IN SITU DETERMINATION OF THE DIGESTIBILITY OF BAMBOO OFFERED TO GIANT PANDAS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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Giant pandas (*Ailuropoda melanoleuca*), are one of the most notable and powerful symbols of species conservation. Research on the species is sparse and especially lacking in the nutrition category. Although they contain a monogastric, carnivorous gastrointestinal tract, they primarily consume a highly fibrous diet of bamboo. In a previous *in vivo* study conducted at the Memphis Zoo, two Giant Pandas were fed four different species of bamboo across five months; July, January, March, May, and October. For the present study, samples from each month's diet were subjected to *in situ* microbial degradation inside bovine rumen for 48 h. Fecal composites from each bear from each month were also digested. Dry matter (DMD) and organic matter (OMD) digestibility of samples was quantified and examined. When pandas consume bamboo, they pick a part the whole bamboo and consume each plant part individually. Primary plant parts analyzed included: leaf, culm, shoot, and cover. As expected, the shoots had the highest values; DMD (68.6%) and OMD (71.8%) while culms were lowest (9.1 to 28.0 and 8.3 to 27.8%; DMD and OMD, respectively). In most months, pandas preferred to consume culm over the other components. Overall, DMD of consumed culm averaged 16.7% for January, slightly higher than the digestibility of culm orts (15.3%). During July; pandas preferred to consume culms although it was leaf season. In July, leaf ort DMD/OMD was averaged 6% higher than the value of what

was fed. Digestibility and diet selection followed the same trend for both bears utilized in this research. This analysis further solidifies the premise that giant pandas selectively consume their diets based on digestibility.

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NOMENCLATURE

AU	*Phyllostachys aureosulcata*
BS	*Phyllostachys bissetii*
CP	Crude Protein
DM	Dry Matter
DMD	Dry Matter Digestibility
GP	Giant Panda
IS	*In situ*
JP	*Pseudosasa japonica*
N	Nitrogen
NU	*Phyllostachys nuda*
OM	Organic Matter
OMD	Organic Matter Digestibility
P.	*Phyllostachys*
PDM	Partial Dry Matter

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CHAPTER I

INTRODUCTION

Captivity and Conservation

Giant Pandas (*Ailuropoda melanoleuca*), are one of the most notable and powerful symbols of species conservation. Giant Panda (GP) native habitat is widespread in southern China from Beijing to southeast Asia specifically in the Qinling Mountains. Populations are fragmented and Pandas reside in several subpopulations; however, the Qinling Mountains are home to the majority. Majority relocation to the Qinling Mountains can be attributed to rapid expansion of human population and industrialization over the years (Swaisgood et al., 2016).

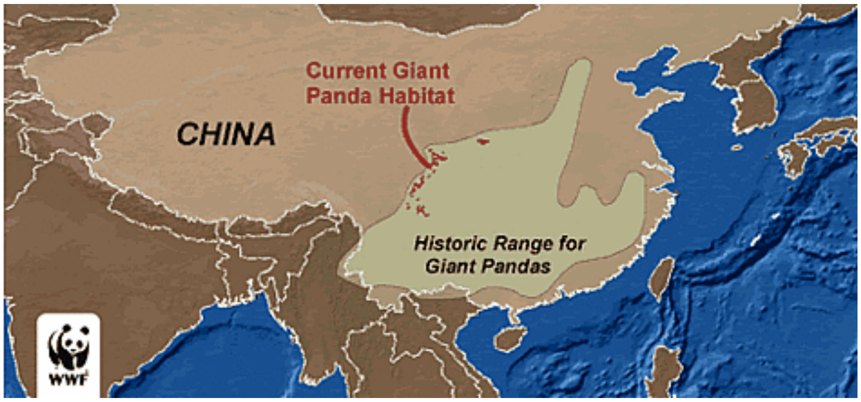


Figure 1: Map detailing range wild pandas used to reside. Red area shows where pandas are currently restricted. (WWF, 2016)

Giant pandas are captive in zoo environments across the globe. Their enclosures are designed to mimic natural habitats. About 16% of the world's GP populations live in captivity (Smithsonian). Zoo animals are bred in captivity. Given that the species is threatened and population is minimal, zoo-breeding efforts assist in preservation of the species.

Research on this species is limited; however, it is extremely important to the long-term survival of this species (Wei et al., 2015). Pertinent research allows professionals to develop habitats, diets and improve husbandry skills to maintain pandas and increase their population. Significant research efforts were initiated for the species when they were labeled as “endangered”. As of the present, they are considered “vulnerable”. This could be attributed to the conservation efforts of researchers and passage of significant laws and bills in China to protect the species (Swaisgood et al., 2016). Major threats to the wild panda population include logging which destroys their shelter and food supply, poaching (illegal killing of animals by hunters) and increases in human population, which decreases forested area. Research over the years has mainly focused on panda habitat (Wei et al., 2015; Hull et al., 2016; Zhang et al., 2011), limited research on their diet has been conducted and published (Swaisgood et, al., 2010, Zhang et. al., 2016).

Nutritional research is pertinent not only because of its relevance to feeding and prolonging the existing pandas, but because of its relevance to reproduction as well. While it is difficult and not practical to control the diet of wild pandas, captive panda diets generally receive 100% of their diet at the discretion of their keepers and nutritionists. Since we provide captive animals with their diets, we are able to carefully create a balanced ration that meets all dietary needs. Therefore, captive pandas should be provided a diet that meets or exceeds their requirements for optimal health, well-being, and reproductive success. However, this is not the current situation as inadequate data is available regarding their requirements and the ability of the offered diet to meet their requirements. Further specific research should be conducted on the bamboo and supplements themselves to determine which species of bamboo is the best fit for these animals depending on the season and location.

Giant Panda Anatomy

Giant pandas consume an entirely vegetarian diet; however, they are not designed with a gastrointestinal tract that is typically associated with efficient utilization of a vegetarian diet. Giant pandas belong to the order, *Carnivora* along with the brown bear, wolves, tigers and other meat-eating animals. However, they possess many unique internal and external features enabling them to consume and, to some extent, digest highly cellulosic plant parts. These attributes include their unique enterobacterio microflora, microbial genomic predisposition for cellulolytic enzymes, intricate pseudo thumb, and a complex skull structure of the mandible, teeth and entangled muscles (Zhu et al., 2010, Endo et al., 1999, Figueirido et al., 2014).

Bacterial Microflora

For quite some time it remained a mystery as to how GPs were able to meet their nutrient requirements from a diet consisting of primarily cellulose. Herbivore animals possess microbial genomes that provide them with an adequate microbiome that can ferment, and produce enzymes to digest cellulose and other common plant structures.

Giant Panda genome codes for all necessary enzymes needed to consume a red meat, carnivorous diet. However, it, like all known mammals, lacks genes for enzymes needed to digest cellulose (Zhu et al., 2010). Pandas have a lower species richness of their microbiome than other herbivores and non-herbivore carnivores. Their reduced microbial species richness has not inhibited them from being able to digest their diets due to combination of adaptations over time (Zhu et al., 2010). When a species lacks necessary resources for long periods of time, they adapt to certain changes to survive. Access to dietary resources and abundance of bamboo feedstuffs has forced the GP to adapt their microflora to a more cellulolytic type of digestion.

Zhu et al. (2010) evaluated fecal samples of 7 wild and 8 captive GP. Overall analysis from the samples showed that the most abundant type of bacteria was *Firmicutes* with *Proteobacteria* being second most abundant. Positive but sparse were findings of *Actinobacteria, Acidobacteria, Bacteriodes and Cyanobacteria. Firmicutes* are gram-positive bacteria belonging to *Ruminococcaceae* and are insoluble carbohydrate metabolizing organisms. Abundance of *Firmicutes* is related to consuming diets rich in fiber. It is believed that *Firmicutes* enhance calorie absorption (Jandhyala et al., 2015). An abundance of *Firmicutes*, at least partially explains why GPs are capable of fermenting cellulose-based diets.

Pandas have high passage rates of digesta to maximize dietary throughput, so microbial populations have a limited amount of time to break down and utilize nutrients. Mean retention time in captive GP have been reported to range from 4-7 hours (Dierenfeld et al., 1982; Finley et al., 2011 and Liu et al., 2015). A panda lacking proper enterobacteria would have their digesta pass straight through the intestines and excreted, with very limited digestion.

Pandas have a unique gut microfluora because it is unlike that of other herbivores (Zhu et al., 2010). Panda cub's microbiome is first established when they are born. They are first exposed to bacteria in the birth canal and further exposed and acquire immunity when nursing and from their environment. Through long-term adaptation to environment and shift of diet over time, their acquired microbiome is rich in bacteria that allow them to be able to utilize their diet (Zhu et al., 2010). Over many years, GP microbiome has evolved and been passed down from generation to generation. Microbiome of relatives are similar because they typically reside in close quarters.

Digestive Enzymes

Another important focus of analysis for Zhu et al. (2010) study was evaluation and identification of pertinent enzymes required for cellulose digestion. The GP values were compared to those of a wallaby, bovine, termite and human. Similar metabolic pathways were found for carbohydrates, amino acids, and DNA metabolism in all species. Oligosaccharide degrading enzymes were found to be similar to that of a human (37%) and significantly lower than those of a bovine (57%). Abundance of cellulases and endohemicellulases was lowest in the pandas for all the herbivores analyzed which would explain the limited digestion of their diet. They detected 13 operational taxonomic units of the analyzed species with 7 of them being unique to GP. This can again be attributed to forced evolutionary adaptation to environment and diet. Overall, Zhu et al. (2010) study was very in depth and provided many specific explanations as to why the carnivorous GP has the ability to digest a highly fibrous diet. Conclusions were that all of theses adaptations are proof that the GP species itself has evolved to overcome the challenge of consuming a diet they were not intended to consume.

Pseudo-Thumb

Giant pandas selectively consume their bamboo diets with selection preferences changing across seasons. With the exception of primates, most animals do not possess the ability to pick apart food with carpus and metacarpus appendages. Extensive imaging including radiographs, computerized tomography (CT) and magnetic resonance imaging (MRI) was utilized by Endo et al. (1999) to generate a thorough explanation as to why GP's pseudo-thumb is able to perform this function. While radial sesamoid bone functions as an active manipulator, images indicated that the bone cannot move independently of its attached bones and rather works as a functional unit of manipulation (Endo et al., 1999). Additionally, the radial sesamoid bone does not possess the same bone and connective tissue as the human thumb; however, it does allow manipulation of bamboo with great dexterity. Limitations to this study were that all imaging was obtained postmortem and not in actual gripped state to avoid interfering with live experimental subjects. As consistent with conclusions from Zhu et al. (2010), and other studies, the adaptation of the radial sesamoid bone can be attributed to evolutionary forced diet changes.

Skull and Mandible

In addition to above discussed morphological adaptations, GPs have an incredibly powerful and mechanical skull and mandible. In a study conducted by Figueirido et al. (2014) the skull biomechanics of both GPs and Red Pandas (*Ailurus fulgens*) were three dimensionally analyzed. It was determined that GP skull and mandible is incredibly strong and can operate with high-level forces (as could be compared with other related species). Mandible operates with high bite forces and stiff structure of the skull allow them to bite in all tooth positions. This ability is what allows them to tear apart the strips and layers of bamboo to access and consume preferred plant part. They concluded that GP's jaw is much stronger than Red Panda and these findings can be attributed to fact that Red Pandas feed primarily on leaves and fruits while GP diet mainly consist of trunks and stems of these same bamboo species. Mandible and skull morphological adaptations are again another long term evolutionary change that the species adapted to in order to survive the environmental and food source shift.

Reproduction

Given that the GP species is threatened and vulnerable, it is clear that they have reproductive difficulties. It is theorized that gastrointestinal upset from consuming an indigestible diet can contribute to lack of *libido* and mating activities. Captive GP have further reproductive difficulties due to unnatural added stressors related to zoo life. Captive panda breeding program began in China in 1955, but it took eight years to actually successfully reproduce a captive GP (Snyder et al., 1996). Over the years, due to development of research and understanding of the estrous cycle and behavior, new strategies such as artificial insemination have been implemented to assist the pandas. The main shortcoming to GP reproduction is that they only experience one estrus per year, of 24-72 hours in duration. Estrus typically occurs mid-July (Palmer et al., 2012). An annual estrus results in short-lived desire for reproductive behavior, and very short window for fertility. Palmer et al. (2012) evaluated male reproductive behavior and its correlation with female estrus. It was observed that male reproductive behavior fluctuates over a protracted interval. Just prior to female estrus, sperm production elevates. Male androgen production fluctuates based on mid-July copulation as well. A unique attribute to GP discovered in this study is cessation of spermatogenesis in August. The only other animal this has been documented in is the Japanese black bear (*Ursus thibetanus japonicas*). Ejaculate also contains high concentration of sperm, high sperm motility, and good morphology, which is advantageous especially given the very brief reproductive window. Kersey et al. (2010) discovered that rising adrenal hormones functioned to prepare males for battles with cohorts to gain access to estrual mates in wild. In addition, olfactory scent marking and detecting behaviors along with vocalization and pacing serve as communication means between potential mates. While captive GP experience an overall greater reproductive difficulty, certain discussed barriers present in the wild are not present in captivity. In contrast, GP are noise sensitive and particular sounds present in a zoo environment are stressors and affect hormones necessary to regulate estrous cycle (McGeehan et al., 2002). Once pandas do successfully reproduce, gestation length can be up to 160 days with a large variance. Variance is due to diapause, which is delayed implantation. Diapause is unique to GP and a few other species. Following fertilization, ova completes several rounds of cell division before development arrests. The blastocyst (fertilized egg at this stage) instead free-floats until uterine conditions become ideal for implantation. Because of this phenomenon, females will not exhibit signs of pregnancy until blastocyst implants, begins development and true pregnancy prevails. While actual gestation length is only 50 days, observed pregnancies vary in length from about 101 to 150 days. The diapause length can vary from year to year even in the same female (Hall, 2011). It's possible that this phenomenon actually assists in fertility and by waiting until uterine conditions are ideal, chance of miscarriage is decreased. Another common complication to GP reproduction is pseudopregnancy. In a pseuodopregnancy, female's body experiences many or all symptoms and physiological changes that occur during a pregnancy although she is not actually pregnant. It's possible for this to occur even in a female that has not been bred. It is not really known why this occurs, one possible theory is miscarriage but with little action taken to detect pregnancy, this has not been proven (Hall, 2011). Pseudopregnancy and pregnancy can also cause low appetite. Further research correlating diet to reproduction could be essential in conservation efforts.

Diet

Wild GP diet consists of only bamboo. Bamboo is a grass and member of family *Graminae* (Hansen et al., 2010). Species of bamboo consumed in the wild are specific to location and time of year. In the wild, GPs migrate to different altitudes to obtain adequate levels of energy for consumption. Level and type of migration (higher or lower altitudes) is dependent on season, location and species of bamboo available (Hansen et al., 2010). On the other hand, in captivity, GPs do not have the option to migrate but this is not necessary since their diet is provided to them and always readily available. In addition, captive animals are supplemented with fruits and other energy sources to ensure all nutritional requirements are being met. However, their main source of energy is still the bamboo. It was observed that captive GPs can eat up to 14 kg of bamboo per day or about 6-15% of their body weight. Only about 17% bamboo DM is actually digested and they spend approximately 19-28% of their time engaging in feeding behaviors. Energy expenditures are reduced by minimal activity and resting when not engaging in feeding or reproductive activities. (Christian et. al., 2015, Dierenfeld et. al., 1982, Dierenfeld et. al., 1997).

Bamboo Parts

Bamboo consists of several different plant parts. Leaf, is green and grows off of stems or branches. Culm is major stem and grows vertically (Hansen et al., 2010). Leaf and culm are observed to be main bamboo parts consumed in various trials. Branch is an indigestible part that grows from stem and is not typically consumed by the animal. Cover is shell of culm that is consumed (Hansen et al., 2010). Shoot is a freshly sprouted cane obtained from under the soil that has firm texture (Grant, 2015). When "complete" is mentioned, this refers to a sample consisting of all plant parts of a particular species.

Selective Consumption Behavior

Giant Pandas choose to eat their bamboo in a very particular way. As mentioned, they tend to consume more of one plant part during different seasons. Selective behavior is related to time since harvest, habitat (climate, captivity), age (plant), species and plant parts (Hansen et al., 2010). When consuming a specific plant part, different behaviors are utilized to obtain a specific part for consumption. When GPs "bite" their bamboo, they are actually using their teeth to separate one part of bamboo from another for consumption. This is typically utilized to separate culm from leaf. When leaves by themselves are consumed, this is done so in a “wad”. Wad is when GPs remove leaves from stems or branches via manipulation of their pseudo-thumb and mandible to form a cluster in their cheek pouch. The collection is then placed into the paw and consumed all at one. Another term for culm consumption is strip, which is when outer layer of culm is removed with teeth (Hansen et al., 2010).

Giant pandas typically select the most nutritious species available to them at that time. The most nutritious species will vary depending on season. Higher protein and higher amino acid content is preferred (Lindburg, 2004). Leaf is observed to be primary plant part consumed during months of June through December (Hansen et al., 2010). Leaf has higher crude protein, lower cellulose and lower lignin content compared to other plant parts (Johnson et al., 1988). Culm is observed to be primary plant part consumed during months of February through May (Hansen et al., 2010). Compared to leaf, culm is significantly lower in crude protein while higher in cellulose and lignin. However, in late winter/early spring it is speculated that cell leaf lignin and cellulose increases, which could explain the shift from leaf to culm consumption (Long et al., 2004). The pith (inner part) of culm is the preferred part for consumption because it is softer. During shift from leaf to culm in winter, shoot is believed to provide additional nutrients in diet as leaf consumption is decreased (Hansen, et al., 2010). Young shoots are preferred as they are tenderer and nutritionally similar to leaves. They are also digested more easily, because they have extremely low lignin content (Lindburg, 2004). During this shifting period, shoots are essential for improvement of body condition and reproduction (Shaller et al., 1985, Long et al., 2004). Shoots are not preferred over leaves because they contain less protein than leaves and become rough when mature. Generally, as shoot height increases, nutritive value decreases (Christian et al., 2015). As with any herbivorous diet, digestibility and plant part selection is dependent on plant species. While many species of bamboo exist, they are all regionally and climate specific.

Bamboo Species

For the purpose of this study, four species of bamboo were selected. These four species were fed to GPs in 5 trials over time. Analyzed bamboo species are *Phyllostachys (P.) aureosulcata*, *P. bissetii*, *P. nuda*, and *Pseudosasa japonica.*

These 4 species were selected based upon location and availability. These are cold tolerant species and typically grown in the United States (Christian et al., 2015).

Phyllostachys species are characterized by horizontal leptomorph rhizomes (Christian et al., 2015). Rhizomes are underground stem and roots that grow horizontally rather than vertically. There are 2 major categories of rhizome in woody bamboo; the leptomorph and pachymorph. The leptomorph is thinner and uniformly long and typically stays underground with further rhizomes branching off at intervals. Morphologically, this rhizome longs like a thin horizontal underground culm with well-developed roots (Stapleton, 1998). *Phyllostachys* are also less fibrous than "clumping" bamboo with pachymorph rhizomes (Christian et al., 2015, Stapleton 1998). Pachymorph rhizomes are thicker and closer together in contrast to the leptomorph species. (Stapleton, 1998). Additionally, *Phyllostachys* species generally are taller at maturity compared to analyzed *Pseudosasa* species.

Table 1 Lab analysis values obtained from Christian, 2016 where samples were obtained. Below are values for dry matter (DM), organic matter (OM), acid detergent lignin (ADL) and crude protein (CP) all expressed in percentages from 4 species of bamboo and their respective parts.

Month	**Species**	**Plant Part**	**Total DM**	**OM**	**ADL**	**CP**
July	AU	Culm	60.1%	98.7%	17.8%	1.3%
July	AU	Leaf	48.6%	84.5%	10.5%	10.6%
July	JP	Culm	42.8%	96.7%	13.4%	5.2%
July	JP	Leaf	52.5%	91.5%	12.5%	15.6%
July	NU	Culm	31.8%	95.6%	14.3%	9.8%
July	NU	Leaf	42.6%	89.5%	10.3%	19.3%
July	BS	Culm	46.1%	97.4%	21.0%	5.2%
July	BS	Leaf	73.7%	89.6%	14.2%	19.9%
January	AU	Culm	53.8%	98.1%	20.9%	4.1%
January	AU	Leaf	44.8%	89.1%	10.1%	19.6%
January	BS	Culm	58.0%	98.8%	17.3%	4.6%
January	BS	Leaf	81.3%	91.7%	8.6%	22.1%
January	JP	Culm	54.2%	98.1%	15.9%	5.3%
January	JP	Leaf	56.4%	88.7%	10.9%	17.2%
March	AU	Culm	60.3%	97.9%	14.0%	4.8%
March	AU	Leaf	53.9%	88.5%	7.0%	19.4%
March	BS	Culm	58.2%	98.2%	14.1%	4.3%
March	BS	Leaf	56.6%	85.2%	8.9%	18.8%
March	JP	Culm	54.4%	98.2%	12.6%	5.5%
March	JP	Leaf	57.1%	88.7%	6.8%	15.8%
March	NU	Culm	56.9%	98.2%	12.4%	4.1%
March	NU	Leaf	57.0%	86.7%	6.6%	16.9%
May	AU	Culm	58.2%	98.5%	16.8%	1.8%
May	AU	Leaf	40.2%	84.7%	9.5%	14.2%
May	BS	Culm	49.4%	98.5%	17.7%	3.1%
May	BS	Leaf	43.7%	88.4%	8.4%	18.6%
May	JP	Culm	47.7%	97.6%	13.9%	4.2%
May	JP	Leaf	45.2%	87.7%	7.9%	13.2%
May	Shoot	Shoot	13.0%	93.7%	1.3%	10.5%
October	AU	Culm	55.2%	98.1%	16.0%	4.2%
October	AU	Leaf	46.7%	86.0%	8.3%	19.4%
October	AU	Complete	49.7%	93.9%	14.4%	7.7%
October	BS	Culm	54.0%	98.8%	15.4%	3.9%
October	BS	Leaf	42.6%	86.4%	7.9%	19.7%
October	BS	Complete	46.1%	97.4%	13.3%	5.1%
October	JP	Culm	49.8%	97.6%	13.4%	5.0%
October	JP	Leaf	44.9%	91.0%	6.1%	15.7%
October	JP	Complete	45.8%	96.4%	12.8%	5.3%
October	NU	Culm	53.4%	98.3%	14.2%	4.0%
October	NU	Leaf	45.6%	86.6%	6.3%	18.6%
October	NU	Complete	50.7%	95.4%	11.0%	7.2%

Insight from Feeding Trial

Analysis by Data from Christian (2016) for January, March and May, culm consumption was consistent with previously established data (Hansen et al., 2010). Given that leaf is the typical plant part of preference in the summer months, majority culm consumption by bears in July (Table 1) is not consistent with existent data. Leaf was primary part of consumption for October trial, which is consistent with previous observations (Hansen et al., 2010). Additionally, shoot was offered during May trial and observed to have moderate crude protein (CP) content (10.5%), low lignin content (1.3%), but also low dry matter content (13.0%). It is believed that if offered more shoots, bears would've consumed more shoot than culm. Fresh shoots are difficult to provide *ad libitum* in captivity because of their limited production in managed stands (Christian, 2016). Daily dry matter intake ranged from approximately 2.1-8.1 kg bamboo per day across all trials. The male GP spent approximately 45% of daytime activity engaging in eating behaviors for all trials except July, which was 28.4%. The female GP also reduced feeding behavior in July but her overall feeding activity was about 40% of her budgeted time (Christian, 2016). Christian also observed that both GP selected for hemicellulose and starch against acid detergent fiber in the bamboo.

In situ **Analysis**

In situ is a Latin term that simply means in place; undisturbed. The *in situ* method is a widely utilized feed analysis method to assess degradation of feed components such as organic matter (OM), dry matter (DM), protein and more (Meyer and Mackie, 1985). This methodology is accomplished by placing feed contents into a synthetic bag that is subsequently incubated in the rumen for a fixed amount of time. This technique assumes that microbial populations within the suspended bags are similar to that of surrounding ruminal contents (Meyer and Mackie, 1985). Extent of material degradation is dependent on amount of nitrogen (N) available to microbes (De Boer et al., 1986); therefore, it is essential that subject animals are supplemented with a proteinaceous feedstuff during trials to ensure ruminal N is not limiting. DMD and CP can be influenced by several factors including porosity of bags, ratio of sample weights and body surface area (De Boer, 1986). For purpose of comparing digestibility of samples, as long as bag size, sample size and porosity are consistent across all samples, variability should be minimized. Vanzant et al. (1998) concluded that utilizing bags with pore sizes between 40 and 60 mm is ideal and grinding all feeds through a 2 mm screen ensures homogeneity. Decreased particle size increases surface area and can lead to greater digestibility or particle loss. It is essential that all samples be of exact same grind size to ensure accurate and consistent results. Bags should be placed in ventral sack of rumen with ability to move freely (Ruiz, 1992). Bottom of the rumen is considered to be best for placement of samples to ensure all samples are thoroughly immersed in rumen fluid and not allowed to migrate to top (Van Soest, 1994). Smooth rocks or other dense material should be utilized to prevent floating. Bags should be fastened so that they are unable to be removed by other cattle or fall out. Protein supplements can be incubated for up to 36 hours, high-quality forages and legumes up to 48 hours and tropical grasses and fibrous residues up to 72 hours. Minimum incubation time is 2 hours for all plant types (Ruiz, 1992). Various methods are existent for rinsing procedure of *in situ* bags such as hand rinsing or machine washing (De Boer, 1986). Water must essentially run clear and no debris may be present on bags for samples to be considered thoroughly rinsed. A standard and consistent incubation and rinsing procedure is imperative for obtaining accurate data and results from an *in situ* study. In addition, samples should always be analyzed in multiples to allow for error and variance (DeBoer, 1986).

Utilization of *in situ* method is a very versatile for a multitude of different digestibility and feedstuff analysis studies. It is an especially useful method when live subjects, such as GP or other exotic species are not readily accessible for research.

CHAPTER II

METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Present study evaluated dry matter digestion and organic matter digestion of bamboo samples. Bamboo samples were collected and originally utilized by a graduate student for her research titled *Seasonal Variations In Bamboo Selection and Utilization By Two Giant Pandas* (Christian et al., 2016). This research was conducted at the Memphis Zoo and sample collection was completed with assistance from zookeepers specially trained in the care of GP and large mammals.

Original Feeding Trials

Five feeding trials were completed with two captive giant pandas housed at the Memphis Zoo (Memphis, TN). Trials 2 and 3 were timed to correspond with period of maximum culm consumption by the giant pandas (January 3-5, 2015; March 23-25, 2015), trials 1 and 5 with leaf consumption (July 21-23, 2014; October 27-30, 2015) and trial 4 with bamboo shoot consumption (May 21-23, 2015), with predicted plant part selection based on previous foraging data (Hansen et al. 2010; Gocinski , 2013 Memphis Zoo personal communication by Christian). During the trials, a male (466, aged 16 years) and female (507, aged 14 years), 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*, and new bamboo was offered several times per day. 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 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.*

Trials 1 through 4 occurred over the course of three days, with sample collection lasting approximately 48 hours, or approximately 4 times the maximum mean retention time of the giant panda (Dierenfeld et al., 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. Trial 5 included an additional day, resulting in approximately 72 hours of diet and ort sampling.

Collection of Experimental Samples

Fresh bamboo samples (approximately 2 kg) from bamboo bundles were randomly drawn and weighed by zookeepers, and the remaining bamboo was fed. Rejected bamboo culms, leaves, branches, and culm coverings (fragments of the culm exterior layer peeled away by the GP), were collected throughout the day when the animals' enclosures were cleaned. After removal from panda 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 course of the trial. Bamboo plant part and fecal samples were dried in a forced-air oven at 60° C until 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.

Laboratory Analyses

Bamboo and fecal samples were homogenized through a Wiley Mill (Model 4). Samples were initially ground to pass a 2 mm screen, then further ground to pass a 1 mm screen. Samples were dried in a forced-air oven at 60° C for 24 hours to determine laboratory DM which was used to calculate dry matter digestion (DMD) following *in situ* procedures. Organic matter (OM) was determined as loss in dry weight upon combustion in a commercial muffle oven at 450° C for 6 hours. This value was used to calculated organic matter digestion (OMD) following *in situ* procedures.

In situ Procedures

Three ruminally cannulated steers were utilized to determine *in situ* disappearance kinetics of bamboo and fecal samples DMD and OMD. Steers were housed in an outside, small fenced pasture. Steers had *ad libitum* access to water, bermudagrass hay, and a trace mineral block (97% NaCl, Min 1.80 % Ca, 1% S, 3000 ppm Mn, 2500 ppm Zn, 1500 ppm Fe, 150 ppm Cu, 90 ppm I, 25 ppm Co, 10 ppm Se, United Salt Corporation, Houston, TX. Approximately 1 kg of dried distillers' grain per steer was supplemented once daily to ensure ruminal nitrogen was not limiting.

Approximately 0.5 grams of an individual bamboo sample was sealed in an Ankom F57 fiber filter bag with 25 micron porosity. Samples were analyzed in triplicate to allow for margin of error and designated as A, B or C. All A samples were analyzed in one steer, B in another, and C in the third steer so one representative of each sample was digested in a different steer to allow for subtle difference in digestion level that may be specific to one steer. Three blank samples (sealed filter bags, no sample) were also analyzed with one in each steer. Within the rumen samples were contained in a 60 × 25 cm mesh laundry bag with weights at the bottom to prevent samples from migrating to top of rumen cavity. Samples were incubated undisturbed for 48 hours. Following removal from rumen, samples underwent a rinsing procedure. First, samples were rinsed while still contained in mesh laundry bags. Once majority of the rumen debris were washed off, individual sample bags were removed and subjected to cold water hand rinsing until all debris were removed and no small particles remained attached to individual sample bag. Following hand rinsing, samples were rinsed in an Ankom agitator for 5 minutes or until water ran clear. Samples were then hand rinsed individually again until bag color was nearly white and all digestive fluids were removed. Samples were then dried in a forced-air oven for 24 hours at 60° C, desiccated for 20 minutes then weighed. Dry weight was used to determine DMD. Organic matter digestion was determined as loss in dry weight upon combustion in a commercial muffle oven at 450° C for 6 hours. The Ankom bag is destroyed in combustion process so removing dry digested samples from Ankom bags was not necessary and would've resulted in lost sample.

Statistical Analysis and Calculations

All calculations were performed, and graphs were created in Microsoft Excel. Total DMD/OMD of diet offered to subject was calculated by multiplying the ratio of species that was provided to the bear, by the DMD/OMD of that species and adding them together. Overall DMD/OMD and ort OMD/DMD was compared for plant parts (leafs, culms and covers). Statistical analysis was conducted using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Dependent variables were DMD and OMD. Diagonal covariance structure, REML estimation method and profile residual variance method was utilized to compare data and test for the random effect. Overall species vs. species were compared along with overall culm vs. leaf and specific species plant part vs. specific species plant part (ex: AU culm vs. AU leaf or AU leaf vs. BS culm).

CHAPTER III

RESULTS AND DISCUSSION

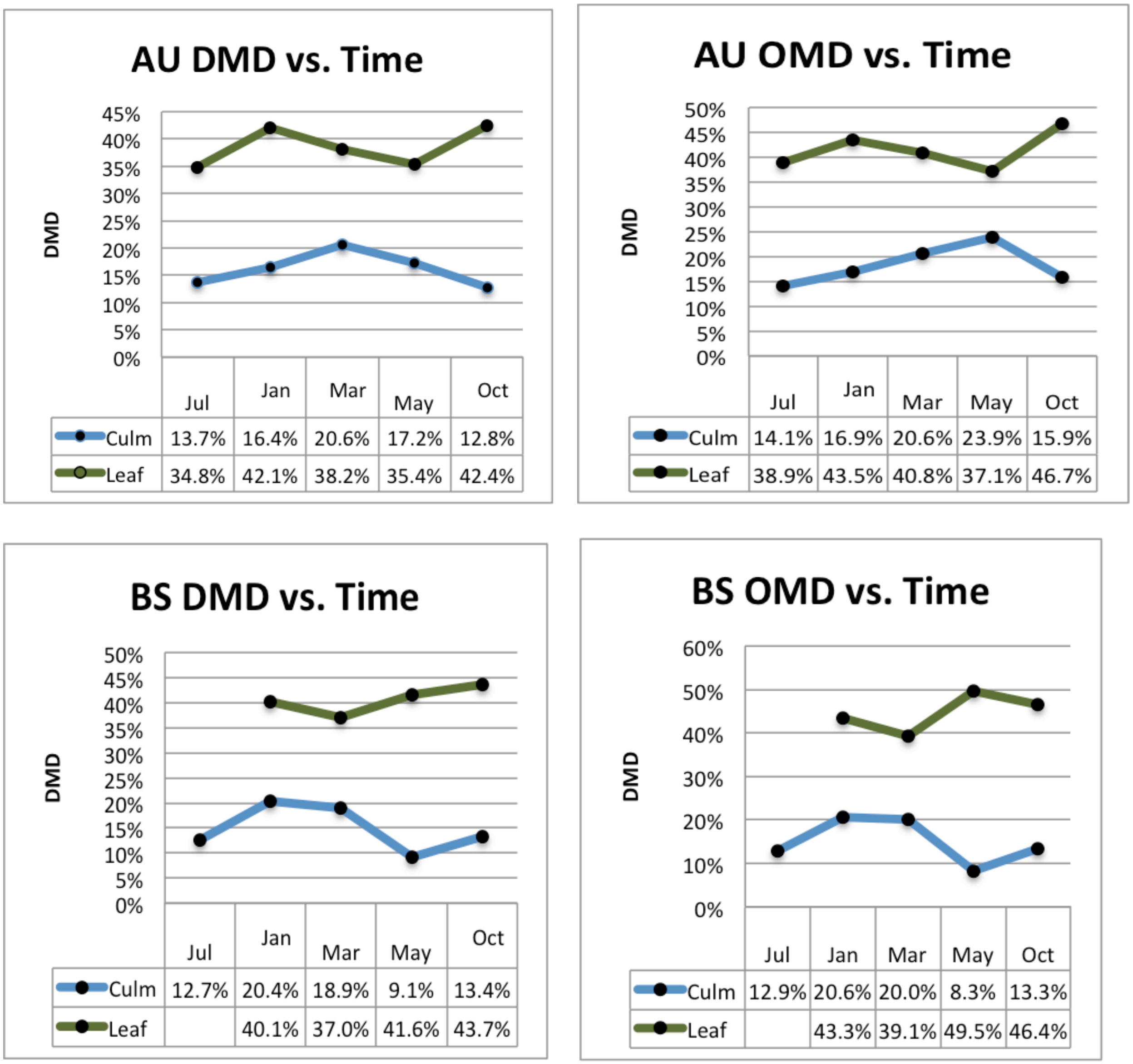
Comparison of DMDs and OMDs

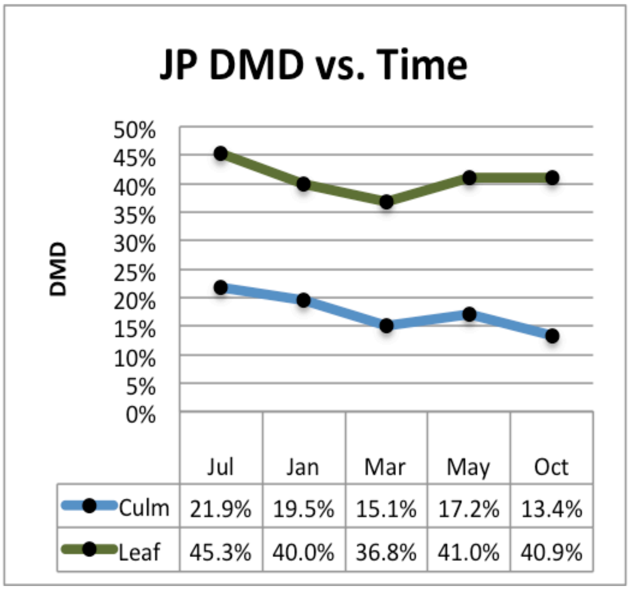
As expected, overall OMD was slightly greater (up to 3%) than DMD. Although only offered in the May trial, shoots displayed the greatest digestibilities 68.6 and 71.8% (DMD and OMD, respectively). Culms were the least digestible with values of 9.1 to 28.0% and 8.3 to 27.8% for DMD and OMD, respectively. Leaf samples had greater digestibility values than culm of 34.8 to 48.2% and 38.9 to 52.4% for DMD and OMD, respectively (Figures 2.1-2.8). For the July trial, although it was designated as leaf season (Hansen et al., 2010), the bears in this study actually preferred to consume culm (Christian, 2016). When digestibility of what was fed to bears was compared with digestibility of orts, it was found that leaf orts were more digestible than what was fed; however, digestibility of culm orts was lower than what was fed. This suggested that bears were selecting for higher-quality culm than the average of what was offered and leaves offered were of lower quality than typical for July. For January, March, and May, typically defined as culm season (Hansen et al., 2010), the bears preferred to consume culm (Christian et al., 2016). For majority of months digestibility of culm fed was greater than orts while digestibility of leaf orts was greater than digestibility of leaf fed which again indicated that bears selectively consumed a more digestible diet. To continue this trend in the final month, October leaf fed was more digestible than the orts while culm orts were more digestible than fed culm (Table 2.1 and 2.2). October is leaf consumption season (Hansen et al., 2010) and bears primarily consumed leaf (Christian et al., 2016).

Digestibility of fecal composite samples (Figure 3.1 and 3.2) was greatest in May (33.7 and 37.6% DMD and 31.0 and 35.4% OMD) and lowest in March (19.5 and 16.4% DMD and

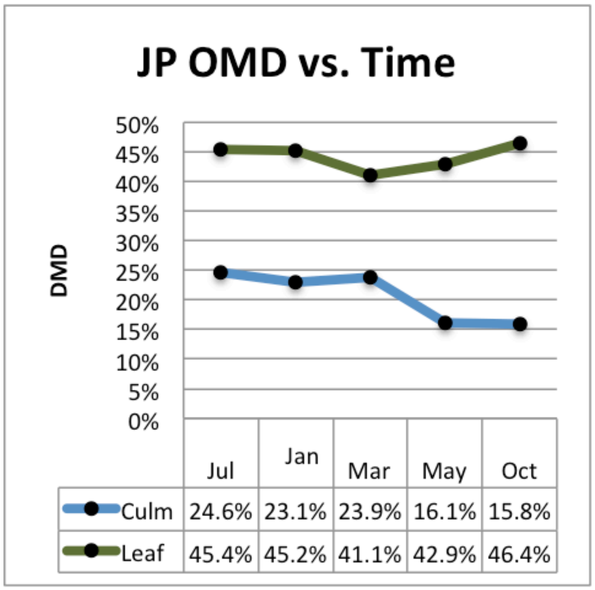
18.5 and 19.7% OMD). A higher fecal digestibility indicates a lower diet digestibility and utilization, which goes along with bamboo digestibility being on the higher end in March and lower end in May.

Figure 2.1-2.8: DMD and OMD over time by species

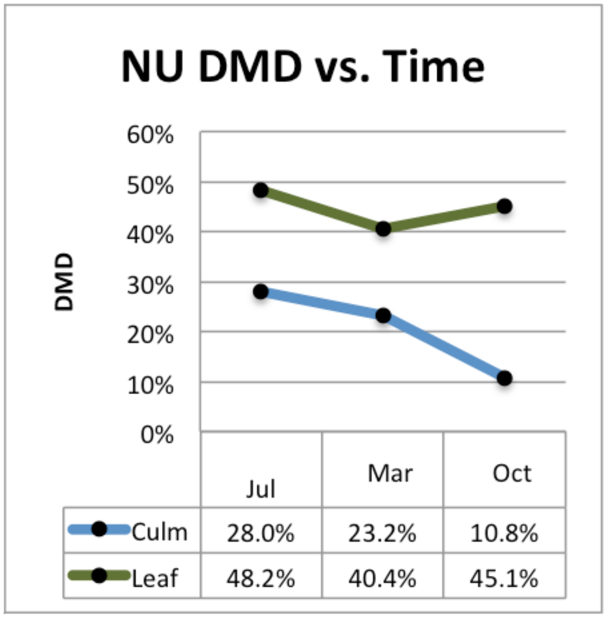




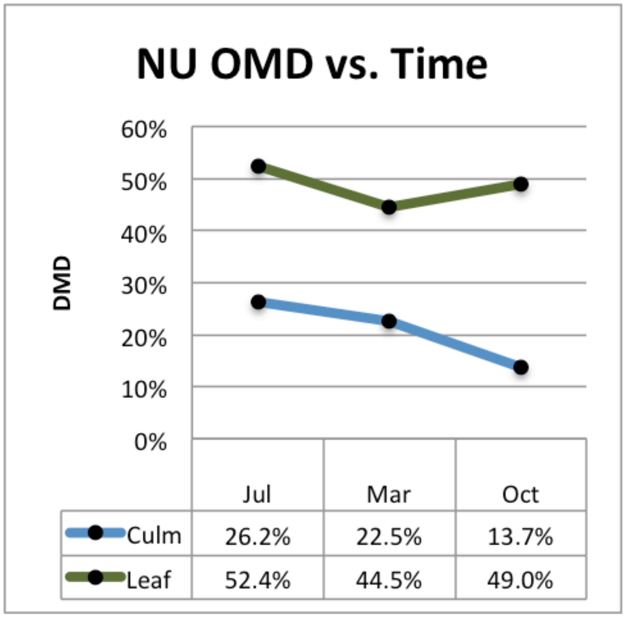
	Jul	Jan	Mar	May	Oct
Culm	21.9%	19.5%	15.1%	17.2%	13.4%
Leaf	45.3%	40.0%	36.8%	41.0%	40.9%



	Jul	Jan	Mar	May	Oct
Culm	24.6%	23.1%	23.9%	16.1%	15.8%
Leaf	45.4%	45.2%	41.1%	42.9%	46.4%

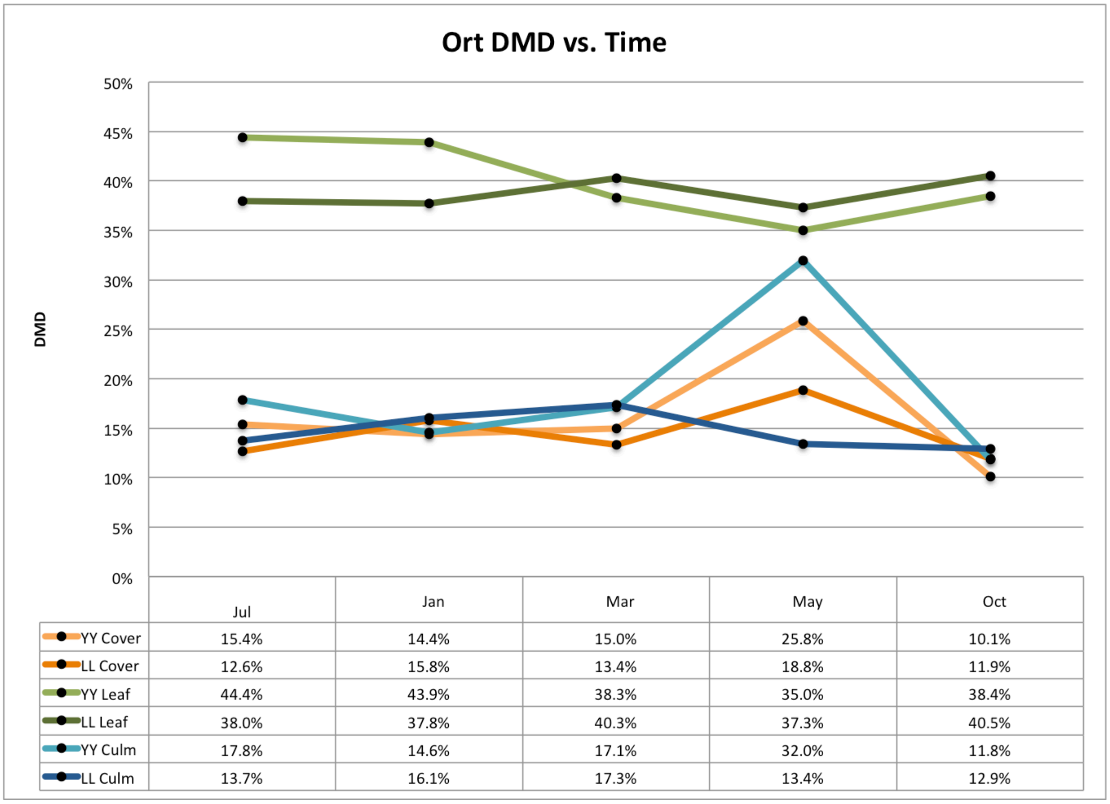


	Jul	Mar	Oct
Culm	28.0%	23.2%	10.8%
Leaf	48.2%	40.4%	45.1%

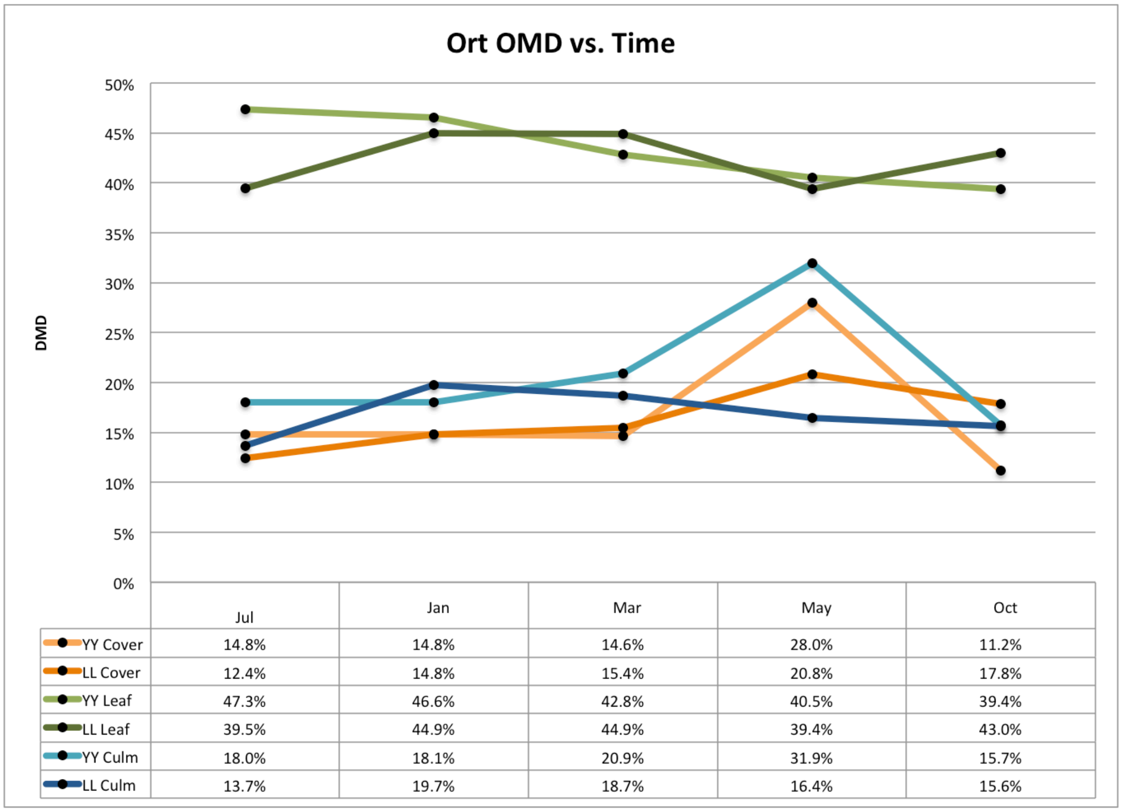


	Jul	Mar	Oct
Culm	26.2%	22.5%	13.7%
Leaf	52.4%	44.5%	49.0%

Figure 3.1 and 3.2: Ort DMD/OMD over time

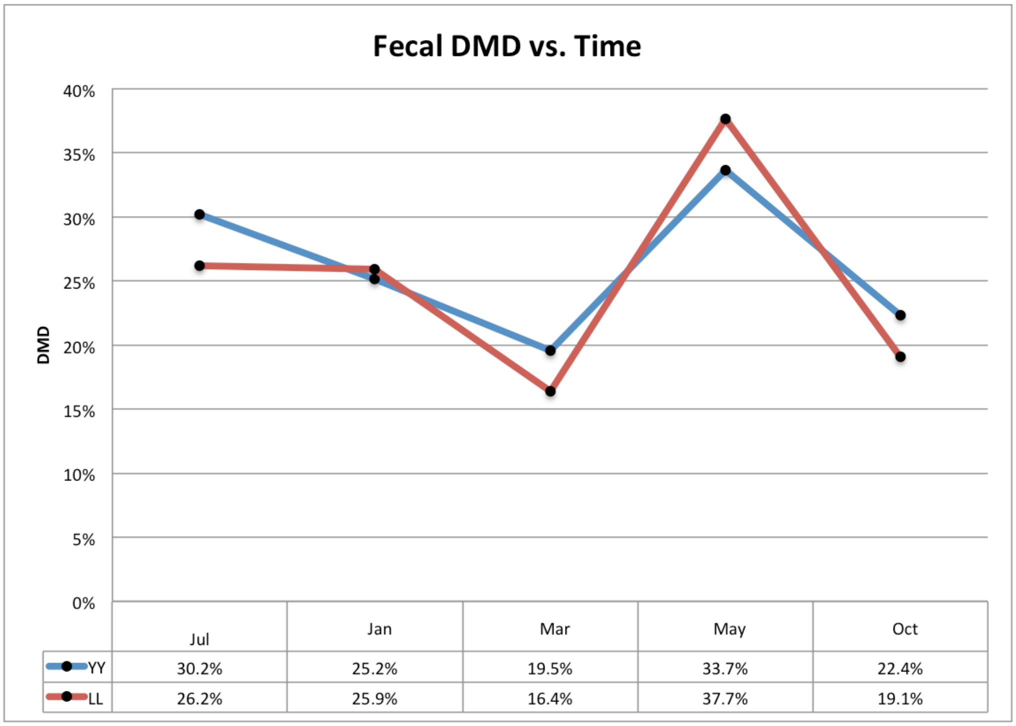


	Jul	Jan	Mar	May	Oct
YY Cover	15.4%	14.4%	15.0%	25.8%	10.1%
LL Cover	12.6%	15.8%	13.4%	18.8%	11.9%
YY Leaf	44.4%	43.9%	38.3%	35.0%	38.4%
LL Leaf	38.0%	37.8%	40.3%	37.3%	40.5%
YY Culm	17.8%	14.6%	17.1%	32.0%	11.8%
LL Culm	13.7%	16.1%	17.3%	13.4%	12.9%



	Jul	Jan	Mar	May	Oct
YY Cover	14.8%	14.8%	14.6%	28.0%	11.2%
LL Cover	12.4%	14.8%	15.4%	20.8%	17.8%
YY Leaf	47.3%	46.6%	42.8%	40.5%	39.4%
LL Leaf	39.5%	44.9%	44.9%	39.4%	43.0%
YY Culm	18.0%	18.1%	20.9%	31.9%	15.7%
LL Culm	13.7%	19.7%	18.7%	16.4%	15.6%

Figure 4.1 and 4.2: Fecal DMD/OMD over time



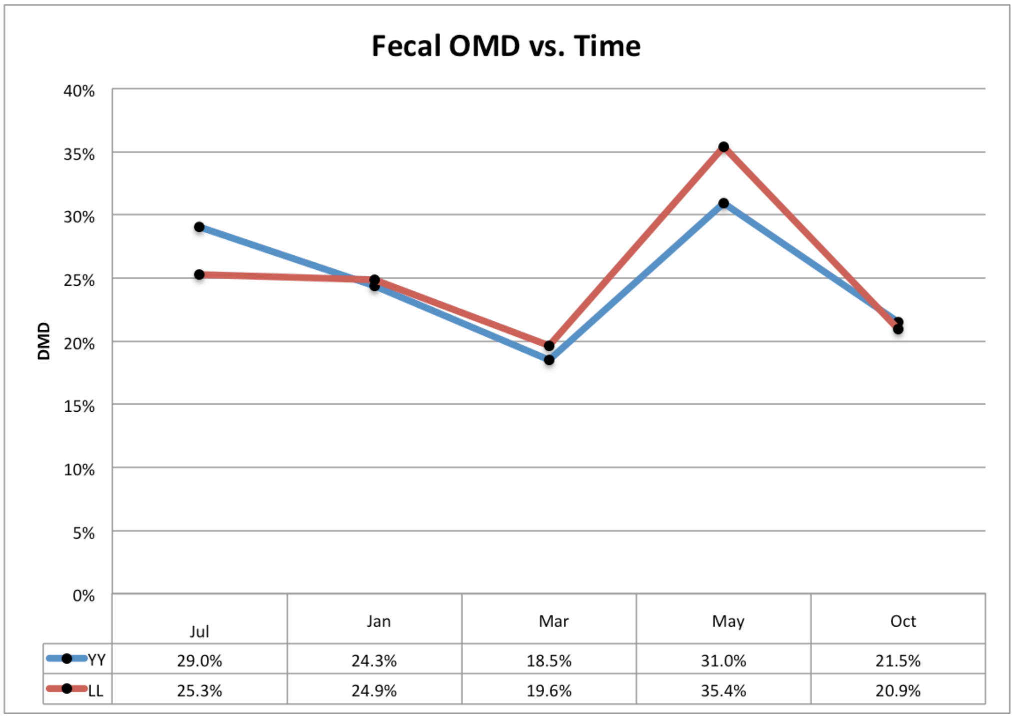


Table 2.1: Fed vs. Ort DMD

Trial	Bear	Plant Part	Ort DMD	Fed DMD
July	LL	Culm	13.7	16.8
July	LL	Leaf	38.0	35.4
July	YY	Culm	17.8	18.9
July	YY	Leaf	44.4	37.6
January	LL	Culm	16.1	16.4
January	LL	Leaf	37.8	42.1
January	YY	Culm	14.6	16.9
January	YY	Leaf	43.9	41.8
March	LL	Culm	17.3	19.8
March	LL	Leaf	40.3	38.0
March	YY	Culm	17.2	19.8
March	YY	Leaf	38.3	37.9
May	LL	Culm	13.4	13.8
May	LL	Leaf	37.3	31.9
May	YY	Culm	32.0	14.3
May	YY	Leaf	35.0	32.9
October	LL	Culm	12.9	12.8
October	LL	Leaf	40.5	42.1
October	LL	Complete	18.4	21.8
October	YY	Culm	11.8	12.3
October	YY	Leaf	38.5	42.9
October	YY	Complete	19.2	20.4

Table 2.2: Fed vs. Ort OMD

Trial	Bear	Plant Part	Ort OMD	Fed OMD
July	LL	Culm	13.7	16.6
July	LL	Leaf	39.4	38.9
July	YY	Culm	18.0	18.5
July	YY	Leaf	47.3	40.4
January	LL	Culm	19.7	16.9
January	LL	Leaf	45.5	45.5
January	YY	Culm	18.1	17.7
January	YY	Leaf	46.5	45.3
March	LL	Culm	18.7	21.1
March	LL	Leaf	44.9	40.9
March	YY	Culm	20.9	20.9
March	YY	Leaf	42.8	40.7
May	LL	Culm	16.4	17.4
May	LL	Leaf	39.4	34.2
May	YY	Culm	31.9	18.4
May	YY	Leaf	40.5	35.2
October	LL	Culm	15.6	15.4
October	LL	Leaf	43.1	46.3
October	LL	Complete	20.7	23.1
October	YY	Culm	15.6	14.8
October	YY	Leaf	39.4	467
October	YY	Complete	23.3	21.9

Statistical Results

There were no plant part × species interactions ($P \geq 0.42$) for either DMD or OMD (Table 3.1 and 3.2). For both DMD and OMD, comparison of the four species tended ($P = 0.10$) to be different. Two species AU and BS were significantly lower than NU for both DMD and OMD ($P \leq 0.03$ and $P \leq 0.04$, respectively). Mean values for DMD of AU, BS, JP, and NU are 27.4, 27.6, 29.1 and 32.6% respectively. Mean values for OMD are 30.4, 29.6, 32.4 and 35.0% respectively. Highest individual value came from NU leaf OMD of 48.6%. Lowest individual value was BS culm DMD of 14.6%. This supports the previous results detailing that NU had highest overall values with AU and BS being lowest and showing similar mean values (Figures 2.1-2.8). Overall, NU species exhibited the highest digestibility over the other species. During feeding trials, bamboo specie ratios were provided based upon region and availability (Christian et al., 2016). Considering that NU had the highest digestibility, if cost effective and available, GP nutritionists may want to consider providing NU in higher amounts or by itself.

However, when plant part alone was compared, there was statistical significance ($P < 0.01$) indicating that there was a large and consistent difference between culms and leaves. Culms displayed average of 17.2% DMD and 18.8% OMD. Leaves exhibited average of 41.2% DMD and 44.7% OMD (Figures 2.1-2.8). Looking at these results alone would indicate that leaves are much more digestible than culms. While it is not definitively known why GP prefer culms over leaves during certain parts of the year, it is speculated that increases in cell leaf lignin and cellulose during late winter/early spring may be cause for shift in consumption from leaf to culm (Hansen et al., 2010 and Long et al., 2004). Additionally, culm is known to have less fat than leaves, but it is also lower in crude protein, higher in fiber and therefore less digestible (He et al., 2000; Long et al., 2004; Sims et al., 2007; Wei et al., 1999, 2000 and Hansen et al., 2010). As now known from above data, the bears selected plant part highest in digestibility to consume, so it is best and necessary to offer them the whole bamboo and allow them to select what they want to consume. Additionally, given that bears pick apart bamboo and engage in unique consumption behavior; if bears were to be provided with just culms or just leaves they would be deprived of an activity necessary to survive in the wild. Enrichment is extremely important for captive zoo animals to prevent boredom and anxiety (Chamove, 1989).

Table 3.1: There was no species x plant part interaction (*P* = 0.80) however; there was significant effect of plant part (*P* < 0.01) and a tendency for an effect of species (*P* = 0.10)

Species	**Part**	**Estimate**	**SEM**
Phyllostachys aureosulcata	Culm	16.3	1.9
Phyllostachys aureosulcata	Leaf	38.6	1.9
Phyllostachys bissettii	Culm	14.6	1.9
Phyllostachys bissettii	Leaf	40.6	2.1
Pseudosasa japonica	Culm	17.4	1.9
japonica	Leaf	40.8	1.9
Phyllostachys nuda	Culm	20.7	2.4
Phyllostachys nuda	Leaf	44.6	2.4

Table 3.2: There was no species × plant part interaction (*P* = 0.43); however; there was significant effect of plant part (*P* < 0.01) and a tendency for an effect of species (*P* = 0.10)

Species	**Part**	**Estimate**	**SEM**
Phyllostachys aureosulcata	Culm	18.3	2.0
Phyllostachys aureosulcata	Leaf	41.8	2.0
Phyllostachys bissettii	Culm	15.0	2.0
Phyllostachys bissettii	Leaf	44.3	2.2
Pseudosasa japonica	Culm	20.7	2.0
Pseudosasa japonica	Leaf	44.2	2.0
Phyllostachys nuda	Culm	21.3	2.5
Phyllostachys nuda	Leaf	48.6	2.5

CHAPTER IV

CONCLUSION

Despite having a carnivorous gastrointestinal tract and consuming a highly fibrous and lowly digestible diet, it is clear that GPs instinctively are able to selectively consume their diet based upon digestibility. If available leaf is less digestible/desirable, the GP will chose to consume primarily culm. Leaf may be undesirable due to seasonal shifts or environmental conditions. Other factors obviously come into play as well such as availability, competition, etc. Given that the species is marked “vulnerable”, significant research efforts aid in the conservation and preservation of GPs. With nutrition research specifically being so limited it is evident that further related research could be imperative for creating new ways to best help both captive and wild GP as well as develop a greater understanding as to how GP select and utilize their diet.

Out of the four analyzed species (selected based upon region and availability) NU exhibited highest digestibility overall indicating potential for optimal diet selection if cost effective and available. Leaves are clearly more digestible than culms but changes in composition that occur in late winter/early spring could be an explanation for shift in plant part consumption. Shoots exhibited highest digestibility overall however it is know that as shoots mature, they become less digestible overall. Comparing digestibility of orts with digestibility of sample from what was fed indicates that during culm season (January, March, May) pandas chose to consume culm because it was highest in digestibility, during October leaf season leaves were preferred based on digestibility and in July it was leaf season but culm was preferred part of consumption based on digestibility.

Giant pandas are able to survive and thrive on a bamboo diet based upon their incredible instinctive ability to pick a part their bamboo and selectively consume it. Without this ability, it is possible that GP would be extinct or more threatened than they are today. Since GP were once "endangered" but promoted to "vulnerable" on the IUCN red list of species and, there is hope that they could potentially move up to "Near Threatened" as a result of research and conservation efforts.

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