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FEED INTAKE, DIGESTIBILITY, DIGESTA PASSAGE, AND
FECAL MICROBIAL ECOLOGY OF THE
RED PANDA (Ailurus fulgens)

presented by

Karen Jaye Warnell

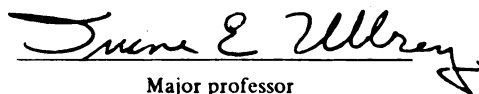
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FEED INTAKE, DIGESTIBILITY, DIGESTA PASSAGE,
AND FECAL MICROBIAL ECOLOGY OF THE
RED PANDA (Ailurus fulgens)

By

Karen Jaye Warnell

AN ABSTRACT OF A THESIS

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ABSTRACT

FEED INTAKE, DIGESTIBILITY, DIGESTA PASSAGE, AND FECAL MICROBIAL ECOLOGY OF THE RED PANDA (Ailurus fulgens)

By

Karen Jaye Warnell

This study was conducted to determine to what extent the captive red panda Ailurus fulgens digests diets consisting of all-bamboo, all-gruel (a cereal-based porridge), gruel with 10% alfalfa meal (mixed) and a gruel with 10% bamboo meal (mixed as above). Five individually-housed red pandas (4.1) were studied at the National Zoological Park (NZP), Washington, DC. Rate of digesta passage was determined by using wheat kernels as the indigestible particulate marker. Feed and fecal samples were analyzed for dry matter, ash, gross energy, crude protein, neutral detergent fiber, acid detergent fiber, and acid lignin. Microbial analyses were conducted to determine if and how the flora of the red panda gut changes in response to diet. Bamboo was retained < 6 hr compared to < 9.5 hr for the gruel and gruel/alfalfa, and < 14 hr for gruel/bamboo meal ($P < 0.10$). The digestibility of dry matter (24%), organic matter (27.5%), gross energy (30%) and crude protein (50%) in bamboo was less ($P < 0.001$) than that in all the other diets. The red panda's digestible energy (DE) requirements for maintenance were estimated to be 4.8 to 5.8 times over the basal metabolic requirement (BMR) using $27.28 \times \text{BW}^{.75}$ for BMR. No cellulolytic bacteria were detected in feces.

DEDICATION

This thesis is dedicated to my parents, Ronald and Joan Warnell. Their eternal faith, trust, love and support throughout my life have made many things possible for me.

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TABLE OF CONTENTS

GENERAL INTRODUCTION	1
I. FEED INTAKE AND DIGESTIBILITY	
A. Introduction	5
B. Materials and Methods	10
C. Results	24
D. Discussion	31
E. References	43
II. RATE OF PASSAGE	
A. Introduction	48
B. Materials and Methods	52
C. Results	55
D. Discussion	58
E. References	61
III. MICROBIAL ECOLOGY	
A. Introduction	63
B. Materials and Methods	67
C. Results	70
D. Discussion	74
E. References	79
APPENDICES	81

LIST OF TABLES

Table 1.	Composition of red panda gruel fed at the National Zoological Park, Washington, DC.	18
Table 2.	10% Alfalfa meal or bamboo meal in gruel fed to red pandas at the National Zoological Park, Washington, DC.	19
Table 3.	1987 Mean red panda dry matter intakes by week with corresponding average temperatures, average relative humidities and average barometric pressures.	25
Table 4.	Weekly means for temperature, relative humidity and barometric pressure during bamboo, gruel and gruel/alfalfa meal dietary treatments.	27
Table 5.	Weekly means for temperature, relative humidity and barometric pressure during all the gruel/bamboo meal dietary treatments.	27
Table 6.	Daily dry matter intake (DMI), and body weight (BW) relationships for red pandas fed 4 diets (n=5).	28
Table 7.	Composition (%) of diets fed to red pandas.	29
Table 8.	Mean apparent digestibility (%) of 4 diets (DMB) fed to red pandas: bamboo, gruel, G/alfalfa and G/bamboo meal.	30
Table 9.	Quantity of food eaten daily by various animal species.	32
Table 10.	Dry matter intakes (% of body weight) of ruminants and non-ruminants, similar in body weight to red pandas.	33
Table 11.	Comparison of digestible energy (DE) consumed on four diets to estimates of energy required by red pandas for basal metabolic functions using $BMR=27.28 \times BW^{0.75}$.	36

kg

LIST OF TABLES (con't.)

Table 12.	Amount of digestible component in 1 g of dry matter.	38
Table 13.	Composition (%) of ingredients in G/alfalfa meal or G/bamboo meal diets.	40
Table 14.	Mean transit times (TT), total retention times (TMRT) and percentages of wheat kernels recovered from red pandas fed 4 different diets.	55
Table 15.	Comparison of the excretion rates of wheat kernels and ytterbium marked bamboo leaves fed to red pandas on an all-bamboo leaf diet.	57
Table 16.	Composition of North American red panda gruel diets (n=10).	64
Table 17.	Aerobic and anaerobic amylolytic bacteria present in the feces of red pandas fed bamboo (X), gruel/bamboo (G/X), gruel (G) and gruel/alfalfa meal (G/Alf) diets.	70
Table 18.	<u>E. coli</u> /g present in the feces of red pandas fed four different diets.	71
Table 19.	Lactobacilli/g present in the feces of red pandas fed four different diets.	71
Table 20.	Molar percentages of volatile fatty acids (VFAs) present in red panda feces.	73
Table 21.	Total concentration of volatile fatty acids (VFAs) present in red panda feces.	73
Table 22.	Nutrient composition of red panda gruel.	81
Table 23.	Summary of coliform and lactobacilli numbers/g present in the feeds provided to red pandas.	84
Table 24.	Summary of individual <u>E. coli</u> counts/g in the feces of red pandas fed four different diets.	84

LIST OF TABLES (con't.)

Table 25.	Summary of individual lactobacilli counts/g in the feces of red pandas fed four different diets.	84
Table 26.	Volatile fatty acids present in the feces of all red pandas over all dietary treatments expressed as molar %.	85
Table 27.	Composition of sterile dilution fluid.	85

LIST OF FIGURES

Figure 1.	Components of red panda gruel.	12
Figure 2.	Components of red panda gruel with alfalfa or bamboo meal added.	13
Figure 3.	Average red panda weights (1986) on bamboo, gruel and gruel/alfalfa meal diets.	17
Figure 4.	Average red panda weights (1987), gruel/10% bamboo meal trial.	20
Figure 5.	Weekly intakes of 5 red pandas, average by week, July 20 to Nov. 27, 1987.	26
Figure 6.	Average time to first appearance of wheat kernel marker.	82
Figure 7.	Total mean retention times of wheat kernel marker in red pandas.	83

General Introduction

The red panda (Ailurus fulgens) is a solitary carnivore native to the bamboo and deciduous forests (3,000-3,800 m) of Nepal and its surrounding areas (Roberts and Kessler, 1979). Although taxonomically classified as a member of the Carnivora, the red panda actually has the feeding strategy of an herbivore, with a large portion of its diet coming from the leaf portion of bamboo grasses (Yonzon, 1987).

The red panda was actually the first 'true' panda to be introduced to the western world. It was discovered some 48 years before Father Pere' David reported in 1869 his observations of a large black and white parti-coloured bear, which later came to be known as the giant panda (Holmgren, 1982).

One species of red panda (Ailurus fulgens) and two subspecies, Ailurus fulgens fulgens and Ailurus fulgens styani, comprise the genus (Roberts and Kessler, 1979). The distinction between subspecies originally was based on morphological and home range characteristics with the styani subspecies generally being larger, of a darker color and occupying the higher elevations (Roberts, 1982). More recently, the subspecies distinction has been corroborated by examining blood proteins. Subspecies specific polymorphisms have been discovered at two loci on a single enzyme protein. Similar traits are used to differentiate between other well-recognized subspecies (Gentz, 1987).

The controversy surrounding the phylogenetic relationship of the red and giant pandas still persist today. This issue of taxonomy has been hotly debated since the animals were first discovered. Originally the red panda was placed in the family Procyonidae and the giant panda was placed with the Ursidae, although those classifications soon changed. Since that time, based on morphological, behavioral and serological evidence, they have both been placed in the Procyonidae, both in the Ursidae, and even in their own separate family, Ailuropodiae (Morris et al., 1966). The most recently published classification, based on molecular biological measures, places the red panda with the Procyonidae and the giant panda with the Ursidae, recognizing the probability of divergence from a common ancestor 50 + million years ago (Mayer, 1986, Gould, 1986). However, a recently completed cladogram by Decker and Wozencraft (pers. comm.) places the red panda with the Ursidae based on the examination of cranial, dental, postcranial and soft morphological characters. Another cladogram by Wozencraft, that examined the phylogenetic relationships among the Ursidae also placed the red panda in the Ursidae family (pers. comm.).

According to the 1987 International Red Panda Studbook 169 red pandas (84 .85) currently are being held in SSP (species survival plan) member institutions worldwide. Presently, the red panda is classified as an Appendix Two species, meaning it is threatened in its country of origin

(Walker, 1983). Unfortunately, censuses of red pandas in the wild have not provided accurate estimates (Roberts, pers. com.). Therefore, the distinction between threatened and endangered is not well defined. It is known that the red panda's habitat is being destroyed through land clearing and habitat infringement by domestic livestock (Yonzon, 1987). Red pandas, like giant pandas, are being forced into smaller areas of suitable habitat, thus increasing the chances of inbreeding problems in wild populations (Conover and Gittleman, 1987). In fact, Veeke and Glatston (1980) determined that the world population of red pandas was headed for extinction, based upon demographic analysis.

If captive breeding populations are to be self-sustaining, many areas of red panda husbandry must be examined and evaluated. Cub mortality has been a critical problem in many red panda management schemes. A survey of pathology reports on 128 red pandas dying within the years 1978 to 1985 revealed that 15% of those deaths occurred in animals < 30 days of age (Latuer 1987). Cub mortality noted in a previous survey of pathology reports ranged as high as 21% (Frankenhuis and Glatston 1982). Fortunately, research into various areas of management has played an important role in the reproductive success enjoyed at many zoological parks, such as the National Zoo in Washington, DC. From 1972 to 1982, 79 young were born into the NZP's breeding program (Roberts, 1982). Breeding season, breeding behavior, growth and cub development and special housing

needs are all research areas that have been or are being examined in a variety of institutions (Roberts and Kessler, 1979). The results of extensive research on the biology of the red panda, both in the wild and in captivity, can be valuable tools in the successful management of such a fragile population.

I. FOOD INTAKE AND DIGESTIBILITY

Introduction

The red panda has been a popular exhibit animal in zoological institutions throughout the world since its discovery in the early 1800's. The majority of research concerning this animal has centered around its reproductive behavior, as successful reproduction is the key to self-sustaining captive populations. Cub growth and development is logically another area of research where considerable time has been invested. Prior to this study, nutrition, a key component in any management plan, had yet to be researched in depth and critically evaluated.

Red pandas are interesting subjects for nutritional study. Classified taxonomically as carnivores, red pandas possess a typical carnivore gut morphology. The stomach is relatively simple, without development of a forestomach region of fermentation, and the overall tract length is relatively short (Flower, 1870; Hume, 1982). The simple design of the carnivore digestive tract is thought to reflect the generally highly digestible nature of the food consumed. The choice of food of the red panda differs from many of its taxonomically related kin.

Red pandas retain few of the traditional carnivorous traits, such as hunting and consuming prey, although they have been observed catching an unwary bird from time to time

(NZP keepers, pers. comm.). They have been characterized as being totally herbivorous in the wild, feeding primarily upon bamboo leaves and native fruits (Yonzon, 1987). A recent study of a radio-collared female in the Wolong Preserve, China revealed a seasonal preference for dietary supplementation with ripe fruits such as raspberries (Rubus sp.), gooseberries (Ribes sp.), cherries (Prunus sp.), cotoneaster (Cotoneaster sp.) and rowan (Sorbus sp.) (Reid, 1988). The dietary choice of bamboo is even more unusual because red pandas have no specialized compartment, such as a cecum, for the digestion of a diet high in plant fiber (Flower, 1870). Although these animals in their natural environment are thought to consume large amounts of bamboo to support maintenance, growth and reproduction, the majority of dry matter consumed by captive red pandas is commonly a cereal/rice-based gruel (Warnell, 1987). The origins of these gruel diets may date to the late 1800s, when the first red panda arrived by ship in England. The following is an early account of dietary preferences published by Bartlett (1900):

...I found however, it would take arrowroot, with the yolks of eggs and sugar mixed with boiled milk; and in a few days I saw some improvement in its condition. I then gave it strong beef-tea well sweetened, adding pea-flour, Indian-corn flour, and other farinaceous food, varying the mixture daily. The appetite of the animal for sweet food was remarkable...

Twenty-one out of 22 North American zoos holding red pandas still feed some sort of gruel to their animals.

Undoubtedly, the original diet was palatable, and the ingredients were readily available on commercial ships. However, the longterm use of such a diet may be questionable.

Twenty-three percent of pathology reports for animals dying between 1978 to 1985 included citations of fatty changes in the livers of these animals. Of course there are many causes of such changes (Latuer, 1987), but improper nutrition may play a causative role. In Latuer's (1987) review, red pandas were noted to have high rates of periodontal disease, ranging from gingivitis to tooth loss and even secondary jaw abscesses. The relationship of a diet high in soluble carbohydrates, with little abrasive texture, causing similar tooth problems has been shown in other captive animals (Vosburgh et al., 1982). The feeding of a cereal-based diet to red pandas has not only raised oral health questions but concern also has been expressed in regard to fecal consistency. Very often ingested dietary items such as gruel, apples, bananas and bamboo are observed in the feces, still retaining much of their original appearance. The question arises whether an animal producing such feces has diarrhea or is simply passing a stool consisting primarily of gruel. Whether or not a soft stool is a sign of illness, this type of stool poses a management problem since feces remain on the anal area, matting the animal's furry tail and encouraging ectoparasites.

In order to better understand how the red panda

utilizes the dietary components offered in a captive management situation, a series of nutritional studies were designed to examine the red panda's ability to digest and utilize commonly fed feedstuffs. By monitoring body weight, overall condition and dry matter consumption, gross energy digestibilities of various diets could be calculated and estimates of digestible energy (DE) required to maintain body weight and condition determined. A second goal for these studies was to find a suitable substitute for bamboo in the diet of the captive red panda. Although bamboo may be important for behavioral reasons as well as a source of nutrients for red pandas, in many areas of the world bamboo may not be available year-round, so that problems associated with gruel stools still remain. Because bamboo also poses numerous problems in its procurement and storage, a replacement for bamboo was sought. Dehydrated alfalfa meal was chosen as a possible substitute since it is readily available nationwide, and it is easy to store and mix. Two studies were conducted to determine the ability of red pandas to digest a diet containing bamboo or alfalfa meal.

The third and final goal of this study was to document the potential effect temperature, relative humidity, and barometric pressure have on the dry matter intakes of red pandas. In this phase, photoperiod became naturally shorter as temperatures decreased, and no attempt was made to separate the two. It has been suggested that daily feed

intake by the red panda increases as enviromental temperatures decrease, resulting in an increase in body weight in preparation for the winter months (NZP keepers, pers. comm.).

An understanding of the relationship between season, food intake, weight gains or losses and the digestibilities of captive red panda foodstuffs should provide a sound, scientific basis for dietary revisions and improve our ability to manage the red panda successfully in the longterm.

Materials and Methods

The intake and digestibility trials comprising this thesis were conducted in 1986 and 1987. For the trials conducted in 1986, two adult (>2 years of age) and three subadult (<2 years) red pandas (4.1) were moved from the Conservation and Research Center (CRC) in Front Royal, VA to the National Zoological Park (NZP) in Washington, D.C. All of the animals were born in captivity.

Prior to shipment, all animals were given a physical examination. Upon arriving at NZP, the animals were randomly assigned to one of five outdoor 'corncrib-type' enclosures in an off-exhibit area belonging to the Department of Zoological Research. The corncribs ranged in size from 12 m to 18 m in diameter and all were 4.6 m in height, not including the roof area to which the animals did not have access. The cages were furnished with elevated logs for climbing as well as planks (7.6 cm x 43.6 cm x 3.7 m) that were used as resting platforms. The flooring throughout the enclosures was made of concrete, and all the cages had an attached 92 x 92 cm nestbox. Food items were provided continually in the nestboxes at approximately 0800 and 1600. Fresh water was available daily in ceramic crocks. All of the enclosures were covered with chicken wire to keep squirrels and other smaller vermin on the outside. Temperature, humidity and barometric pressure were recorded twice daily at feeding times as well as maximum and minimum temperatures throughout a 24 hr time period.

The dietary trials conducted in 1987 were actually a portion of a larger study which is beyond the scope of this thesis (see Montali et al., 1987 and Warnell et al., 1987). One animal, #3b, an adult female, was housed in the same enclosure at NZP that was utilized by #3 in the previous year. The remaining two 1987 trial animals, #10b, a subadult female and #11b, an adult male, were housed individually at CRC. Cages were of similar size, being 4.6 m x 9 m x 4.6 m and rectangular in shape. Each cage contained two wooden nestboxes (91 cm x 61 cm x 61 cm) used primarily for sleeping. Food and fresh water were usually provided at 0800 and 1530 hours.

The intake and digestibility trials conducted in 1986 examined the red pandas' response to an all bamboo diet (leaves), an all gruel diet (see Fig. 1) and a gruel/alfalfa meal diet, with alfalfa comprising approximately 10% of the diet on an as fed basis (see Fig. 2). The dietary trials conducted in 1987 examined the digestibility of an all gruel diet with bamboo meal added as 10% of the diet on an as fed basis.

All Bamboo Diet

Previous attempts (by this researcher) to quantify the intake of bamboo by red pandas had met with difficulties. In most instances, when bamboo is fed to red pandas, it is offered on the stalk, although the animals select only the leaf portion (Yonzon, 1987), unlike the giant panda which consumes the leaves, culms and stems (Dierenfeld, 1981).

Figure 1. Components of Red Panda Gruel

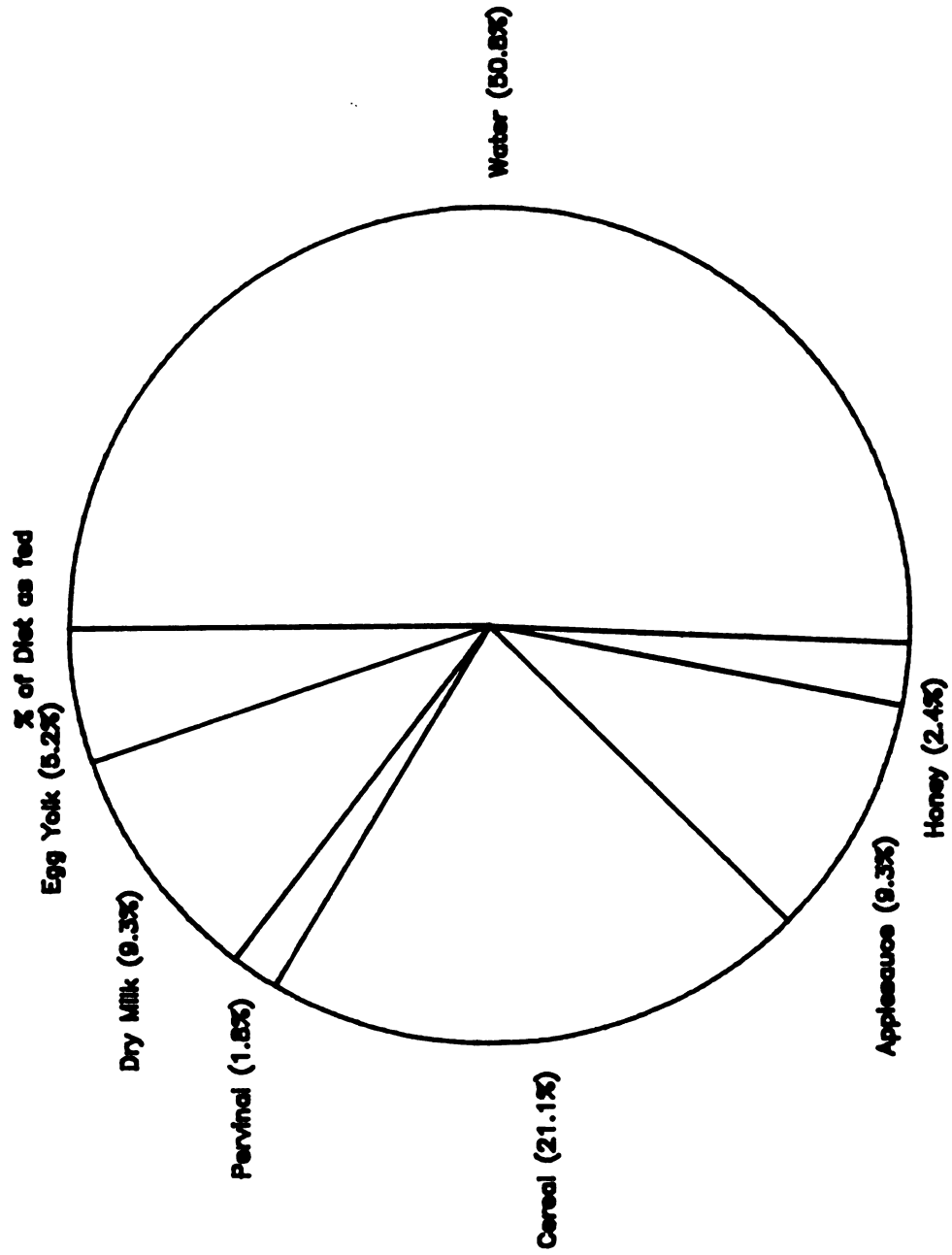
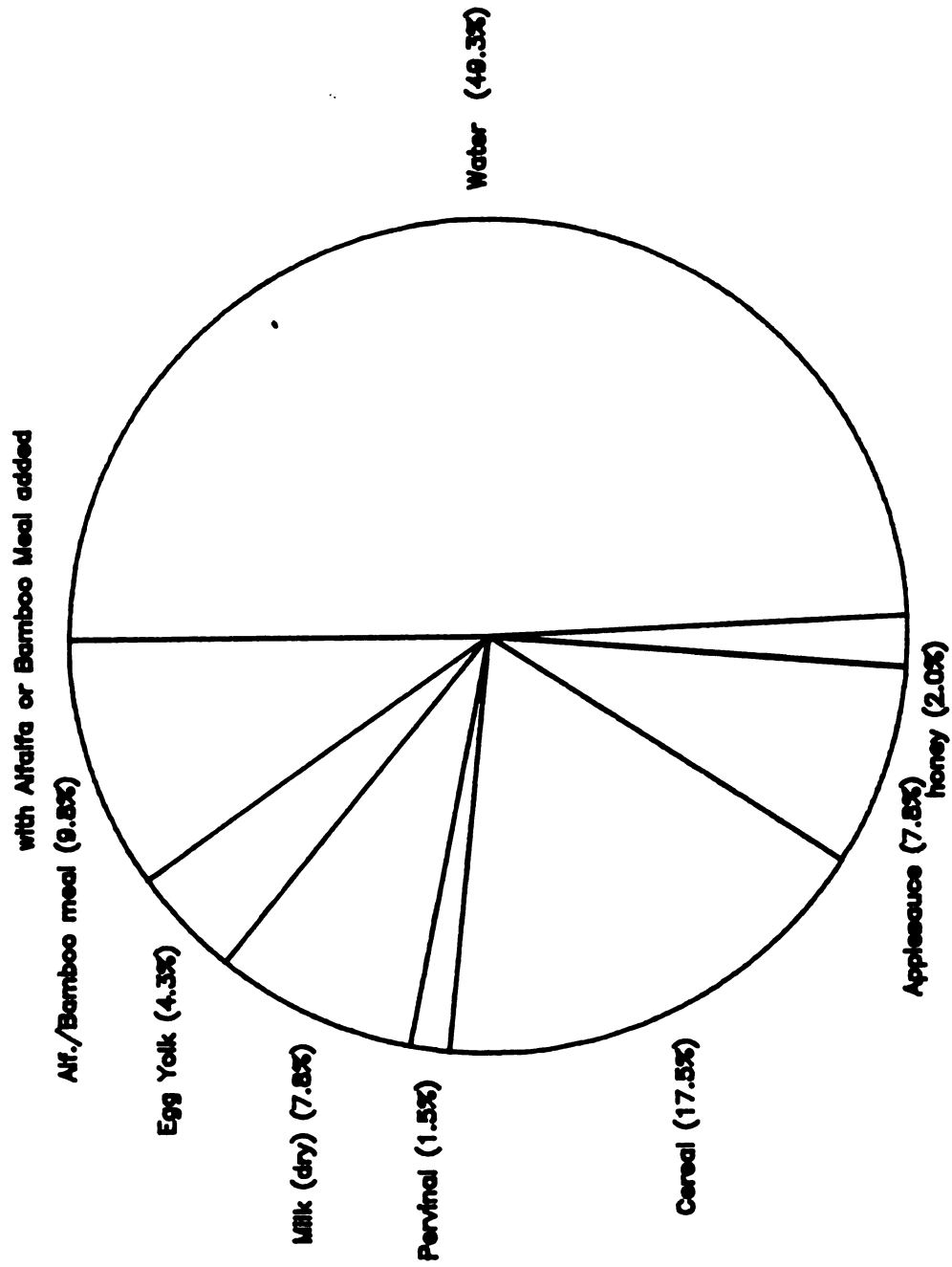


Figure 2. Components of Red Panda Gruel



Therefore, it is difficult to get accurate intake measurements by weighing the entire stalk, as the various components of the stalk have different dry matter concentrations, and certain components (particularly the leaves) lose moisture faster (Dierenfeld, 1981).

Gittleman (1987) developed a technique to quantify intake using such measures as bite frequency, and estimations of the number of leaves ingested. Although these methods proved useful for behavioral studies, because of the labor intensity in the number of observers required, they were not deemed practical for this nutritional study.

In an attempt to make the quantitative measurement of bamboo intake as accurate as possible, a different technique was tried. Bamboo (Pseudosasa japonica) was collected on the grounds of the NZP, Washington, DC, and the leaves were manually removed from the stems and branches. If the leaves could not be removed on the day of harvest, the stalks of bamboo were kept fresh under a outside sprinkler system designed by the NZP research keepers. Bamboo leaves, after picking, were thoroughly washed and stored in plastic bags in a refrigerator at 10 C until they were fed. The plastic bags containing the leaves also contained a small amount of water to keep the leaves from drying. In general, leaves were stored no longer than seven days before feeding.

One week after arrival at NZP, while still being fed bamboo on the stalk, the red pandas were presented with

small amounts (10-15 g as fed) of bamboo leaves in a ceramic crock filled with approximately 30 g of water. The water helped to prevent the leaves from drying out. These crocks were located inside their nestboxes, next to their gruel food bowls and their ceramic water bowls. Slowly, over the next two weeks, less bamboo was offered on the stalk and more leaves were offered in the crock. After the initial two weeks, any bamboo offered was presented as leaves in the crock with water.

Six days prior to the start of the bamboo digestibility trial, the amounts of gruel, apples and bananas offered to the animals were decreased, and the amount of bamboo was increased. Because the collection and processing of bamboo was so labor intensive, the amount of bamboo offered was determined for each individual, based upon the amount of leaves remaining after a day's intake.

On day one of the trial, all the animals were given whole wheat kernels, offered in gruel, apples or bananas (approx. 20-30 kernels in each food item). These wheat kernels served as indigestible particle markers. After wheat kernel consumption, the animals were also offered bamboo which had been soaked in ytterbium as a comparison to wheat kernels (see rate of passage materials and methods). Fecal samples were collected hourly post-dosing for 14 hr and then at 24 hr. After 24 hr post-dosing, the feed and fecal samples were collected daily at 0900 hr in plastic bags and stored at -20 C until further analyses were

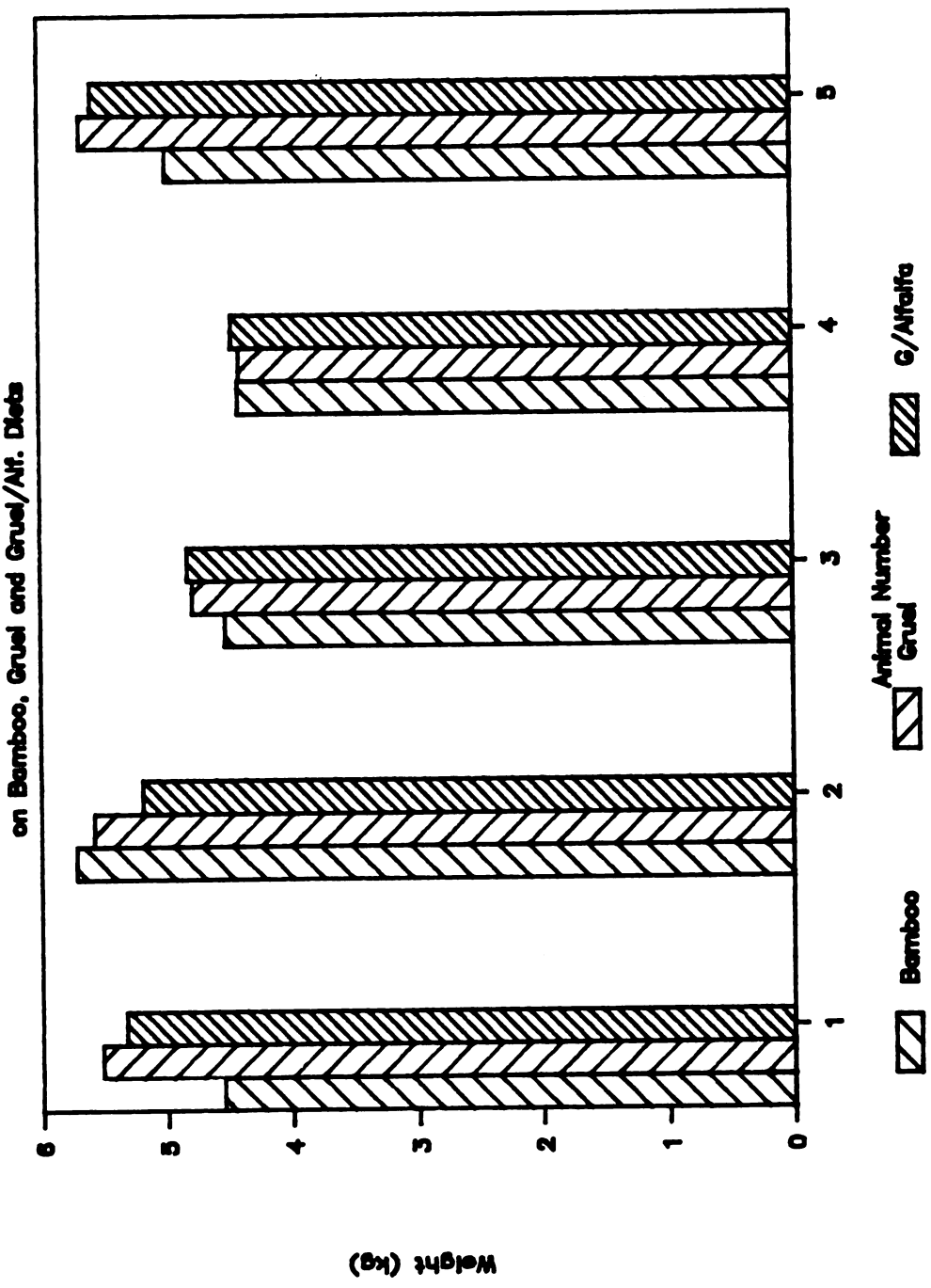
performed. Feed intake was monitored daily. Food bowls were collected at 1400 hr and 0730 hr the following morning. Uneaten food samples were weighed and a 4 g daily subsample of both the fresh and remaining food was taken to correct intake for moisture losses. Daily feed and fecal samples were not pooled. Feed and fecal samples were weighed on an electronic Mettler balance (Mettler Instruments, Hightstown, NJ) to the nearest 0.01 g. After seven days of collection, wheat kernels were again placed in the feed; appearance of wheat kernels in feces were used to indicate the completion of the trial.

Animal #2 was taken off trial since, by the second day he had failed to consume adequate amounts of bamboo to maintain body weight. He was then put back on a gruel, apple and banana diet which he readily consumed. Therefore, his pattern of body weight change (Fig. 3) served as a control for this trial.

All Gruel Diet

The same five animals were used in this trial. All animals were weighed prior to and after the trial (Fig. 3). The red pandas were given a dietary adaptation period of 8 days before the trial in which the amount of bamboo previously offered was decreased so that by day 4 before the start of the study the animals were consuming an all-gruel diet (Table 1). The start and finish of this study used wheat kernels as a marker (see rate of passage materials and methods).

Fig. 3. Ave. Red Panda Weights (1986)



The animals were fed daily at 0800 and 1600 hr, and fresh water was provided in ceramic crocks located in their nestboxes. Feed and fecal samples were collected daily for 5 days. Feed and fecal samples were prepared in exactly the same manner as stated for the all bamboo trial.

Table 1. Composition of red panda gruel fed at the National Zoological Park, Washington, DC.

Ingredients	Amount (g) (as fed)
Water	2708
Honey, natural, unrefined	129
Applesauce, canned, sweetened	498
Cereal, Gerber's Baby, mixed ^a	1125
Pervinal ^b	97
Dry milk, whole	498
Egg yolks, chicken, raw	277

^aFreemont, MI.

^bSt. Aubrey Pet Products,
New York, NY.

Gruel and 10% Dehydrated Alfalfa Meal

This trial was conducted in 1986 to determine the digestibility of a gruel diet with a source of fiber added. Prior to this trial the animals were maintained on an all-gruel diet. Over the period of 2 weeks, prior to the start of the digestibility trial, the amounts of dehydrated alfalfa meal were increased in the original gruel recipe (see Fig. 2). Alfalfa meal as 10% of the gruel ingredients on an as fed basis proved to be a level where the gruel still remained palatable to the animals, did not lose moisture quickly when fed and produced uniform stools. Alfalfa meal levels as high as 21% were tested; however, any

level above 10% decreased intake and caused the stools to be excessively hard.

The animals were fed the gruel/10% alfalfa meal (Table 2) 10 days before the start of the trial. The start and finish of this trial were marked in the same manner as outlined in the all- gruel trial. Feed and fecal samples were collected daily for 5 days at 0900 hr. All samples were prepared as described previously.

Table 2. 10% Alfalfa meal or bamboo meal in gruel fed to red pandas at the National Zoological Park, Washington, DC.

Ingredients	Amount (g) (as fed)
Water	6329
Honey, natural, unrefined	58
Applesauce, canned, sweetened	996
Cereal, Gerber's Baby, mixed ^a	2250
Pervinal ^b	195
Dry milk, whole	996
Egg yolks, chicken, raw	555
Alfalfa meal, dehydrated or	1264
Bamboo meal, dehydrated ^c	

^aFremont, MI.

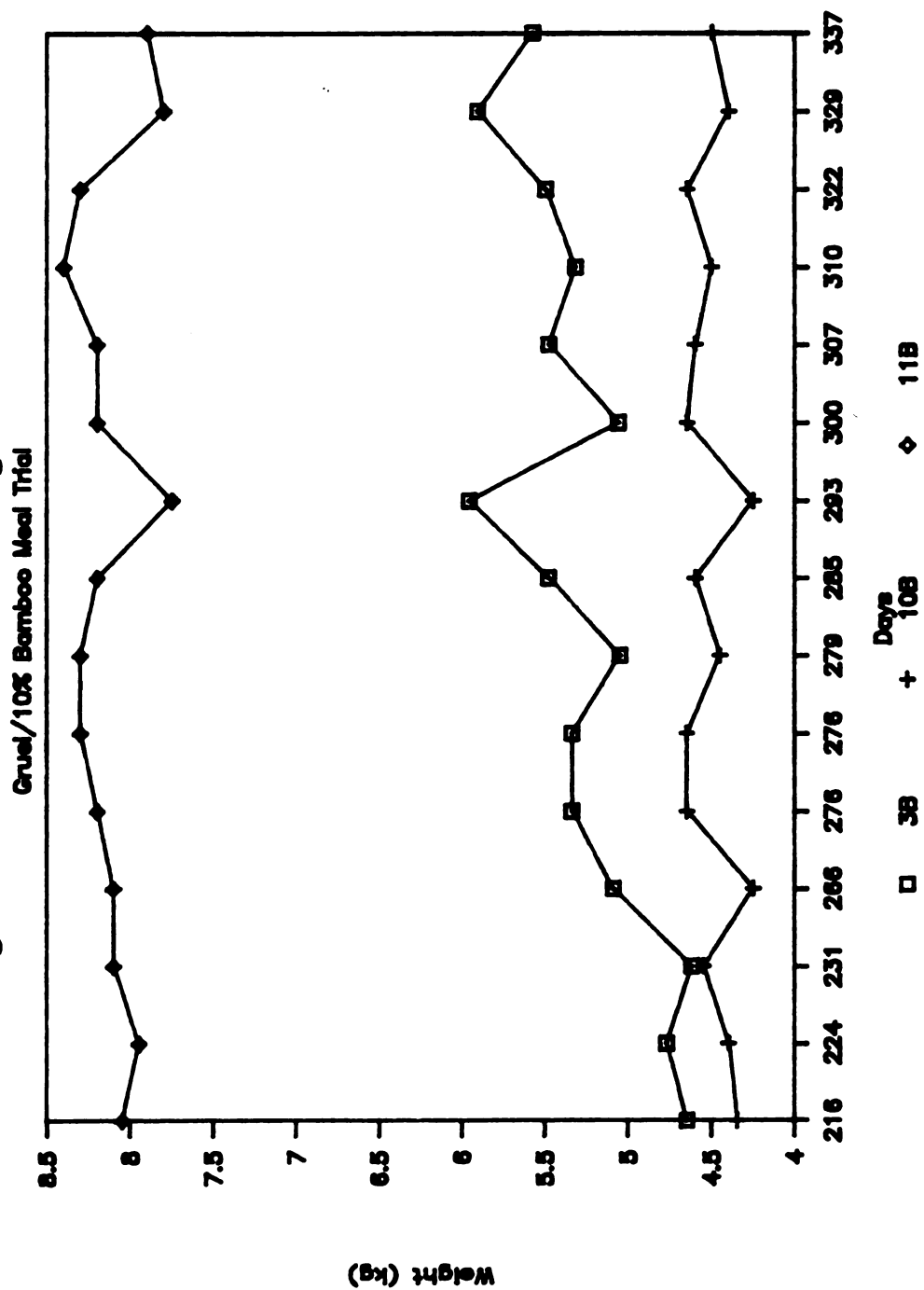
^bSt. Aubrey Pet Products,
New York, NY.

^cPsuedosasa japonica leaves.

Gruel and 10% Bamboo Meal

This final trial was conducted in 1987 using three different animals. One of those animals, #3b was studied over three different time periods (see Fig. 4 for weights), and animals #10b and 11b were studied during the same time frame. Because bamboo meal is not commercially available it was necessary to collect and process it at NZP. Due to the

Fig. 4. Red Panda Weights 1987



large quantities of bamboo needed, collections also were made outside the Zoo from a variety of locations throughout greater Washington, DC while still making an attempt to keep the species homogeneous. The stalks with leaves were collected and thoroughly washed. The leaves were manually removed then dried in a forced draft oven at 60 C to a constant weight. The leaves were then ground in a Wiley mill through a 2 mm screen and stored in sealed plastic bags until feeding.

The animals on this trial remained on the same dietary treatment (Table 2) throughout. All of these animals were on this diet a minimum of 30 days prior to the first collection. Wheat kernels served to mark the start and completion of this trial as they did in the previous trials. Daily feed and fecal samples were collected over a 5-day time period. Sample preparation was performed in a manner identical to that described for the previous trials.

Prior to and after each trial, the weights of all the red pandas were recorded (see Fig. 3). The red pandas were coaxed into their nestboxes and were locked inside for a short time. A portable plastic kennel (Dorskocil Mfg. Co. Inc., Arlington, TX), approximately 92 cm x 62 cm x 92 cm, was then placed at the entrance to the tunnel connecting the nestbox to the cage, and the pandas were individually coaxed into it. Weights were recorded to the nearest gram after the animal became quiet. The animals were returned to their cages, and the kennel was reweighed. Animal weights were calculated by difference.

Laboratory analyses

Feed and fecal samples were analyzed for dry matter (DM), ash, organic matter (OM) calculated as DM-ash, gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid lignin (ALIG) and crude protein (CP). All samples of feed and feces were weighed and dried in a forced draft oven at 60 C for 24 hrs to determine dry matter. Feed and feces were then ground in a Wiley Mill through a 2mm screen, and stored in sealed plastic bags until further analyses were performed. All analyses were performed in duplicate. Ash concentrations in the samples were determined by igniting approximately 0.800 g of sample in a muffle furnace at 600 C for 14 hr. Gross energy concentrations of the samples were determined by complete combustion in a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Company, Moline, IL). Residues were titrated with .0725 N sodium carbonate to correct for the heat of formation of nitric acid. NDF and ADF components of the diet were calculated by using the procedures of Goering and Van Soest (1970) as modified by Robertson (1978). Approximately 0.500 g and 1.000 g of subsamples were used for the NDF and ADF procedures, respectively. A separate subsample was used for the NDF and ADF procedures. For those diets containing gruel in any amount, 0.200 (NDF) or 0.300 (ADF) g of pepsin (Catalog No. P-7000, Sigma Chemical Co., St. Louis, MO) was placed in each sample then 10 ml of 0.1 N HCl were added to each sample, and the mixtures were

incubated at room temperature for 2 hr. After the initial 2 hr, 10 ml of NaH_2PO_4 buffer and 0.200 or 0.300 g of Alpha-amylase (Catalog No. A-6880, Sigma Chemical Co., St. Louis, MO) were added to the samples, followed by 2 hr of additional incubation at room temperature before digestion with NDF or ADF solution (Van Soest, 1982). ALIG was determined utilizing the ADF digested sample to which 72% sulfuric acid was added (Goering and Van Soest, 1970). A semi-automated micro-Kjeldahl method was used to determine crude protein (CP) content of the feed and feces using approximately 0.150 g of subsample (AOAC, 1984). Apparent digestibilities were calculated based on individual daily intakes and total fecal collections, using Lotus 1-2-3 software (Lotus Development Co., Cambridge, MA) and an MS-DOS (version 3) operating system.

Statistical analyses were completed using the Statistical Analysis Systems (SAS) analysis of variance by general linear models and regression procedures (SAS, 1982).

Results

Seasonal Intake

Five animals studied from July 27 to November 27, 1987 were significantly different ($P < 0.01$) in the average amount of dry matter consumed by the group, during each week of the 19-week monitoring period, (Table 3; Fig. 5) although no significant body weight differences were detected by using the SAS general linear model analysis of variance and comparing means by the least squares means statement (SAS, 1982). A step-wise regression (SAS) of average daily intake on ambient temperature, barometric pressure and relative humidity revealed that temperature ($P < 0.001$) and barometric pressure ($P < 0.08$), accounted for the greatest variation in daily dry matter consumption.

Intake and Digestibility

Mean ambient temperatures and relative humidities differed ($P < 0.001$) between periods when the bamboo diet and the gruel/alfalfa meal diet were fed and between the periods when the gruel diet and gruel/alfalfa meal diet were fed. There were no significant differences among barometric pressures among periods. There were significant differences in temperature ($P < 0.001$) and relative humidities ($P < 0.05$) among the 4 dietary periods during the gruel/bamboo meal trials. The mean trial temperatures, relative humidities and barometric pressures are presented in Tables 4 and 5.

Table 3. 1987 Mean red panda dry matter intakes by week with corresponding average temperatures¹, average relative humidities¹ and average barometric pressures¹.

Week	Intake (g)	SEM	Temperature (c)	Relative humidity	Barometric pressure (mm of Mercury)
1	68.51	37	33.3	87	119.0
2	87.72	32	29.4	72	117.3
3	143.53	41	29.4	84	117.7
4	180.32	26	26.7	84	118.1
5	128.83	17	28.3	79	118.1
6	189.79	21	26.7	73	118.5
7	195.87	20	23.3	88	118.5
8	76.73	11	24.4	79	119.7
9	118.71	28	25.0	83	120.5
10	185.49	10	19.4	91	118.1
11	180.77	10	18.3	83	117.7
12	217.28	7	13.9	73	118.5
13	197.67	18	10.6	60	118.5
14	204.12	19	12.2	62	118.5
15	247.49	17	8.9	55	118.9
16	208.19	22	13.9	52	118.9
17	258.13	21	9.4	57	119.3
18	238.35	27	13.3	61	118.1
19	224.66	20	11.1	34	118.5

¹Values presented are the averages of 10 readings (2 readings per day)

Weeks listed below are significantly different at ($P < 0.01$)

Week 1	from weeks 3-7 and 10-19	Week 10	from weeks 1+2+8
2	4, 6+7 and 10-19	11	1+2+8
3	15-19	12	1+2+5+8+9
4	1+2+8+17	13	1+2+8+9
5	12+15-19	14	1+2+5+8+9
6	1+2+8	15	1+2+5+8+9
7	1+2+8	16	1+2+5+8+9
8	4+6+7+10-19	17	1-5+8+9
9	6+7+12-19	18	1-3+5+8+9
		19	1-3+5+8+9

Fig. 5. Weekly Intakes of 5 Red Pandas

Ave. by week, July 20 to Nov. 27, 1987

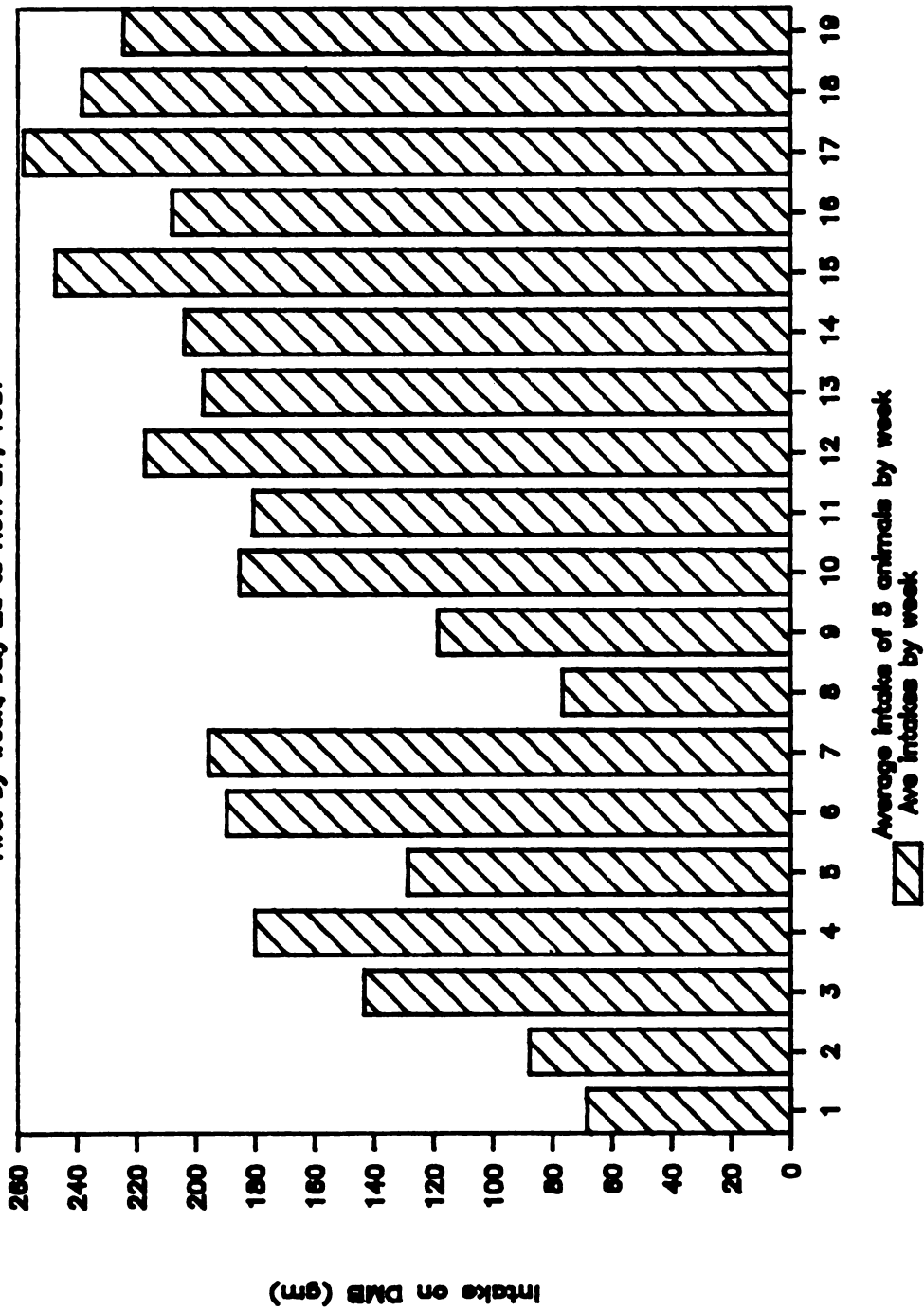


Table 4. Weekly means for temperature, relative humidity and barometric pressure over bamboo, gruel and gruel/alfalfa meal dietary treatments.

Diet	Temperature (C)	SEM	Relative humidity (%)	SEM	Barometric pressure (mm of Mercury)	SEM
Bamboo	23.3 ^a	1.3	83.1 ^a	4.9	118.1	0.1
Gruel	24.7 ^a	1.5	86.2 ^a	5.7	118.5	0.1
G/Alf.	14.4 ^b	1.6	47.5 ^b	6.2	118.9	0.1

^{ab} Means within a column having different superscripts are significantly different ($P < 0.001$).

Table 5. Weekly means for temperature, relative humidity and barometric pressure over all the gruel/bamboo meal dietary trials.

Diet	Temperature (C)	SEM	Relative humidity (%)	SEM	Barometric pressure (mm of Mercury)	SEM
G/bam t1	26.9 ^a	1.9	70.8	3.1	122.4	0.3
G/bam t2	24.8 ^a	1.9	70.8	3.1	120.5	0.3
G/bam t3	14.7 ^b	2.1	71.3	3.5	118.1	0.4
G/bam t4	13.2 ^b	1.9	72.8	3.1	118.9	0.3

^{ab} Means within a column having different superscripts are significantly different ($P < 0.001$).

Animal body weight differed significantly ($P < 0.001$) between the bamboo trial and both the gruel and gruel/bamboo meal trials. Mean daily dry matter intake significantly differed ($P < 0.01$) across treatments (Table 6) with differences occurring between the bamboo and both the gruel/alfalfa and gruel/bamboo meal trials, and the gruel trial differed ($P < 0.001$) from the gruel/bamboo meal trial. Dry matter intakes as a percent of body weight differed significantly ($P < 0.05$). Mean daily dry matter intake per metabolic body size were 32.93, 32.83, 42.83 and 42.77

$\text{g/BW}^{0.75}$ for the bamboo, gruel, G/alf. and G/bamboo meal trials, respectively. Only the bamboo and G/alf. meal diets were significantly different ($P < 0.01$).

Table 6. Daily dry matter intake (DMI) and body weight (BW) relationships for red pandas fed 4 diets (n=5).

Factor	Treatment mean				SEM	P< ANOVA
	Bamboo	Gruel	G/Alf	G/Bam		
BW (kg)	4.62 ^a	5.19 ^b	5.08 ^{ab}	5.69 ^c	0.134	0.001
DMI (g)	104.3 ^a	113.1 ^{ab}	145.2 ^{bc}	153.7 ^c	9.32	0.001
DMI(% of BW)	2.24	2.18	2.85	2.80	0.184	0.05
DMI/MBS (gm/kg ^{0.75})	32.93 ^a	32.83 ^a	42.83 ^b	42.77 ^b	2.74	0.01

^{abc}Means within a row with different letter superscripts are significantly different ($P \leq 0.01$).
MBS=Metabolic body size ($\text{BW}^{0.75}$)

Mean dry matter, organic matter and gross energy digestibilities of the G/alf. and G/bamboo meal diets were not significantly different. As the percentage (of the dry matter) of NDF and ADF increased in the diet, the apparent digestibility of dry matter, organic matter, gross energy and crude protein decreased (Tables 7).

Table 7. Composition (%) of diets fed to red pandas.

Dietary Components	Bamboo ¹ % of DM	Gruel ² % of DM	G/Alf. ² % of DM	G/Bam. ² % of DM
Dry matter	24.1	38.4	43.1	42.2
Organic matter	90.9	95.9	94.7	94.5
Ash	9.1	4.1	5.3	5.5
Gross energy ³	4.35	4.61	4.46	5.2
Ether extract	2.7	10.7	9.4	9.8
Crude protein	12.7	15.2	17.1	15.8
NDF	76.8	2.0	12.8	20.6
ADF	48.9	1.9	11.5	10.3
ALIG	8.4	0.0	4.0	1.9

¹n=4 animals²n=5 animals³kcal/g

Table 8. Mean apparent digestibility (%) of 4 diets (DMB) fed to red pandas: bamboo, gruel, G/alfalfa and G/bamboo meal.

Factor	Treatment mean				SEM	P< ANOVA
	Bamboo	Gruel	G/Alf	G/Bam		
Dry matter	24.0 ^a	84.7 ^b	68.0 ^c	67.4 ^c	0.025	0.001
Organic matter	27.5 ^a	86.5 ^b	70.1 ^c	69.7 ^c	0.022	0.001
Gross energy	30.3 ^a	87.0 ^b	70.4 ^c	71.0 ^c	0.022	0.001
Crude protein	50.4 ^a	83.5 ^b	77.9 ^{bd}	67.3 ^{be}	0.024	0.001
NDF	24.7	14.8	26.7	18.5	0.067	NS
ADF	31.8 ^d	44.4 ^a	40.3 ^a	8.4 ^{be}	0.055	0.001
ALIG	50.1 ^{ad}	0.0 ^b	61.1 ^{ae}	-5.55 ^b	0.047	0.001

^{abc} Means within a row with different superscripts are significantly different (P < 0.001).

^{de} Means within a row with different superscripts are significantly different (P < 0.01).

Discussion

Seasonal Intake

The previously presented results lead to the conclusion that the red panda must increase food consumption to maintain body weight as the seasons progress toward colder weather. Most of the significant changes ($P < 0.01$) in dry matter intake occurred between weeks 1-10 and weeks 11-19, and those were the weeks when significant changes in temperatures and relative humidities occurred with no significant differences in barometric pressure. Mc Nab (1987), through his work with red pandas in metabolism chambers, reported that these animals are unusually well-suited for life in a cold environment and are able to maintain body homeostasis down to ambient temperatures of 5 C. Below 5 C, the animals decreased their metabolic rate and began to decrease their peripheral circulation. This decrease may not be as detrimental to the red panda as to some other mammals because red pandas are covered heavily in thick fur, even on their paws. Part of the red pandas' adaptation to cold weather, along with plentiful fur, may be to increase dry matter intake to compensate for increased energetic demands.

Intake and Digestibility

Bleijenberg (1984) has suggested that red pandas consume a greater percentage of body weight in dry matter per day than other carnivores (Table 9) , but his

comparison fails to recognize that dry matter intake is a function of body size. In general larger animals consume a smaller percentage of body weight per day because their mass-specific energy requirements (kcal/kg) decrease with increasing body size (Van Soest, 1982).

Table 9. Quantity of food eaten daily by various animal species.

Animal	Food Intake (g DM/kg BW)
Tiger	12
Domestic cat	12
Dog	20
Cow	30-40
Red panda (Rotterdam)	45
Red panda (NZP Bamboo)	22.6
Red panda (NZP Gruel)	21.8
Red panda (NZP G/Alf.)	28.6
Red panda (NZP G/Bam.)	27

Bleijenberg's contention was that the red pandas he observed had a feeding pattern (g DM/kg BW) more closely related to that of a strictly herbivorous animal, the cow, than to that of carnivores. It is unfortunate that the energy densities used in his comparison were not available, as a more accurate relationship to this study could be established. The animals used in the present study, according to Bleijenberg's figures, actually resemble the dog most closely in the relationship of intake to body weight. Of course, the values obtained for the red pandas might vary on a free-choice diet, especially if they chose a less energy-dense diet. Table 10 shows animals, both ruminants and non-ruminants, of similar body weight to the red panda and their corresponding dry matter intakes as a percent of body weight.

Table 10. Dry matter intakes (% of body weight) of ruminants and non-ruminants, similar in body weight to red pandas.

	Body wt (kg)	DMI (% of BW)	Diet
Dik Dik ^a (<u>Madoqua kirki</u>)	4.23	2.85	Alfalfa leaf
Suni ^b (<u>Nesotragus mochatatus</u>)	3.21	3.50	Alfalfa leaf
Blk. & whi. colobus ^{cd} (<u>Colobus quereza</u>)	8.68 7.26	1.89 2.09	High fiber monkey biscuits and mixed fruits and vegetable
Koala ^e (<u>Phascolarctos cinereus</u>)	5.78 6.77	2.00 1.80	Eucalyptus
Domestic dog ^e	5.00	2-2.50	Dry and moist dog food, 1800 and 550 kcal/lb
Red panda (<u>Ailurus fulgens</u>)	4.62 5.19 5.08 5.69	2.24 2.18 2.85 2.80	Bamboo Gruel G/alf.meal G/bam.meal

^aBaer, 1987.

^bHoppe, 1977.

^cWatkins et al., 1985.

^dOftedal et al., 1982

^dUllrey et al., 1981.

^eNRC, 1962.

Table 10 shows that the red panda consumes an amount of dry matter as a percent of its body weight similar to that of the dik dik and slightly higher than that of the koala or the colobus and most closely resembles the domestic dog. The results of this study indicate that the red panda does not consume a higher dry matter intake on an all forage diet as compared to gruel or the gruel mix diets. However, the animals on the all bamboo diet lost an average of 0.69 kg

over the trial period, indicating the animals were not consuming enough dry matter to maintain body weight, not digesting enough energy or both. The gross energy concentrations (kcal/g) of the various feedstuffs in this study were 4.35, 4.61, 4.46 and 5.20 for the bamboo, gruel, G/alfalfa meal and G/bamboo meal diets, respectively and the corresponding apparent gross energy digestibilities (%) were 30.3, 87.0, 70.4 and 71.0, respectively.

Although the dry matter consumed by the red panda was not significantly different among dietary treatments ($P > 0.05$), the low amount of DE consumed on the all bamboo trial may be attributed to its low apparent gross energy digestibility (30.3%). During the bamboo trial, the animals were all thought to be at ad libitum intake, using the amount of orts as an indicator. However, when offered more leaves, the red pandas consumed them, leaving a similar amount of orts. The reason for the low consumption of leaves, even when in a negative energy balance for support of basal metabolism has not been established. A high concentration of secondary plant compounds, found in bamboo leaves, such as phenolic compounds (Baer, 1987) or tannin complexes (Horvath, 1982) have been shown to decrease palatability. Higher concentrations of lignin in leaves of many plants have been found to adversely affect palatability (Van Soest, 1982). Ullrey et al. (1981) found that the ether extract fraction was elevated in eucalyptus browse rejected by koalas. In order for these red pandas to

consume enough digestible energy to support basal metabolic functions, they would have needed to consume approximately 1,000 to 1,100 g of bamboo leaves as fed. At this rate, the red pandas would be consuming dry matter at between 6 and 6.25% of their body weight, a much greater amount than was actually the case (Table 10). This high rate of intake, as compared to many of the animals in Table 11, was related to the low digestibility of the bamboo.

Red panda body weights, on the average, remained relatively constant throughout all trials except the bamboo trial. In trials other than the bamboo trial animals displayed small weight gains or losses. The positive and negative relationships may be explained in part by fluctuations in daily consumption and the corresponding weight changes. Body weights, although accurately measured, varied daily even for the same animal. Defecation and food consumption prior to weighing would affect weight measures, although an attempt was made to keep error to a minimum.

McNab (1988) calculated the red panda's basal metabolic rate (BMR) to be $27.28 \text{ kcal BW}^{0.75}$ which is lower than that of the koala, $36 \text{ kcal per kg W}^{0.75}$ per day (Degabriele and Dawson, 1979). This value, is only 39% as great as the interspecific number ($70 \text{ BW}^{0.75}$) used by Kleiber (1961). Table 11 demonstrates the relationship between the digestible energy consumed and the estimate of energy required for basal metabolic functions.

Table 11. Comparison of digestible energy (DE) consumed on four diets to estimates of energy required by red pandas for basal metabolic functions using $BMR = 27.28 \times BW^{0.75}$ kg

Diet	1	2	Animal 3	4	5
Bamboo					
DE consumed kcal/d	126.3	no val.	79.3	152.6	191.9
Animal weight (kg)	4.99	"	4.99	4.99	5.45
Basal energy req.kcal	91.09	"	91.09	91.09	97.31
+ or - basal	+35.2	"	-11.8	+61.5	+94.6
DE kcal/BW ^{.75}	37.8		23.8	45.7	53.8
Ave.daily wt.change(kg)	-0.09		-0.08	-0.07	-0.07
Gruel					
DE consumed kcal/d	588.3	322.0	287.3	467.7	596.7
Animal weight (kg)	5.53	5.6	4.9	4.5	5.8
Basal energy req.kcal	98.38	99.31	89.85	83.72	101.97
+ or - basal	+490.0	+222.7	+197.5	+384.0	+494.7
DE kcal/BW ^{.75}	163.1	88.5	87.2	151.4	159.7
Ave.daily wt.change(kg)	+0.004	+0.007	-0.01	-0.003	-0.009
Gruel/alfalfa meal					
DE consumed kcal/d	543.1	431.4	400.2	402.5	510.1
Animal weight (kg)	5.21	5.44	4.61	3.95	5.23
Basal energy req.kcal	94.07	97.17	85.82	76.44	94.35
+ or - basal	+449.0	+334.2	+314.4	+326.1	+415.8
DE kcal/BW ^{.75}	157.5	121.1	127.2	143.7	145.5
Ave.daily wt.change(kg)	+0.03	-0.07	+0.009	-0.04	0.0
Gruel/bamboo meal¹					
DE consumed kcal/d	488.7	632.1	711.4	474.4	529.4
Animal weight (kg)	5.28	5.28	5.28	4.51	8.12
Basal energy req.kcal	95.02	95.02	95.02	84.43	131.22
+ or - basal	+393.7	+537.1	+616.4	+390.0	+398.2
DE kcal/BW ^{.75}	140.3	181.5	204.3	153.3	110.1
Ave.daily wt.change(kg)	+0.004	-0.01	+0.02	+0.02	+0.03

¹Animal numbers 1-3 are the same animal, #3b and animal numbers 4 and 5 represent animals #10b and #11b.

Animals fed the all gruel diet, consumed DE in amounts that exceeded the calculated energy requirement for basal metabolism by an average of 4.7 times. Red pandas fed the

gruel/alfalfa meal diet consumed DE in amounts that exceeded the basal energy requirement an average of 5.1 times. The gruel/bamboo meal trial provides interesting information for comparison because one animal was studied over 3 different time periods, with 3 different temperature ranges. The results suggest that during the first trial, which had the highest ambient temperature, she consumed DE 5.1 times above her estimated basal requirements, then up to 6.7 times above basal and finally to 7.5 times over her basal requirements as the weather became colder. These results are in agreement with the data from the study of seasonal intakes of DM by red pandas that suggest that although intake increases with the cold weather, body weight does not change significantly.

Given the results of this study, using the BMR as calculated by McNab, red panda maintenance DE requirements may be estimated at 4.8 to 5.8 times above BMR. The maintenance energy requirement of many eutherian mammals is commonly estimated using Kleiber's value of $2 \times 70 \text{ BW}^{0.75}$.
 kg
 The digestible energy intake measured in this study suggests that red pandas are similar in their daily DE intake to other eutherian mammals, although it appears they have a low BMR as calculated by McNab.

When comparing alfalfa meal to bamboo meal as a source of fiber in the diet of the red panda, the significant differences between the two diets were in the digestibility of crude protein ($P < 0.01$), NDF ($P < 0.001$) and ADF ($P < 0.001$) (alfalfa being higher in each case). However, when

comparing the amount of digestible component in 1 g of dry matter the differences become less apparent (Table 13). The calculated grams of acid detergent fiber digested for G/alf. meal actually exceeds the calculated grams of neutral detergent fiber digested, which is probably an artifact of analysis. The NDF portion of the analysis recovers the insoluble cell matrix which is digested in the ADF portion, therefore the NDF amount recovered should exceed the ADF portion although the differences really are trivial in this instance. No real differences were detected between the apparent digestibilities of dry matter, organic matter and gross energy in gruels containing alfalfa meal or bamboo meal, thus implying that alfalfa meal would be an acceptable substitute for bamboo meal.

Table 12. Amount of digestible component in 1 g of dry matter¹.

Dietary component	Bamboo	Gruel	G/Alf.	G/Bam.
Dry matter	.24	.85	.68	.67
Organic matter	.25	.83	.66	.66
Gross energy ²	.01	.04	.03	.04
Crude protein	.06	.13	.13	.11
NDF	.19	.01	.03	.04
ADF	.16	.01	.04	.01
ALIG	.04	.00	.02	-.00

¹Calculated by multiplying the percent of component, DMB, by the apparent digestibility of the component.

²kcal

Urine nitrogen contamination of feces was not accounted for in these studies due to the difficulty in the separation of feces from urine. Red pandas tend to urinate on or very close to their feces at the time of defecation, making separation of urine from feces impossible given the housing constraints. Crude protein digestibility was significantly higher for the gruel/alf. meal trial ($P < 0.01$), than for the G/bamboo trial, which may be related to the higher crude protein (17%) concentration and the lower ADF (33.5%) levels in the alfalfa meal as compared to the corresponding values for bamboo meal (14.5% CP and 48.9% ADF) (Table 13). Given the red panda's inability to utilize the more fibrous component of diets fed, a diet with lower amounts of less soluble components and a higher protein content would be desirable.

Table 14. Composition (%) of ingredients in
G/alfalfa meal or G/bamboo meal diets.

Dietary components	Gruel	Alfalfa meal (dehyd)	Bamboo meal (dehyd)
Ash	4.1	8.5	7.5
Gross energy ¹	4.6	4.4	4.3
Crude protein	15.2	17.0	14.5
NDF	2.0	30.4	82.0
ADF	1.9	33.5	48.9
ALIG	0.0	7.6	8.4
¹ kcal/g			

So alfalfa meal would be the feed of choice if the desire is to increase the total digestible protein content of the ration.

The importance of fiber and its form in the diet of the red panda is difficult to determine. Behavioral data obtained from field research indicate that the red panda has adapted to a diet high in fiber and low in digestibility. The importance of abrasiveness in the diet of the giant panda for proper GI functioning (gut sloughing) has been suggested (Nouvel, 1974). Whether there is a need for such a texture in the giant or red panda's diet has not been determined. It is now known that the red panda, through careful manipulation, can be adapted to a mixed feed and will maintain body weight and condition without bamboo although the form of the diet (gruel) may not be particularly desirable. Animals on these trials were studied for 7 and 6 months with no aberrant stool

consistency problems, other than those expected on the all-gruel dietary treatment.

The development of a complete diet, with the consistency appropriate to support good oral health as well as a suitable energy level to support maintenance, is possible. This study has shown that, although the red panda is initially resistant to dietary change, it is possible to manipulate the red panda diet to provide a substitute source of fiber as an alternative to bamboo. Thus, rather than serving as an essential ingredient of the red panda diet, bamboo may be used, as it is available, as a source of behavioral enrichment and as an occasional supplementary source of nutrition.

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II. RATE OF PASSAGE

Introduction

The red panda, classified as a carnivore, has a gastrointestinal tract very similar to other carnivores such as the raccoon, cat, mink, and dog (Clemens and Stevens, 1979; Stevens, 1977). However, red pandas have been observed to have feeding strategies ranging from strictly herbivorous to omnivorous, both in the wild and in captivity (Reid, 1988).

Many animals that rely on plants as a major source of energy and nutrients have specially adapted compartments such as complex foreguts or sacculated hindguts (Van Soest, 1982) to slow the passage of digesta and allow for greater microbial breakdown of plant materials. Generally, the longer the food consumed remains in the digestive tract, the greater the opportunity for microbial degradation (Van Soest, 1982).

Red pandas have adapted to a diet high in plant components although their digestive tract lacks anatomical features such as a sacculated stomach or cecum that could facilitate the retention of digesta (Flower, 1870).

Several factors that affect the passage of digesta have been documented in ruminant and non-ruminant species. These properties include the level of intake, physical form of the feed (particle size), specific gravity (density) and environmental temperature (Balch and Campling, 1965).

Animal body size and the structure of the gastrointestinal tract will also affect the flow of digesta (Demment and Van Soest, 1983).

In order to accurately measure digesta passage, dietary markers must be used to characterize the passage of particulate and fluid components of the digesta.

Properties of an ideal marker as defined by Kobb and Luckey (1972) include:

1. Inert with no toxic , physiological or psychological effects.
2. Neither absorbed nor metabolized within the alimentary tract, and has no appreciable bulk.
3. Mixes with the digesta and has no influence on alimentary secretion, digestion, absorption, normal motility of the digestive tract or excretion.
4. No influence on the microflora of the alimentary tract.
5. Can be precisely and quantitatively measured, and has physical-chemical properties that make it discernable throughout the digestive process.

The marker chosen for these studies was wheat kernels, because they are largely consistent with the aforementioned criteria, as well as being acceptable to the staff at the National Zoological Park (NZP). To serve as a check on particulate marker passage, ytterbium was used in the bamboo trial. This element is one of the heavier rare earth elements with a small ionic radius that tends to form strong complexes with dietary fiber, making it a desirable solid

marker. Liquid markers were not used due to the difficulty in their administration to red pandas. Passage of liquid ingesta may be assumed to be somewhat faster than passage of solids in the red panda, based on passage rate studies in the raccoon and dog (Clemens and Stevens, 1979; Banta et al., 1979).

Transit time (TT) and total mean retention time (TMRT) are terms commonly applied in measuring the rate of digesta passage. Transit time is defined as the time it takes for the first appearance of a given meal to pass through the digestive tract. Mean retention time may therefore be defined as the weighted average time for the appearance of marker in the feces (Faichney, 1975; Parks, 1969). Other terms used to describe a marker excretion curve include peak time (time of maximal fecal marker concentration), the natural log slope of the declining marker phase and time of disappearance (Van Soest et al., 1983).

Digesta flow through the red panda digestive tract has not been previously quantitated. Passage rate measurements have been conducted on other carnivores such as the raccoon, dog and mink (Clemens and Stevens, 1979; Banta et al., 1979, Bleavins and Aulerich, 1980). Clemens and Stevens (1979) observed that a fluid marker moved more rapidly through the raccoon digestive tract than did a particulate marker. Fifty-four percent of the fluid marker was recovered in the first 12 hr, and 95% was recovered in 24 hr. Both fluid and particulate markers were recovered from the feces by 24 hr.

Banta et al. (1979) reported a slower mean retention time in dogs than was found in the above study with raccoons, with most of the fluid marker excreted within 38 hr. Particulate marker movement was also slower through the dog's digestive tract when compared to the raccoon, especially for larger particle sizes between 10-20 mm. Between 8-16 hr, 30% of the 2 mm particles had been excreted. Blevins and Aulerich (1980) reported an average particulate transit time of just over 3 hr for both mink and ferret with total mean retention time between 15 and 20 hr.

Transit times and mean retention times were measured to characterize the rate of digesta passage in 5 red pandas and to use these values for comparison with other carnivores to better evaluate the feeding strategy of the red panda. The transit time studies also served to mark the start and finish of digestion trials.

Materials and Methods

The series of transit and total retention time trials that comprise this work utilized the same animals, during the same years, housed under the same conditions as were described in the previous chapter. The markers were used to determine the beginning and end of the digestion trials as well as providing information on rate of digesta passage.

All Bamboo Trial

This study utilized two solid markers, wheat kernels as an indigestible particulate marker and bamboo leaves treated with ytterbium. The wheat kernels facilitated the quantification of marker recovery as the wheat kernels were easily distinguished from fecal material. The bamboo leaves soaked with ytterbium served in this instance as a comparison to the wheat kernel marker. Comparisons were made between TT and TMRT of both the kernel and ytterbium markers to determine if passage rates were similar, thus validating the use of kernels as following a representative particulate portion of the digesta.

The bamboo leaves were marked with ytterbium by first manually separating the fresh leaves from the stalk. A known quantity of bamboo leaves, approximately 230 g, was added to a solution of 5 g YbCl_3 dissolved in 1000 ml of distilled water. The leaves were soaked for 6 hr, drained, then soaked for 1 hr in 1000 ml distilled water. The leaves were drained and soaked (1 hr) a second time in 1000 ml

distilled water. The leaves were drained and placed in a sealed plastic bag in a refrigerator at 10 C until they were fed the next day.

Intakes had been monitored prior to the start of the digestion trial and food intakes gauged so that maximum dry matter intake could be achieved. In the morning of the first study day the animals' cages were thoroughly cleaned, and the previous day's orts were removed and weighed as described in the earlier chapter. At approximately 0730, the animals were presented with their usual food bowl containing a piece of apple, banana and a quarter-size drop of gruel. All of these items were weighed, and no one item exceeded 20 g in weight. Each of these food items contained a known number of wheat kernels, usually 25, that were counted by hand a minimum of three times and placed in each individual food item.

The bowls were placed in the nestbox and were checked every 5-10 minutes for food consumption. Care was used to avoid frightening the animals and, thus, inhibiting them from feeding. After the first consumption occurred, usually within 15-20 minutes, the bowls were removed and the concrete floor of the nestbox and the connecting runway were carefully examined for any kernels that might have been dropped. At this point, the ytterbium marked bamboo leaves (20 g as fed) were offered in a ceramic crock, with a small amount of tap water to keep the leaves from drying prematurely and adversely affecting intake. The animals

were monitored visually and given one half hour from the start of leaf consumption before the leaves were removed and the orts weighed. The regular AM feeding of leaves then took place. At this time the dosed food items removed earlier were examined, and the number of kernels ingested was verified and recorded.

The cages were checked every hour following the first record of kernel intake. If feces were found, they were collected by hand, using rubber surgical gloves, and placed in a ziplock freezer bag marked with the animal's number and time of collection. The feces were examined through the plastic bag and the kernels counted while all the fecal materials and kernels remained inside the bag. The cages were checked every hour for a minimum of 14 hr and then again at 24 hr.

Gruel, G/alf and G/bam trials

The procedures used for both transit and total retention time studies were identical to those described for the all bamboo trial except only wheat kernels were used as the particulate marker. Samples were collected, labeled, examined and stored in the same manner as previously described.

Statistical analyses were performed using the Statistical Analysis Systems (SAS) analysis of variance by general linear models and least square means procedures.

Results

No significant differences were detected at the 0.05 probability level in the transit times (time to first appearance) of the marker over the four dietary treatments. Trends were detected, however, for the all bamboo diet to pass through faster ($P < 0.10$) than the all gruel diet and the gruel/alfalfa meal diet. Differences were found ($P < 0.05$) between the total mean retention times across all dietary treatments. The all bamboo diet differed from the G/bam diet ($P < 0.01$). Although not significantly different from either the all bamboo or all gruel/bamboo diets ($P > 0.01$), the gruel and gruel/alfalfa diets had intermediate retention times. No significant differences were found in the percent of marker recovered across dietary treatments.

Table 14. Mean transit times (TT), total retention times (TMRT) and percentages of wheat kernels recovered from red pandas fed 4 different diets.

Diet	TT (min)	SEM	TMRT (min)	SEM	Rec (%)	SEM
Bamboo	300 ^b	93	340 ^a	109	96.7 ^a	1.9
Gruel	566 ^a	93	574 ^{ab}	109	95.8 ^a	1.9
G/alf.	311 ^a	93	578 ^{ab}	109	93.7 ^a	1.9
G/bam.	365 ^{ab}	83	800 ^b	97	92.8 ^a	1.7

^{ab} Means within a column with different superscripts are different if a $P < 0.10$ is used.

Similar trends were found when comparing the excretion rates of the wheat kernel particulate markers to the bamboo leaves soaked with ytterbium (Table 15). In most instances, the majority of both the kernel and ytterbium marker in the all bamboo trial was excreted in the first defecation. With the exception of animal #1, during the start of the digestion trial, the greatest concentration of ytterbium marker was found at the time of greatest wheat kernel concentration (Table 14). In a few instances (Table 15), the percent of Yb recovered was rather low, however all the animals were observed for the same amount of time. The low recovery rates may have been a result of the animals failure to defecate within the collection period. The results suggest that wheat kernels were, in fact, a suitable marker by comparison to another particulate marker.

Table 15. Comparison of the excretion rates of wheat kernels and ytterbium marked bamboo leaves fed to red pandas on an all-bamboo leaf diet.

Animal #1								
Start of digestion trial								
Def. after feeding, hr ¹	3.0	5.5	6.5	8.5	9.5	10.5	11.5	12.5
Recovery of wheat kernels,%	92.3	7.7	0.0	0.0	0.0	0.0	0.0	0.0
Recovery of ytterbium,%	31.0	58.0	2.7	1.6	1.3	0.1	1.1	0.1
Animal #1								
End of digestion trial								
Def. after feeding, hr	3.3	4.5	5.5	6.5	7.5	8.5	9.5	
Recovery of wheat kernels,%	100.0	0.0	0.0	0.0	0.0	0.0	0.0	
Recovery of ytterbium,%	76.0	8.9	3.8	1.5	1.9	1.3	2.2	
Animal #3								
Start of digestion trial								
Def. after feeding, hr	2.0	5.5	14.0					
Recovery of wheat kernels,%	0.0	100.0	0.0					
Recovery of ytterbium,%	4.6	43.0	18.0					
Animal #3								
End of digestion trial								
Def. after feeding,hr	3.0	5.0						
Recovery of wheat kernels,%	0.0	100.0						
Recovery of ytterbium,%	6.1	64.2						
Animal #4								
Start of digestion trial								
Def. after feeding,hr	4.5	5.5	6.5	7.5	8.5	9.5	10.5	
Recovery of wheat kernels,%	100.0	0.0	0.0	0.0	0.0	0.0	0.0	
Recovery of ytterbium,%	61.3	2.9	2.7	3.0	3.1	2.5	2.5	
Animal #4								
End of digestion trial								
Def. after feeding,hr	4.0	5.0	6.0	7.0	8.0	9.0		
Recovery of wheat kernels,%	100.0	0.0	0.0	0.0	0.0	0.0		
Recovery of ytterbium,%	83.9	2.5	1.8	3.1	1.9	1.8		
Animal #5								
Start of digestion trial								
Def. after feeding,hr	5.0	7.0	8.0	9.0	10.0			
Recovery of wheat kernels,%	100.0	0.0	0.0	0.0	0.0			
Recovery of ytterbium,%	81.0	2.8	1.6	1.0	1.3			
Animal #5								
End of digestion trial								
Def. after feeding,hr	4.5	6.5						
Recovery of wheat kernels,%	99.8	0.2						
Recovery of ytterbium,%	85.1	3.0						

¹Cages were checked hourly, up to 14 hr, for defecations.
Times shown are those at which defecations occurred.

Discussion

Red pandas on the all-bamboo trial had a transit time only 39 minutes faster than the total mean retention time. The relative concentrations of ytterbium were much higher at the time of the highest kernel marker concentration, supporting the conclusion that most kernels pass through the red panda digestive tract quickly, and in a single mass. This high rate of passage is indicative of a diet low in digestibility, where there is limited digestion of soluble plant components, making it necessary for a high turnover of ingesta.

Results from the study of gruel diet show a transit time only slightly faster than the total mean retention time, similar to the results of the all-bamboo trial. However, the time required for digesta passage when gruel was fed was almost twice that when bamboo was fed, indicating a more digestible substrate (GE apparent digestibility of 87% vs 30%).

The gruel/alfalfa meal and gruel/bamboo meal diets resulted in digesta transit times intermediate to those found for the bamboo and gruel diets. The TMRT tended to be longer for these diets but not significantly at the $P > 0.10$ level, and the gross energy digestibilities were significantly lower than for the gruel diet ($P < 0.001$) and significantly greater than for the bamboo diet ($P < 0.001$). Decreased digestibilities for the G/alfalfa and G/bamboo meal diets compared to the gruel diets are probably due to

the red panda's limited ability to efficiently digest fiber, which also explains the lower digestibility of the all-bamboo diet. The results of this particulate marker passage study are similar to those of Dierenfeld (1981) who found that in the giant panda mean retention times for the liquid and particulate markers 5-14 and 6-11 hr, respectively. Regardless of diet, the red pandas used in this study, appeared to be more rapid in their particulate transit and total retentions times when compared to dogs used by Banta et al., 1979 (8-16 hr; 38 hr), and raccoons studied by Clemens et al., 1979 (12 hr; 24 hr). By contrast the time to first appearance was faster in mink and ferrets (3 hr) but these animals had slower total mean retention times ranging from 15 to 20 hr (Bleavins and Aulerich, 1982). Given the low apparent gross energy digestibility of bamboo, 30.3%, it appears that the red panda has adapted to such a diet by digesting a low percentage of the total mass and passing the undigested portion totally through the gut in less than 6 hr.

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III. MICROBIAL ECOLOGY

Introduction

The red panda is an animal adapted to a diet consisting largely of plant materials. Although research is underway to establish in detail the dietary profile of the red panda in the wild, previous reports have characterized the diet as being primarily composed of bamboo and a limited quantity of fruits (Bleijsberg, 1982). However, in captivity these animals are not fed solely bamboo. A survey conducted in 1987 of 22 North American zoos holding red pandas revealed that, while 17 of the 20 responding zoos fed some amount of bamboo, an equal, if not larger portion of the diet consisted of a gruel (Warnell, 1987). The proportion of ingredients (see Table 16) in this gruel may vary among institutions, but the basic types of ingredients remain similar. How efficiently the red panda digests bamboo, how these apparently 'unnatural' ingredients affect the digestibility of the red panda's diet, and the type and quantity of microbial flora are unknown.

The effect of diet on rumen microbial populations has been well-documented, although relatively little information is available on the effects of diet on the intestinal microorganisms of nonruminant animals (Varel et al., 1982). A few studies have reported on the species and numbers of bacteria in the feces or lower intestine of normal pigs (Moore et al., 1987). Volatile fatty acid production and

Table 16. Composition of North American red panda gruel diets (n=10)¹.

Ingredients ²	%		Average
	Min	Max	
Water	29.8	50.8	45.8
Cereals	8.7	53.0	25.3
Milk products	0.7	41.6	14.4
Fruit/vegetables	9.0	49.0	26.2
Fiber/complete ³	0.2	19.8	5.7
Vit./min.	0.1	9.6	2.4
Eggs	2.2	5.2	3.7
Other	1.2	25.0	8.6

¹Results of an unpublished red panda diet survey by Warnell (1987).

²Ingredients expressed as a % (by weight) of diet on an as fed basis.

³Feeds intended to be fed alone.

excretion have been quantified in a few nonruminant species, particularly the horse, rabbit and pig (Marty and Vernay, 1984). The red panda poses an interesting subject for study since, though taxonomically belonging to the order Carnivora (Roberts and Kessler, 1979), it subsists on an essentially herbivorous diet. This is remarkable since the red panda gut has no special adaptations for forage digestion, including no cecum (Flower, 1870). In fact, the red panda gut most closely resembles that of a true carnivore, the cat, with respect to the ratio of body length to gut length: cat 1:4.2 vs red panda 1:4.7 (Bleijsberg, 1982).

The influence of diet on the microbial population in the red panda gut has not been evaluated. The goal of this study was to characterize the bacteria present in the feces of red pandas, to determine if the populations shift when different diets are fed, and to relate any shifts to changes in digestibility. The numbers of both aerobic and anaerobic bacteria were measured as well as the presence or absence of cellulolytic and amylolytic bacteria. Volatile fatty acids (VFAs) are the end products of microbial fermentation in herbivores. The concentrations of VFAs in gut contents are often used to characterize the amount of fermentation, which is a function of substrate availability and retention time (Annison et al., 1970). Since it was not feasible to sample ingesta from various points along the panda digestive tract, VFA concentrations in the feces were chosen as indices of VFA production. One might expect that diets which contain

large concentrations of soluble carbohydrates would result in higher rates of microbial fermentation in the gut and large concentrations of VFAs in the feces. Esherichia coli and lactobacilli numbers in panda feces were counted and served as a check on the VFA levels. Lactobacilli grow favorably in an environment high in VFAs (low pH) while coliforms are inhibited.

Materials and Methods

Four red pandas were used in this study (3 males, 1 female), two < 2 yr and two > 2 yr. All of the animals were captive-born in zoos. The animals were fed four diets sequentially, with at least 7 days between sampling periods, and microbial analyses of fecal and feed samples were made for each of the four treatments. The treatments consisted of an all-bamboo diet (X), an all-gruel diet (G), a diet of gruel with bamboo ad lib (G/X) and a diet of gruel with alfalfa meal (G/Alf.) added in amounts providing the same neutral detergent fiber (NDF) levels as those consumed by each animal during the gruel and bamboo trial.

The animals were individually housed in outdoor metal corn-crib-type enclosures with concrete floors at the National Zoological Park (NZP) in Washington, DC. The enclosures ranged in size from 12 m to 18 m in diameter, and all were 4.6 m in height, not including the roof area where the animals had no access. The cages were furnished with logs for climbing as well as 7.6 cm x 43.6 cm x 3.7 m planks that were used as resting platforms. The flooring throughout the enclosures was made of concrete, and all the cages had an attached 92 x 92 cm nestbox. The animals were given a dietary adaptation period of 5 to 7 days before fecal samples were collected. After the adaptation period, on the day of sample collection, the animals were observed continuously, and freshly-voided feces (within 3 min of

defecation) were collected using sterile gloves and a sterile collection bag. Because timing was of critical importance, two animals were usually collected on one day and the remaining animals the day after.

Post-collection, the fecal samples were taken to the Nutrition Laboratory at NZP, weighed and a 1 g sample immediately diluted, using anaerobic techniques, with 9 ml of sterile dilution fluid (Appendix C, Table 29) under nitrogen gas passed through a copper column. It was determined through preliminary trials that it was necessary to dilute the fecal sample as soon as possible to ensure a representative microbial count. The dilution procedure was carried out to 10^{-3} at NZP, then continued to 10^{-7} using carbon dioxide passed through a copper column at the Ruminant Nutrition Laboratory at USDA in Beltsville, MD.

For preparation of the sample for VFA analysis at Beltsville, 1 ml of the sample which had been diluted to 10^{-1} was acidified with 0.09 ml 50% (vol/vol) H_2SO_4 and centrifuged at 2 C and $3,000 \times g$ for 5 min. Then 0.5 ml of the supernatant was diluted with 0.2 ml of an internal standard that contained 2 ethylbutyric acid (6 g/l). A 1 μl amount of the mixture was injected into a column (ID, 3.175 mm) packed with 10% SP-1200 and 1% H_3PO_4 on Chromosorb W/AW (80/100 mesh) and maintained isothermally at 122 C (Slyter, 1979).

MacConkey's agar plates were prepared and inoculated with 1 ml from the 10^{-3} to 10^{-7} dilutions (4 plates

at each dilution). After 24 hr, the plates were read and the average of the four plates at each dilution was used as the representative count for E. coli. The LBS (Rogosa et al., 1951) and 98-5 media (Bryant and Robinson, 1961) were prepared using the anaerobic roll tube technique of Hungate (1950). After 24 hr, the average viable colony count of 4 tubes each at 10^{-2} and 10^{-3} dilutions were used to determine the average number of lactobacilli present.

The most probable number test (Bryant and Burkey, 1953) was carried out using three aerobic and three anaerobic tubes of starch-cellulose broth (3 ml) at each dilution. The medium used was similar in ingredients to the RGCA medium of Bryant and Burkey (1953), except that agar, glucose and cellobiose were excluded, and 0.1% ball-milled cellulose filter paper (Bryant and Burkey, 1953), 0.05% starch, and 0.1% trypticase were included (Slyter et al., 1970). Inoculations of 0.1 ml per tube were made between 10^{-1} and 10^{-5} dilutions and assayed for hydrolysis of cellulose by visual examination after 7 days incubation at 39 C. Inoculations of 0.1 ml per tube were made between 10^{-3} through 10^{-8} dilutions for hydrolysis of starch. These assays were performed to determine the number of anaerobic cellulolytic and amylolytic bacteria as well as aerobic cellulolytic and amylolytic bacteria in the feces. Where an anaerobic roll tube viable bacterial count was made, the 98-5 medium of Bryant and Robinson (1961), a CO₂ gas phase and the anaerobic roll tube technique of Hungate (1950) was used.

Results

The results of these microbiological tests indicated that the red panda is likely to be a poor digester of cellulose. The most probable numbers test revealed that none of the inoculated starch/cellulose broth tubes showed any sign of cellulose hydrolysis, implying that the red panda's gut lacks the crucial microbes necessary for efficient fiber digestion. Amylolytic bacteria were present in the feces, but the relative numbers of aerobes and anaerobes varied (see Table 17). Differences ($P < 0.05$) were detected in the numbers of anaerobic amylolytic bacteria found in the feces of red pandas fed bamboo and those fed gruel/bamboo or all-gruel diets. Differences ($P < 0.05$) were also detected in the numbers of aerobic amylolytic bacteria found in the feces of red panda fed bamboo and those fed the other diets.

Table 17. Aerobic and anaerobic amylolytic bacteria ($10^7/g$) present in the feces of red pandas fed bamboo (X), gruel/bamboo (G/X), gruel (G) and gruel/alfalfa (G/Alf) diets.

Diet	<u>Aerobic</u>		<u>Anaerobic</u>	
	Mean	SEM	Mean	SEM
X	4.0	2.9	7.0	2.1
G/X	0.0024	0.0022	1.36	0.54
G	0.00023	0.0	3.2	1.1
G/Alf	0.032	0.0051	4.8	3.2

Significant differences ($P < 0.02$) in E. coli numbers were found between the means of the bamboo and gruel/alfalfa meal treatments, with the bamboo treatment producing the highest counts (Table 18). Although no significant differences were found between lactobacilli concentrations in the feces of pandas fed different diets, fewer of these bacteria were present in the feces of red pandas fed the bamboo diets (Table 19).

Table 18. E. coli/g present in the feces of red pandas fed four different diets.

	Bamboo	G/Bamboo	Gruel	G/Alfalfa meal
Mean	4.2×10^8	1.9×10^8	2.3×10^8	0.2×10^8
SEM	16.1×10^7	7.6×10^7	10.5×10^7	13.8×10^6

Table 19. Lactobacilli/g present in the feces of red pandas fed four different diets.

	Bamboo	G/Bamboo	Gruel	G/Alfalfa meal
Mean	1.0×10^3	4.5×10^4	2.3×10^7	1.6×10^8
SEM	1.0×10^3	14.5×10^3	21.9×10^6	147.9×10^6

Significantly ($P < 0.05$) lower acetate concentrations were found in feces of red pandas fed the G/Alf diet when compared to the other dietary treatments (Table 20). However, no significant differences were detected between the remaining treatments. In the all-bamboo trial, the levels of propionate tended ($P = 0.10$) to constitute a higher proportion of the total VFAs compared with the other treatments. Fecal isobutyrate levels resulting from the

gruel/alfalfa diet tended to be higher ($P = 0.11$) when compared to the other treatments. Butyrate levels in the feces of pandas fed gruel/bamboo and gruel/alfalfa diets differed ($P < 0.02$), and there was a marked trend for the gruel/bamboo and all-gruel diet to differ ($P = 0.12$). Isovalerate in the all bamboo treatment tended to differ ($P = 0.14$) across all diets, and valerate levels were significantly higher ($P < 0.001$) in the gruel/alfalfa meal diet. The total concentration of the VFAs varied dependent on the diet fed to the pandas (Table 21). Thus, the proportions (molar %) of the individual VFAs do not reflect differences in acid quantities being voided, but instead show the relationship of acids within a given dietary treatment. The molar percentages also reflect different types of fermentation, or relative absorption from or secretion into the intestines.

Table 20. Molar percentages of volatile fatty acids (VFAs) present in red panda feces.

-----Diets-----								
	Bamboo		G/Bamboo		Gruel		G/Alfalfa	
VFAs ¹	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acetate	64.9	3.6	78.0	4.8	78.8	4.0	31.3	18.1
Propionate	9.4	3.7	1.6	0.7	4.1	2.3	0.0	0.0
Isobutyrate	13.5	4.4	9.4	1.5	11.7	1.3	26.1	9.5
Butyrate	3.2	1.9	9.7	2.5	4.6	3.0	1.1	1.1
Isovalerate	6.1	4.5	1.2	0.2	0.9	0.5	0.0	0.0
Valerate	14.5	5.9	0.0	0.0	5.7	5.7	41.5	9.3

¹Individual animal values are presented in Appendix C, Table 28.

Table 21. Total concentration of volatile fatty acids (VFAs) present in red panda feces.

Diet	Volatile fatty acids (uM/ml)	
	range	total
Bamboo	21-55	30
Gruel/bam.	35-74	52
Gruel	32-93	52
Gruel/alf.	0.4-1.31	2

Discussion

The purpose of these experiments was to characterize the bacterial flora of the red panda gut. In order to minimize animal stress, digesta samples were obtained only as they were voided as feces, thus limiting the opportunity to gain information about the entire digestive system. Based on examination of fecal samples, the gut microbial population of the four red pandas on this study did change in response to dietary changes. As shown in Table 17, the larger numbers of anaerobic bacteria associated with consumption of bamboo, as compared to the other three diets, indicate that there was a relatively greater opportunity for anaerobic fermentation, possibly due to more indigestible residues and substrate. Lactobacilli grow favorably in an environment high in volatile fatty acids, while coliforms react in an opposite manner. An all-bamboo diet produced the lowest levels of lactobacilli, due to the low soluble substrate availability, while the coliform count remained the highest. In this way the two additional tests helped to verify that VFA production on an all-bamboo diet was low relative to the other diets, probably due to reduced fermentation as indicated by the high aerobic bacterial counts.

The results presented in Table 19 show that the gruel/bamboo diet produced the next highest levels of lactobacilli, then the all gruel diet and finally the gruel/alfalfa meal diet. The aerobic, anaerobic and

coliform bacterial counts in the respective diets followed in a manner similar to the levels of lactobacilli present. When comparing these bacterial numbers to the VFAs measured, the total VFAs followed a pattern which might have been predicted, given the previous data. The all-gruel and gruel/bamboo diet resulted in feces higher in total VFAs than the all-bamboo diet. The unexpected similarity in molar proportions between VFAs produced on the all-gruel and gruel/bamboo diets may be explained in part as an artifact of the time at which various dietary components were ingested and when the fecal samples were taken. The bamboo was not physically mixed with the gruel. Therefore, it is conceivable that the fecal sample taken may have been more representative of an all-gruel diet than a mixture as intended. The exceptionally low total VFAs in feces from red pandas fed gruel/alfalfa mixture may be explained if more VFAs were being absorbed from the intestine and not present in the fecal sample. It is not likely that a malfunction within the chromatograph caused a false reading as the samples were rerun on different days. Because the total VFAs were so low in the feces of red pandas fed the gruel/alfalfa meal diet, relative molar percentages provide little reliable data. The levels of individual VFAs across diets remained fairly consistent but with a higher proportion of acetic acid than any other VFA. In ruminants, a lower concentration of propionate would be expected on a high fiber diet, whereas the literature is conflicting on

the effect of fiber on the acetate-propionate ratio in the gastrointestinal tract of pigs (Varel et al., 1982).

Although the acetate:propionate ratios were not significantly different, there was a trend toward the opposite of what one might expect in a ruminant. The propionate levels were somewhat higher, although not significant ($P < 0.05$) on the all bamboo diet. Because no bacterial cellulolytic activity was detected by the most probable numbers test, the amylolytic bacteria may have been responsible for the production of the majority of the VFAs.

The red panda is an animal that has evolved to use a particular feedstuff in the wild, bamboo. However, based on the bacteria present in the feces, it appears that although the total numbers of anaerobic bacteria may increase on an all-forage diet, growth of cellulolytic bacteria may be hindered by the environment of the gut and by a rapid transit time and short total retention time (see previous chapter). One consequence of ineffective cellulose degradation (as no bacteria with cellulolytic activity were detected) is that the more soluble plant components must be utilized.

Cellulolytic bacteria have been detected in human feces in only a small portion of individuals (Betian et al., 1977). Wedekind et al. (1988), in a recently published paper, attributed the low apparent concentrations of cellulose-degrading bacteria in human feces to the type of cellulose used in the assay procedure. A comparison of

Whatman no. 1 filter paper, PMC (used in the red panda assay), a relatively crystalline type of cellulose, to AHP-WS, a hydrated cellulose in spinach and in wheat straw pretreated with alkaline hydrogen peroxide, revealed that human cellulolytic bacteria were able to repeatably degrade the AHP-WS cellulose, while little or no activity was found when human feces were incubated with PMC. Perhaps the isolates take longer to breakdown the PMC cellulose making the cellulolytic bacteria more susceptible to competition by non-cellulolytic bacteria. Wedekind et al. (1988) concluded that AHP-WS cellulose is a readily fermentable substrate that may serve as a more appropriate medium for recovering cellulolytic bacteria that are stressed during collection. Although no cellulolytic bacteria were detected in red panda feces by the tests used, the question remains, in light of this new evidence, whether cellulolytic bacteria are not present or whether those that are may not be able to degrade the type of cellulose (PMC) provided. The neutral and acid detergent fiber digestibility estimates reported in the previous chapter suggest that some cell wall degradation was occurring, and this digestion may have been performed by cellulolytic organisms that were simply not detected by the methods used.

The rate of digesta passage through the red panda's GI tract is rapid, with the majority of marker excretion occurring in less than 6 hr on an all-bamboo diet. This rapid passage rate may limit the establishment of a

substantial cellulolytic bacterial population. The red panda may have adapted to a diet in the wild that is high in plant components. However, the results of this study indicate that the use of bamboo by the red panda is inefficient, particularly when compared to the use of a gruel diet (GE digestibilities of 30.3 vs 87%, respectively). To compensate for a diet low in digestible energy (DE), the red panda appears to have evolved to pass the less digestible plant components through its digestive tract faster than if fed a diet higher in DE such as gruel. As a consequence, the red panda realizes more DE from a gruel diet. However, the long term effects of such a diet on oral health, digestive tract function, animal health and hygiene still remain in question.

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Appendix A

Table 22. Nutrient composition of red panda gruel.

Nutrient	Concentration (%) (dry matter basis)
Crude protein	16.1
Fat	13.6
Crude Fiber	0.0
Calcium	0.7
Phosphorous	0.5
Vit. A	3.19 ¹
Vit. D ₂	0.11 ¹
Vit. E	8.47 ²

¹IU/g
²Mg/kg

Appendix B

Fig. 6. Ave. Time to First Appearance
of Wheat Kernel Marker

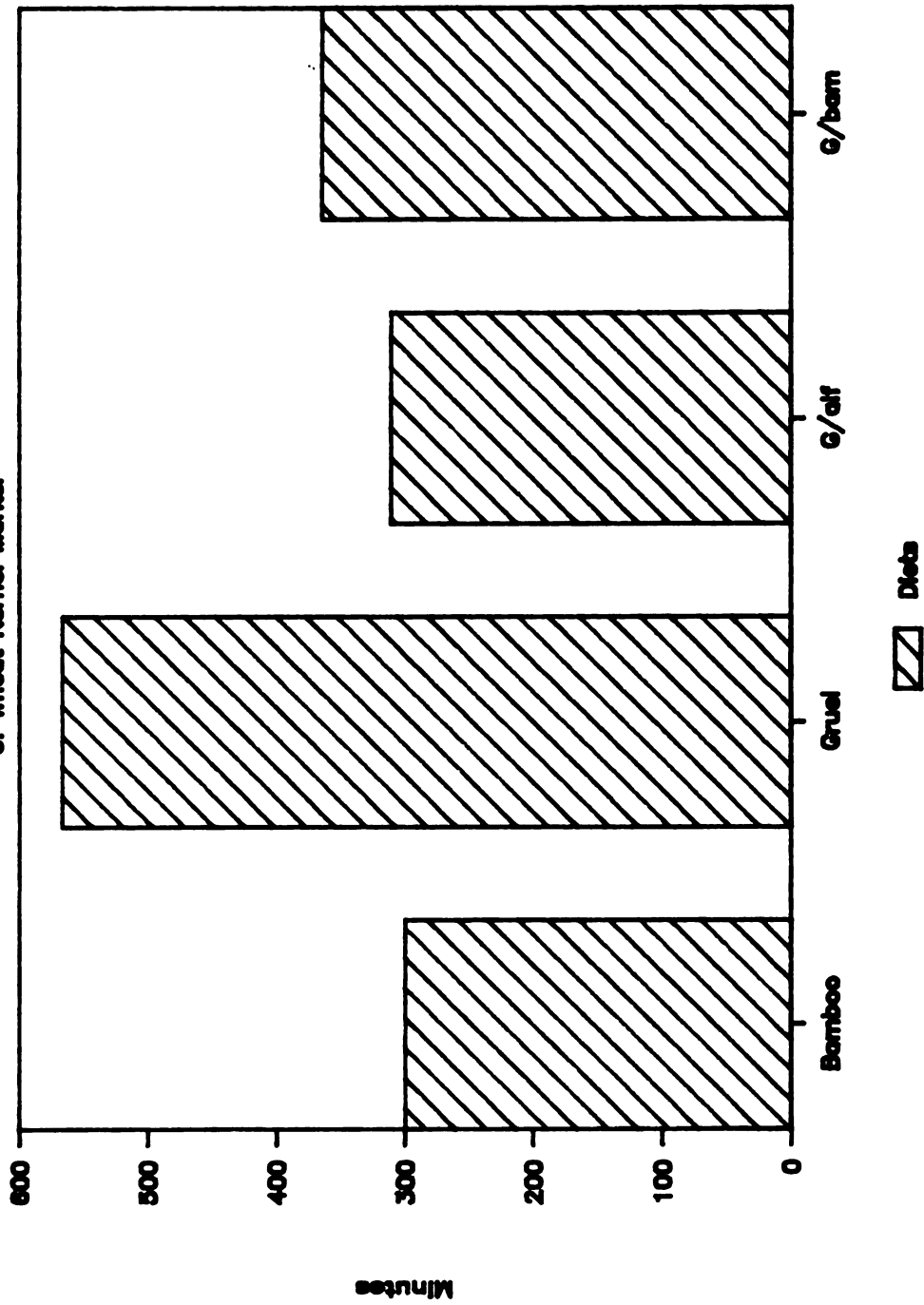
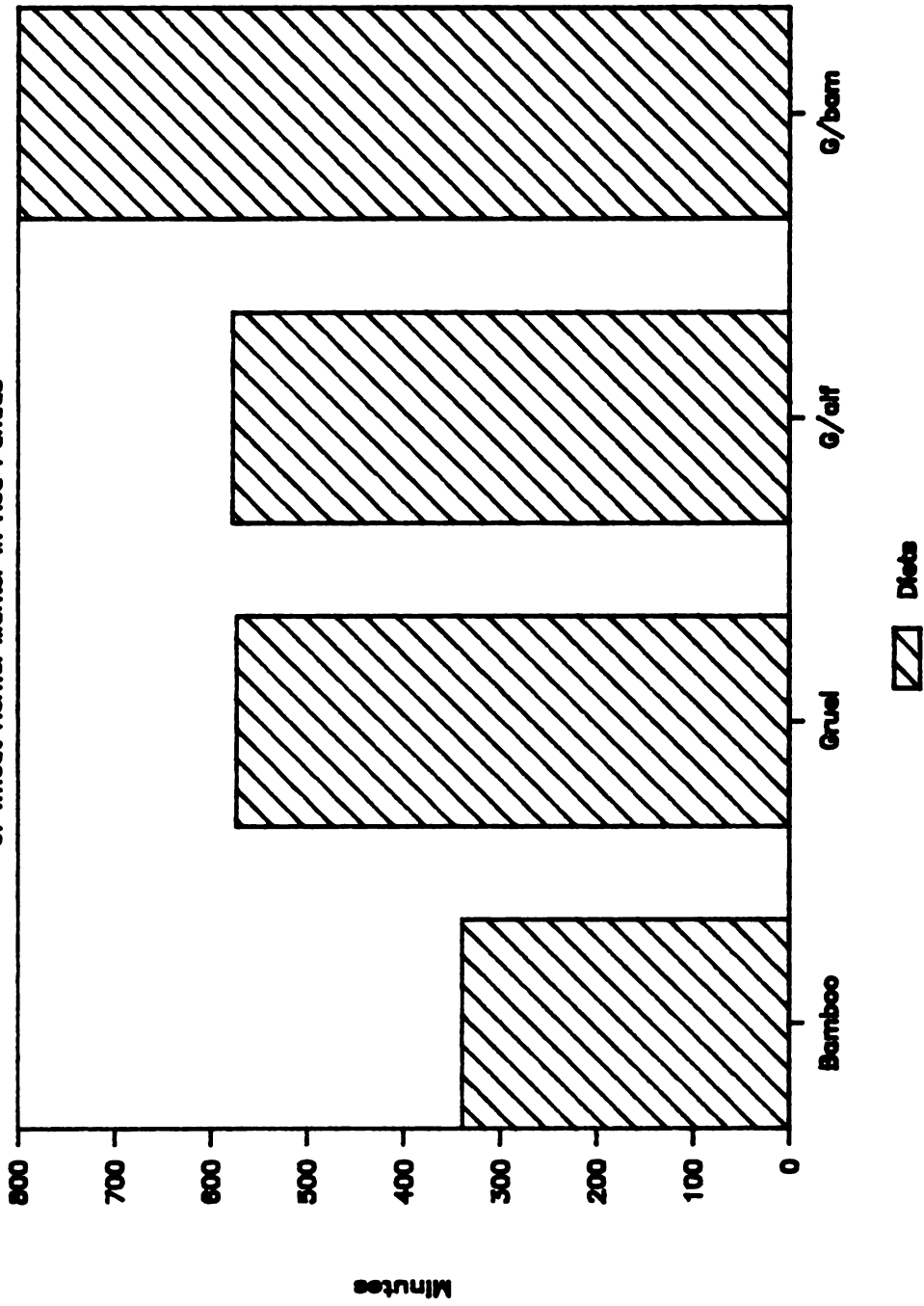


Fig. 7. Total Mean Retention Times
of Wheat Kernel Marker in Red Pandas



Appendix C

Table 23. Summary of coliform and lactobacilli numbers/g present in the feeds provided to red pandas.

Feedstuff	<u>E. coli</u>	Lactobacilli
Bamboo leaves	3.5×10^4	0.0
Gruel	4.0×10^4	8×10^1
Alfalfa meal	0.0	0.0

Table 24. Summary of individual E.coli counts/g in the feces of red pandas fed four different diets.

Animal #	Diets			
	X	X/G	G	G/Alf
1	27×10^7	2×10^5	3×10^7	13.5×10^6
3	86×10^7	34×10^7	51×10^7	63.5×10^6
4	11×10^7	29×10^7	12×10^7	6.0×10^6
5	44×10^7	16×10^7	26×10^7	7.0×10^6

Table 25. Summary of individual lactobacilli counts/g in the feces of red pandas fed four different diets.

Animal #	Diets			
	X	X/G	G	G/Alf
1	0.0	5.25×10^4	11.5×10^4	605×10^6
3	0.0	1.75×10^4	19.0×10^5	28×10^6
4	4×10^3	27.75×10^3	89.0×10^6	11.7×10^3
5	0.0	8.25×10^4	20.0×10^5	14×10^6

Table 26. Volatile fatty acids present in the feces of all red pandas over all dietary treatments expressed as molar %.

Animal #	Diet	C-2	C-3	IC-4	C-4	IC-5	C-5
1	X	74.36	5.60	8.52	6.95	4.57	0.0
3	X	64.25	1.14	25.04	0.0	0.0	9.58
4	X	64.22	17.77	15.55	0.0	0.65	1.72
5	X	56.65	13.13	4.89	6.02	19.31	0.0
1	G/X	73.67	1.36	10.84	12.50	1.63	0.0
3	G/X	91.35	0.0	5.14	2.87	0.63	0.0
4	G/X	78.00	1.58	9.82	9.05	1.54	0.0
5	G/X	69.16	3.43	11.99	14.33	1.09	0.0
1	G	85.36	0.0	12.37	0.0	2.27	0.0
3	G	82.70	3.11	3.92	8.27	2.01	0.0
4	G	67.51	9.80	8.91	12.95	0.83	0.0
5	G	83.26	0.91	10.65	4.96	0.47	0.0
1	G/Alf	60.39	0.0	7.07	4.50	0.0	28.05
3	G/Alf	64.83	0.0	12.41	0.0	0.0	22.76
4	G/Alf	0.0	0.0	42.25	0.0	0.0	57.75
5	G/Alf	0.0	0.0	42.73	0.0	0.0	57.27

Table 27. Composition of sterile dilution fluid

Ingredient	Amount to make 100 ml
Mineral 1	7.5 ml
K ₂ HPO ₄ 6 g/l	
Mineral 2	7.5 ml
KH ₂ PO ₄ 6 g/l	
NaCl 12 g/l	
MgSO ₄ 1.2 g/l	
CaCl ₂ 1.2 g/l	
Resazurin (0.01 %)	0.1 ml
(0.01 %)	
Na ₂ CO ₃	5.1 ml
(8.0 %)	
Cysteine hydrochloride	3.3 ml
(2.5 %)	

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