rejected bamboo plant part and giant panda fecal samples were ground through a Wiley Mill (Model 4) to pass a 1 mm screen. Subsamples were weighed dried at 100º C for 24 hours to determine DM, and the following nutrient concentrations were determined in respect to DM. Organic matter was considered as the content lost during combustion at 450º C for six hours. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were extracted according to Van Soest's method (1963) and performed with an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Hemicellulose (HC) was considered as the difference between NDF and ADF. Gross energy (GE) was determined using a Parr 300 model bomb calorimeter (Parr Instrument Co., Moline, IL). Crude protein (CP = %total nitrogen × 6.25) was assessed by a Leco Nitrogen Analyzer (model FP-2000, Leco Corporation, St. Joseph, MI; AOAC, 2000). Total starch content was determined by two-stage α-amylase, glucoamylase digestion of subsample and subsequent recording of absorbance at 510 nm (Hall, 2009; Thivend, 1972). Nutrient analyses were performed in duplicate with variance less than 5%, and nutrient averages were reported.

Determination of daily dry matter intake (DMI) and daily fecal output (DFO)

In Chapter II, we concluded that daily dry matter intake of bamboo by giant pandas was too variant to be determined over a 3-day observation period. Therefore, for determination of nutrient intake and digestibility, we elected to use mean DMI calculated from daily husbandry records spanning one week before the start and following the end of the trial (total = 17 days). Daily DMI values were estimated from the daily husbandry reports of bamboo offered and rejected, which were converted to DM basis using coefficients for bamboo species and orts determined during the corresponding feeding trial. Daily fecal output was also recorded by the zookeepers during this time, and multiplied by the corresponding feeding trial fecal DM coefficient to estimate fecal output on a DM basis over the same 17 days.

Diet and feces composition and calculation of apparent nutrient digestibility

Offered and rejected bamboo plant parts were used to characterize the nutrient and plant part composition of the individual animal's selected diet for each trial. Plant part (culm, leaf, branch) proportions of bamboo samples collected by species represented the total amount of plant part respective to species offered, and total nutrient offered (kg/day) was the sum of the nutrient (kg) contributed by each plant part, specific to species, offered. Quantity of a nutrient or plant part offered (on a DM basis) and composition of the diet was calculated for each animal and trial using the following formulae:

$$\begin{aligned}&Total\ plant\ part\ offered\ (kg\ DM)\\&\qquad = \{sp.A\ offered\ (kg\ DM) \times \%plant\ part_{\ sp.A}\} + \{\cdots\}_{sp.B} + \{\cdots\}_{\ sp.C} + \{\cdots\}_{\ sp.D}\end{aligned}$$

$$\begin{aligned}&Total\ nutrient\ offered\ (kg)\\&\quad = \big\{\%nutrient_{leaf} \times leaf\ offered\ (kg\ DM) + \%nutrient_{culm} \times culm\ offered\ (kg\ DM)\\&\qquad\qquad + \%nutrient_{branch} \times branch\ offered\ (kg\ DM)\big\}_{sp.A} + \{\cdots\}_{sp.B} + \{\cdots\}_{sp.C} + \{\cdots\}_{sp.D}\end{aligned}$$

Plant parts and nutrients (kg DM) in rejected bamboo were calculated similarly, with plant part proportions of rejected bamboo samples representing the quantity of plant parts or nutrients rejected. We corrected for any negative plant part intake values by assuming that the giant panda did not consume that plant part during the trial, and rejected quantity of that plant part was substituted as the offered quantity so that total plant part intake was zero kg DM.

$$Total\ plant\ part\ rejected\ (kg\ DM) = total\ bamboo\ rejected\ (kg\ DM) \times \%plant\ part_{rejected}$$

$$\begin{aligned} Total\ nutrient\ rejected\ (kg) = {} & \big\{\%nutrient_{leaf} \times leaf\ rejected\ (kg\ DM) \\ & + \%nutrient_{culm} \times culm\ rejected\ (kg\ DM) \\ & + \%nutrient_{branch} \times branch\ rejected\ (kg\ DM) \\ & + \%nutrient_{culm\ cover} \\ & \times culm\ cover\ rejected\ (kg\ DM)\big\}_{sp.A} + \{\cdots\}_{sp.B} + \{\cdots\}_{sp.C} \\ & + \{\cdots\}_{sp.D} \end{aligned}$$

Consumption of the nutrient or plant part by the giant pandas was determined as the difference of the quantity in offered and rejected bamboos, and the concentration in the diet consumed was calculated in respect to total bamboo daily intake, as estimated by mean DMI.

$$Total\ nutrient\ intake\ \left(\frac{kg}{day}\right) = Total\ nutrient\ offered\ (kg) - total\ nutrient\ rejected\ (kg)$$

Nutrient concentrations in fecal samples were considered representative of total feces collected, and were used as coefficients to calculated total nutrient output (kg/day).

$$Total\ nutrient\ output\ \left(\frac{kg}{day}\right) = \ DFO\ \left(\frac{kg\ DM}{day}\right) \times \%nutrient_{feces}$$

Apparent nutrient digestibility was determined as nutrient disappearance in consumed and excreted nutrients.

$$Apparrent\ nutrient\ digestibility\ (\%) = 100 \times \frac{Total\ nutrient\ intake\ \left(\frac{kg}{day}\right) - Total\ nutrient\ output(\frac{kg}{day})}{Total\ nutrient\ intake\ (\frac{kg}{day})}$$

Statistics and data evaluation

Least squares means (LSM) and standard error for apparent digestibilities of DM, OM, NDF, ADF, CP, and starch during four trials (January, March, May, October) were determined using SAS Mixed Procedure, with animal as replicate within trial (SAS 9.3, SAS Institute, Cary, NC). Significant changes in digestibility between trials were determined at 0.05 level. Tables and figures were prepared in Microsoft Excel (Microsoft Office 2016, Microsoft Corporation, Redmond, WA).

Results

Between both animals, bamboo DMI ranged from 2.1 to 7.4 kg DM (2.5% to 8.3% BW), with maximum DMI occurring in May (male: 7.1 kg/day or 6.3% of BW; female: 7.4 kg/day or 8.3% BW; Figure 5.1). The female (507) exhibited low DMI during January compared to other months (DMI = 2.5% vs 6.2% - 8.3% BW), while the male's DMI remained between 4.7% and 6.3% BW throughout all trials. Diet selection and composition was described in detail in the previous chapter (Ch. IV), and is summarized in Table 5.2.

Bamboo digestibility in January was numerically the lowest of all the four time periods for DM, OM, ADF, and GE (LSM = 19.2%, 19.1%, -33.7%, and 16.1% for DM, OM, ADF, and GE, respectively; Table 5.3; Figure 5.2). However, hemicellulose digestibility was significantly higher in January than March and October (55.5%; $P = 0.04$).

Figure 5.1— Bamboo dry matter digestibility (DMD) and dry matter intake (DMI) for two giant pandas at the Memphis Zoo. Dry matter digestibility (DMD) and daily DMI (as %body weight) is represented individually for the male (466; 5.1.a) and female (507; 5.1.b) giant pandas over four months (January, March, May, October).

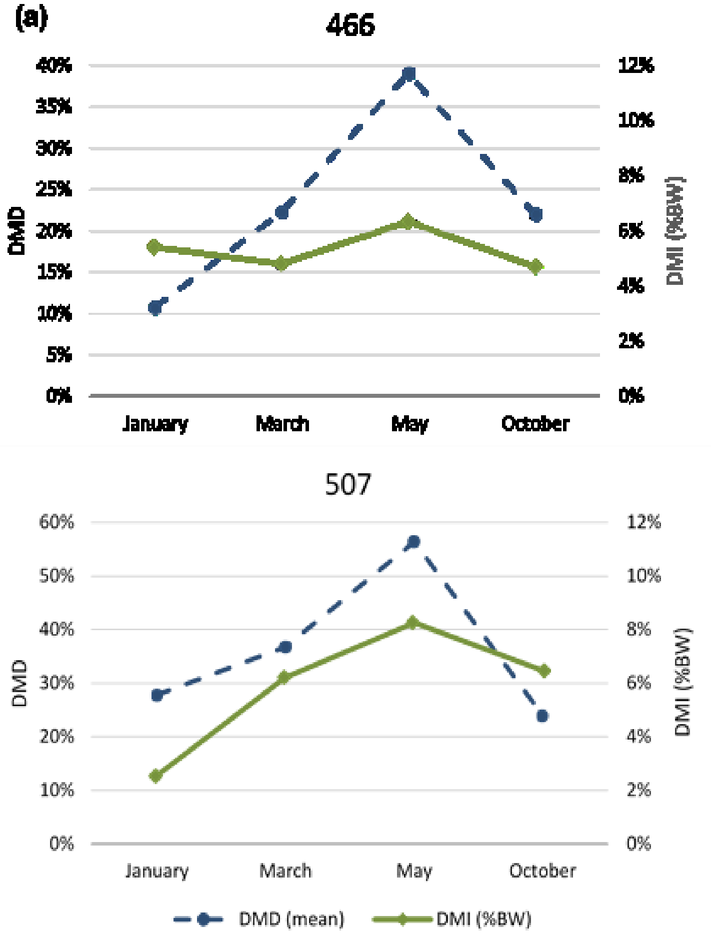


Table 5.1— Nutrient composition of bamboo diets consumed by two giant pandas (male, 466; female, 507) over four months (January, March, May, October).

Month	Animal ID	Nutrient Concentration (% DM)					
		OM	NDF	ADF	HC	CP	Starch
January	466	98.7%	85.9%	30.6%	55.3%	7.3%	17.9%
	507	93.8%	86.0%	30.2%	55.8%	8.6%	14.0%
March	466	97.8%	83.7%	47.1%	36.6%	8.1%	22.2%
	507	98.2%	85.3%	50.2%	35.1%	3.7%	16.4%
May	466	96.3%	77.9%	50.1%	27.8%	5.6%	4.2%
	507	97.2%	79.5%	56.3%	23.1%	3.9%	2.8%
October	466	96.5%	82.3%	53.0%	29.2%	9.2%	6.8%
	507	91.3%	76.4%	46.8%	29.6%	14.6%	4.5%

Table 5.2— Individual animal and mean apparent digestibilities of dry matter (DM), organic matter (OM), gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HC), starch, and crude protein (CP) by two giant pandas at the Memphis Zoo (466 and 507). Nutrient digestibilities were recorded over four months (January, March, May, and October). Superscripts indicate Student's T categorization of apparent digestibility values that displayed significant differences among months ($P < 0.05$).

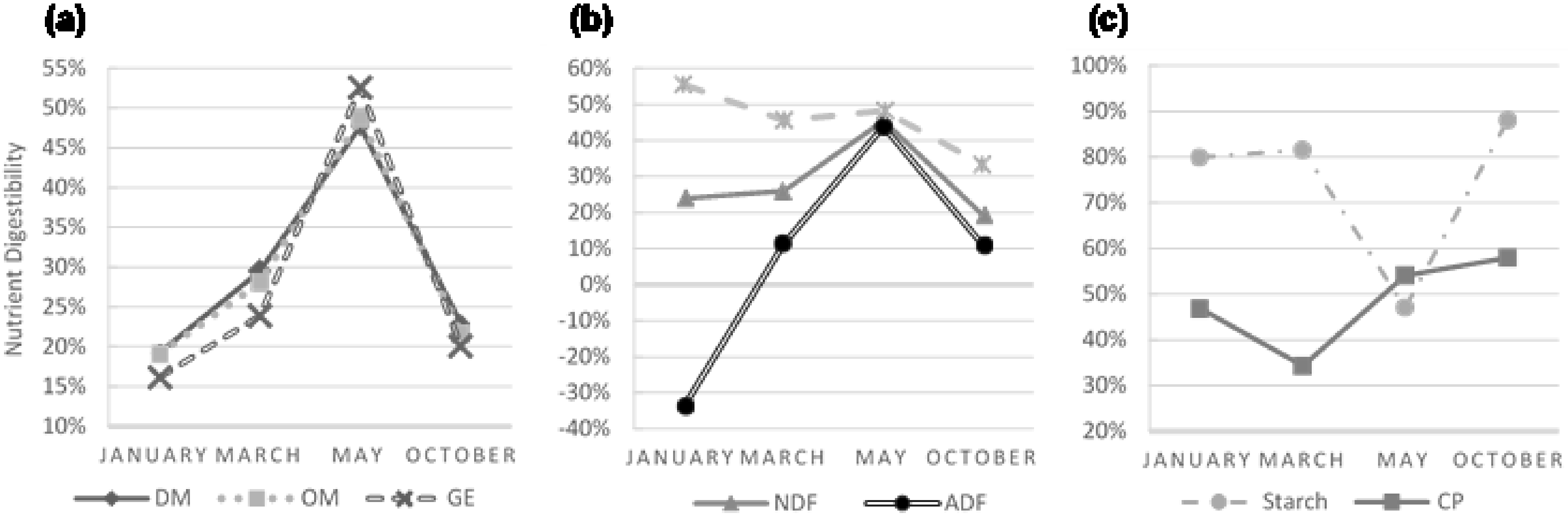
Trial	Animal ID	Nutrient digestibility (%)							
		DM	OM	NDF	ADF	HC	Starch	CP	GE
January	466	10.7%	12.2%	15.3%	-50.2%	51.5%	80.5%	39.0%	11.6%
	507	27.7%	25.9%	32.5%	-17.3%	59.5%	79.4%	54.6%	20.7%
	Mean	19.2%^{a}	19.1%^{a}	23.9%	-33.7%^{a}	55.5%^{a}	80.0%^{a}	46.8%	16.1%^{a}
March	466	22.3%	22.7%	18.9%	0.3%	42.8%	79.0%	61.8%	19.7%
	507	36.8%	33.4%	33.1%	22.4%	48.3%	84.2%	6.8%	28.1%
	Mean	29.5%^{a}	28.1%^{a}	26.0%	11.3%^{b}	45.6%^{b}	81.6%^{a}	34.3%	23.9%^{a}
May	466	39.0%	40.0%	35.6%	31.9%	42.3%	52.0%	51.1%	44.1%
	507	56.4%	57.4%	55.3%	55.7%	54.1%	42.1%	57.1%	61.0%
	Mean	47.7%^{b}	48.7%^{b}	45.4%	43.8%^{c}	48.2%^{ab}	47.1%^{b}	54.1%	52.5%^{b}
October	466	22.0%	21.8%	18.2%	9.7%	33.7%	82.9%	56.5%	20.0%
	507	23.9%	21.5%	20.1%	12.1%	32.9%	93.3%	59.4%	20.1%
	Mean	22.9%^{a}	21.7%^{a}	19.2%	10.9%^{b}	33.3%^{c}	88.1%^{a}	58.0%	20.1%^{a}
	P	0.036	0.033	0.056	0.013	0.035	0.014	0.696	0.014
	SE	7.1	6.1	7.5	11.6	3.8	3.8	14.4	5.2

In general, DM, OM, ADF, and GE digestibilities were greater in March relative to January (Table 5.3; Figure 5.2). Acid detergent fiber (ADF) digestibility was significantly greater during March than in January, and significantly less in March than May ($P = 0.01$; Table 5.3). However, ADF digestibility ranged from -50% to 56% across all observations, leading to a high degree of error (SE = 12%). Crude protein (CP) digestibility was lowest during March, however, CP digestibility estimates were also variable and not considered significant different among trials ($P = 0.7$; SE = 14%; Table 5.3; Figure 5.2).

For both animals, bamboo DMD was significantly higher during May than in all other months (47.7%; $P = 0.04$; Table 5.3; Figure 5.2). Digestibilities of OM, NDF, ADF, and GE also peaked at this time, with changes in OM, ADF, and GE considered significant ($P < 0.05$). On the other hand, starch was significantly less digestible during May than all other trials (47.1%; $p = 0.01$).

Digestibilities of DM, OM, NDF, starch, CP, and GE in October were similar to those observed during January and March (Table 5.3; Figure 5.2). Hemicellulose digestibility in October was significantly lower than all other trials ($p = 0.04$; LSM October: 33.3%).

Figure 5.2—Mean apparent nutrient digestibilities of dry matter (DM), organic matter (OM), gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HC), starch, and crude protein (CP) by giant pandas at the Memphis Zoo. Apparent digestibilities were recorded over four months (January, March, May, October). Significant differences in apparent digestibility across trials were observed for DM (*P* = 0.04), OM (*P* = 0.03), GE (*P* = 0.01), ADF (*P* = 0.01), HC (*P* = 0.04), and starch (*P* = 0.01).



Discussion

Earliest reports of giant panda ecology described seasonal transitions in bamboo plant part selection, which have since been confirmed in numerous groups of free-ranging and captive giant pandas (Hansen et al. 2010; Long et al. 2004; Schaller et al. 1985). Recently, compositional changes in the giant panda's intestinal microbiota were found to accompany these dietary shifts (Williams et al. 2013). Our aim was to examine a different physiological response to changes in plant part selection: that is, its impact on digestion of key energy-providing nutrients.

Monthly plant part selection by giant pandas has been an ongoing study at the Memphis Zoo since the animals' arrival in 2003. In general, historical foraging behavior data spanning 2003 – 2013 depicted a recurring pattern of leaf selection during the late summer through autumn, culm selection during late winter through spring, and preferential consumption of bamboo shoots when offered during late spring and early summer (Williams et al. 2012; Hansen et al. 2010). We timed our trials accordingly, so as to capture digestibility data during at least one of each culm-consumption, shoot-consumption, and leaf-consumption seasons. Plant part and nutrient selection by giant pandas in the present study was discussed in the previous chapter (Ch. IV). Feeding trials were categorized into feeding seasons by the predominant plant part consumed, with January and March falling within culm-consumption season, May being shoot-consumption season, and October occurring in leaf-consumption season, though October leaf intake levels were lower than historical values for both animals (Figure 4.6). Nevertheless, changes in plant part composition of diet incurred changes of in diet nutrient composition between trials, with culm-consumption season diets highest in starch and leaf-based diets highest in CP (relative to concentrations of other trials; Table 5.2). Interestingly, shoot selection did not appear to influence diet composition relative to the nutrients we examined; though both giant pandas exhibited greatest DMI during the May trial. Upon investigation of nutrient digestibility, we found that digestion of DM, OM, NDF, ADF, and most notably, GE were considerably higher at this time (Figure 5.2). This increase in digestibility was expected, and indicates differences in the composition of young bamboo shoots compared to mature culm and leaves (Christian et al. 2015; Liu et al. 2015). In general, digestibilities of OM, NDF, ADF, and GE reflected trends in overall DM digestibility, as plant structural carbohydrates and lignin (which together comprise dietary fiber) constituted over 80% of mature bamboo dry biomass offered to giant pandas. While dietary fiber is energy-bearing, giant pandas lack necessary enzymes to degrade its complex structures, and thus cannot utilize the majority of GE in bamboo. However, bamboo shoots have undeveloped cell walls, and consequently lower concentrations of plant structural carbohydrates, while lignin is virtually nonexistent. The majority of dietary fiber present in bamboo shoots was hemicellulose (33% of total shoot DM; Figure 4.4.c), which is known to be partially digestible by giant pandas (Dierenfeld et al. 1982; Long et al. 2004; Sims et al. 2007). Furthermore, bamboo shoots elongate at a rapid rate, and accordingly are concentrated in readily mobilizable simple sugars required to promote plant growth (Liese and Weiner 1996; Thammawong et al. 2009). Though we did not analyze specifically for specific simple sugars, these carbohydrates likely caused overall shoot GE to be more digestible than GE of culm- or leaf-based diets. Digestible protein also contributed to GE digestibility at this time, as CP digestion was increased relative to the trials occurring in culm-consumption season (54% in May vs. 47% and 34% in January and March, respectively; Figure 5.2.). Protein in plant cells of bamboo shoots would be more highly bioavailable to giant pandas, as opposed to mature culm, due to the decreased indigestible fiber content associated with the cell wall. Previously, we noted that bamboo shoot emergence in late spring coincides with the time of mono-estrous and early gestation in the female giant panda (Ch. IV; Christian et al. 2015). High levels of DMI, and corresponding digestible, energy intake during shoot-consumption season resonates with the period of hyperphagia preceding denning and parturition observed in other ursid species (Brody and Pelton 1988; Elowe and Dodge 1989; Lopez-Alfaro et al. 2013; Nelson et al. 1983). Although giant pandas do not exhibit hibernation (as it pertains to other ursids), they are known to spend an increased amount of time resting, while time foraging or engaging in activity is reduced, during the summer (Hansen et al. 2010; Mainka and Zhang 1994; Schaller et al. 1985). It is likely that excessive bamboo shoot intake during late spring and early summer provides the basis of energy required to sustain pregnancy and induce embryo implantation in female giant pandas.

Though digestive efficiencies for DM and GE were low ($< 30\%$) in culm- and leaf-consumption seasons, starch was apparently highly utilized, with digestibilities $\approx 80\%$ in all but the May trial. This is consistent with the giant pandas' high level of starch selection during those same trials, and lack of selection during shoot season. It is possible that starch digestibility was underestimated in May, as starch digestibility was still relatively high (compared to digestibilities of structural carbohydrates), and shoots contained negligible concentrations of starch which would introduce error when determining nutrient intake and excretion. Nevertheless, this high degree of digestibility further suggests that starch content influences selection and utilization of bamboo by giant pandas.

Throughout all four trials, hemicellulose was more digestible than other fractions of dietary fiber (NDF and ADF; Figure 5.2). This was expected based on previous reports of hemicellulose disappearance in giant pandas, though hemicellulose digestibility in the present study was greater than values reported in the literature (Present study: 33% - 56% digestible, vs literature: ≈ 20% digestible; Table 5.1). In bamboo, hemicellulose is comprised mainly of branched glucose and xylose polysaccharides, which are resistant to enzymatic digestion in the mammalian small intestine. However, several studies have identified fecal microflora unique to the giant panda which may aid in fiber degradation in their large intestine (Hirayama et al. 1989; Zhu et al. 2011; Fang et al. 2012; Williams et al. 2012). As gut microorganisms digest structural carbohydrates, volatile fatty acids are produced and absorbed by the host to be used as metabolic substrates, namely energy sources for epithelial cells (Case et al. 2000; Van Soest 1996). While the giant panda gut microbiome has only recently begun to be investigated, it is very probable that gastrointestinal health is linked to this symbiotic relationship between microorganisms and host. In multiple reports, instances of mucoid feces and apparent discomfort occur in giant pandas consuming low levels of fiber, or experiencing an overall change in diet composition (Mainka et al. 1989; Williams et al. 2016). The association between digestible fiber and gut health, as well as heightened digestibility of hemicellulose, are in keeping with the apparent selection for diets of increased hemicellulose concentrations discussed in the preceding chapter (Figure 4.1).

Previous reports of protein digestibility by giant pandas range from relatively low (30% [Sims et al. 2007]) to near complete disappearance (90% [Dierenfeld et al. 1982]). Digestibility values from our feeding trials fell intermediate to reported values, ranging from 34% in March to 58% in October. Variance in CP digestibility among feeding trials could be attributed to low initial concentrations of CP in offered and rejected bamboo, which likely incited the high degree of error associated with mean CP digestibility LSM values (Table 5.2). Individual giant panda foraging preferences likely also resulted in variability relating to CP selection and utilization. While overall trends in plant part selection across trials were similar between animals, individual incorporation of bamboo leaves and shoots, the plant parts most concentrated in CP, were different between animals (Figure 4.3). However, the general trends in CP digestibility observed between trials are plausible when considering the corresponding shifts in plant part selection. Maximum CP digestibility occurred during May and October, when both giant pandas integrated bamboo shoots or leaves into their diets. Compared to culm, shoots and leaves are greatly reduced in indigestible cellulose and lignin (ADF during May and October ≈ 66%, 42%, and 37% for culm, shoot, and leaf, respectively; Table 4.1). Lower concentrations indigestible dietary fiber would allow intracellular proteins, as well as proteins associated with the cell wall structure, to be more bioavailable to the giant panda, resulting in increased CP digestibility. Additionally, increased CP digestibility during shoot-consumption and leaf-consumption seasons is reflects selection of bamboo plant parts based the availability of digestible sources of energy, rather than fluxes of GE.

Previously, we determined that giant pandas selected bamboo diets based on fluctuations of digestible energy, namely starch and hemicellulose, in different plant parts, which consequently caused shifts in plant part preference (Ch. IV). Despite low overall bamboo digestibility by giant pandas, digestion of key energy-bearing nutrients reflected nutrient selection tendencies. Rather than select bamboo to formulate a diet that remains consistent in nutrient balance year-round, giant pandas appear to seek out different nutrients at times and in plant parts when they are either abundant or digestible. Our data suggests that giant pandas prioritize digestible energy intake in order to thrive on a near-exclusive diet of bamboo. If this interpretation of giant panda foraging strategy is correct, it falls in accordance with the many adaptations for energy management and conservation that define this species' unique biology. Giant panda activity patterns are largely characterized by behaviors which reduce energy expenditure. For instance, studies of giant panda foraging habits describe animals reclining against a hill or tree during bouts of bamboo consumption, preferring to forage on gently-sloped terrain, and consuming large amounts of bamboo in one sitting (Hansen et al. 2010; Long et al. 2004; Schaller et al. 1985; Wei et al. 2015). Morphological features specific to giant pandas, such as an elongated "pseudo-thumb" and prominent zygomatic arches, are centered upon improving the efficiency of bamboo intake (Schaller et al. 1985). Recently, Nie et al. reported daily energy expenditure of giant pandas to be approximately 38% of what was expected in terrestrial mammals of similar size (2015). In the same study, a species-unique genetic mutation was identified and linked to circulation of thyroid hormones T_3 and T_4 at levels lower than those found in hibernating black bears, thereby suppressing metabolic activity (Nie et al. 2015). Seasonal plant part selection and nutrient utilization could be considered an additional adaptation developed by the giant panda to meet energetic requirements on a largely indigestible diet.

Bamboo was once an abundant and widespread resource in the giant panda's historical range of southeastern China, providing the quantity and flexibility of diet necessary to sustain a stable giant panda population (Lindburg and Baragona 2004). The relatively recent and rapid decline in giant panda numbers is largely attributed to habitat reduction and fragmentation. However, there have been significant efforts to preserve and restore areas of giant panda habitation, such as China's "Grain to Green" reforestation program and a commercial logging ban covering giant panda habitats (Lindburg and Baragona 2004; Swaisgood et al. 2011). Future conservation activities would undoubtedly benefit from a deeper knowledge regarding utilization of bamboo as a feed resource by giant pandas, and its implications for species' nutritional requirements, migration patterns, and habitat carrying capacity (Swaisgood et al. 2011). Our data supplements available information regarding giant panda nutritional ecology by relating foraging decisions to metabolic processes. We propose that the basis of giant panda foraging strategy is maximization of digestible energy intake, often from carbohydrate sources, which has applications in the aforementioned areas of giant panda conservation, in addition to management of animals in captive scenarios.

CHAPTER VI

CONCLUSIONS

Bamboo was once an abundant and widespread resource in the giant panda's historical range of southeastern China, providing the quantity and flexibility of diet necessary to sustain a stable giant panda population (Lindburg and Baragona 2004). The relatively recent and rapid decline in giant panda numbers is largely attributed to habitat reduction and fragmentation. However, there have been significant efforts to preserve and restore areas of giant panda habitation, such as China's "Grain to Green" reforestation program and a commercial logging ban covering giant panda habitats (Lindburg and Baragona 2004; Swaisgood *et al.* 2011). Future conservation activities would undoubtedly benefit from a deeper knowledge regarding utilization of bamboo as a feed resource by giant pandas, and its implications for species' nutritional requirements, migration patterns, and habitat carrying capacity (Swaisgood *et al.* 2011).

Conclusions from Ch. II and III of this report are meant to aid and standardize the planning of future feeding trials with giant pandas, and identify scenarios where different methods of examining giant panda intake and digestibility are justified. We determined that in giant pandas, day-to-day DMI varies broadly while fecal output remains relatively consistent. Thus, a period longer than three days is necessary to evaluate bamboo digestibility in giant pandas. The mean DMI taken over a 17-day period best represented bamboo intake, and predicted reliable DMD values. The internal dietary markers ADL, AIA, and ADIA could not accurately calculate DMI and DMD in the two giant pandas, likely due to difficulty quantifying the intake of each marker. Describing plant part selection by giant pandas is also difficult, as differences in water content and bamboo intake rate between bamboo plant parts preclude behavioral observations from accurately predicting actual dry matter consumed. Image analysis for green pixilation in giant panda feces was highly correlated to leaf dry matter intake for two giant pandas (9 observations), and has potential field applications as a non-invasive, simple procedure to evaluate giant panda feeding behavior.

After a reliable estimation of bamboo intake was estimated, we conducted an investigation of seasonal variances in digestibility and utilization of macronutrients, with an emphasis on energy, for captive giant pandas consuming a bamboo-based diet (>95%). Chapters IV and V aim to tie together available data concerning bamboo utilization by the giant panda, while also examining whether varying nutrients in bamboo culms and leaves impact dietary shifting behaviors. Despite apparent higher nutritive value and likely digestibility of leaves, culm was the predominant plant part consumed by giant pandas during the majority of trials. Maximum bamboo culm intake coincided with times of high starch concentration in culm. However, starch and hemicellulose content in culm was relatively low during October, when both giant pandas incorporated considerable amounts of leaves into their diets. Bamboo shoots were preferentially consumed when offered during May, likely due to their reduced proportion of plant part structural compounds (ADF and NDF) and increased CP content. Giant pandas have a very limited ability to utilize diets primarily comprised of mature bamboo culm and leaves, with total dry matter digestibility being less than 30%. Though the majority of bamboo dry biomass are plant-structural compounds (fiber), giant pandas can apparently access and utilize non-fiber nutrients, such as starch, to a greater extent. When bamboo shoots were included in the diet, giant pandas achieved higher digestion rates for DM, OM, NDF, ADF, HC, and GE. Rather than select bamboo to formulate a diet that remains consistent in nutrient balance year-round, giant pandas appear to seek out different nutrients at times and in plant parts when they are either abundant or digestible. Our data suggests that the basis of giant panda foraging strategy is maximization of digestible energy intake, often from carbohydrate sources, which has applications in the aforementioned areas of giant panda conservation, in addition to management of animals in captive scenarios.

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APPENDIX A

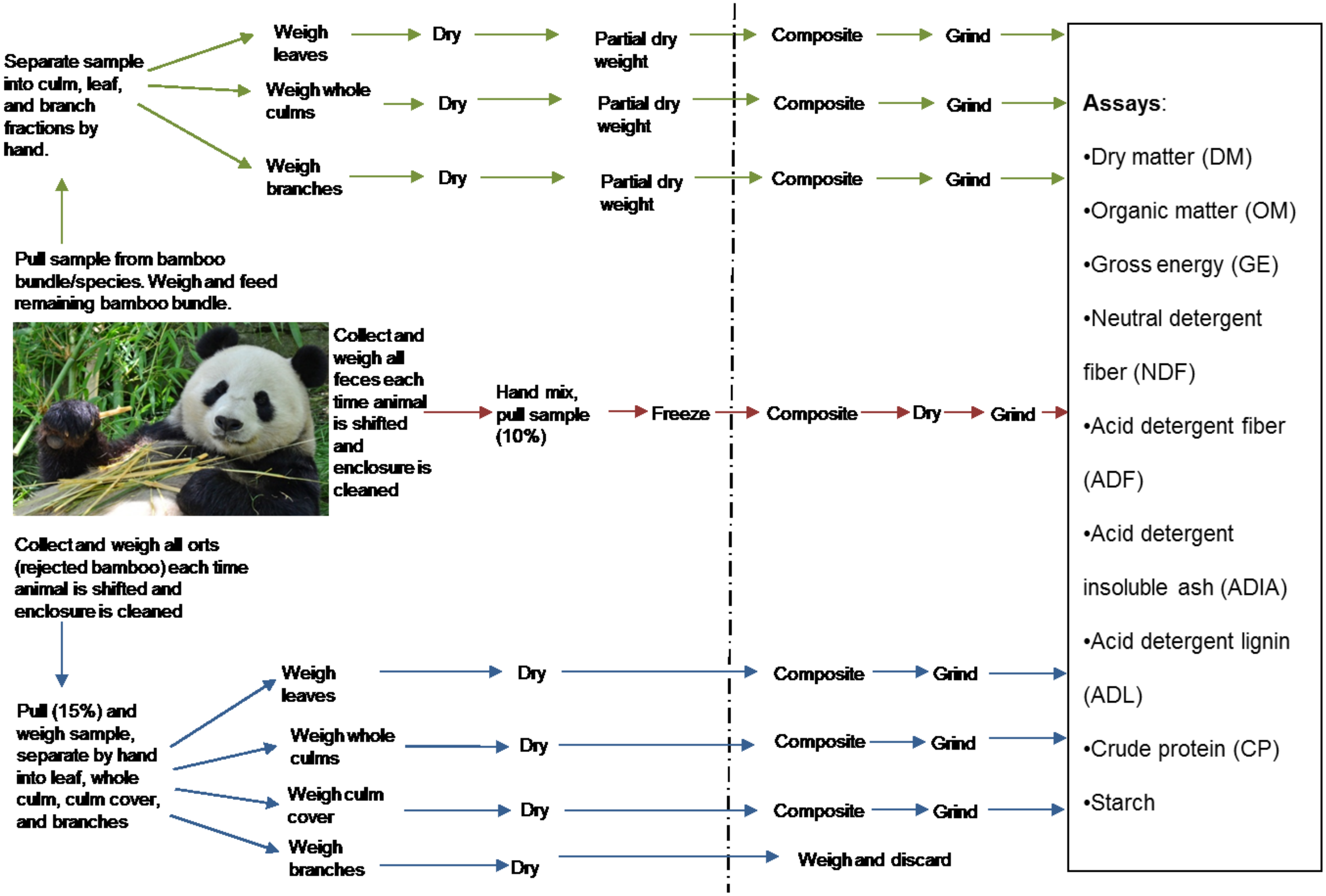
NOMENCLATURE

ADF	Acid detergent fiber
ADIA	Acid detergent insoluble ash
ADL	Acid detergent lignin
AH-fat	Acid-hydrolyzed fat
AIA	Acid insoluble ash
BW	Body weight
Cell.	Cellulose
CF	Crude fiber
CP	Crude protein
DE	Digestible energy
DFO	Daily fecal output
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
EE	Ether extract
GE	Gross energy
GIT	Gastrointestinal
HC	Hemicellulose
LSM	Least squares means
MBW	Metabolic body weight

MRT	Mean retention time
NDF	Neutral detergent fiber
NFE	Nitrogen-free extract
NRC	National Research Council
NSC	Non-structural carbohydrates
OM	Organic matter
P	*Phyllostachys*
PDM	Partial dry matter
RPI	Relative preference index
SD	Standard deviation
TDF	Total dietary fiber
USFWS	United States Fish and Wildlife Service
VFA	Volatile fatty acids

APPENDIX B

BAMBOO SAMPLING DESIGN



APPENDIX C

INDIVIDUAL TRIAL SUMMARIES

1. *Feeding Trial 1 (7/21/14 - 7/23/14)*

   Diet sample collection began the evening of 7/21 with den loading for overnight, and ended early afternoon of 7/23, lasting approximately 48 hours. Fecal and ort collections began the morning of 7/22, and continued until approximately 6 hours following the last diet sampling on 7/23. The giant pandas were offered four species of bamboo*: Pseudosasa japonica, Phyllostachys (P.) aureosulcata, P. nuda,* and *P. bissetii.* Supplemental fruits (apples, bananas, grapes), hard-boiled eggs, leafeater biscuits (Mazuri, Marion), sugarcane, and an in-house prepared "panda bread," were also given to the bears, although they constituted less than 4% of the total diet. Bamboo bundles weighed approximately 5 to 9 kg, and giant pandas were provided fresh bamboo 4 to 6 times per day, at based on apparent animal interest in eating as determined by zookeepers. From visual examination of feces, the male bear consumed both culm and leaves, while the female consumed primarily culm. Male and female body weights, taken on the second day of the trial (7/22/14), were 107.3 and 105 kg, respectively. At this time, the female subject was potentially gestating, though ultimately losing the fetus, or experiencing pseudopregnancy, in which she was exhibiting the hormonal and behavioral changes associated with gestation. As such, the female bear was allowed access to a maternity den and displayed nesting behaviors.

2. *Feeding Trial 2 (1/3/15 – 1/5/15)*

Bamboo species offered during Digestibility Trial 2 was primarily *P. aureosulcata*, although the male giant panda also received *P. bissetii* and *Pseudosasa japonica*. Supplements provided (Mazuri leafeater biscuit, apple, grape, sugarcane, panda bread, hard-boiled egg) were less than 2% of the total diet offered for both bears. The female bear was experiencing a second rise in progesterone, indicative of the start of an estrous cycle, which was believed to cause reduced intake and increased time spent resting. Visual examination of fecal material and periodic behavioral assessments indicated that both bears consumed primarily bamboo culm during the trial. Average male and female body weights, taken all three days of the trial, were 105 and 84 kg, respectively.

3. *Feeding Trial 3 (3/23/15 – 3/25/15)*

   Giant pandas were offered four species of bamboo*: Pseudosasa japonica, Phyllostachys (P.) aureosulcata, P. nuda,* and *P. bissetii*, with *P. aureosulcata* and *P. bissetii* being the most abundantly provided to both bears. Supplements (Mazuri leafeater biscuit, apple, grape, hard-boiled egg) comprised less than 2% of the offered diets, and less than 5% of the consumed diets for both bears. Visual examination of fecal material and periodic behavioral observations indicated that both bears consumed primarily bamboo culm during the trial. Average male and female body weights, taken all three days of the trial, were 109 and 86 kg, respectively.

4. *Feeding Trial 4 (5/11/15 – 5/13/15)*

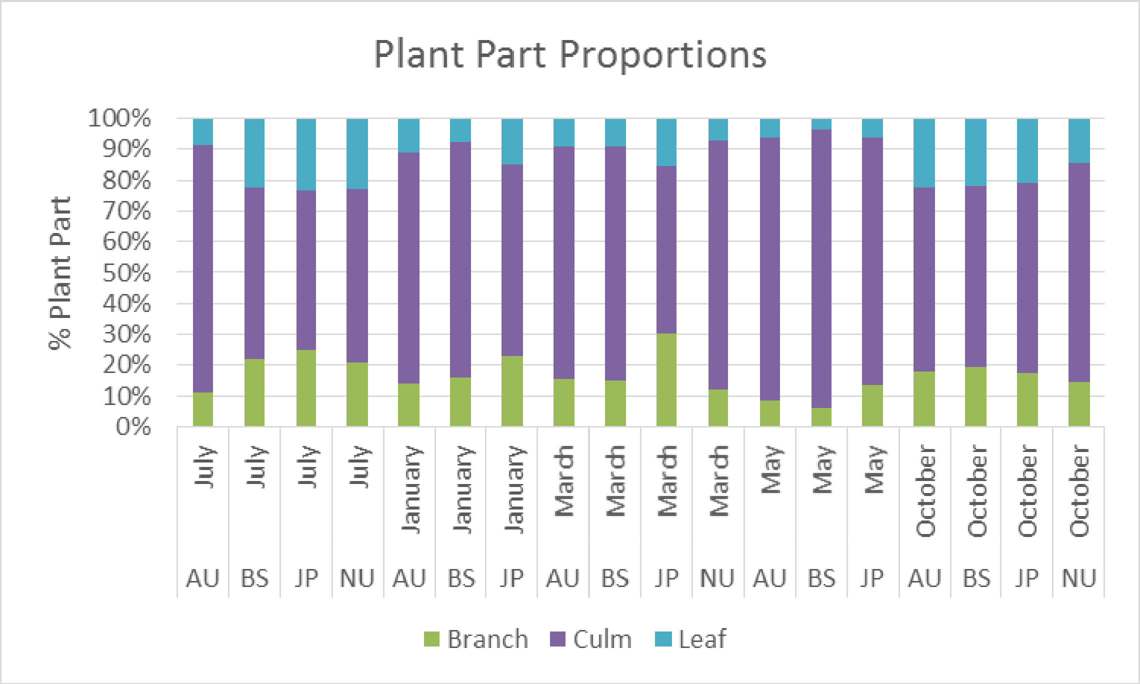
   Giant pandas were offered *Pseudosasa japonica, Phyllostachys (P.) aureosulcata, P.* and *P. bissetii* bamboo, as well as bamboo shoots of varying sizes and species collected. Bamboo shoots constituted 35% and 43% of the diets (fresh weight) for the male and female, respectively, and were almost completely consumed. Both bears were also frequently provided large, branchless culms, and leaf consumption was not observed. At this time, it also was noted that leaves on offered bamboo were very small and had high water content. Average male and female body weights were 113 and 90 kg, respectively. Following the feeding trial, an additional, brief culm-rejection trial was performed with the male, however he showed no interest in any of the offered culms. The female bear was artificially inseminated two weeks prior to the start of the trial, and may have been experiencing early gestation.

5. *Feeding Trial 5 (10/27/15 – 10/30/15)*

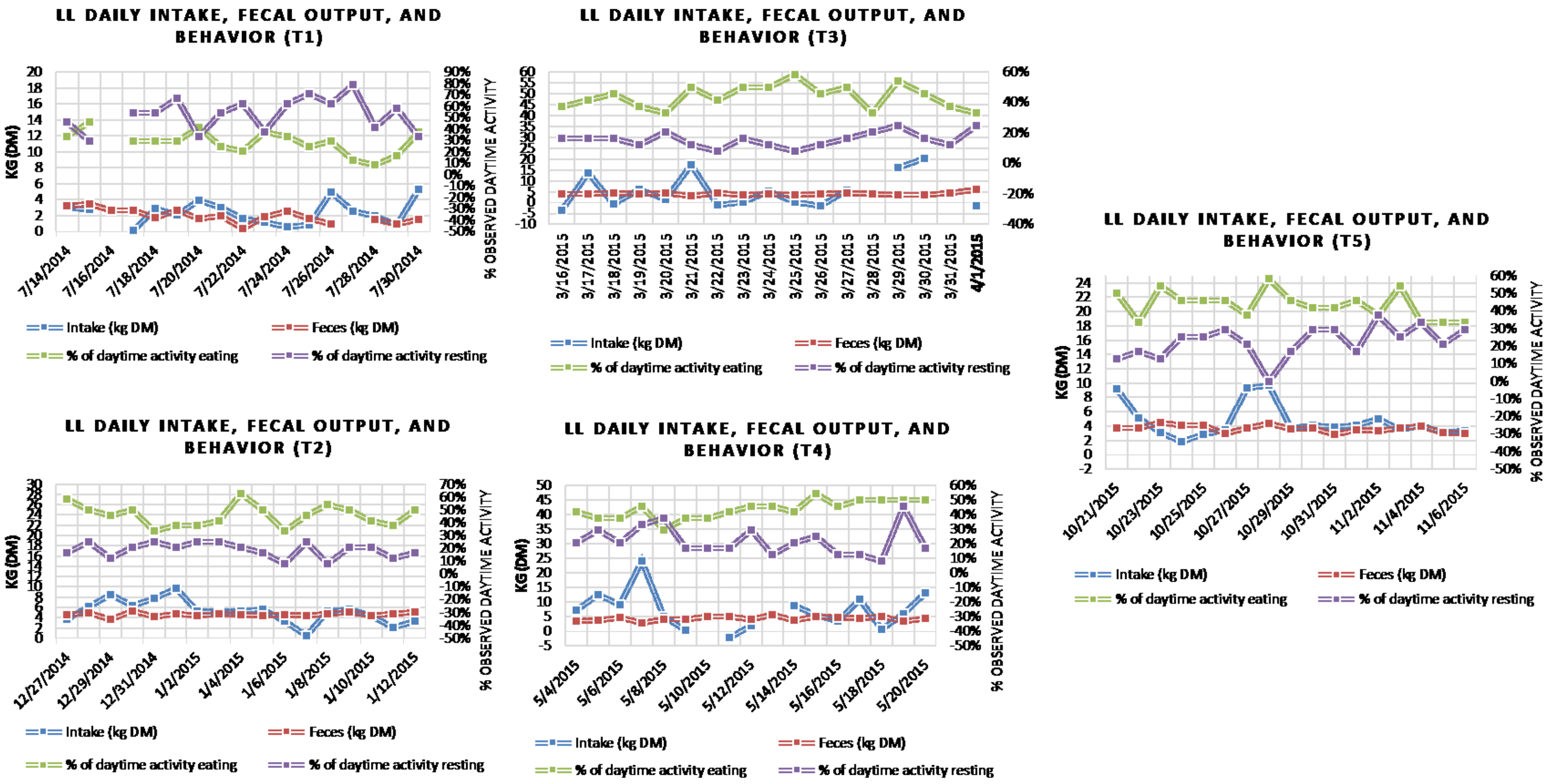
   Trial 5 was extended compared to the previous trials, and lasted approximately 72 hours. At the beginning and end of diet collection, whole corn kernels were fed to the male bear to measure gastrointestinal passage rate. Total orts and fecal collection began after the passage of corn kernels fed at the beginning of the trial, and ended after the passage of corn kernels consumed at the final diet collection. The average of the two gastrointestinal transit trials for the male bear was 5 hrs and 47 min. In addition, complete bamboo diet and orts samples were taken and processed as whole bamboo through chipper shredder to calculate dry matter. The female bear was eating leaves and culm, and passed a mucoid in the weeks prior to the beginning of the trial. The male bear had shifted to eating primarily culm, and had two mucoid fecal instances during the trial with the passage of the corn kernels. Bamboo was always offered as a mixed bundle of culms with branches and leaves, and the following species were provided: *Pseudosasa japonica, Phyllostachys (P.) aureosulcata, P. nuda,* and *P. bissetii*. Average male and female body weights were 99 and 81 kg, respectively.

C.1—Bamboo species composition of diets of diets offered to the male (a) and female (b) giant panda during each trial.

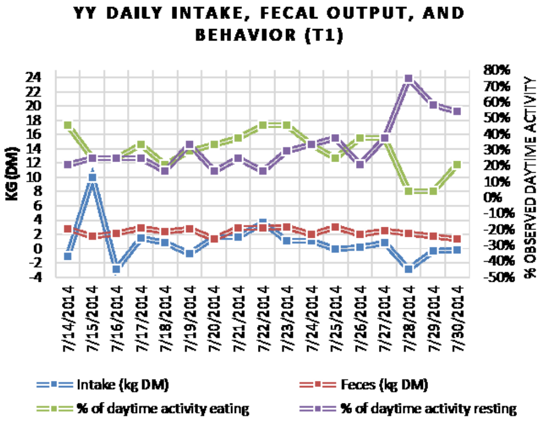
C.2— Average proportions of bamboo plant parts of species offered to giant pandas at the Memphis Zoo over five feeding trials. *Phyllostachys nuda* (NU) was only fed in T1, T3, and T5. Proportions presented as percent of total bamboo sample on a PDM basis.

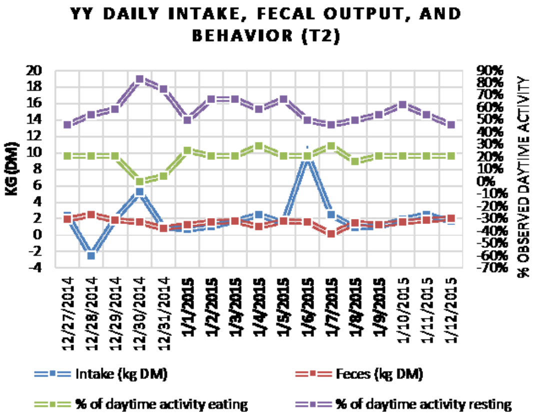


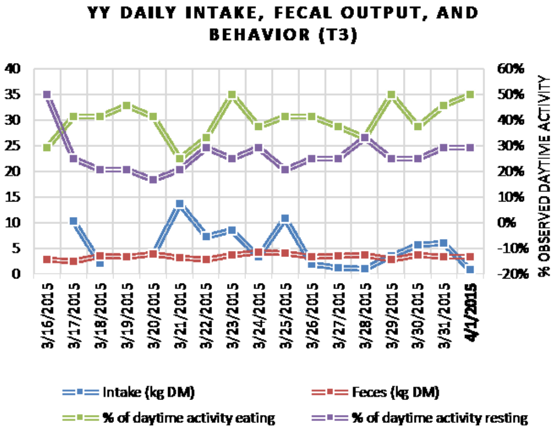
C.3—Daily dry matter intake (DMI), fecal output (DFO), daytime eating activity, and daytime resting activity for the male (LL, 466) and female (YY, 507) adapted from keeper records taken over 17 days

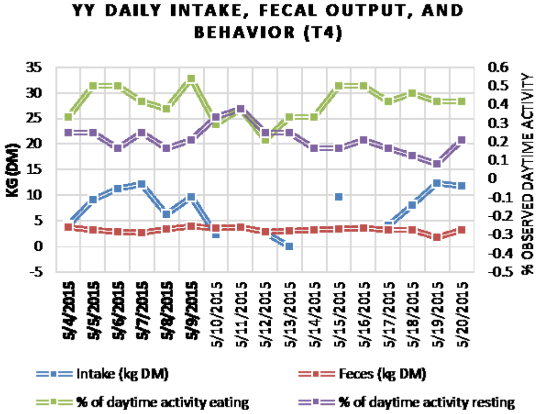


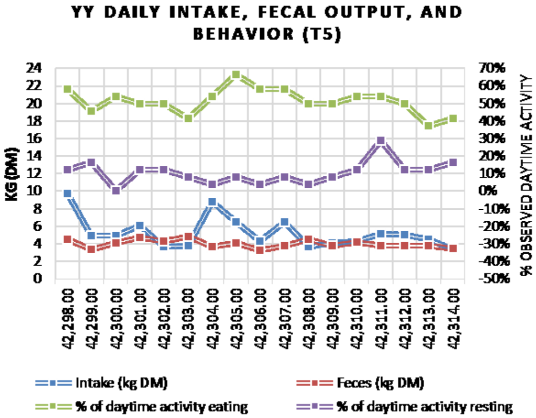
C.3—continued (YY, 507)











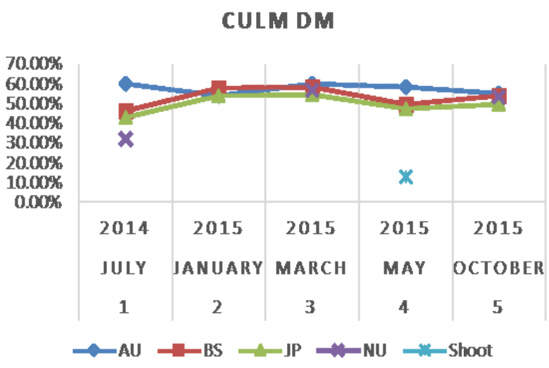
C.4— Individual animal relative preference index (RPI)

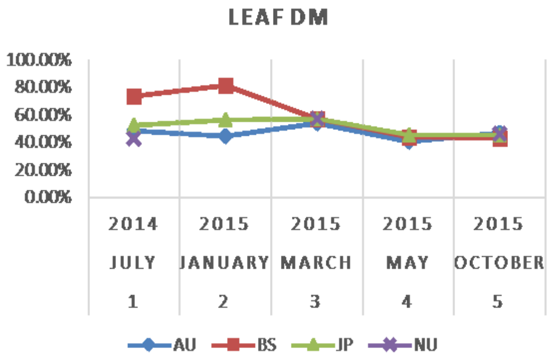
Month	Animal	Bamboo	Nutrient Concentration (% DM)						
			OM	NDF	ADF	HC	ADL	CP	Starch
January	466	Offered	96.85%	83.54%	55.23%	28.31%	19.19%	6.22%	5.53%
		Consumed	98.73%	85.88%	30.62%	55.26%	35.58%	7.32%	17.90%
		RPI	1.02	1.03	0.55	1.95	1.85	1.18	3.23
	507	Offered	96.78%	83.13%	54.83%	28.30%	19.82%	6.15%	5.68%
		Consumed	93.77%	86.02%	30.18%	55.83%	29.24%	8.60%	14.02%
		RPI	0.97	1.03	0.55	1.97	1.48	1.40	2.47
March	466	Offered	96.47%	82.56%	55.22%	27.35%	12.76%	6.47%	7.76%
		Consumed	97.79%	83.71%	47.12%	36.59%	7.23%	8.14%	22.23%
		RPI	1.01	1.01	0.85	1.34	0.57	1.26	2.86
	507	Offered	96.66%	82.90%	56.03%	26.86%	12.94%	5.86%	8.19%
		Consumed	98.20%	85.32%	50.23%	35.09%	11.95%	3.73%	16.39%
		RPI	1.02	1.03	0.90	1.31	0.92	0.64	2.00
May	466	Offered	96.62%	83.50%	58.91%	24.59%	14.00%	4.42%	4.03%
		Consumed	96.31%	77.91%	50.08%	27.83%	10.12%	5.61%	4.20%
		RPI	1.00	0.93	0.85	1.13	0.72	1.27	1.04
	507	Offered	96.14%	82.54%	57.44%	25.11%	13.16%	5.16%	3.65%
		Consumed	97.20%	79.46%	56.34%	23.12%	15.79%	3.86%	2.79%
		RPI	1.01	0.96	0.98	0.92	1.20	0.75	0.77
July	466	Offered	95.91%	81.28%	54.33%	26.94%	15.88%	5.23%	2.61%
		Consumed	96.36%	65.93%	31.27%	34.66%	2.68%	3.53%	9.31%
		RPI	1.00	0.81	0.58	1.29	0.17	0.67	3.57
October	466	Offered	95.76%	84.12%	59.63%	24.48%	13.41%	6.99%	2.28%
		Consumed	96.49%	82.27%	53.03%	29.24%	12.25%	9.15%	6.83%
		RPI	1.01	0.98	0.89	1.19	0.91	1.31	2.99
	507	Offered	95.40%	83.59%	59.00%	24.59%	13.27%	7.39%	1.94%
		Consumed	91.28%	76.40%	46.80%	29.60%	9.18%	14.64%	4.52%
		RPI	0.96	0.91	0.79	1.20	0.69	1.98	2.33

APPENDIX D

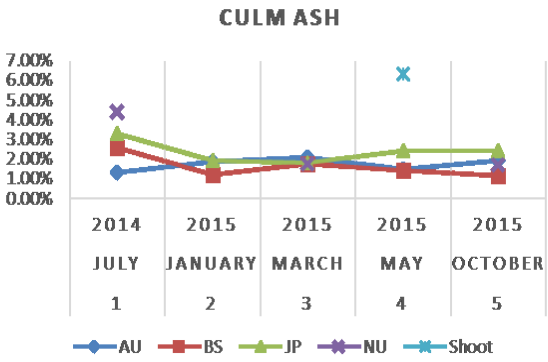
NUTRIENT CONCENTRATIONS OF BAMBOO PLANT PARTS BY SPECIES

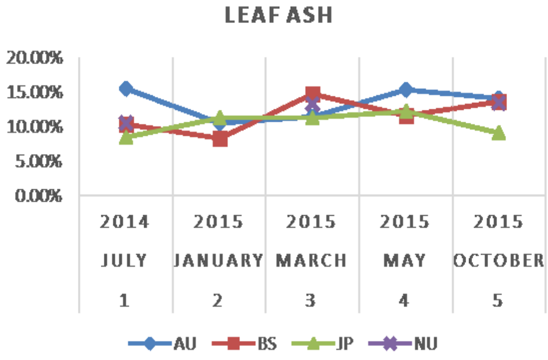
D.1— Dry matter (DM)



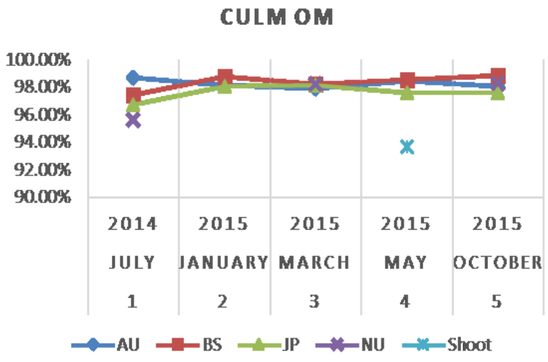


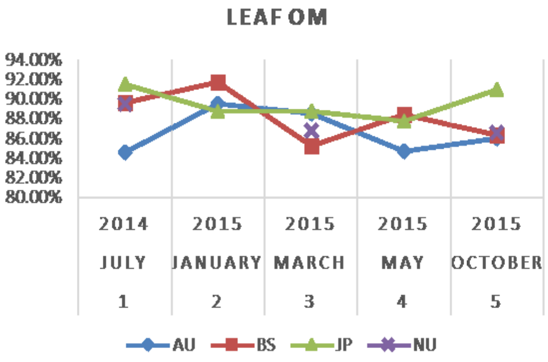
D.2— Ash



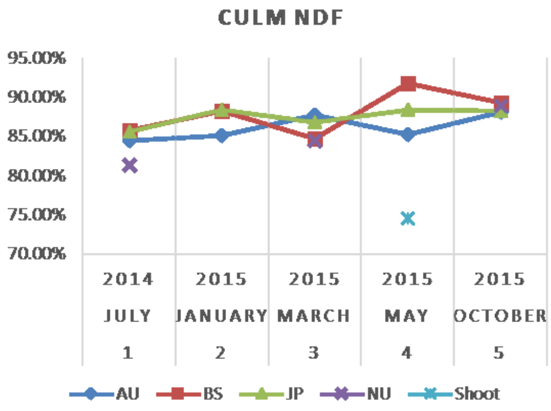


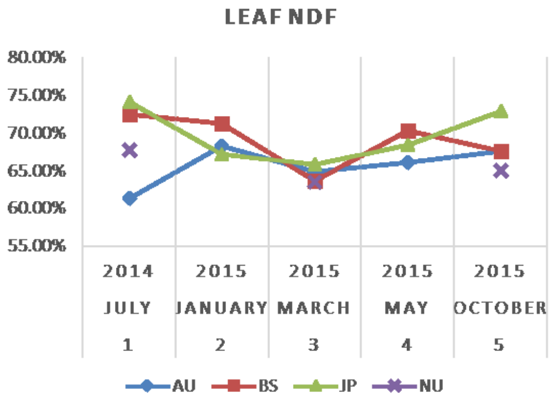
D.3— Organic matter (OM)



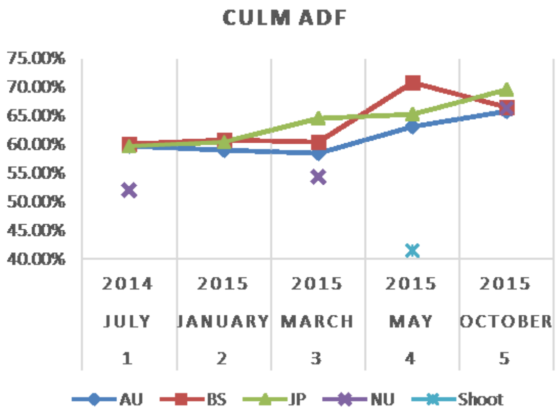


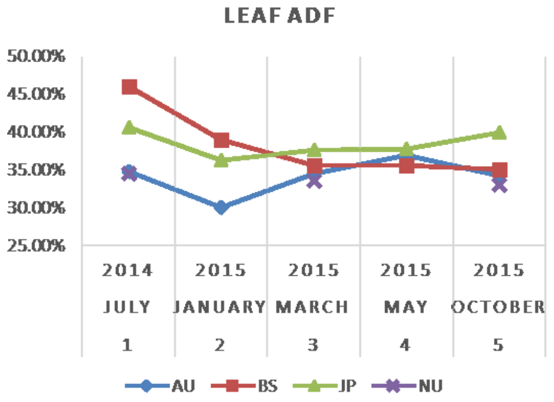
D.4— Neutral detergent fiber (NDF)



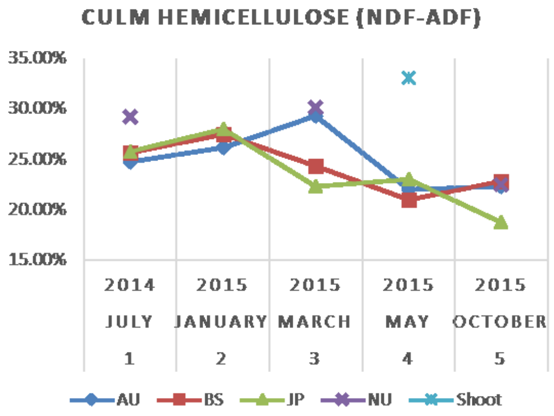


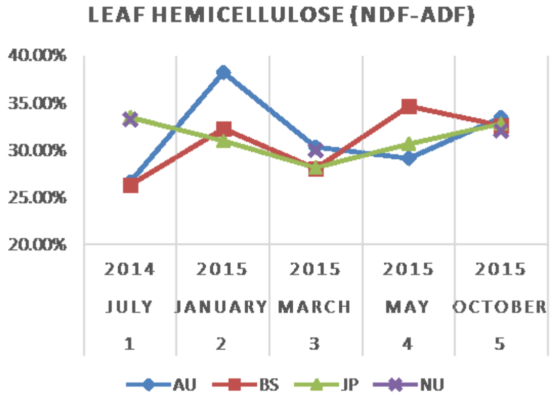
D.5— Acid detergent fiber (ADF)



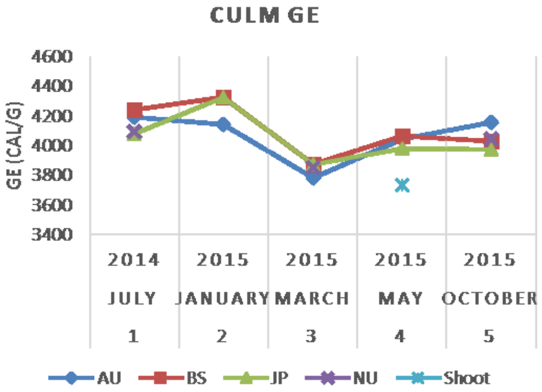


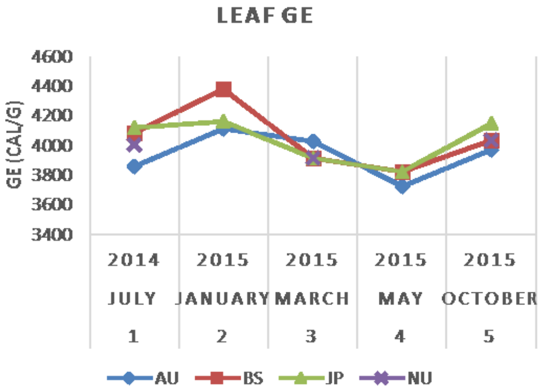
D.6— Hemicellulose



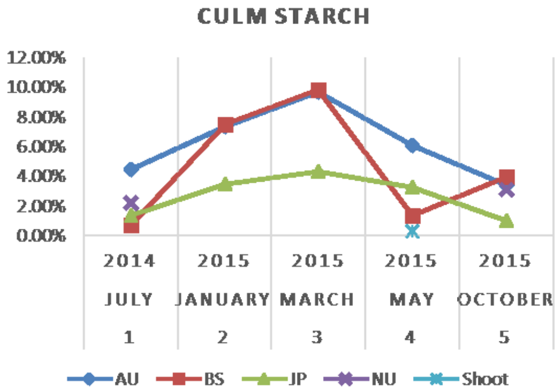


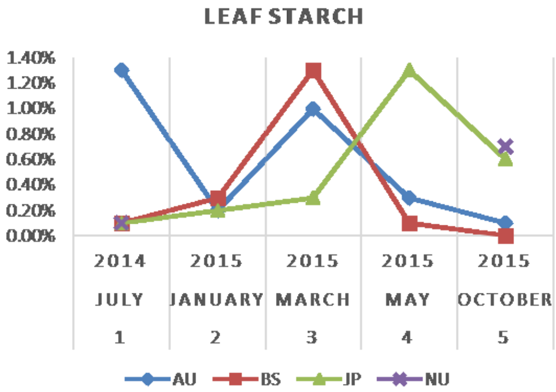
D.7— Gross energy (GE)



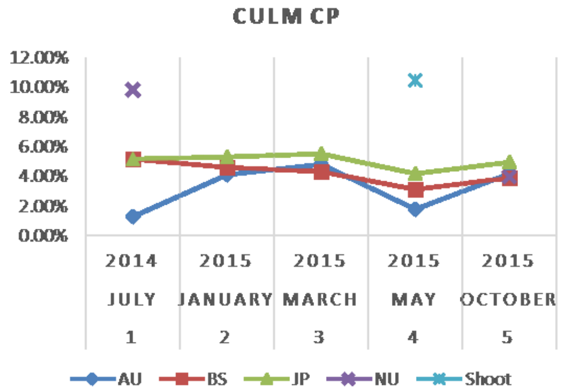


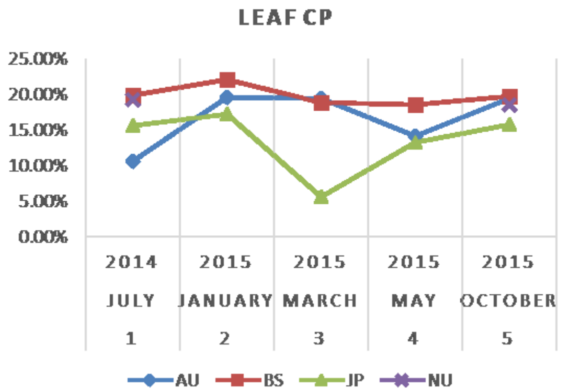
D.8— Starch



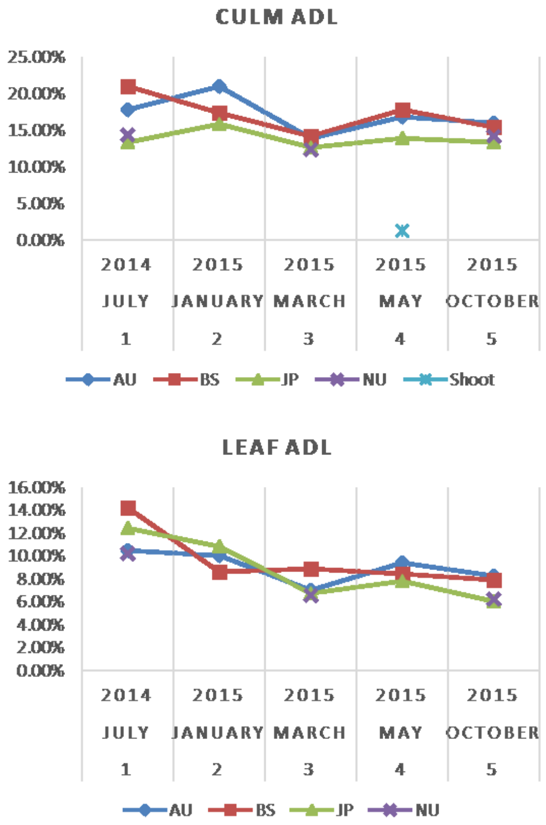


D.9— Crude protein (CP)

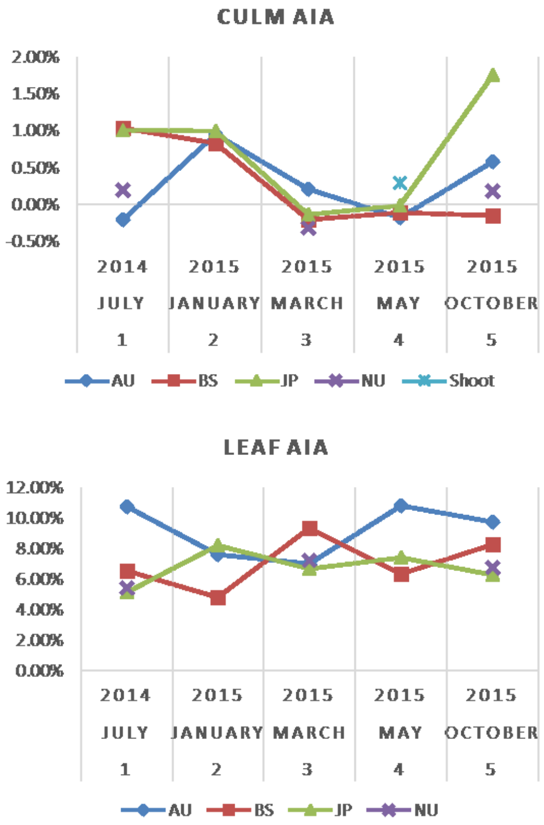




D.10— Acid detergent lignin (ADL)



D.11— Acid insoluble ash (AIA)



D.12—Acid detergent insoluble ash (ADIA)