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APPARENT DIGESTIBILITY OF DIET DOMINANTLY COMPRISED OF *PHYLLOSTACHYS VIVAX* BAMBOO SHOOTS FOR CAPTIVE GIANT PANDAS

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ABSTRACT

This study, using the method of direct collection feces, investigated captive giant pandas daily intakes and apparent digestibility coefficients of diet dominantly comprised of *Phyllostachys vivax* bamboo shoots. The results showed that the captive giant pandas daily ingested ~2818 g (dry mass) of bamboo shoots (corresponding to ~42 kg of fresh bamboo shoots with shells). Based on this daily intake of bamboo shoots, we further analyzed the daily intakes of dietary components, including energy, crude protein, lipids, hemicelluloses, neutral detergent fiber (NDF), and acid detergent fiber (ADF). The results demonstrate that: i) the trials bamboo shoot diet provides giant pandas with sufficient and readily digestible, energy and crude protein; ii) the lipid (in low content) was efficiently utilized by giant pandas; and iii) the digestibility of fiber was very low in giant pandas.

Keywords: apparent digestibility; bamboo shoot; daily intake; giant panda; nutrient.

INTRODUCTION

The giant panda (*Ailuropoda melanoleuca*) is one of the relict species of high ornamental and scientific values. They have subsisted in the wild for 8~9 million years. Today, wild giant pandas only inhabit in southwestern China and are in danger of extinction. Numerous conservation efforts, throughout the world, have the goal of protecting giant pandas. Not only are a suitable habitat and ecology required to efficiently conserve giant pandas, but an intimate knowledge of their feeding habits, diet selectivity, and utilization is also needed.

Although giant pandas belong to the order *Carnivora*, they are highly specialized bamboo feeders, and bamboo makes up 99% of a wild giant panda's diet (Schaller *et al.* 1985). Chinese researchers reported wild giant pandas in Wolong Mountains daily consumed 12.8 kg of fresh bamboo (leaf and culm), or 38 kg of *Fargesiarobusta Yi* bamboo shoots; giant pandas in Tsinling Mountains daily consumed 10-15 kg of *Bashaniafargesii* bamboo or 45.2-57 kg of bamboo shoots; giant pandas in Liangshan Mountains daily consumed 21.3 kg of bamboo in the summer and 17.37 kg of *Qiongzhueamacrophylla* bamboo in the autumn; In the case of captive giant pandas, it was reported that they daily takes about 15 kg of bamboo (Hu 2001). In general, farm cultivated bamboo, rather than wild type bamboo, is fed to captive giant pandas. In addition, some dietary supplements are supplied based on the digestion and utilization of the bamboo.. The bamboo breeds typically fed to captive giant pandas are selected based on the preference of wild giant pandas. Therefore, investigation has mainly focused on the breeds of bamboo that are distributed near or in the habitats of wild giant pandas (Hu *et al.* 1994a; Hu *et al.* 1994b; Liu *et al.* 2002a; Liu *et al.* 2002b; Wang and Zhang 2002; Yang *et al.* 2005a; Yang *et al.* 2005b). There were only a few studies on specific breeds of bamboo growing outside of the wild giant pandas habitats (Dierenfeld *et al.* 1982; Sims *et al.* 2007);

It was recommended to develop sufficient bamboo supplies to support the growing population of captive giant panda. Selecting appropriate bamboo breeds and producing these in high quality are critical to the long-term viability of captive giant pandas (Edwards *et al.* 2006). In order to screen high quality bamboo shoots for giant pandas, we have previously assessed varieties of bamboo shoots. *Phyllostachys vivax* bamboo shoot was one of preferred breeds for the captive giant pandas in Zhejiang Province (unpublished data). The present study was designed to evaluate the daily intake and apparent digestibility coefficient of diet dominantly comprised of *Phyllostachys vivax* bamboo shoots for the captive giant pandas.

MATERIALS AND METHODS

Trial animals: This study was approved by College of Animal Sciences, Zhejiang Agriculture & Forestry University. The procedures followed the guidelines of the National Institutes of Health for the experimental use of animals. Four captive giant pandas were in *ex situ* conservation at bamboo exposition park of Anji County, Zhejiang Province (located at 30°38' N,119°53' E; the south of the lower reaches of the Yangze River, P.R. China). They were Shulin (male sub-adult, 4 years old, 102.4 Kg body weight (BW)), Kelin (female sub-adult, 4 years old, 89 Kg BW), Jiaojiao (male adult, 9 years old, 93.6 Kg BW), and Bingbing (female senior, 26 years old, 98 Kg BW). All of them were housed individually in residential farms.

Trial diet: During the process of assessment, each giant panda was daily fed with *Phyllostachys vivax* bamboo shoots supplemented with 800 g of concentrate (steamed corn bread; formula: 39% maize meal, 22% wheat bran, 12% bean meal, 15% rice meal, 12% wheat flour, and some multivitamin tablets.). The corn bread was fed twice a day (*i.e.* 8:30 and 18:00).

Preparation of bamboo shoots: The experiments were carried out in May, when *Phyllostachys vivax* bamboo shoots were abundantly available. The bamboo shoots were collected from the Bamboo Exposition Park and nearby hills, and the breed of bamboo shoots was identified by a bamboo consultant. The bamboo shoots were daily collected in the previous afternoon, and the shoots were sub-grouped into 4 pieces (corresponding to the 4 giant pandas) and weighted after cleaning up mud, then washed and stored in the underground shed with relatively constant temperature of 15°C. All collected bamboo shoots were just fed in the next day.

Procedures of direct collection feces: The trial duration was 13 days including 6 days of adaptation period, and 7 days for assessment/experimental period.

During the entire experiments, the giant pandas were fed with *Phyllostachys vivax* bamboo shoots supplemented with steamed corn bread. They were fed with bamboo shoots 5 times per day at 8 am, 10 am, 2 pm, 5 pm and 8 pm. Before feeding bamboo shoots every time, the remainders and shells of previous bamboo shoots were completely collected and weighted immediately; at the same time, the feces was collected and weighted promptly.

The daily intakes of bamboo shoots and corn bread, and daily excretion of faces were recorded for each giant panda. The average daily feed intake and excretion were calculated for each giant panda.

Sampling procedures: During the 7-day formal experiments, about 3-4 Kg of bamboo shoot flesh and a corn bread were respectively sampled daily. The samples were then immediately sliced and dried at 105 until constant weight to measure the dry matter (DM) content in each sample. Next, seven bamboo shoot samples and 7 concentrate samples were individually grounded, and equally pooled to prepare mixed bamboo shoot and concentrate samples, respectively

The fecal sample of each giant panda was collected daily and dried at 105°C until constant weight, and the DM content of each sample was calculated. Prior to laboratory analysis, seven fecal samples of each animal were individually powdered, and four groups of fecal samples were respectively mixed for each animal according to the ratio of daily excretive DM.

Analytical methods: Diet and fecal samples were analyzed in triplicate for following nutrient composition.

Nitrogen was determined by the Kjeldahl method using an Auto Kjeldahl System (FOSS, Denmark) and a coefficient of 6.25 was used for conversion into crude protein; *i.e.* crude protein (% DM) = 6.25×Nitrogen (% DM). Caloric content (energy) was determined with an Oxygen Bomb Calorimeter. Lipid was determined following petroleum ether extraction method (Bligh and Dyer, 1959) through 10454 Soxtec System.

Detergent fiber analysis was performed following Van Soest (1994). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined sequentially for each sample using an Auto Fiber Analyser (FOSS, Denmark), while hemicelluloses were calculated by weight difference (NDF–ADF).

The apparent digestibility coefficients of DM, energy, crude protein, lipid, hemicelluloses, NDF, and ADF were calculated as follows:

Apparent digestibility % = 100 *[1-(F/D)]

Where D = nutrient content of diet; F = nutrient content of feces.

Statistical analysis: In the present study, we regarded the formal 7-day collection as one trial unit, and calculated average daily intakes and apparent digestibility for each panda; then estimated the averages of daily intakes and apparent digestibility for all 4 pandas, which were expressed as “mean ± standard error (SE)”.

RESULTS AND DISCUSSION

Nutrient composition of the trial diet: The nutrient composition of experimental diets has been presented in Table 1.

Daily intakes of food for the giant pandas: Using bamboo shoots as predominant food, the 4 giant pandas ingested 2525-3245 g of dietary DM per day (Table 2). Moreover, for all 4 animals, the mean daily intakes of other various components, (energy, crude protein, lipid, NDF, ADF, and hemicelluloses) were 11688±640 kcal,

730±43 g，65±4 g，703±45 g，327±21 g, and 376±24 g, respectively (Table 2).

Considering the effect of animal body sizes on daily ingestion, the data in Table 2 were converted on body weight basis (% BW) (Table 3). Table 3 shows the average daily intakes of DM, bamboo shoot DM, energy, crude protein, lipid, NDF, ADF, and hemicelluloses were 2.84-3.2% BW, 2.35-2.7% BW, 117.8-131.3 kcal/kg BW, 0.73-0.83% BW, 0.065-0.073% BW, 0.67-0.81% BW, 0.32-0.38% BW, and 0.37-0.43% BW, respectively (Table 3). The results show that the male sub-adult (Shulin) showed greatly higher intakes across all parameters than other three animals, suggesting the male sub-adult panda consumed larger amounts of bamboo shoots to meet nutrient requirements. In contrast, the intakes of other various components between the female sub-adult (Kelin), male adult (Jiaojiao), and female adult (Bingbing) were very similar, while the female sub-adult performed slightly lower intakes than other 2 adult pandas.

Previous studies had investigated the daily DM intakes for giant pandas fed with bamboo (leaf and culm) as the roughage. Under the wild condition, the daily DM intakes ranged 3800-9460 g, or 4.5-9.5% BW (Edwards *et al.* 2006), while for the captive adult giant pandas fed with bamboo and supplements, the daily intakes of DM were 1600-2400 g (Yang *et al.* 2005a); when bamboo comprised 54-75% of diet, the pandas consumed 1800-5200 g of bamboo DM equivalent to 1.6-4.2% BW (Dierenfeld*et al.* 1982). In our present study, daily DM intakes for the giant pandas (Table 2, 3) were close to above captive giant pandas' results, which meant bamboo shoots could provide captive giant pandas similar DM ingestion as bamboo leaf/culm. However, the daily dietary DM intakes of captive giant pandas were obviously lower than wild giant pandas; which may caused by various factors, i) captive giant pandas have significantly reduced activities, and, thus, have lower energy requirements; and ii) under captivity, supplemental concentrate could efficiently improve dietary digestible energy density, and reduce the DM ingestion to meet energy requirements (Van Soest 1994; Edwards *et al.* 2006).

Under captivity conditions, it was reported that the daily crude protein intakes was 283-575 g for the sub-adult giant pandas (Liu *et al.* 2002b), and 350-550 g for the adults (Zhang and Wei 2006). These values were significantly lower than current results, 648-849 g/d, or 0.73-0.83% BW). For the wild giant pandas in the Wolong Nature Reserve of China, Hu (2001) reported that the pandas daily ingested 246.1 g of crude protein from bamboo in spring, 645.6 g in summer/fall, and 401.9 g in winter; and ingested 606.7 g of crude protein in the seasons rich in bamboo shoots. It was estimated that the crude protein requirement of giant pandas is 100 g per day (Schaller *et al.* 1985), or 75.3-93.0 g per day for an individual of 80-kg body weight (Pan 1988). We previously found that the daily crude protein intakes ranged from 1074.14 to 1397.53g from the diet dominantly comprised of *Phyllostachys praecox* bamboo shoots (Table 5; Li *et al.* 2012).

Regarding the crude fiber intakes, the daily intakes of NDF for the giant pandas ranged from 619 to 825g (mean ± SE, 703 ± 45g), which is lower than previously reported results. Liu and coworkers (2002a) reported that the captive sub-adult giant pandas (2.5-4.7 years old) daily intake 456.5-1954.4 g of crude fiber from the diet of bamboo with concentrate supplement. Our previous results showed the daily NDF intakes were ranging 953.98-1253.23 g (mean ± SD, 1046.65 ± 149.52 g) from *Phyllostachys praecox* bamboo shoots with concentrate supplemental (Li *et al.* 2012). It is known that dietary fiber is one of facultative nutrients, proper crude fiber consumption may improve animal gastrointestinal health and capability of digesting dietary nutrients (Edwards 2006). It needs to be further investigated to determine whether ~700 g of daily fiber ingestion is the proper amount for giant pandas.

The apparent digestibility of DM: The apparent digestibility coefficients of dietary DM and other components for giant pandas were analyzed based on the 7-day trial.

The apparent digestibility of dietary DM for the 4 giant pandas was 53-61% (Table 4). Individually, we found that the apparent DM digestibility coefficients were very similar for the 3 young giant pandas, male sub-adult (Shulin), female sub-adult (Kelin), and male adult (Jiaojiao); while the digestibility for the old giant panda (Bingbing) was significantly lower than that for the other 3 young pandas.

The DM digestibility to wild giant pandas in the Wolong Nature Reserve of China ranged from 12.5 to 23.3% (Schaller *et al.* 1985). The digestibility of bamboo DM for adult giant pandas was 10.8~30.5％ (Hu *et al.* 1994a) when bamboo as the only food. The DM digestibility was 35.44-48.95% for sub-adult giant pandas (Yang *et al.* 2005b), or 51.44-54.91% for adult giant pandas (Wang and Zhang 2002) when fed with bamboo and concentrated supplement. The results suggest that the DM digestibility for giant pandas fed with mixed diet is higher than those giant pandas fed with only bamboo. The concentrated supplement might optimize the quality of diet and improve DM digestibility (Liu *et al.* 2002a; Yang *et al.* 2005a). In present study, the average apparent DM digestibility (Table 4) is higher than the previous reported results, implying the DM of trial bamboo shoots is more digestible.

The apparent digestibility of energy: The apparent digestibility of dietary energy was 59-67% (Table 4). The results showed that the apparent energy digestibility coefficient for the female sub-adult (Kelin) was highest,

while the old giant panda (Bingbing) was significantly lower than other 3 young pandas (Table 4).

Finley *et al*. (2011) reported the energy digestibility of bamboo for giant pandas was 26.07-38.9% (apparent energy digestibility). In present study, bamboo shoots contributed 84% of total consumed energy corresponding to 59-67% apparent energy digestibility (Table 2, Table 4). The apparent energy digestibility in present study was much higher than that of giant pandas fed with bamboo (Dierenfeld *et al*. 1982; Finley *et al*. 2011), suggesting that the present trial diet provided more efficient energy for giant pandas.

The apparent digestibility of crude protein: The apparent digestibility of crude protein for the 4 giant pandas was 84-88% (Table 4). The male sub-adult (Shulin) showed highest apparent digestibility, and the old female panda (Bingbing) showed much lower digestibility. These data also indicate that the digestibility coefficient of crude protein for male pandas was higher than female pandas.

In the case of feeding bamboo leaf/culm and concentrated supplement, it was reported that the apparent crude protein digestibility for sub-adult giant pandas ranged 30.2-50.91% (Yang *et al*. 2005b; Sims *et al*. 2007), and 50.33-61.99% for adult pandas (Yang *et al*. 2005a). These values are lower than the apparent crude protein digestibility from bamboo shoots in present and previous study (Table 5; Li *et al*. 2012). It was suggested that consuming a highly fibrous diet may reduce nutrient digestibility and absorption (Edwards *et al*. 2006). The major fibrous contributor in present diet was bamboo shoots, and the bamboo shoot NDF was 29% of DM (Table 1), which was much lower than that in other bamboo parts (71.2% of leaf DM, 81.0% of branch DM, and 85.8% of stem DM; Schaller *et al*. 1985); therefore, an improved crude protein digestibility might be attributed to the low fiber content in diet.

The apparent digestibility of lipid: The apparent lipid digestibility for the 4 giant pandas was 76-82% (Table 4). The apparent lipid digestibility for the male sub-adult (Shulin) was 82%,, which was obviously higher than the other 3 giant pandas. The other 3 giant pandas showed very similar apparent digestibility of lipid (Table 4).

In the present study, the apparent lipid digestibility (76-82%) was higher than other captive giant pandas fed with bamboo leaf/culm as main food: *i.e*. 3.97-19.17% (Yang *et al*. 2005b), or 53.76-63.62% for sub-adult giant pandas (Wang and Zhang 2002). It has been proposed that dietary crude fat concentration should be approximately 5% (dry matter basis) for the captive giant panda (Dierenfeld 1997). In present study, the lipid contents in bamboo shoots and supplemental corn bread were lower than 5% (Table 1).The low lipid diet may affect the lipid ingestion for giant pandas.

The apparent digestibility of hemicelluloses, NDF, and ADF: The average apparent digestibility coefficients of hemicelluloses, NDF, and ADF were 2.5±9.6%, 1.1±7.5%, and -0.5±5.3, respectively (Table 4), which means the utilization of bamboo shoot fibers for the giant pandas is poor. In particular, fibers seemed almost indigestible to the older female panda (Bingbing).

When feeding pandas with bamboo and supplements, it was reported that the apparent digestibility coefficients of hemicelluloses, NDF, and cellulose for the sub-adult giant pandas were 28.90-47.60%, 21.17-34.88%, and 18.20-25.60%, respectively (Yang *et al.* 2005b); for the adult giant pandas were 43.39-48.42%, 28.20-32.80%, and 15.64-22.09%, respectively (Yang *et al.* 2005a). Current data were much lower than the results from the reported studies (Yang *et al.* 2005a; Yang *et al.* 2005b; Sims *et al.* 2007; Li *et al.* 2012). Furthermore, the present study also showed the order of digestibility coefficients of hemicelluloses, NDF, and ADF for the male adult (Jiaojiao): hemicelluloses < NDF < ADF (Table 4), which was in agreement with the reported order (5.4% hemicelluloses < 10% NDF < 13% ADF) (Sims *et al*. 2007).

Table 1. Contents of DM, energy and other components in bamboo shoots and corn bread (%)

Nutrient components	DM	energy (kcal/g)	crude protein	lipid	NDF	ADF	Hemicellulose
bamboo shoots	9.2	4.1	27.9	2.4	28.7	13.5	15.2
corn bread	54.1	4.3	14.8	2.0	4.4	1.0	3.4
bamboo shoot contribution to totally intake	84.5±0.8	83.8±0.8	91.2±0.5	86.8±0.7	97.3±0.2	98.7±0.1	96.0±0.2

Note: The content of DM in bamboo shoots or bread was the ratio of DM to fresh weight, while the contents of other nutrients were expressed on % DM basis. In the last line, the proportion of bamboo shoot component to its total intake was expressed as mean of 4 individuals ± standard error (SE)

As a member of *Carnivora*, giant panda has evolved to be herbivorous. During evolution, herbivorous animals have evolved various adaptation strategies, one adaptation is large volumes consuming. However, higher

intakes of herbivorous animals tend to promote faster digesta passage rates, the digesta passage time for captive giant pandas ranged 4-7 hours (Dierenfeld *et al.* 1982; Finley *et al.* 2011); while too rapid digesta passage in pandas is not conducive to microbial fermentation of digesta, and cellulose digestibility is not significantly above zero (Van Soest 1994). Therefore, the low fiber digestibility in present study might caused by much faster digesta transmit, however, it is regret there were no transmit records in present study.

In conclusion, present trial bamboo shoot diet provided the giant pandas with sufficient and readily digestible, energy and crude protein. The lipid (low in content) was efficiently utilized and the digestibility of fibers was very low in giant pandas. The information should be useful for dietary and nutritional management for giant pandas with high-quality bamboo.

Table 2. Daily intakes of DM, energy and other components for the giant pandas fed with mixed diet (unit: g·d^{-1})

Giant panda	dietary DM	bamboo shoot DM	energy (kcal·d^{-1})	crude protein	lipid	NDF	ADF	hemicellulose
Shulin	3245	2812	13442	849	75	825	384	441
Kelin	2525	2093	10483	648	58	619	287	332
Jiaojiao	2667	2235	11067	687	61	660	306	353
Bingbing	2836	2403	11761	735	65	708	329	379
Means	2818.±156	2386±156	11688±640	730±43	65±4	703±45	327±21	376±24

Table 3.Daily intakes of DM, energy and other components in Table 2 expressed on body weight basis (BW) (unit: %BW).

Giant panda	dietary DM	bamboo shoot DM	energy (kcal/kg BW)	crude protein	lipid	NDF	ADF	hemicellulose
Shulin	3.19	2.75	131.3	0.83	0.07	0.81	0.38	0.43
Kelin	2.84	2.35	117.8	0.73	0.07	0.67	0.32	0.37
Jiaojiao	2.85	2.39	118.2	0.74	0.07	0.71	0.33	0.38
Bingbing	2.89	2.45	120.0	0.75	0.07	0.72	0.34	0.39
Means	2.94±0.08	2.49±0.09	121.83±3.2	0.76±0.02	0.07±0.00	0.73±0.03	0.34±0.01	0.39±0.01

Table 4. Apparent digestibility for the giant pandas fed with mixed diet (unit: %)

Giant panda	DM	energy	crude protein	lipid	hemicellulose	NDF	ADF
Shulin	60.7	65.9	87.9	82.2	12.2	8.8	4.9
Kelin	61.0	66.6	86.6	76.7	17.9	11.1	3.3
Jiaojiao	60.9	64.9	86.4	76.1	5.3	5.7	6.2
Bingbing	53.0	59.3	84.0	76.5	-25.2	-21.1	-16.4
Means	58.9±2.0	64.2±1.7	86.2±0.8	77.9±1.5	2.5±9.6	1.1±7.5	-0.5±5.3

Table5. Daily intakes and nutrient digestibility for giant pandas with *Phyllostachys praecox* bamboo shoots as predominant food (unit: g·d^{-1}; %)(Li *et al*., 2012).

Giant panda	CP intake	CP digestibility	Lipid digestibility	NDF intake	NDF digestibility	ADF digestibility	HC digestibility
Shulin	1398±49	90.0±0.6	83.3±0.9	1253±45	16.2±4.9	8.1±0.5	24.3±4.4
Kelin	1074±102	90.7±3.0	85.1±4.8	954±94	33.4±3.9	20.6±4.7	42.4±3.3
Jiaojiao	1076±157	88.5±0.7	68.6±1.9	956±145	17.3±4.7	7.0±1.6	26.8±4.2
Bingbing	1149±33	88.5±0.2	67.9±0.7	1024±30	24.4±1.6	11.6±1.9	33.6±1.4
Means	1174±162	89.4±1.7	76.2±8.7	1047±150	21.9±7.4	11.8±5.7	30.8±7.5

Note: values expressed as Mean ± standard deviation (SD); the means in the final line represented grand means of all data collected. CP represented crude protein, HC represented hemicellulose.

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