USE OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) TO INVESTIGATE SELECTION AND NUTRIENT UTILIZATION OF BAMBOO AND TO MONITOR THE PHYSIOLOGICAL STATUS OF GIANT PANDAS (*AILUROPODA MELANOLEUCA*)

A Thesis

by

ERIN WIEDOWER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2008

Major Subject: Rangeland Ecology and Management

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Approved by:

Chair of Committee,	X. Ben Wu
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ABSTRACT

Use of Near Infrared Reflectance Spectroscopy (NIRS) to Investigate Selection and Nutrient Utilization of Bamboo and to Monitor the Physiological Status of Giant Pandas (*Ailuropoda melanoleuca*). (August 2008)

Erin Wiedower, B.S., Texas A&M University

Chair of Advisory Committee: Dr. X. Ben Wu

The objective of this study was to develop near infrared reflectance spectroscopy (NIRS) calibration equations from bamboo and fecal samples to predict diet composition and the physiological status of giant pandas.

Discrimination between branch, culm, and leaf parts of bamboo resulted in an R-square (R^2) of 0.88. The calibration equation for discriminating between 4 species of bamboo had an R^2 of 0.47. Calibration equations were created for all bamboo species combined to determine the ability of NIRS to predict the nutrient constituents of CP, NDF, ADF, DM, and OM. No R^2 was lower than 0.96, with the exception of DM at 0.63, which was consistently difficult to accurately predict due to variation in factors relating to difference in location of lab work (humidity, shipping, methods, etc.).

Giant panda diets vary between seasons from eating primarily leaf to eating almost only culm. When bamboo part samples were compared between March and October, all resulting R^2s were above 0.80. The sensitivity analyses for leaf and culm samples within diet season produced inconclusive results, but sensitivity analyses for

fecal samples yielded an ability to more greatly discriminate between months that were further apart.

For giant panda physiological status calibrations, fecal samples were collected from the Memphis Zoo, Smithsonian's National Zoo, Zoo Atlanta, and San Diego Zoo from 2006 to 2007. One-hundred fecal spectra were used to develop discriminant equations with which to predict between adults and juveniles. The resulting calibration was 100% correct for both age classes. Predictions between 252 male and female fecal spectra were 89% correct for females and 90% correct for males. A small number of samples (N= 60) were used to create a discriminant equation to differentiate between pregnant and non pregnant females. The exercise resulted in an R^2 of 0.68 and a prediction of 100% for both pregnant and not-pregnant.

It has been determined through these studies that NIRS has the potential to determine nutrient composition of bamboo and giant panda fecals, but increased sampling and equation development is needed before these calibrations are applicable in a captive or wild giant panda setting.

NOMENCLATURE

ADF	Acid Detergent Fiber
AOAC	Association of Official Analytical Chemists
CP	Crude Protein
CV	Cross Validation
DM	Dry Matter
DR	Difference Ranked
GAN	Grazingland Animal Nutrition
H	Mahalanobis Distance
NDF	Neutral Detergent Fiber
NIRS	Near Infrared Reflectance Spectroscopy
OM	Organic Matter
PLLAR	*Phyllostachys aurea*
PLLAU	*Phyllostachys aureosulcata*
PLLGL	*Phyllostachys glauca*
PLS	Partial Least Squares
PSSJA	*Pseudososa japonica*
R	Reflectance
R^2	Coefficient of determination
SD	Standard Deviation
SEC	Standard Error of Calibration

SECV	Standard Error of Cross Validation
SEP	Standard Error of Prediction
SNV	Standard Normal Variate
VR	Variance Ratio

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CHAPTER I

INTRODUCTION

While the number of applications of near infrared reflectance spectroscopy (NIRS) has been expanding, there has currently been no NIRS research applied to a species as unique and endangered as the giant panda (*Ailuropoda melanoleuca*). Giant pandas are the only member of the family Ursidae that subsists entirely on vegetative material. The only other species in the order Carnivora that also has such a unique diet is the red panda (*Ailurus fulgens*) (Wei et al. 2000). The diets of giant pandas are highly specialized, feeding almost entirely on bamboo (Schaller et al. 1985). It is this dependence, combined with deforestation of their habitat in China and their charismatic nature, that is causing the giant panda to be one of the most recognized endangered species in the world (Liu et al. 1999).

NIRS has also been applied to vegetative material, both to determine the direct nutrient composition of vegetation (Park et al. 1998) and to determine forage quality through fecal analysis (Stuth et al. 2003). Many plant species have been analyzed using NIRS, from species used as forage for livestock (Welle et al. 2003) to fossil peat (McTiernan et al. 1998). Lawler et al. (2006) analyzed seagrass, a marine plant, to assess the diet of the dugong (*Dugong dugon*). However, there has yet to be published NIRS research on the nutritional and seasonal qualities of bamboo. Since bamboo is the primary dietary component of giant pandas, it would be of great use to determine the

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This thesis follows the style and format of the Journal of Wildlife Management.

nutritional value of bamboo in order to better understand its digestibility and nutrient density for the giant panda. It is known that giant pandas consume up to 6% of their body weight in bamboo per day, but only digest about 20 % of this diet (Dierenfeld et al. 1982).

Furthermore, it has been observed that pandas exhibit seasonal shifts in their diet (Schaller et al. 1985). Throughout most of the year, pandas both in captivity and in the wild eat almost solely leaves, due to greater nutritive value (Dierenfeld et al. 1982). However, during the spring months of March – May, pandas shift to eating almost solely inner culm (Taylor and Qin 1987). Conventional methods were used by Tabet et al. (2004) to analyze four species of bamboo and determine the macronutrient content during these shifts. In order to determine the concentrations of these components, four different techniques had to be applied. It would be more efficient to apply NIRS in situations such as these, especially since all concentrations could be determined in a timely manner using only one NIRS instrument. Monitoring these seasonal changes in bamboo nutrient content will aid in better understanding panda diet selection.

If NIRS is calibrated for use with giant pandas, it can become an invaluable tool for monitoring this species both in the wild and in captivity. NIRS equations could be developed to discriminate between males and females, age classes, pregnant and non-pregnant, and even perhaps between individuals. This would allow researchers to use giant panda fecal samples to non-invasively discern a great deal about an individual or population.

CHAPTER II

LITERATURE REVIEW

Near Infrared Reflectance Spectroscopy (NIRS)

Near-infrared light is located in the region between 700 and 2500 nm on the electromagnetic spectrum (Figure 1). NIRS quantifies the relationship between light and the chemical bonds of nitrogen, carbon, and oxygen with hydrogen (Foley et al. 1998). Near infrared (NIR) instruments measure the absorption and reflectance of these bonds. In the research described herein, a spectroscopy instrument was used that scans across both visible (400-700nm) and NIR light, but my calibrations specifically utilize the wavelengths between 1100 and 2500 nm. The absorbance of light is recorded as log 1/R, using the following formula:

$$A_{\lambda} = \log (1/\text{reflectance}_{\lambda}).$$

Every sample will have a unique spectrum reading, similar to a fingerprint, which will vary depending on the properties of the sample.

Although more than one bonding group can be found at any one wavelength, certain compounds present in samples have consistent absorption bands, or peaks, located on the spectra. Water peaks are generally found at 1450 and 1930 nm (Shenk et al. 2001) and protein has many peaks throughout the NIR range (Shenk et al. 2001, Williams 2005). Forage materials specifically have been found to have particular bond absorption bands in the NIR region (Workman and Shenk 2004).

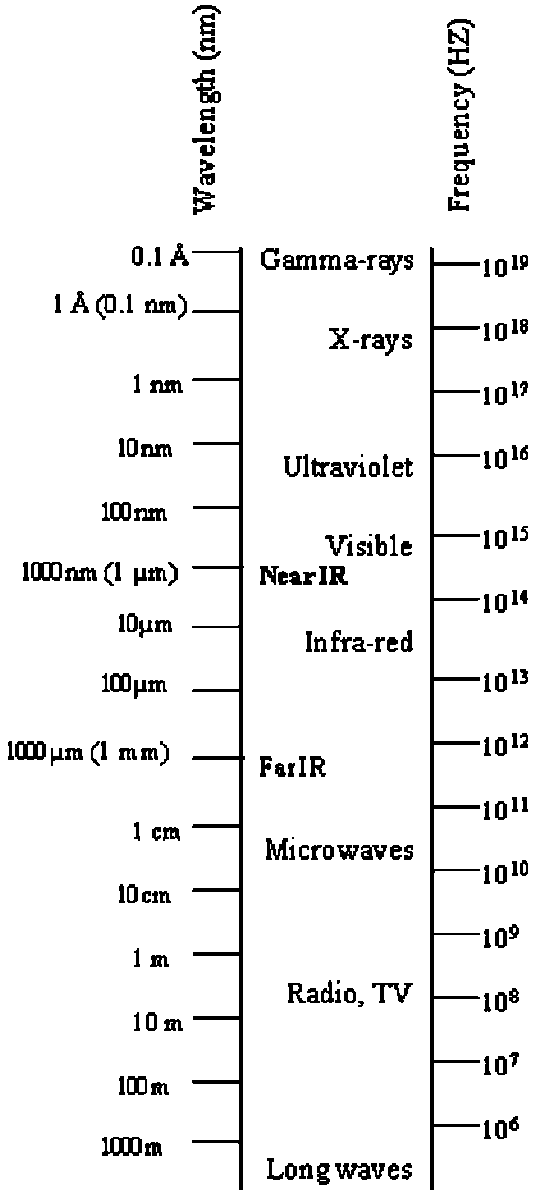

Figure 1. Electromagnetic spectrum showing near IR region.

Regression equations are created by comparing the sample spectra within a designated set, with a coefficient of determination value (R^2) statistically representing the level of variance explained by the equation. This method of applying math and statistics to the chemistry within organic compounds is termed chemometrics (Landau et al. 2006*a*).

There are several other parameters that are commonly used to judge the quality of a calibration. The standard error of calibration (SEC) is the standard deviation of the errors between the reference analysis values and the NIRS analysis values. SEC decreases as R^2 increases (Hrushka 1987). Standard error of cross validation (SECV) is the result of individually predicting each sample from the calibration set using the calibration of the remaining n-1 samples (Stone 1974). Some authors believe that SECV is a preferable parameter to SEC because it avoids over-fitting (Cozzolino and Moron 2004). It is also a method that is generally used when there are not enough samples to withhold from the calibration and then validate separately. The standard error of prediction (SEP) is used during validation when using an independent sample set from calibration. It is the standard deviation of the errors between the reference analysis values and the NIRS analysis values for the validation sample sets (Williams 1987)

Varying math pre-treatments can be applied to the calibration spectra in order to further develop differences between the individual spectra, for example "1,4,4,1" or "2,4,4,2." The four numbers, respectively, represent the derivative, the nm width the derivative is calculated from, the number of points used in the first smoothing procedure, and the nm interval over which the second smoothing is applied. Random variation

effects caused by water molecules and different particle sizes in samples can be controlled by applying the standard normal variate (SNV) to each spectrum. Application of the SNV also decreases undue collinearity between samples (Barnes et al. 1989). Detrend is another scatter correction function that can be added alone or in conjunction with SNV.

Historically, near infrared reflectance spectroscopy (NIRS) was established for use in agriculturally-related applications. What once began as a method for monitoring captive livestock, has now expanded into applications for free-ranging livestock (Leite and Stuth 1995), due to fast processing times, low maintenance costs, and sample preservation. NIRS has been successfully used to determine diet quality (Leite and Stuth 1995, Lyons et al. 1993), selection (Walker et al. 1998), and digestibility (Purnomoadi et al. 1996) of livestock using both vegetative (Shenk and Westerhaus 1985) and fecal matter (Walker et al. 1998). NIRS can measure a variety of nutrient components of diet and feces (e.g. crude protein, neutral detergent fiber, acid detergent fiber, dry matter, and organic matter). Some have used NIRS to measure minerals, but they do not have absorption bands that are within the near infrared wavelength region. Any measurements made in the NIR region are due to bonding with organic constituents (Shenk et al. 1979).

Despite a focus on livestock, applications of fecal NIRS to wildlife are not unprecedented as more researchers have recognized the potential of the technology. NIRS has been applied to free-ranging wildlife (Tolleson et al. 2005, Greyling 2002), marsupials (McIlwee et al. 2001), and endangered species such as the hairy-nosed

wombat (*Lasiorhinus krefftii*) (Woolnough and Foley 2002). One avian species has been examined by Landau et al. (2006*b*) who predicted the nutrient content of ostrich diets. There has also been a limited amount of research done with NIRS applied to carnivores. Dahl et al. (2000) predicted the digestibility of mink diets and Kaneko and Lawler (2006) used NIRS to predict the diets of two species of marine mammals. These are just preliminary studies, however, and much more effort in these areas is needed before NIRS use can be opened up to a wider array of species.

A more comprehensive review of NIRS, its techniques, and its applications, can be found in Shenk and Westerhaus (1994) and Foley et al. (1998).

Bamboo

Bamboo is the tallest grass in the world and there are nearly 1,000 species found all over the world (Keeley and Bond 1999). It has been studied extensively and much is known about its structure and functional processes (McClure 1993). However, a couple of important questions about bamboos remain that are relevant to the behavior and success of giant panda populations.

The first important question is: "Why do giant pandas switch from eating primarily leaf to primarily less nutritious culm during the spring months?" In winter, pandas feed on tall, thick culms and during the summer they feed on primarily leaf or short, thin culms (Reid and Hu 1991, Liu et al. 2005). There are several explanations that have been suggested for this diet seasonality, and it may be one or a combination of all of these that hold the answer. Bissell et al. (Memphis Zoo, unpublished data) found that there are higher soluble carbohydrate levels in the culm during the spring months

than during summer months. Reid and Gong (1999) believe these animals switch to a less nutritious culm diet during the winter because most of the leaves are dead. The final suggestion of this dietary shift is that leaves have a higher level of unpalatable silica during spring, thereby driving the pandas to eat the relatively more palatable culms (Schaller et al. 1985, Tabet et al. 2004).

The second question that has long been debated is: "Why do most semelparous bamboos species mast flower and then die with long intermast intervals?" Since whole stands of bamboo can flower and die at once, mast flowering can have an impact on panda populations if their main food source has been depleted. There are several explanations given for this question, as well. One of the most notable is the predator satiation hypothesis proposed by Janzen (1976). He suggested that the timing of seeding among cohorts is genetic and not affected by environmental conditions, and that the large number of seeds are created to overcome predation by small animal predators. Plant mortality after flowering occurs most likely because all resources are used up by the plants in creating such a high number of seeds. A second major hypothesis, the bamboo fire cycle hypothesis (Keeley and Bond 1999), specifically discounts Janzen's in favor of a disturbance model. Mass mortality of the bamboo creates a large fuel load which encourages wildfire that kills woody plant seedlings but not their own. Wildfire also opens up the tree canopy which aids in bamboo seedling propogation and mature bamboo growth.

The following thesis does not directly attempt to answer either of these questions. Rather than researching the physiological changes in the bamboo that cause the giant

panda diet shift, it attempts to create a method of monitoring the leaf to culm change, both within the bamboo itself and also within the giant panda fecal samples.

The Giant Panda

The giant panda is an endangered member of the family Ursidae. They are herbivorous, feeding almost completely on bamboo. Since radiating from the other bears and becoming prevalent in China during the early Pleistocene about 3 million years ago (Schaller et al. 1985), they have developed some specializations for a diet restricted almost solely to bamboo. They have no carnassial teeth and their molars are wide with heavy cusping for crushing bamboo (Reid and Gong 1999). They also have an enlarged radial sesamoid bone in their wrist that acts as a surrogate "thumb" and is used in manipulating bamboo with great efficiency (Anton et al. 2006). Their unusually high bite force and craniodental morphology are specialized for herbivory, but is still considered unspecialized when compared with other herbivores (Christiansen 2007).

Despite these adaptations, the giant panda is still rather ill equipped for a bamboo diet. Their gastrointestinal tract is comparable in length to other carnivores and they possess no rumen, no caecum, and a short colon (Dierenfeld et al. 1982). As stated in Demment and Van Soest (1985), the gut size of herbivores determines the capacity to digest food into nutrients, therefore, a smaller GI tract in pandas equates to a lower ability to process diet into necessary nutrients. Food items move quickly through the gastrointestinal tract, with passage rates ranging from as few as 5 hours to a maximum of only 11 hours (Dierenfeld et al. 1982). All of these traits limit microbial digestion, thereby limiting the cellular components of bamboo a panda is able to assimilate to only

about 17-20% of dry matter (Dierenfeld et al. 1982). While they are known to be omnivorous, 99% of their diet is made up of bamboo due to a lack of alternative food availability (Dierenfeld 1997, Reid and Gong 1999). Because of the low nutrition quality of bamboo, in order to maintain their body weight, the pandas must consume very large amounts of forage each day. In fact, these animals daily consume up to 6% of their body weight (Dierenfeld et al. 1982) or 10-18kg (Reid and Gong 1999).

Due to the unique characteristics and behavior of giant pandas, their taxonomic placement has been disputed. While none have disputed that they belong in the order Carnivora, their familial status has been up for debate. Some have proposed them to be grouped with the red panda (*Ailurus fulgens*) in the family Procynidae (Morris and Morris 1966). Some propose that the giant panda is more closely related to bears in Ursidae (Davis 1964, Sarich 1973), while others suggest they belong in a family of their own called Ailuropodidae (Wurster-Hill and Bush 1980, Zhang and Ryder 1993). However, it has since been concluded that giant pandas are most appropriately placed in the Ursid family based on life histories, morphology, and genetic data (Chorn and Hoffmann 1978, O'Brien et al. 1985, Wayne et al. 1989, Gittleman 1994).

Due to the large amounts of bamboo they must consume, giant pandas spend most of their time each day performing feeding behaviors (average of 10.5 hours/day) and rest frequently to conserve energy (Johnson et al. 1988). As mentioned previously, panda bamboo selection preferences vary seasonally throughout the year and can also shift after a bamboo species flowers and dies (Taylor and Qin 1987, Schaller et al. 1985). They are elevational migrants within their habitat, moving to lower elevations

during the winter months and to higher elevations during summer months (Loucks et al. 2003, Liu et al. 2005).

Giant pandas maintain territories within the few provinces in China in which they can still be found. Female home range sizes are determined by food availability while male home ranges are determined by the availability of mature females (Reid and Gong 1999). Since only a small portion of an individual's territory is used at any one time, territories of the same sex may overlap (Swaisgood et al. 2003). Pandas are solitary animals except during mating season or when the females are caring for their young (Brambell et al. 1969).

There is some disagreement in the literature as to when male and female pandas become sexually mature. Schaller et al. (1985) state that both sexes become mature between 5.5 and 6.5 years of age. However, it has been observed that pandas can become mature as early as 4.5 years (Mainka et al. 1990, Pan et al. 2004). Mating occurs between the months of April and May and the female will give birth after a gestation period of 97 to 161 days, with an average of 135 days (Schaller et al. 1985). It is believed that females have delayed implantation of the fertilized blastocyst, due to the longer gestation period yet undeveloped nature of the infants (Mead 1989, Sandell 1990). Giant pandas have the most altricial babies of any other eutherian mammal (Swaisgood et al. 2003). This most likely contributes to the high infant mortality rate of 59% in the wild (Wei et al. 1990) and between 40% and 70% in captivity (Jinchu 1990, Schaller et al. 1985). The mother panda may have twins, but only one of the young is fed and raised (Peng et al. 2001). The young start to be weaned from their mother's milk at

about 6 months old and will be completely eating solid foods by 24 months of age (Edwards et al. 2006). However, the young remain dependent on their mothers from 1.5 to 2 years (Peng et al. 2001). Therefore, the female birth interval is an average of 2 years (Schaller et al. 1985). If the infants die within the first year, the female may come into estrous again the following year (Reid and Gong 1999). The giant panda has a reproductive life span of around 19 or 20 years of age (Zhu et al. 2001), and life span of 25-30 years (Schaller et al. 1985).

CHAPTER III

USE OF NIRS TO DISCRIMINATE BETWEEN AND PREDICT THE NUTRIENT COMPOSITION OF DIFFERENT SPECIES AND PARTS OF BAMBOO

Synopsis

Giant pandas (*Ailuropoda melanoleuca*) are obligate feeders, dependent upon bamboo as their main dietary resource. Due to the decline of their habitat, it is important to be able to readily quantify the quality of bamboo. Near infrared reflectance spectroscopy (NIRS) has been used previously as a tool to measure forage quality for both domestic and free-ranging species. The objective of this study was to determine the capability of NIRS to 1) discriminate between bamboo parts, 2) discriminate between bamboo species, and 3) to predict the nutrient composition of bamboo.

All bamboo samples were received from the Memphis Zoo Bamboo Farm (Memphis, TN) then were dried at 60°C and ground to pass through a 1mm screen before NIRS procedures were applied. Discrimination between branch, culm, and leaf resulted in an R^2 of 0.88 and standard error of cross-validation (SECV) of 0.18. Spectra from a total of 756 samples of 4 different species were used to create a discriminant equation between bamboo species. This resulted in a disappointing R^2 of 0.47 and SECV of 0.29. Calibration equations for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter (DM), and organic matter (OM) were created using all bamboo samples. No R^2 was no lower than 0.96 for any nutritional constituent, with the exception of DM at 0.63. The SECV ranged from 0.31 for OM to

1.94 for NDF. NIRS was successful in discriminating between bamboo plant parts within species and predicting the nutrient parameters of bamboo. The preliminary inability of NIRS to discriminate between bamboo species is most likely due to a close physiological similarity between at least 2 of the species.

Introduction

The giant panda is one of the most well known endangered species in the world. There are only about 1,100 pandas left in the wild (National Conservation Management Plan 1989). They are limited to only three provinces in China (Reid and Gong 1999) and are fragmented into 24 small subpopulations, some of which may be too small to be genetically viable (O'Brien et al. 1994). Pandas are obligate feeders and about 99% of their diet consists of bamboo (Schaller et al. 1985, Dierenfeld 1997). Since the ability of pandas to digest bamboo is limited, they must consume up to 6% of their body weight or 10-18kg (Dierenfeld et al. 1982, Reid and Gong 1999) per day. However, as with many other wildlife species, pandas are able to select for the more nutritious plant parts in any given season (Howery & Pfister 1990, Reid and Hu 1991).

The dependency of pandas on bamboo indicates that not only the amount, but also the quality of their habitat is key to sustaining their current populations. Therefore, it would be of great use to have a reliable method of assessing bamboo quality. Traditional methods of evaluating forage quality are both time and financially expensive. However, near infrared reflectance spectroscopy (NIRS) can be applied with just as much precision as these methods, sometimes more, but without these time and money constraints (Foley et al. 1998, Cozzolino et al. 2000). NIRS has been applied with

varying degrees of success to several different aspects of wildlife forage, including nutrients (Dorgeloh et al. 1998, Showers et al. 2006), foliage moisture (Gillon et al. 2002), and browsing damage predictions (Stolter et al. 2006). We hypothesize that NIRS can also be a useful tool in quantifying the nutritional quality of bamboo.

The objectives of this study were to evaluate the ability of NIRS to discriminate between bamboo species and parts and, more importantly, to predict the nutrient composition of various bamboo species.

Methods

Bamboo samples of 4 species, *Phyllostachys aurea* (PLLAR), *P. aureosulcata* (PLLAU), *P. glauca* (PLLGL), *and Pseudosasa japonica* (PSSJA), were grown and cut opportunistically at the Memphis Zoo Bamboo Browse Farm in Memphis, Tennessee, USA beginning in 2003. The samples were dried in a forced-air oven at 60°C overnight, ground in an Udy mill to pass uniformly through a 1mm screen, and kept at room temperature (20-22ºC) until processing, at which time wet chemistry analysis was performed on all of the bamboo samples by the Department of Animal and Dairy Sciences at Mississippi State University. Nutritional constituents include crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), dry matter (DM), and organic matter (OM). The wet chemistry procedures are Association of Official Analytical Chemists (AOAC 2003) 2001.11, AOAC 973.18, AOAC 2002.04, AOAC 930.15, and AOAC 942.05 (Ash procedure), respectively. OM is calculated by subtracting Ash from DM.

The same bamboo sample catalog was then sent to the Grazingland Animal Nutrition (GAN) Lab at Texas A&M University and kept at room temperature (20-22ºC) until they were to be scanned. Then they were put in a forced-air oven at 60°C for at least 3 hours and then into a dessicator for one hour to return to ambient temperature while preventing ambient moisture from entering the sample. Ground samples were then manually packed in sample cups with quartz cover glass at a consistent level and compression. Quartz glass is used instead of traditional glass because quartz maintains a more uniform thickness. Each sample was scanned using a Foss NIRS Systems 6500 Spectrometer (Foss North America, Eden Prairie, MN, USA) with spinning drawer attachment and WinISI II v. 1.04a software. Measurements of light are made at 8-nm intervals over the visible and near infrared range (400 – 2500nm).

The NIRS spectra were paired with the corresponding wet chemistry to develop a calibration set and to make predictive equations for each species and constituent. The procedures performed in these studies are the standard NIRS calibration and validation procedures similar to those described in such sources as Tolleson et al. (2005) and Li et al. (2006). All samples used in validation sets were chosen randomly using the WinISI II software for randomization using a 75% for calibration to 25% for validation ratio.

All equations had a mathematical pre-treatment of a second order derivative 2,4,4,1 (derivative, gap, smooth, gap of smooth) with scatter corrections of Standard Normal Variate (SNV) and Detrend (D), unless stated otherwise. Regression equations for calibration were created using the partial least squares (PLS) procedure (Wold et al. 1983). Discriminant equations use a two-block PLS procedure. The PLS method uses

the full near-infrared light spectrum (Martens and Naes 1989), as opposed to the previously popular multiple linear regression (MLR) method which used only a few wavelengths (Foley et al. 1998). Simultaneously, a cross-validation procedure is performed to validate the discriminant equations. Calibration spectral outliers were to be identified as those with a global Mahalanobis distance (GH) of 10.0 or greater units from the average of all calibration spectra, which is the default set in the WinISI software. A coefficient of determination (R^2) value of 0.80 or greater was considered applicable with caution for most applications (Williams 2005). Once the discriminant equation has been created for differentiating between 2 species, the validation set will be predicted using the discriminant equation, i.e. those samples will be grouped by species. Correct categorical prediction of at least 80% of samples from a given category into its proper grouping will be considered satisfactory ability of NIRS to discriminate between groups.

Prediction equations will also be created to determine the ability of NIRS to predict these nutrient constituents of bamboo. For prediction equations, outliers were removed by hand and a new prediction equation created (Williams 1987). Samples with a *t* value of greater than 2.5 were considered outliers (Shenk and Westerhaus 1991). These outliers are those with estimated values that differ statistically significantly from the reference chemistry values (Azzouz et al. 2003). Extreme GH outliers (H > 10.0) will also be removed if there is evidence that scanning error has occurred. It is suggested that these samples be removed from the calibrations.

Calibration sets should ideally be diverse in order to be useful in the field (Foley et al. 1998). Therefore our bamboo samples are representative of different bamboo

species with temporal variation of collection in order to get the most widely applicable equation within our means.

Results

The ability to discriminate between all bamboo species was low, with an R^2 of only 0.475 (Table 1). After performing predictions on all samples of each species and within individual species validation sets (Tables 2 and 3), it became clear that PLLAR was the poorly predicted species and was responsible for the low correlation between spectra and species identity in the calibration set. The calibration equation was subsequently recreated, this time leaving out PLLAR samples, which resulted in a new R^2 value of 0.712 and SECV of 0.243. This still does not meet the minimum R^2 standard of 0.80 (Williams 2005), but can still be a useful calibration for screening or rough classification. Removing only the PLLGL samples from the equation produces similar results (Table 1).

In the species predictions, PLLAR was most often incorrectly classified as PLLGL. It can also be seen that when each pair of species was discriminated, the only pair that clearly didn't meet calibration standards was PLLAR and PLLGL (Table 2). This indicates that there may be some spectral similarity between the 2 species which is confounding the ability of NIRS to distinguish between them. Discriminations between PLLAR and PLLGL with the other two bamboo species, PLLAU and PSSJA, produced similar results (Table 2).

The ability to discriminate between bamboo parts was high with an R^2 value of 0.881 and a relatively low SECV of 0.176 (Table 1). Predictions of all plant part

samples to the part discriminant equation were also high, with no prediction lower than 94% (Tables 3 and 4).

Table 1. Statistics of the calibration equations discriminating between bamboo species and plant part.

Discrimination	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Species	756	0.381	0.276	0.475	0.291	0.406
Species w/out PLLAR	496	0.426	0.229	0.712	0.243	0.675
Species w/out PLLGL	419	0.383	0.212	0.693	0.235	0.624
Part	722	0.459	0.158	0.881	0.176	0.853

[1] Standard deviation
[2]Standard error of calibration
[3]Coefficient of determination
[4]Standard error of cross validation
[5]One minus variance ratio

Table 2. Statistics of the validation equations discriminating between pairs of bamboo species.

Discrimination	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
PLLAR vs. PLLGL	201	0.462	0.348	0.433	0.401	0.246
PLLAR vs. PLLAU	360	0.378	0.178	0.778	0.214	0.678
PLLAR vs. PSSJA	121	0.500	0.176	0.876	0.209	0.825
PLLGL vs. PLLAU	437	0.466	0.207	0.804	0.230	0.757
PLLGL vs. PSSJA	198	0.457	0.170	0.861	0.214	0.782
PLLAU vs. PSSJA	357	0.371	0.119	0.898	0.136	0.865

Table 3. Prediction of all samples of each bamboo species and plant part against the respective calibration equation.

Discrimination		# Correct Out of Total	Percent Correct
Species	PLLAR	5/83	6
	PLLAU	400/411	97
	PLLGL	153/188	81
	PSSJA	63/74	85
Part	Branch	177/181	98
	Culm	267/270	99
	Leaf	260/260	100

Table 4. Prediction of validation samples of each bamboo species and plant part against the respective calibration equation.

Discrimination		# Correct Out of Total	Percent Correct
Species	PLLAR	2/21	10
	PLLAU	98/100	98
	PLLGL	36/45	80
	PSSJA	11/15	73
Part	Branch	31/33	94
	Culm	46/46	100
	Leaf	44/44	100

Calibration equations and validations for the 5 most commonly measured nutrient constituents are given below (Tables 5 - 19). Very consistently throughout the equations, NIRS had a lower ability to predict DM in this study than for any of the other 4 constituents. Also, NDF and ADF consistently have higher standard errors than the other constituents. The R^2 values for all bamboo samples combined together (regardless of species) do not go below 0.963, with the exception of DM with 0.628 (Table 5). The predictive equations had similar results (Table 6), with no R^2 below 0.916, except for DM with 0.536. Calibration and predictive equations by bamboo part were similar, but a bit more variable, with leaf in particular showing a lower level of prediction (Tables 7-12). The leaf calibrations for NDF, ADF, and DM resulted in R^2 values that did not reach the minimum of 0.80 for calibration (0.674, 0.518, and 0.606, respectively) (Table 11). Leaf samples had similar predictive results with R^2 values of 0.715, 0.448, and 0.510, respectively. In this case, it was ADF that did more poorly than DM.

Nutrient predictions for individual bamboo species behaved in the same way as all bamboo samples combined and bamboo parts. All R^2 values for both calibration and

predictive equations were above 0.90 except for NDF predictive equation of PSSJA at 0.894 (Table 20) and the DM values. The lowest DM value was 0.409 for the PLLGL calibration (Table 17).

The relationships between the actual amount of the designated nutrient constituent in a branch bamboo sample, as determined by wet chemistry procedures, and the predicted amount for that same sample using the NIRS calibration equations are shown in the figures below (Figures 2-6). Keeping in mind that outliers were removed, all of the parameters for branch samples have a positive linear regression, with the exception of DM (Figure 4), which had consistently poor calibrations and predictions.

Table 5. Statistics of the nutrient parameter calibration equations for all bamboo samples combined.

Constituent	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	708	5.960	0.319	0.997	0.374	0.996
NDF	710	8.677	1.662	0.963	1.942	0.950
ADF	713	13.235	1.825	0.981	1.901	0.979
DM	734	1.552	0.947	0.628	1.061	0.533
OM	722	3.912	0.262	0.996	0.312	0.994

Table 6. Statistics of the predictive equations for all bamboo samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	147	0.992	0.516	2
NDF	145	0.933	2.484	5
ADF	145	0.945	3.214	5
DM	148	0.502	1.018	2
OM	147	0.995	0.282	3

[6]Standard error of prediction

Table 7. Statistics of the nutrient parameter calibration equations for bamboo branch samples of all bamboo species combined.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	172	0.739	0.175	0.944	0.226	0.906
NDF	172	3.585	1.397	0.848	1.859	0.731
ADF	172	5.344	1.958	0.866	2.239	0.824
DM	181	1.325	1.015	0.413	1.102	0.309
OM	175	0.886	0.156	0.969	0.250	0.920

Table 8. Statistics of the predictive equations for bamboo branch samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	30	0.952	0.190	3
NDF	29	0.876	1.225	4
ADF	29	0.903	1.616	4
DM	30	0.183	1.009	3
OM	26	0.973	0.159	1

Table 9. Statistics of the nutrient parameter calibration equations for bamboo culm samples of all bamboo species combined.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	250	0.536	0.145	0.927	0.218	0.835
NDF	258	3.947	1.461	0.863	1.869	0.775
ADF	257	5.582	1.868	0.888	2.517	0.796
DM	267	1.544	0.778	0.746	0.942	0.628
OM	263	0.581	0.129	0.951	0.166	0.918

Table 10. Statistics of the predictive equations for bamboo culm samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	45	0.903	0.297	1
NDF	37	0.854	1.678	9
ADF	38	0.916	2.454	8
DM	44	0.767	0.774	2
OM	32	0.943	0.129	2

Table 11. Statistics of the nutrient parameter calibration equations for bamboo leaf samples of all bamboo species combined.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	252	2.425	0.425	0.969	0.502	0.957
NDF	252	3.088	1.762	0.674	2.031	0.567
ADF	251	2.145	1.489	0.518	1.602	0.444
DM	250	1.491	0.936	0.606	1.072	0.484
OM	254	1.889	0.333	0.969	0.437	0.946

Table 12. Statistics of the predictive equations for bamboo leaf samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	42	0.975	0.367	2
NDF	43	0.711	2.148	1
ADF	42	0.505	1.589	2
DM	40	0.510	0.941	4
OM	38	0.981	0.212	1

Table 13. Statistics of the nutrient parameter calibration equations for *P. aurea* species of bamboo.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	70	5.688	0.271	0.998	0.534	0.992
NDF	77	9.546	2.584	0.927	3.018	0.904
ADF	74	14.779	3.080	0.957	3.940	0.933
DM	81	1.426	0.700	0.759	0.988	0.516
OM	76	3.670	0.683	0.965	0.698	0.966

Table 14. Statistics of the predictive equations for *P. aurea* bamboo samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	18	0.998	0.265	1
NDF	20	0.949	2.131	1
ADF	20	0.973	2.547	1
DM	21	0.864	0.562	0
OM	20	0.988	0.415	1

Table 15. Statistics of the nutrient parameter calibration equations for *Phyllostachys aureosulcata* species of bamboo.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	392	6.224	0.243	0.999	0.286	0.998
NDF	392	8.190	1.445	0.969	1.604	0.962
ADF	396	12.623	1.420	0.987	1.504	0.986
DM	395	1.625	0.814	0.749	0.977	0.639
OM	390	3.835	0.183	0.998	0.256	0.996

Table 16. Statistics of the predictive equations for *P. aureosulcata* bamboo samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	98	0.996	0.387	2
NDF	99	0.970	1.421	1
ADF	98	0.988	1.332	2
DM	98	0.777	0.728	2
OM	100	0.996	0.242	0

Table 17. Statistics of the nutrient parameter calibration equations for *P. glauca* species of bamboo.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	170	5.818	0.288	0.998	.385	0.996
NDF	177	9.338	1.730	0.966	2.104	0.949
ADF	174	14.219	2.289	0.974	2.821	0.961
DM	182	1.333	1.025	0.409	1.097	0.319
OM	171	4.034	0.235	0.997	0.390	0.991

Table 18. Statistics of the predictive equations for *P. glauca* bamboo samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	42	0.997	0.340	3
NDF	44	0.968	1.692	1
ADF	42	0.979	2.214	3
DM	44	0.573	0.845	1
OM	41	0.995	0.270	4

Table 19. Statistics of the nutrient parameter calibration equations for *Pseudosasa japonica* species of bamboo.

Prediction	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
CP	68	4.470	0.391	0.992	0.575	0.984
NDF	70	7.402	1.527	0.957	2.025	0.925
ADF	69	11.754	2.259	0.963	3.366	0.918
DM	72	1.452	0.670	0.787	1.056	0.479
OM	68	2.885	0.237	0.993	0.590	0.958

Table 20. Statistics of the predictive equations for *P. japonica* bamboo samples withheld for validation.

Prediction	N	RSQ[3]	SEP[6]	# *t* outliers removed
CP	15	0.993	0.392	0
NDF	14	0.920	2.016	1
ADF	13	0.945	2.723	2
DM	15	0.883	0.572	0
OM	13	0.995	0.320	2

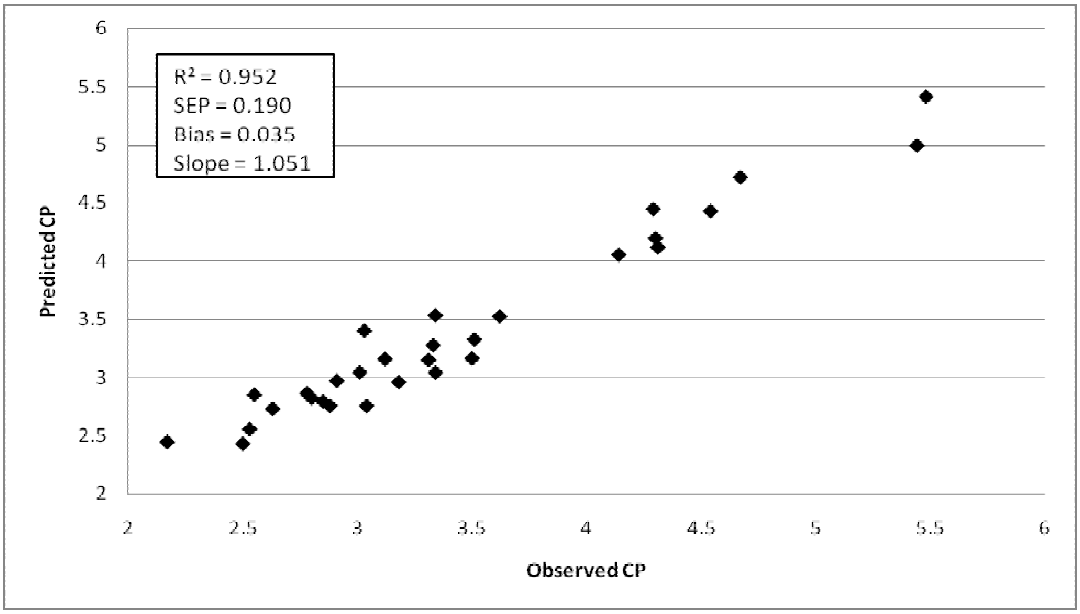

Figure 2. The relationship between observed and predicted CP in the branch of bamboo using NIRS calibration equations. The data represent 30 validation samples taken as a subset of the calibration samples and not used to create predictive equations. 3 *t* outliers were removed.

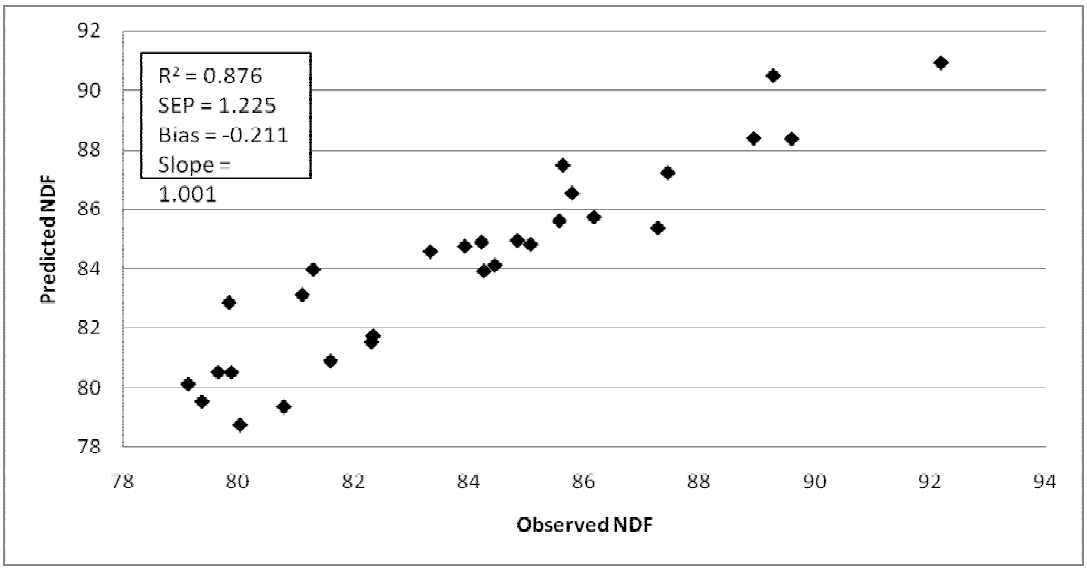

Figure 3. The relationship between observed and predicted NDF in the branch of bamboo using NIRS calibration equations. The data represent 29 validation samples taken as a subset of the calibration samples and not used to create predictive equations. 4 *t* outliers were removed.

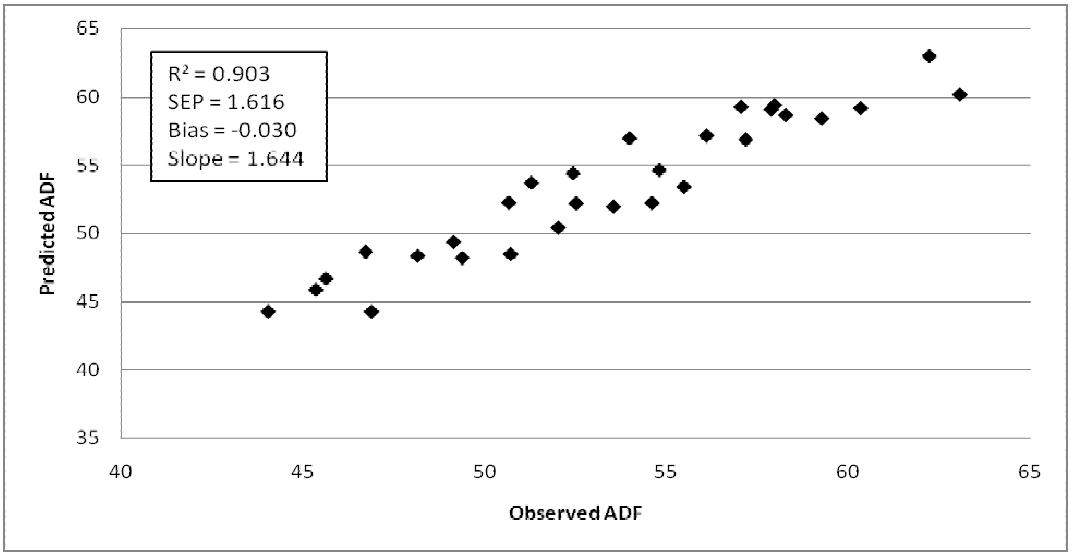

Figure 4. The relationship between observed and predicted ADF in the branch of bamboo using NIRS calibration equations. The data represent 29 validation samples taken as a subset of the calibration samples and not used to create predictive equations. 4 *t* outliers were removed.

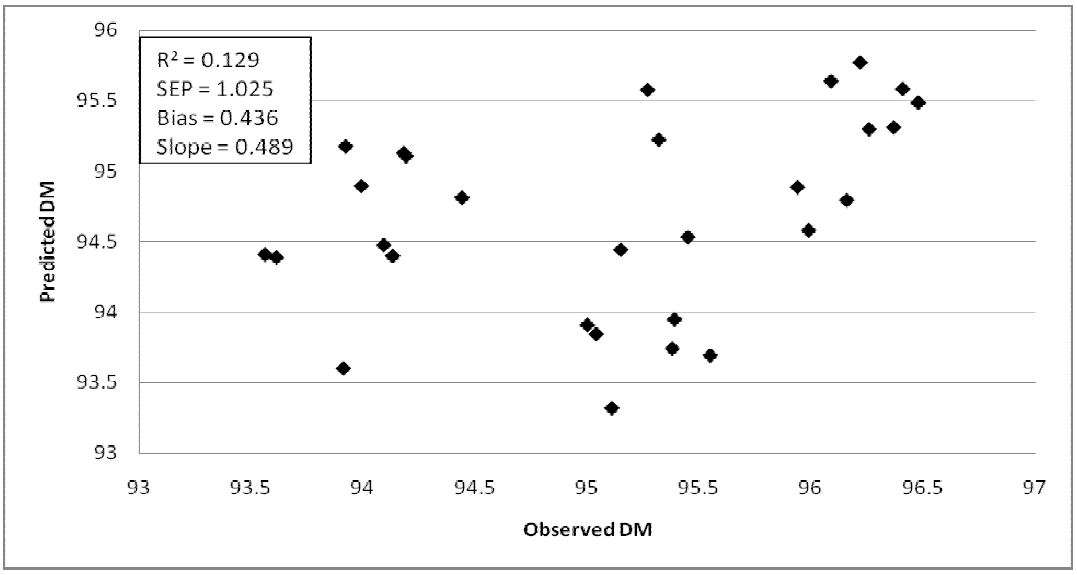

Figure 5. The relationship between observed and predicted DM in the branch of bamboo using NIRS calibration equations. The data represent 29 validation samples taken as a subset of the calibration samples and not used to create predictive equations. 3 *t* and 1 global H outliers were removed.

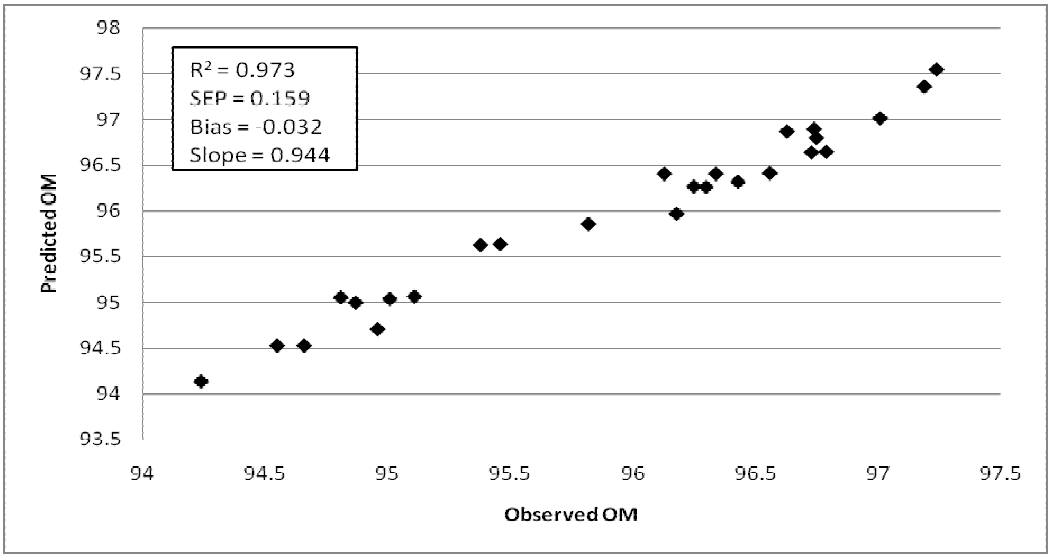

Figure 6. The relationship between observed and predicted OM in the branch of bamboo using NIRS calibration equations. The data represent 26 validation samples taken as a subset of the calibration samples and not used to create predictive equations. 1 *t* outlier was removed.

Discussion

NIRS had a higher ability to distinguish between the four bamboo species when the PLLAR samples were removed. Originally it was thought that all of the species were physiologically similar, therefore making it difficult to discriminate between them. However, now it seems that the species PLLAR and PLLGL are the only two that are physiologically similar. The individual plant parts within a single species have a higher degree of discrimination, indicating that they are more physiologically different. The leaves are generally known to have a higher level of crude protein (Dierenfeld et al. 1982) and the culms as being more nutritionally devoid (Bissell et al 2006). This produces spectrally different samples that are more readily distinguishable by NIRS.

NIRS was successfully able to predict CP, NDF, ADF, and OM in bamboo samples of all species and parts. The R^2 values of CP were similar to the findings of other authors (0.99, Norris et al. 1976; 0.97-0.98, Marten et al. 1984; 0.93-0.95, Garcia-Ciudad et al. 1993; 0.96, Berardo 1997; 0.95-0.97, Hsu et al. 2000). The SEP values, were also similar to the findings of other studies (0.95, Norris et al. 1976; 0.57-0.70, Garcia-Ciudad et al. 1993; 2.7, Berardo 1997), but SEC and SECV were lower than that found by Berardo (1997; 3.0, 4.2, respectively).

The R^2 results for NDF were also similar to other findings (0.98, Norris et al. 1976; 0.95-0.98, Marten et al. 1984; 0.92-0.93, Garcia-Cuidad 1993; 0.73-0.92, Hsu 2000). SEP values were similar to those of Garcia-Ciudad et al. (1993; 2.06-2.37), but were higher than those in Berardo (1997; 0.33). However, our findings for SEC and

SECV were lower than those in Berardo (1997; 3.7 and 4.7, respectively). Similarly for ADF, other authors found R^2 values comparable with our results (0.96, Norris et al. 1976; 0.85-0.87, Garcia-Ciudad et al. 1993; 0.95, Berardo 1997), SEP (1.42-1.56, Garcia-Ciudad; 3.1, Berardo 1997), with SEC and SECV (3.3, 4.2, respectively, Berardo 1997) being generally higher than our results.

NIRS was not successfully able to predict the DM of bamboo. Dry matter is difficult to accurately predict when the wet chemistry and the NIRS scanning are done at different locations. In this case, samples had wet chemistry analysis performed by the Department of Animal and Dairy Sciences at Mississippi State University and then scanning at the Grazingland Animal Nutrition (GAN) Lab at Texas A&M University. This creates too many variables in the moisture content which can result in a low ability of NIRS prediction, which is what most likely occurred with these samples.

The bamboo species that were analyzed in this study are species originating from China. However, the species grown at the Memphis Zoo Bamboo Farm are not the native forage species of giant pandas in China (Taylor and Qin 1987, Reid and Gong 1999) nor are they grown in the same climatic and soil conditions. For these reasons I would hesitate to extrapolate the equations presented here to other bamboo species. Therefore, for these equations to be applied to free-ranging panda populations, they will need to be updated with samples of bamboo species native to giant pandas, such as *Fargesia spathacea* Franchet and *Bashania fangiana* Yi.

CHAPTER IV

USE OF NIRS TO MONITOR SEASONAL CHANGES OF BAMBOO PLANT PHYSIOLOGY IN THE DIET AND FECES OF GIANT PANDAS (*AILUROPODA MELANOLEUCA*)

Synopsis

Giant pandas (*Ailuropoda melanoleuca*) demonstrate seasonal preferences for different parts of their primary food source, bamboo. During most months pandas eat almost solely bamboo leaf. However, during the winter months they begin to progressively eat more culm until during the months of March through May they are eating culm almost exclusively. Since the giant panda is an endangered species with declining habitat due to deforestation and fragmentation it would be of great use to be able to monitor these seasonal diet shifts, and ideally to anticipate them before they even begin to occur. Near infrared reflectance spectroscopy (NIRS) has been used to determine the forage quality and fecal composition of domestic and free-ranging species. NIRS may also prove to be useful in monitoring these seasonal shifts and aiding in giant panda management. All bamboo samples were harvested from the Memphis Zoo Bamboo Farm and fecal samples were collected from the male and female panda being housed at the zoo. Bamboo and fecal samples were grouped and calibration equations were created comparing samples between and within diet seasons. All three of the bamboo parts (branch, leaf, and culm) when compared between March and October, resulted with an R^2 above 0.80 and SECV below 0.41. The sensitivity analyses for leaf

and culm samples within diet season produced inconclusive results, with R^2 ranging from 0.459 to 0.972 and SECV ranging from 0.167 to 0.530. Sensitivity analyses for fecal samples yielded an ability to more greatly discriminate between months that were more temporally distant. In the leaf-consumption season, October versus February had an R^2 of 0.864, SECV of 0.252 and in the culm-consumption season April versus July had an R^2 of 0.886, SECV of 0.226. NIRS has the ability to discriminate between bamboo plant parts from different seasons, but further calibration equation development is needed to predict bamboo changes before the giant pandas exhibit a diet shift.

Introduction

Giant pandas are obligate feeders with a diet consisting of about 99% bamboo (Schaller et al. 1985, Dierenfeld 1997). Due to the poor ability of pandas to digest bamboo, they must consume up to 6% of their body weight (Dierenfeld et al. 1982), equivalent to 10-18kg (Reid and Gong 1999) each day. However, as with many other wildlife species, pandas prefer certain forages and are able to select for certain plant species and parts in any given season (Howery & Pfister 1990, Reid and Hu 1991, Lister et al. 1997). During a large portion of the year (June to December), pandas consume mostly the leaf matter of bamboo, which has a higher amount of crude protein (Dierenfeld et al. 1982, Reid and Hu 1991). Throughout the remainder of the year (January to May) pandas consume mostly culm (stem) which is a normally less nutritious part of bamboo (H. Bissell et al., Memphis Zoo, unpublished data). The proportion of parts eaten by the two pandas at the Memphis Zoo between the years 2003 and 2006 is shown below (Figure 7).

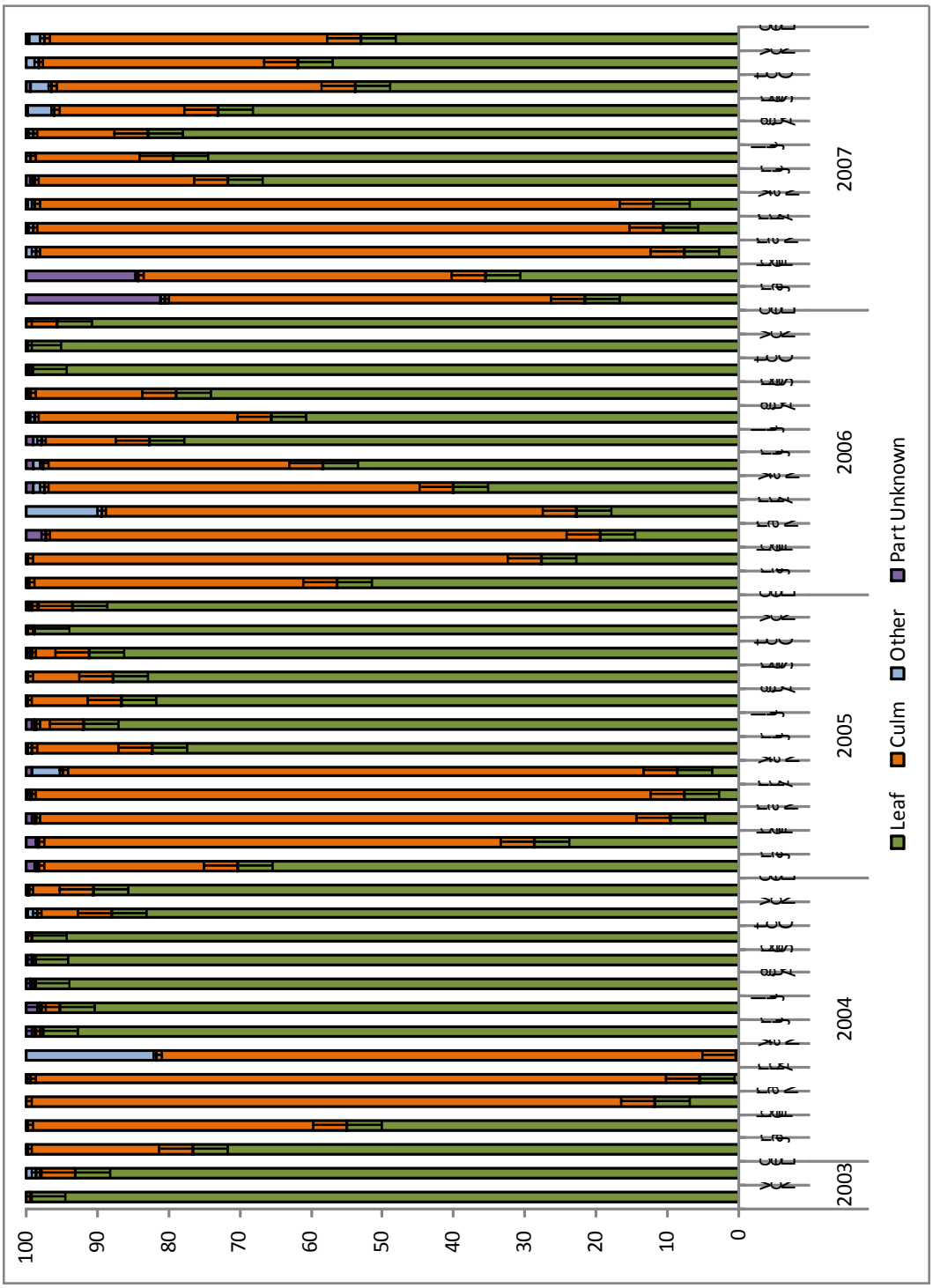

Figure 7. The proportion of bamboo plant part consumed for both Ya Ya (F507) and Le Le (M466) combined for years 2003 to 2007, including standard errors.

The exact cause for this shift is not known with certainty (Reid and Hu 1991, Liu et al. 2005). Several theories have been postulated. The most basic of these is that panda selection is based on the nutrition of the plant parts themselves, and they will select for the most nutritious part at any given time (Warnell et al. 1989). Bissell et al. (Memphis Zoo, unpublished data) suggest that there is no seasonal change in bamboo crude protein, but instead that starches accumulate in the inner culm during spring months, making the culm more nutritious for pandas during these months. Reid and Gong (1999) state that the shift is driven simply by the fact that leaves are more numerous in summer months and culms more abundant in the winter when the leaves are dead. However, this would most likely be a theory that could only apply to wild pandas, considering caretakers of captive populations have methods of providing fresh bamboo year round and the diet shifts are still observed. It has also been documented that there is a higher silica content of leaves during summer months, possibly making them less palatable to pandas and forcing them to switch to a less nutritious culm diet (Dierenfeld 1997, Tabet et al. 2004).

Giant pandas also feed on bamboo shoots whenever possible, which is generally during late spring/early summer months (Taylor and Qin 1987, Reid and Gong 1999). It has been suggested that there is a possibility of a reproductive stimulant being found in new growth causing giant panda preference for shoots (Dierenfeld 1997). Berger et al. (1981) have previously shown that the plant derivative, 6-methoxybenzoxazolinone, can stimulate reproduction in rodents. If the same plant derivative, or some other, were

found in bamboo shoots, it would be an interesting implication of extended panda dependence on bamboo in their diet.

Conventional methods were used by Tabet et al. (2004) to analyze four species of bamboo and determine the macronutrient content during these panda diet shifts. In order to determine the concentrations of these components, four different techniques had to be applied. It would be more efficient to apply NIRS, since all concentrations could be determined in a timely manner using only one NIRS instrument and without terminal consumption of the sample.

Monitoring seasonal changes in bamboo nutrient content will aid in better understanding panda diet selection. The objective of this study was to evaluate the ability of NIRS to effectively detect spectral changes in leaf and culm composition from one season to the next and to detect a similar shift in fecal sample composition.

Methods

In 2003, collaboration was established with the Memphis Zoo and the Grazingland Animal Nutrition (GAN) Lab at Texas A&M University. The Memphis Zoo currently houses two adult giant pandas on loan from China, Le Le (male, studbook #M466) and Ya Ya (female, studbook #F507). All of the fecal samples used in this study came from these two pandas and all of the bamboo samples came from the Memphis Zoo bamboo browse farm where the zoo grows their own bamboo diets. Fecal samples were collected opportunistically and shipped to the GAN Lab frozen and in labeled plastic bags. The bamboo samples were dried and pre-ground, placed in coin envelopes and also shipped to the GAN Lab.

At the lab, frozen fecal samples were thawed, dried in a forced-air oven at 60°C for 24 hours, and ground in an Udy mill to pass uniformly through a 1mm screen for higher precision (Norris et al. 1976). After grinding, they were placed in the oven again at 60°C for at least 3 hours, to remove moisture collected during the handling procedures. The fecal samples were then placed in a dessicator for one hour in order to bring the samples back to room temperature while keeping out ambient moisture. Pre-ground bamboo samples were kept at room temperature until processing, at which time they were put in a forced-air oven at 60°C for at least 3 hours. These samples were also placed in a dessicator for one hour prior to scanning.

All samples, both bamboo and fecal, were scanned using a Foss NIRS Systems 6500 Spectrometer (Foss North America, Eden Prairie, MN, USA) and WinISI II software. Measurements of light are made at 8-nm intervals over the visible and near infrared range (400 – 2500nm).

In order to observe seasonal changes of bamboo nutrient composition, culm and leaf spectra of multiple species and years from the database were combined and sorted by month. One season included March to June samples, when culm ingestion is highest, and the second included October to December samples, when culm ingestion is lowest. Two-block partial least squares (PLS) regression techniques were applied to create discriminant equations between spring leaf samples and fall leaf samples. A sensitivity analysis of leaf samples within season was also conducted in order to find the point at which the spectra are significantly different and therefore, the earliest that we might possibly detect a nutrient composition change. Due to a low number of samples

available for by-month comparisons, no calibration samples were withheld for validation and only cross-validation was used. However, all of the samples from two given months were individually predicted against their respective calibration equations to determine what percentage of samples were correctly predicted per month. The same procedures were used for culm samples.

Second, to observe seasonal changes of panda fecal composition, spectra obtained from fecal samples from the two pandas were grouped and compared in the same way as above. In addition to the predictions previously described, approximately 25% of the samples were withheld from the calibration samples to be used for validation. Calibration equations were created using the remaining 75% of the samples. Fecal spectra from the spring season were discriminated against calibration fecal spectra from the fall season to establish the PLS regression equations. Validation fecals, containing both spring and fall spectra, were predicted against the regression equations of their respective season groups. The same procedure was done for samples within season.

All equations used a mathematical pre-treatment of 2,4,4,1, (derivative, gap, smooth, gap of smooth) unless otherwise indicated, and scatter corrections of Standard Normal Variate (SNV) and Detrend (D). According to Williams' (2005) guidelines for coefficient of determination (R^2) interpretation, a value of 0.80 or greater is considered usable with caution for most applications. R^2 values of about 0.65-0.80 are suitable for screening and some approximate calibrations. R^2 values lower than this demonstrate poor correlations and have little application.

Similarly, correct prediction of at least 80% of samples from a given category into its proper grouping will be considered satisfactory ability of NIRS to discriminate between groups.

It is also possible to observe differences in spectra of given categories. A new method of discerning similarities between spectra, known as difference ranked (DR) spectra, is being developed by NIRS researchers at Texas A&M University (Earlywine et al. 2008, Rater et al. 2008). In this technique, the difference in absorbance at each wavelength is measured by subtraction (Hruschka 2001) and the pairs can be ranked in order of similarity. This allows for a visual representation of how similar or dissimilar a group of samples may be. Here, DR spectra were calculated by subtracting fecal samples of progressive months (October, December, February, April, June, and August, respectively) from a constant October fecal sample to observe spectral changes of fecal composition throughout the year.

Results

Bamboo samples

All three calibration equations of the bamboo parts, when compared between March and October, resulted with an R^2 above 0.80 (Table 21). Likewise, all of the predictions of the individual months using their respective calibration equations were 100% correct (Table 22). These predictions are different from the validation predictions done for fecal samples because these samples were not withheld from the calibration equation; they are only classified into their respective categories.

The sensitivity analyses for leaf and culm samples within diet season yielded varying results. The regressions between October leaf samples and leaf samples from other months within the leaf consumption season show high levels of discrimination, with the exception of December, which is most likely the result of some sampling error, such as improper labeling (Table 23). Comparisons between adjacent months within the leaf consumption season resulted in lower R^2 values and higher SECs. Leaf sample discriminations between months during the culm consumption season had varying R^2 values (Table 24). Discriminating April against the three months following it gave an increasing ability to discriminate between months. June and July also showed a high level of discrimination while May to June did not. The regressions of culm samples with the leaf consumption season all have an R^2 value above the minimum 0.80 except for November to December (Table 25). Unfortunately, the regressions of culm samples within the culm consumption season are incomplete due to a very small sample size within the month of June (N=1) (Table 26). The two comparisons possible (April to May and April to July) are both below the minimum R^2 of 0.80.

Fecal samples

The sensitivity analyses for fecal samples produced a trend similar to the bamboo sensitivity analyses. Within the leaf consumption season, the ability to discriminate between October and the four following months increases with each subsequent month (Table 27). The November versus December and December versus January discriminations had R^2 values above 0.80, but January versus February was surprisingly low. This most likely indicates that there is a shift in diet, and therefore also fecal,

composition in the preceding months, but between January and February the shift ends. The validation predictions for these months follow a similar pattern as the calibration equations (Table 28). October versus January and October versus February validation sets had the highest prediction percentages (92% October, 100% January and 100% October, 87% February) and the January versus February had the lowest (88% January, 29% February).

Within the culm consumption season, April discriminations against May and June do not get above R^2s of 0.58, but April discriminated against July has an R^2 of 0.89 (Table 29). May versus June and June versus July also have lower R^2 values with 0.63 and 0.55, respectively. This is to be expected as the shift stops occurring and the samples become more consistent in content. As with the leaf consumption season, the validation set predictions using the corresponding calibration equations provide results that follow the same pattern as the calibration equations themselves (Table 30). The highest ability to predict was April versus July (86% April, 96% July) and the overall least ability to predict was June versus July (83% June, 68% July).

When averaging all the fecal sample spectra from October and March together, it can be seen that specific regions of these fecal spectra have differences due to diet composition (Figure 8). It is these differences in spectra that make the technique of DR spectra possible. The difference in spectra between an October fecal sample versus progressive months are also shown (Figure 9). The DR spectra graph uses the absolute values of the differences (Figure 10). This figure shows that there is a general change in fecal composition that progresses from one season (leaf consumption) to the next (culm

consumption). Fecal samples from a similar annual time period (two from October) have similar spectra readings and, therefore, less of a difference ranking. Fecal samples from two different annual time periods (one from October, one from August) have the greatest spectral differences, indicating that there is, in fact, a change of fecal composition between these periods.

Table 21. Statistics of the calibration equations discriminating all March samples of the given part to all October samples of the given part.

Part	Math[1]	N	SD[2]	RSQ[3]	SECV[4]	1-VR[5]
Leaf	1,4,4,1	16	0.4635	0.9162	0.4062	0.2321
Culm	1,4,4,1	18	0.4969	0.8034	0.2464	0.7542
Branch	2,4,4,1	10	0.4583	0.9117	0.2155	0.7788

[1]Derivative, gap, smooth 1, gap of smooth
[2]Standard deviation
[3]Coefficient of determination
[4]Standard error of cross validation
[5]One minus variance ratio

Table 22. Prediction of bamboo samples by individual month using their respective calibration equations.

Part	Month	# Correct Out of Total	Percent Correct
Leaf	March	11/11	100
	October	5/5	100
Culm	March	10/10	100
	October	8/8	100
Branch	March	7/7	100
	October	3/3	100

Table 23. Sensitivity analysis of bamboo leaf samples during the giant panda leaf-consumption season.

Discrimination	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Oct to Nov	15	0.4714	0.0844	0.9680	0.4578	0.0567
Oct to Dec	26	0.3941	0.2878	0.4669	0.4421	-0.2586
Oct to Jan	63	0.2703	0.0728	0.9274	0.2347	0.2464
Oct to Feb	40	0.3307	0.0788	0.9433	0.2202	0.5567
Nov to Dec	31	0.4675	0.2272	0.7638	0.4421	0.1057
Dec to Jan	79	0.4418	0.2367	0.7130	0.2733	0.6174
Jan to Feb	93	0.4845	0.2317	0.7713	0.3153	0.5765

[1]Standard deviation
[2]Standard error of calibration
[3]Coefficient of determination
[4]Standard error of cross validation
[5]One minus variance ratio

Table 24. Sensitivity analysis of bamboo leaf samples during the giant panda culm-consumption season.

Discrimination	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Apr to May	50	0.3666	0.1583	0.8136	0.3323	0.1785
Apr to June	11	0.4454	0.1658	0.8615	0.2788	0.6082
Apr to July	28	0.4518	0.1293	0.9180	0.2198	0.7633
May to June	45	0.2494	0.1836	0.4585	0.2447	0.0376
June to July	23	0.3368	0.0580	0.9703	0.2117	0.6047

Table 25. Sensitivity analysis of bamboo culm samples during the giant panda leaf-consumption season.

Discrimination	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Oct to Nov	25	0.4665	0.1576	0.8859	0.4737	-0.0311
Oct to Dec	28	0.4518	0.0753	0.9722	0.1670	0.8633
Oct to Jan	66	0.3264	0.0947	0.9157	0.1782	0.7017
Oct to Feb	39	0.4038	0.0910	0.9492	0.2419	0.6411
Nov to Dec	37	0.4984	0.2651	0.7170	0.5303	-0.1325
Dec to Jan	78	0.4367	0.1059	0.9412	0.2226	0.7402
Jan to Feb	89	0.4764	0.2109	0.8041	0.2793	0.6564

Table 26. Sensitivity analysis of bamboo culm samples during the giant panda culm-consumption season.

Discrimination	N	SD[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Apr to May	53	0.3386	0.2235	0.5643	0.2843	0.2950
Apr to June	Not enough June samples for analysis (N=1)					
Apr to July	30	0.4230	0.1900	0.7983	0.3223	0.4194
May to June	Not enough June samples for analysis (N=1)					
June to July	Not enough June samples for analysis (N=1)					

Table 27. Sensitivity analysis of fecal samples during the giant panda leaf-consumption season using calibration PLS regression equations.

Discrimination	Math[1]	N	SD[2]	SEC[3]	RSQ[4]	SECV[5]	1-VR[6]
Oct to Nov	2,4,3,1	127	0.4955	0.3487	0.5046	0.4261	0.2604
Oct to Dec	2,4,3,1	97	0.4374	0.2198	0.7474	0.3488	0.3639
Oct to Jan	2,4,3,1	95	0.4284	0.1933	0.7964	0.3045	0.4948
Oct to Feb	2,4,4,1	93	0.4181	0.1540	0.8643	0.2515	0.6382
Nov to Dec	2,4,3,1	80	0.4635	0.1999	0.8139	0.3752	0.3446
Dec to Jan	2,4,3,1	48	0.4996	0.1882	0.8581	0.3946	0.3760
Jan to Feb	2,4,4,1	44	0.4995	0.3786	0.4253	0.4956	0.0154

[1] Derivative, gap, smooth 1, gap of smooth
[2]Standard deviation
[3]Standard error of calibration
[4]Coefficient of determination
[5]Standard error of cross validation
[6]One minus variance ratio

Table 28. Prediction of validation fecal sample sets using their respective calibration equations from leaf-consumption season.

Discrimination	Month	# Correct Out of Total	Percent Correct
Oct to Nov	Oct	17/25	68
	Nov	14/18	78
Oct to Dec	Oct	22/25	88
	Nov	4/7	57
Oct to Jan	Oct	23/25	92
	Jan	8/8	100
Oct to Feb	Oct	25/25	100
	Feb	6/7	87
Nov to Dec	Nov	15/18	83
	Dec	4/7	57
Dec to Jan	Dec	4/7	57
	Jan	7/8	88
Jan to Feb	Jan	7/8	88
	Feb	2/7	29

Table 29. Sensitivity analysis of fecal samples during the giant panda culm-consumption season using calibration equations.

Discrimination	Math[1]	N	SD[2]	SEC[3]	RSQ[4]	SECV[5]	1-VR[6]
Apr to May	2,4,4,1	108	0.3958	0.2627	0.5593	0.3705	0.1239
Apr to June	2,4,3,1	110	0.3930	0.2551	0.5786	0.3397	0.2530
Apr to July	2,4,4,1	99	0.4088	0.1381	0.8859	0.2258	0.6949
May to June	2,4,4,1	176	0.5000	0.3021	0.6349	0.3836	0.4114
June to July	2,4,3,1	167	0.4989	0.3349	0.5495	0.4164	0.3033

Table 30. Prediction of validation fecal sample sets using their respective calibration equations from culm-consumption season.

Discrimination	Month	# Correct Out of Total	Percent Correct
Apr to May	Apr	4/7	57
	May	29/30	97
Apr to June	Apr	5/7	71
	June	28/30	93
Apr to July	Apr	6/7	86
	July	24/25	96
May to June	May	24/30	80
	June	25/30	83
June to July	June	25/30	83
	July	17/25	68

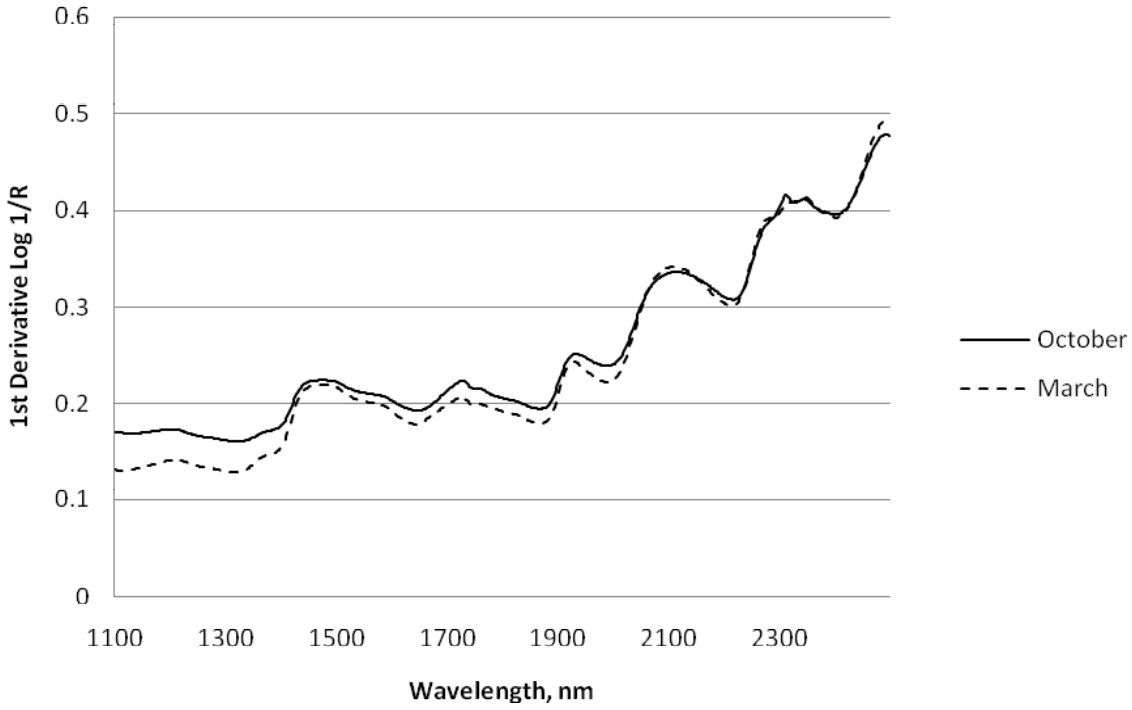

Figure 8. Comparison of fecal NIRS spectra from October and March.

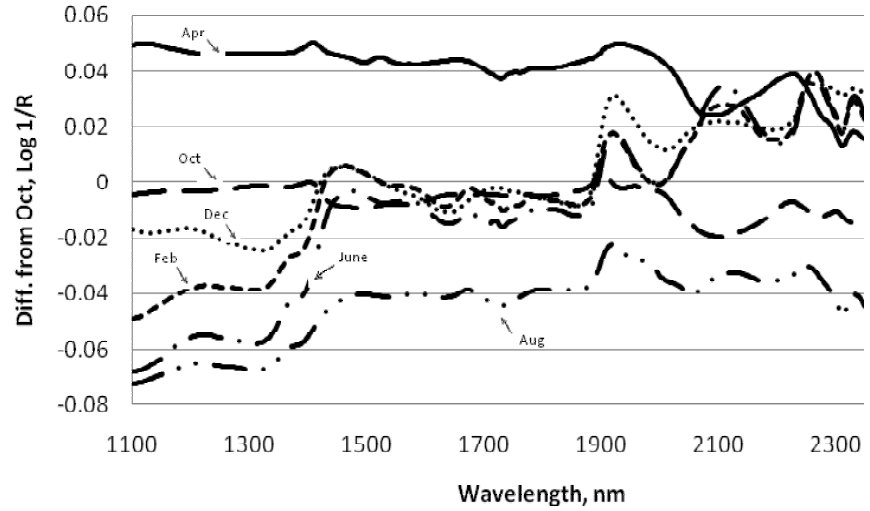

Figure 9. Fecal NIR difference spectra (Oct fecal - $Fecal_x$).

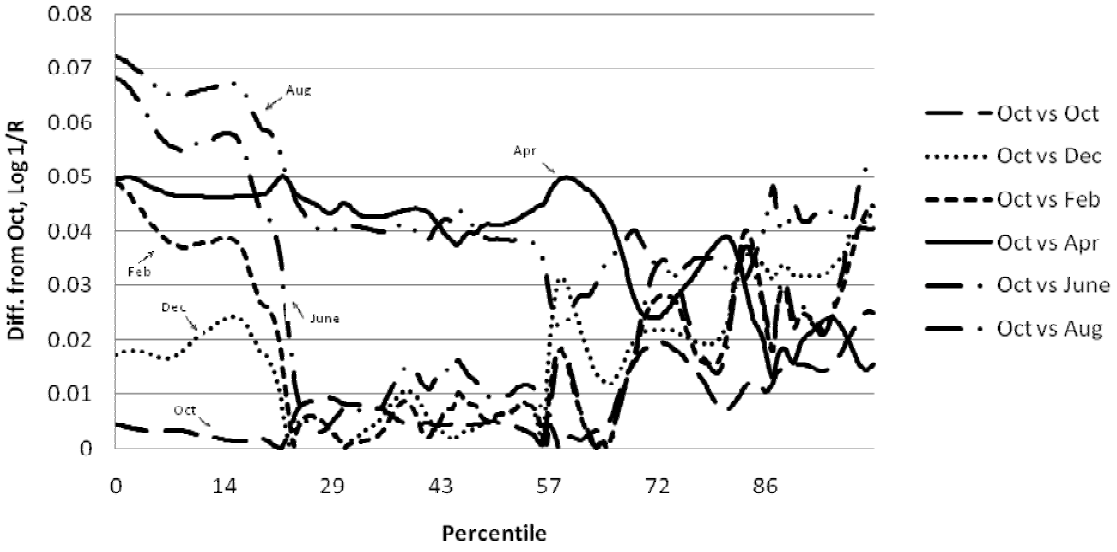

Figure 10. Fecal composition similarity to October as indicated by difference (ranked) spectra.

Discussion

NIRS was able to both distinguish between and correctly predict parts of bamboo from March and October, extreme months in the culm and leaf consumption seasons, respectively. This indicates that there are spectral seasonal changes occurring in these bamboo species, perhaps changing diet quality, which is a phenomenon not uncommon in forages (Dorgeloh et al. 1998, Woolnough and Foley 2002). This supports the current thinking that there are bi-annual physiological shifts in bamboo and that giant pandas are able to select for these changes (Reid and Hu 1991).

Overall, the sensitivity analyses of bamboo composition change for both the leaf and culm consumption seasons produce inconclusive results. It was expected that using NIRS we would be able to distinguish at what point the bamboo changed physiology, and therefore be able to predict when the giant panda diet would change. However, there seems to be no consistent trends between the two seasons that would lead us to believe that the use of NIRS would be able to accurately determine bamboo composition changes.

This is surprising since it has been determined that NIRS can determine the nutrient content of bamboo (E. Wiedower, Texas A&M University, unpublished data). The difference could be due to the varying number of bamboo samples from each month used in calibrations in predictions. Variable sample sizes could cause discriminations and predictions to be improperly weighted and, therefore, mask the ability of NIRS to track bamboo composition changes.

The sensitivity analysis of fecal samples showed more promise. The progression of the ability of NIRS to distinguish between samples of different months indicates that a change in fecal composition is occurring. This change is most likely caused by a diet shift, since Walker et al. (2007) found that diet created the largest spectral variation, more so than sex, breed, and age in goats. The low R^2s of the January to February and June to July discriminations indicate that the sharp change in bamboo physiology between seasons has already occurred (possibly in January or June) and therefore the samples in the following months (February and July) would have very similar spectra and, therefore, lower NIRS ability to discriminate between them.

However, the utility of being able to detect giant panda diet shifts through fecal NIRS is limited for several reasons. One, the diet shift has already occurred, so there is no way for managers and caretakers to prepare for the change in diet ahead of time. Two, due to the low level of digestion of giant pandas, diet composition of fecal samples is visually evident, making the use of NIRS unnecessary. It would be more useful to be able to detect the changes within the bamboo itself in order to predict the giant panda diet shifts, both in the wild and captivity, before they begin. For this to happen, further samples need to be collected to add to the existing leaf and culm season discriminations. Sampling would preferably be of a wide variety of species and locations to broaden the equations applicability. To apply these equations to wild populations, forage species of giant pandas, such as *Fargesia spathacea* Franchet and *Bashania fangiana* Yi should be included in development (Taylor and Qin 1987, Reid and Hu 1991).

The difference ranked spectra method is an ideal way to view fecal or forage composition differences between individuals or time periods. Trends, or lack thereof, can be more readily observed. In this study, it could be seen that there was decreasing similarity between fecal samples further apart in time, which is in line with our findings, and also expected considering the seasonal bamboo composition changes. It is also possible to create a predictive equation for percent diet similarity using a certain percentile on the DR spectra (Earlywine et al. 2008, Rater et al. 2008). This is something that could be applied if further bamboo sampling is done to create more effective NIRS equations above.

CHAPTER V

USE OF NIRS TO DISCRIMINATE BETWEEN THE SEX, AGE CLASS, AND REPRODUCTIVE STATUS OF GIANT PANDAS (*AILUROPODA MELANOLEUCA*)

Synopsis

Giant pandas (*Ailuropoda melanoleuca*) are an increasingly endangered species due to deforestation and habitat fragmentation within their home ranges of China. Creating a reliable population count in the wild is difficult because visual sightings of pandas are rare and DNA analysis of fecal samples is time and financially costly. Reproduction among captive populations is also a challenge due to breeding difficulties and low offspring survival. Near infrared reflectance spectroscopy (NIRS) may be a tool to help overcome both of these hurdles. NIRS has been demonstrated to be able to differentiate between sex, age class, and reproductive status of both domestic and wildlife species. The objective of this study was to determine if NIRS could be successfully applied similarly to giant pandas. Fecal samples were collected opportunistically from pandas at the Memphis Zoo, Smithsonian's National Zoo, Zoo Atlanta, and San Diego Zoo from 2006 to 2007. All samples were dried in a forced-air oven at 60°C, ground to pass through a 1mm screen, and dried again until scanning. Canonical discriminations were used to predict between a total of 252 male and female fecal sample spectra and were 89% correct for females and 90% correct for males. One hundred fecal spectra used to predict between adults and juveniles was 100% correct for both age classes. A limited number of samples were used to create a two-block PLS

discriminant equation between pregnant and non pregnant females which resulted in an R^2 of 0.68 and SECV of 0.360, and a prediction of 100% for both pregnant and not-pregnant. It has been determined that NIRS has the potential to be a valuable tool for differentiating between the sex, age class, and reproductive status of giant pandas. However, further sample collection to increase spatial and temporal variability needs to be done before reliable discriminant equations can be applied to wild populations.

Introduction

The giant panda (*Ailuropoda melanoleuca*) is one of the most well-known critically endangered species in the world. According to the Ministry of Forestry of China/World Wildlife Fund panda survey conducted from 1985-1988, there are approximately 1,100 free-ranging pandas (National Conservation Management Plan 1989). There are also currently about 165 captive individuals kept in breeding facilities and zoos (Giant Panda Studbook 2006). Much research has been done on this species, ranging from nutrition, digestion, behaviors, and reproduction. Understanding of their behaviors and habitats has increased recently, especially in the area of captive panda reproduction (Swaisgood et al. 2003).

However, most of the methods currently used for panda population counts and for detecting female pregnancies are flawed and time consuming. Taking a census of wild populations requires long field observation hours or expensive radio-collars that are invasive to the animal. Bite size of bamboo found in fecal samples can be used as an indicator of the age class of a panda (Schaller et al. 1985, Pan et al. 2004), but this method is labor intensive. Detection of pregnancy in female pandas is difficult because

they sometimes experience pseudopregnancies, which closely resemble a true pregnancy (Mainka et al. 1990). Pregnancy determination via ultrasound is invasive and the fetus can be difficult to detect since panda infants are so little developed. Sutherland-Smith (2004) state that more work is needed in order to develop a method to differentiate between pseudo- and true pregnancies, document changes before and after implantation, and determine pregnancy loss.

Near infrared reflectance spectroscopy (NIRS) is a tool that has previously been used to differentiate between sex in domestic (Tolleson et al. 2000, Godfrey et al. 2001), Walker et al. 2007) and wildlife species (Tolleson et al. 2005, Osborn 2002). Similarly, age has been determined in white-tailed deer (Osborn 2002) and physiological status of females within a species (not pregnant, pregnant, or lactating) has been determined as well (Tolleson et al. 2001*a*, Godfrey et al. 2001). Although currently the start up costs for NIRS can be expensive and initial calibrations can be lengthy, once the calibrations are created the rewards can be great. In this study we wanted to calibrate NIRS for use on giant pandas in order to create a viable tool for collecting panda population census data and determining the physiological status of females while minimizing the invasive and time consuming drawbacks of traditional methods.

We hypothesized that biological differences occur in the feces of male versus female giant pandas, as well as between age classes and females of varying physiological states. The objective of this study was to determine whether NIRS can be used as an effective tool for determining the sex and age class of individual pandas, and/or pregnancy status of females.

Methods

Fecal samples were collected opportunistically from all adult and juvenile giant pandas housed in the United States between 2006 and 2007. This included 2 adults at the Memphis Zoo (1F, 1M), 2 adults and 1 juvenile at Zoo Atlanta (2F, 1M), 2 adults and 2 juveniles at the San Diego Zoo (2F, 2M), and 2 adults and 1 juvenile at the Smithsonian's National Zoo (1F, 2M). This was a total of 8 adults and 4 juvenile pandas. All samples were frozen after collection and mailed to the Grazingland Animal Nutrition (GAN) Lab at Texas A&M University (College Station, TX) in sealed styrofoam coolers.

These samples were processed in a method similar to Lyons and Stuth (1992). Samples were dried in a forced-air oven at 60°C at least overnight and ground in an Udy Mill to pass uniformly through a 1-mm screen for greater precision of NIRS results (Norris et al. 1976). Ground samples were re-dried at 60°C for at least 3 hours and put in a dessicator for one hour to stabilize sample temperature and moisture. Ground samples were then manually packed in sample cups with quartz cover glass at a consistent level and compression. Quartz glass is used instead of traditional glass because quartz maintains a more uniform thickness. Each sample was scanned using Foss NIRS Systems 6500 Spectrometer (Foss North America, Eden Prairie, MN, USA) with spinning cup attachment. Measurements of reflectance were made over the visible and near infrared range (400 – 2500nm).

Spectra were grouped by sex, age class, or physiological status for the appropriate discriminations. Under the same guidelines created by Schaller et al. (1985),

juveniles were considered to be between 1.5-5 years because at this time they are being weaned onto solid foods, but are not yet sexually mature, and adults were considered to be 5 years and older. Pregnancy in female pandas was determined by the respective zoos using a combination of observation, hormone level monitoring, and ultrasound.

Discriminant equations were created for all 3 categories using WinISI II v. 1.04a software, which utilizes the two-block partial least squares (PLS) method (Martens and Martens 2001). A coefficient of determination (R^2) value of 0.80 or greater was considered usable with caution for most applications (Williams, 2005). All equations had a mathematical pre-treatment of a second order derivative 2,4,4,1 (derivative, gap, smooth, gap of smooth) with scatter corrections of Standard Normal Variate (SNV) and Detrend (D), unless stated otherwise. Cross-validation was applied to the equations yielding a standard error of cross-validation (SECV). Cross-validation uses the same samples for validation as it does for calibration, individually predicting each sample using the calibration of the remaining n-1 samples (Stone 1974). This method is generally used when there are not enough samples to withhold from the calibration and then validate separately. Sex discriminations were made from 168 male and 164 female samples. Discriminations for age class were created using 239 adult and 93 juvenile panda samples, while physiological status used 42 samples from pregnant females and 42 from not-pregnant females.

A SAS 9.1 program (SAS Institute, Inc., Cary, NC, USA) was written to create multivariate canonical discriminations for the same 3 categories above. While both WinISI and SAS create equations and predictions, they do so using two different

statistical methods. Canonical discriminations have proven useful when the data are classified by non-numerical variables (e.g. male and female) (Glaser 1999). This can sometimes produce differing results between the two methods, but consistency between the methods can act as another form of validation. Sex discriminations were made from 120 male and 120 female samples. Discriminations for age class were created using 50 adult and 50 juvenile panda samples, while physiological status used 30 samples from pregnant females and 30 from not-pregnant females.

Results

Experiment 1: Sex discrimination

Initially, samples were only available from the two adult Memphis Zoo pandas (Studbook #s 507 and 466). This calibration produced an R^2 value of 0.68 and SECV of 0.405 (Table 31). It was thought that increased variation, i.e. samples from more individuals, would increase the R^2 value. However, the opposite occurred and as sample size was increased the R^2 value decreased to 0.43 and SECV increased slightly to 0.434.

A validation sample set was removed (25 validation: 75 calibration) and re-calibration produced the discriminant equations (Table 32). Predicting the withheld samples against the new calibration equation resulted in 89% of the females and 90% of the males predicted correctly (Table 33). Predictions using a canonical discrimination between a total of 120 male and 120 female fecal sample spectra identified 87% females correctly and 84% of the males correctly (Table 34).

Experiment 2: Age class discrimination

The two-block PLS discriminant equation for age class resulted in an R^2 of 0.59 and SECV of 0.309, which, according to Williams (2005), would only be adequate for rough screening of samples (Table 31). A re-calibrated equation with validation samples removed resulted in an increased R^2 of 0.66 and slightly reduced SECV of 0.297. It seems that some samples may have influenced the calibration, but had less of an effect when used in a smaller validation set.

The 25% validation set predicted against the 75% calibration equation correctly predicted 92% of the juveniles and 97% of the adults (Table 33). A total of 200 fecal spectra were used to create a canonical discrimination between adults and juveniles (Table 34). The output identified both age classes 100% correctly.

Experiment 3: Physiological status discrimination

The two-block PLS discriminant equation developed between pregnant and non pregnant females resulted in an R^2 of 0.68 and SECV of 0.360 (Table 31). When the validation set was removed, the R^2 improved to 0.83 with a slightly lower SECV of 0.375 (Table 32). The prediction of the validation set using the re-calibrated equation was 100% correct for both pregnant and not pregnant females (Table 33). The prediction of 30 pregnant and 30 not pregnant female samples using a canonical discriminant equation identified 97% of both physiological groups correctly (Table 34).

Table 31. Statistics of the two-block PLS discriminant equations for sex, age class, and pregnancy status.

Discrimination	N	SEC[1]	RSQ[2]	SECV[3]	1-VR[4]
Sex (Memphis only)	160	0.283	0.679	0.405	0.346
Sex (all pandas)	332	0.386	0.434	0.434	0.248
Age class	332	0.289	0.585	0.309	0.528
Pregnancy	84	0.285	0.675	0.360	0.481

[1]Standard error of calibration
[2]Coefficient of determination
[3]Standard error of cross validation
[4]One minus variance ratio

Table 32. Statistics of the two-block PLS discriminant equations for sex, age class, and pregnancy status with validation sets removed.

Discrimination	N	SEC[1]	RSQ[2]	SECV[3]	1-VR[4]
Sex (all pandas)	252	0.387	0.401	0.458	0.160
Age class	247	0.260	0.661	0.297	0.558
Pregnancy	65	0.207	0.828	0.375	0.439

[1]Standard error of calibration
[2]Coefficient of determination
[3]Standard error of cross validation
[4]One minus variance ratio

Table 33. Predictions of validation sets using the respective two-block PLS discriminant equations.

Prediction	Constituent 1 correct	Constituent 2 correct
Sex	89% females	90% males
Age class	92% juveniles	97% adults
Pregnancy	100% pregnant	100% not pregnant

Table 34. Canonical discriminant equation predictions for sex, age class, and pregnancy status.

Prediction	Constituent 1 correct	Constituent 2 correct
Sex	87% females	84% males
Age class	100% juveniles	100% adults
Pregnancy	97% pregnant	97% not pregnant

Discussion

It has been shown in previous studies that fecal spectra from different sexes of the same species were significantly different (Tolleson et al. 2000, 2005; Godfrey et al. 2001). The results of this study are not as clear. One explanation for this difference in results is that giant pandas show less size dimorphism between the sexes than do other species, including other bears. While other male bear species are about 40% larger than the females, male giant pandas are only 18% larger than females (Schaller et al. 1985). The lower level of sexual dimorphism could be representative of other physiological similarities between male and female giant pandas which would result in a less reliable ability of NIRS to discriminate between the two. Lyons and Stuth (1992) also found that there was no effect of physiological status of female cattle on their calibrations. The varying conclusions among studies could indicate that NIRS when applied to sex discrimination could be species specific.

The relatively high R^2 values for the pregnant/not pregnant discriminant equations seem promising given the small number of samples used to create them. However, given that the equations for gender determination resulted in lower R^2 values as more variation was added, it cannot be assumed that the pregnancy equations will be improved with a greater number of samples. Prior research has found that pregnancy in cattle is more consistently predicted in later pregnancy stages rather than earlier ones (Tolleson et al. 2001*a*, 2001*b*). This is something that may be relevant to giant pandas, as well, due to their altricial level of fetal development.

The endangered status of giant pandas makes panda reproduction a highly anticipated and delicate issue. As such, it has proven difficult to obtain fecal samples from contributing zoos during pregnancies because of their focus on the health of the female. In later stages of the pregnancy, as parturition nears, the female panda does not eat or drink (Schaller et al. 1985). During this time, pregnancy would be clear and therefore samples would only be required before the female begins to fast. In order to further the applicability of this equation, a relationship with a panda-holding institution must be established and priority given to sample collection during and after pregnancy.

As with any study, there are possible sources of error that could have been introduced into this research. Each zoo provided their pandas with supplemental treats, such as apples and carrots, in addition to their largely bamboo diets. This creates unaccounted for variability in the sample spectra (Lyons et al. 1993). However, since all four of the institutions provided similar treat regimens, there is consistency across all of the fecal samples that should not interfere with the discriminations. Inconsistency in sample packing of the scanning cups could create variation in spectra reading and that it why all cups were packed to as close to a standard level and pressure as possible. Furthermore, different ambient conditions during reading (such as temperature and humidity) can introduce scanning error. Before any scanning is done, the machine is tested using a sealed check sell with a constant, known spectra reading to maintain a level of accuracy. All of these efforts, and more, were taken throughout the length of this study in order to maintain the highest level of accuracy possible.

Management Implications

It has been demonstrated that NIRS has the potential of being a useful tool for giant panda management. Currently, the equations are not ideal for immediate application or field use, but with increased sample collection from pandas outside of the United States, all of these equations could be improved to satisfactory standards.

All three of these discriminations will be extremely valuable for field research and assessing wild population demographics. By having a more accurate idea of the current condition of wild panda populations, managers can more effectively tailor their management plans under their specific circumstances. Being able to determine the pregnancy status of a female giant panda also has a very important implication for captive populations. This method would be a non-invasive tool to augment the sometimes unreliable pregnancy monitoring methods in place today.

It must be noted that, as has already been demonstrated with this study, NIRS is always a work in progress and a discriminant equation should never be considered "complete." More sample diversity, both spatial and temporal, should be continually added as it becomes available in order to broaden the relevance of an equation. All of the pandas from the four zoos used in this study were fed diets of similar bamboo species (such as *Phyllostachys spp*.). Fecal samples from pandas consuming different diets, such as pandas from China, would increase the sample variability in all three of the discriminations here. This study was intended to set the foundation for future work in this particular area and to encourage others to continue where this study began.

CHAPTER VI

CONCLUSIONS

Summary of Contributions

The studies presented here have shown the potential of NIRS to be used to determine the nutrient constituents of giant panda diets, monitor leaf to culm shifts in bamboo, and determine the physiological status of pandas. Data from these calibration equations produced mixed results. NIRS was able to perform some discriminations with an acceptable degree of quality. Others require further sampling before they can be used with captive and wild populations with confidence.

NIRS was clearly able to accurately predict CP, NDF, ADF, and OM, but not DM for multiple bamboo species. In order to improve the predictive ability of NIRS for DM, both the reference values and NIRS values need to be calculated in the same location to avoid confounding variables of different locations. NIRS was also able to distinguish between bamboo parts and, to some extent, bamboo species. It will need to be determined what it is that makes species PLLAR and PLLGL produce similar spectra, thereby lowering the ability of NIRS to distinguish between the two.

NIRS was also able to discriminate between bamboo samples of different seasons, indicating that there are, in fact, seasonal differences. However, the sensitivity analysis calibrations will need to be further developed in order to detect the point at which the bamboo actually changes. NIRS could accurately detect the point at which the

giant panda fecal samples showed a shift in diet, but this is not practically useful because diet shifts are visually observable.

It has been demonstrated that NIRS may not be able to distinguish between male and female and juvenile and adult pandas. It is possible that wider panda variation may lead to an increase in calibration equation quality. The calibration equation for discriminating between pregnant and not pregnant female pandas shows potential. It does not currently meet the minimum requirements for a quality equation, but the low number of samples and moderate ability to distinguish between the two states is promising.

Future Research

The current equations should be expanded to include more variation in pandas, panda diet, and spatial and temporal factors. It is hypothesized that this will increase the quality of equations created here and also lend more applicability to a broader number of situations.

Use of portable NIRS will also help to accomplish this goal. Portable NIRS is in its beginning stages of use, but the technology and software exists and is currently being applied. This allows for real-time analysis in the field and for calibration equations to be created even more quickly than before with only a stationary system. While using portable NIRS, giant panda populations in the wild can be monitored efficiently and perhaps with more accurate results than the Chinese Ministry of Forestry and WWF population estimate of 1989 can be obtained.

This technology may also help investigate the possibility of using NIRS to identify individual giant pandas. Initially, calibration equations would need to be created using known animals, such as from zoos used in these studies. Then they would need to be validated with an independent sample set, and then, finally, they could be applied to wild populations and used to create giant panda population estimates in China.

Lastly, NIRS can be used to determine the diet quality of pandas. Instead of only looking at the nutrient parameters of bamboo itself, digestibility trials compare fecal spectra with matching reference chemistry (of the diet) to create calibration equations of diet quality for a given species. Diet quality generally includes the amount of crude protein and level of digestibility. Since pandas are known to have such a low ability to digest their food, results of diet: fecal pairs should be very interesting.

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Experience

Guest lectured for Wildlife and the Changing Environment (WFSC 301), General Mammalogy (WFSC 401), and Natural History of the Vertebrates lab (WFSC 302) during Fall 2006 and Spring 2007. Senior author of 2 abstracts, both presented at the Southwestern Association of Naturalists (SWAN) annual meeting in April 2007. Social coordinator for the Graduate Student Organization for the Ecosystem Science and Management department.