Effects of Altitude on Nutritional Components and Amino Acid Content of Staple Bamboo for Giant Pandas in the Min Mountains (Postprint)

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Abstract

Fargesia denudata is the staple food bamboo for giant pandas in the Minshan Mountains, and its edibility is of great significance in evaluating the nutritional quality of giant panda diets. To reveal the effects of temperature variation along altitudinal gradients on the nutritional components and their contents in giant pandas' staple food bamboo, we investigated the nutritional components and amino acid contents in shoots, culms, and leaves of Fargesia denudata naturally growing at different altitudes (2600, 2850, and 3100 m) in the core area of the Minshan Mountains—Wanglang National Nature Reserve, Sichuan. The results showed: (1) At the same altitude, crude protein and crude fat contents in Fargesia denudata followed the pattern leaf > shoot > culm, with significant differences in nutritional component contents among shoots, culms, and leaves ($P < 0.05$); (2) Total crude protein and amino acid contents increased significantly with increasing altitude ($P < 0.05$), whereas the effects on crude fat and crude fiber were not significant ($P > 0.05$); (3) Except for threonine, glycine, and cysteine in shoots, serine and methionine in culms, and cysteine in leaves, altitude had significant effects on the contents of all other amino acids ($P < 0.05$); (4) Altitude significantly affected the ratios of crude protein/crude fat and crude protein/crude fiber ($P < 0.05$), which were highest at 3100 m in both shoots and culms, while its effects on cellulose/crude fiber and crude fat/crude fiber ratios were not significant ($P > 0.05$). The nutritional component contents in leaves and shoots of Fargesia denudata were significantly higher than those in culms; high altitude favored the accumulation of crude protein and amino acids in various organs of Fargesia denudata, but was unfavorable for crude fiber accumulation, which may alter giant pandas' feeding selection, making them more inclined to feed on shoots and leaves at high altitudes.

Full Text

Effects of Altitude on the Nutrient and Amino Acid Contents of *Fargesia denudata*, Staple Food of the Giant Panda in Minshan, Sichuan, China

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Abstract

Nutritional components of plant species (e.g., contents of crude protein, crude fat, and amino acids), as well as fiber contents that influence animals' food digestibility, collectively determine animal food preferences. Animals prefer to feed on leaves or stems with higher contents of crude protein, crude fat, and amino acids and lower fiber content. Many studies have demonstrated that nutritional components or fiber contents are differentially impacted by various environmental factors. It is possible that differences in the nutrient or fiber content of vegetation under different conditions would potentially affect animal distribution through food choice. Dwarf bamboo species dominate the understory layer of subalpine forests. However, the distribution or growth of these bamboo species is predicted to be markedly affected by global warming. As the main food source for the panda, changes in temperature may influence their preference for bamboo by altering bamboo nutrition. Although the temperature difference along vertical altitudinal gradients is obvious, there have been few studies focusing on the effects of different temperatures on bamboo due to altitudinal gradients, which could potentially influence panda food preference and distribution.

We designed an experiment to examine the effects *in situ* of different altitudes on the contents of nutrients, such as crude protein and amino acids, and parameters related to animal food digestibility, such as fiber content, of dwarf bamboo (*Fargesia denudata*) in Wanglang Nature Reserve in northwest Sichuan Province, China. Samples (leaf, shoot, and culm) of *F. denudata* were selected from three altitudes (2600 m, 2850 m, and 3100 m) to study differences that may be attributable to differences in altitude. The results showed different change patterns among the different altitudes. Our main findings were as follows:

1. At the same altitude, nutrients of different *F. denudata* tissues (leaf, shoot, and culm) were significantly different from each other. For instance, the crude protein and crude fat contents of the leaf were the highest, whereas

those of the stem were the lowest. In the shoot, these parameters were in the middle range.

2. Most interestingly, the amount of crude protein and total amino acids were markedly influenced by the altitudinal gradient, being significantly higher at 3100 m than at 2600 m. For example, the contents of crude protein and total amino acids of bamboo shoots were highest at 3100 m. However, crude fat and crude fiber showed no significant differences among the different altitudes.
3. In addition to threonine, glycine, and cysteine in shoots, serine and methionine in culms, and cysteine in leaves, contents of the other amino acids were significantly affected by the altitudinal gradient. For example, the histidine content in shoots increased with increasing altitude.
4. Altitude had significant effects on the ratios of crude protein/crude fat and crude protein/crude fiber; however, no significant effects were observed on the ratios of cellulose/crude fiber and crude fat/crude fiber.

Our results demonstrate that at higher altitude, *F. denudata* accumulates higher contents of crude protein and total amino acids. Warmer climates would probably affect the distribution of *F. denudata* and characters related to panda food preference, such as crude protein content, which may, to a certain extent, determine panda movement or migration to higher altitudes to feed on relatively nutritious food.

Keywords: *Fargesia denudata*; altitude; nutrient; amino acid; giant panda

Introduction

Global warming and plant responses to environmental changes are among the most important scientific issues currently concerning ecologists [1]. Plants in high-altitude regions are extremely sensitive to climate warming. Altitude affects other ecological factors, particularly temperature [2], which greatly influences plant distribution, allocation, and physiological metabolism [3]. Numerous studies have shown that plants are undergoing obvious migrations from low to high latitudes [4] and from low to high altitudes [5-7] due to climate warming. In northern Japan, the dwarf bamboo *Sasa kurilensis* has also been observed migrating to higher altitudes [8]. The migration of dwarf bamboo to higher altitudes will inevitably lead to changes in habitat quality, thereby affecting the survival and reproduction of animals that feed on it.

Over the past few decades, simulations using CART (classification and regression tree) models [9] and MAXENT (maximum entropy) models [10] have indicated that under future climate change scenarios, the suitable habitat for *Ailuropoda melanoleuca* (giant panda) will decrease with increasing mean annual temperature, and the distribution range of pandas will shrink [11-13]. Temperature increase will be one of the main threatening factors for the future survival and reproduction of giant pandas [11-13].

Giant pandas select food based on nutritional components and digestibility [14-15]. They prefer bamboo species and parts with higher nutrient content and easier digestion. The higher the contents of crude protein, crude fat, and amino acids, and the lower the fiber content, the more pandas prefer to feed on them [15]. Lignin in bamboo, which is almost indigestible in crude fiber, can increase plant hardness and coarseness, affecting gastrointestinal digestion and absorption. Therefore, the higher the lignin content, the less pandas feed on it [15].

Plant crude fiber and nutritional components vary with plant species and nutrient category. Under warming conditions, *Morus alba* shows significantly reduced crude fiber content [16], while warming benefits the accumulation of cellulose in *Triticum aestivum* [17]. Low temperatures are unfavorable for nitrogen and amino acid accumulation in *Nicotiana tabacum* and *Hordeum vulgare*, but high altitudes benefit protein and amino acid accumulation in *Kobresia humilis* [18-21]. The response of staple bamboo food for pandas to altitude is of important scientific significance for panda survival, reproduction, and conservation.

Fargesia denudata is mainly distributed in the Minshan mountain range at altitudes between 1900-3200 m and is an important staple bamboo species for pandas in this mountain system [22]. It is also one of the most important dominant understory layers in the Minshan mountain system. This study examined *F. denudata* growing at different altitudes in the core panda habitat of Wanglang National Nature Reserve in the Minshan mountain system. By analyzing the nutritional components and amino acid composition in shoots, culms, and leaves of *F. denudata* at different altitudes, we aimed to answer the following scientific questions: (1) Does altitude cause changes in the nutritional component content of *F. denudata*? (2) How do the types and contents of amino acids in *F. denudata* respond to altitude?

Study Area

The study area is located in Wanglang National Nature Reserve in Pingwu County, Sichuan Province, with geographic coordinates of 32°49' N, 104°03' E. The reserve has a relative elevation difference of 2300-4980 m and covers an area of 322.97 km^2. The region belongs to the Danba-Songpan semi-humid climate zone. Due to monsoon influence, the climate is characterized by concentrated rainfall (May-October), forming distinct wet and dry seasons. The climate features strong sunshine and dry air. The mean annual temperature is 2.9°C, with mean temperatures of 12.7°C in July and -6.1°C in January. The annual precipitation is 859.9 mm, and the frost-free period is 195 days.

The vertical vegetation zones are: coniferous-broadleaf mixed forest or deciduous broadleaf forest (2300-2600 m), *Sabina saltuaria* forest and *Abies faxoniana* forest (2600-3500 m), subalpine shrub meadow (3500-4400 m), alpine scree vegetation (4400-4900 m), and alpine desert zone above 4900 m. Some areas also have small distributions of river terrace seabuckthorn forests and scree slope larch forests.

Materials and Methods

Experimental Materials

Fargesia denudata mainly grows under coniferous-broadleaf mixed forests and dark coniferous forests at altitudes of 1900-3200 m in Wanglang National Nature Reserve. The bamboo is a clonal plant of the Poaceae family and *Fargesia* genus, with clustered or nearly scattered culms. The underground stem is sympodial, consisting of rhizome and culm base. The growth period of *F. denudata* is March to May. Mid-to-late March is the root emergence period, root emergence basically ends in early April, late April to mid-May is the seedling growth period, growth stops in mid-May, and seedlings enter the tillering period after June.

Sampling and Testing Methods

Sampling sites were established at three altitudes (2600 m, 2850 m, and 3100 m), with three replicate plots at each altitude. At each sampling point, fresh samples of shoots and one-year-old clone ramets (culms and leaves) were randomly collected, with 1.0 kg of fresh samples for each component. The selected sites had consistent soil type, tree species composition, and canopy density. Samples were passed through a 1 mm sieve and stored in a low-temperature refrigerator for testing.

Nutritional components and amino acids were determined using the following methods [24]: Crude protein was measured using the Kjeldahl distillation method (total nitrogen multiplied by 6.25) with a KDY-9820 Kjeldahl nitrogen analyzer (Beijing Tiancheng Litong). Crude fat was measured using the Soxhlet extraction method. Crude fiber was measured using the acid detergent fiber method. Cellulose was measured using the weight method. Total amino acids were measured using the ninhydrin colorimetric method with a visible spectrophotometer. Amino acid components were analyzed using an L-8800 automatic amino acid analyzer.

Data Processing and Statistical Analysis

Data analysis was completed using SPSS 22.0 statistical software. One-way ANOVA was performed on nutrient component changes among different groups, followed by Tukey' s post-hoc test. All data are expressed as mean ± standard deviation. The significance level was set at $= 0.05$.

Results

Crude Protein

At the same altitude, crude protein content in shoots, leaves, and culms of *F. denudata* differed significantly ($P < 0.05$). Leaf crude protein content was highest, followed by shoots, while culm crude protein content was lowest. Shoot crude protein content at 3100 m was significantly higher than at 2600 m and

2850 m ($P < 0.05$). Leaf crude protein content at 3100 m was significantly higher than at 2600 m ($P < 0.05$). Culm crude protein content at 3100 m was highest, significantly higher than at 2600 m ($P < 0.05$).

Crude Fat

At the same altitude, crude fat content in shoots, leaves, and culms of *F. denudata* differed significantly ($P < 0.05$). Leaf crude fat content was highest, culm crude fat content was lowest, and shoot crude fat content was intermediate. Altitude had no significant effect on crude fat content in shoots and leaves ($P > 0.05$). Culm crude fat content at 3100 m was significantly higher than at 2600 m ($P < 0.05$).

Crude Fiber

In the 2600 m zone, culm crude fiber content was significantly higher than leaf crude fiber content ($P < 0.05$). In the 2850 m and 3100 m zones, culm crude fiber content was significantly higher than both shoot and leaf crude fiber content ($P < 0.05$), while shoot and leaf crude fiber content showed no significant difference ($P > 0.05$). Altitude had a significant effect on shoot crude fiber content ($P < 0.05$), with the lowest crude fiber content at 3100 m, but had no significant effect on culm and leaf crude fiber content ($P > 0.05$).

Cellulose

In the 2600 m zone, cellulose content in shoots and culms was significantly higher than in leaves ($P < 0.05$), with no significant difference between shoots and culms ($P > 0.05$). In the 2850 m and 3100 m zones, culm cellulose content was significantly higher than in shoots and leaves ($P < 0.05$), and shoot cellulose content was significantly higher than leaf cellulose content ($P < 0.05$). Altitude had a significant effect on shoot cellulose content ($P < 0.05$), with the lowest cellulose content at 3100 m. Leaf cellulose content at 3100 m was significantly lower than at 2600 m ($P < 0.05$). Culm cellulose content at 3100 m was significantly higher than at 2600 m and 2850 m ($P < 0.05$).

[Figure 1: see original paper] Differences in nutrient component content in shoots, culms, and leaves of *Fargesia denudata* and altitude effects on shoots, culms, and leaves. Lowercase letters (a, b, c) indicate differences in a given nutrient component among shoots, culms, and leaves at the same altitude; uppercase letters (A, B, C) indicate effects of altitude on a given nutrient component in shoots, culms, and leaves.

Nutrient Ratios

The ratio of crude protein to crude fat in shoots was significantly higher at 3100 m than at 2600 m ($P < 0.05$). In culms, the ratio of crude protein to crude fat at 3100 m was significantly higher than at 2600 m ($P < 0.05$). In leaves, crude protein and crude fat contents at 3100 m were significantly higher than at 2850 m and 2600 m ($P < 0.05$). Altitude had no significant effect on cellulose/crude fiber ratios in shoots and leaves ($P > 0.05$). The cellulose/crude fiber ratio in culms was lowest at 3100 m. Altitude had significant effects on crude protein/crude fiber ratios in shoots and culms ($P < 0.05$), but no significant effect in leaves ($P > 0.05$).

[Figure 2: see original paper] Differences in nutrient ratios in shoots, culms, and leaves of *Fargesia denudata* and altitude effects on shoots, culms, and leaves. Lowercase letters (a, b, c) indicate differences in nutrient ratios among shoots, culms, and leaves at the same altitude; uppercase letters (A, B, C) indicate effects of altitude on nutrient ratios in shoots, culms, and leaves.

Amino Acids

Total Amino Acid Content At different altitudes, total amino acid content followed the pattern: shoots > leaves > culms. Altitude had a significant effect on total amino acid content in shoots ($P < 0.05$), with the highest content at 3100 m. Total amino acid content in shoots at 3100 m was significantly higher than at 2600 m ($P < 0.05$). Total amino acid content in culms at 3100 m was significantly higher than at 2600 m ($P < 0.05$). Total amino acid content in leaves at 3100 m was significantly higher than at 2600 m and 2850 m ($P < 0.05$).

Amino Acid Types *Fargesia denudata* shoots, culms, and leaves contained 17 natural amino acids, including 7 essential and 10 non-essential amino acids. At the same altitude, various amino acid contents differed significantly among shoots, culms, and leaves ($P < 0.05$). The top four amino acids in shoots, culms, and leaves were aspartic acid, glutamic acid, alanine, and leucine, while the bottom four were cysteine, methionine, tyrosine, and histidine.

The effects of altitude on the 17 amino acid types were complex and variable. The response of essential and non-essential amino acids to altitude is described below. Altitude had significant effects on lysine, leucine, and phenylalanine contents in leaves, showing the pattern: 3100 m > 2850 m > 2600 m. Altitude had significant effects on the other six essential amino acids, all showing the pattern: 3100 m > 2850 m > 2600 m. Except for threonine, altitude had significant effects on essential amino acid contents in shoots ($P < 0.05$). Except for methionine, altitude had significant effects on essential amino acid contents in culms ($P < 0.05$).

Except for glycine and cysteine, altitude had significant effects on non-essential amino acid contents in shoots ($P < 0.05$), with aspartic acid, histidine, arginine, and proline all highest at 3100 m. Except for serine, altitude had significant effects on non-essential amino acid contents in culms ($P < 0.05$), with aspartic acid, histidine, and arginine contents all highest at 3100 m. Except for cysteine, altitude had significant effects on non-essential amino acid contents in leaves (P

< 0.05), with other non-essential amino acids all highest at 3100 m.

[Figure 3: see original paper] Differences in total amino acid content in shoots, culms, and leaves of *Fargesia denudata* and altitude effects on shoots, culms, and leaves. Lowercase letters (a, b, c) indicate differences in total amino acid content among shoots, culms, and leaves at the same altitude; uppercase letters (A, B, C) indicate effects of altitude on total amino acid content in shoots, culms, and leaves.

Amino acid types and their contents in shoots, culms, and leaves of *Fargesia denudata* (mg/g). The table shows amino acid contents across three altitudes (2600 m, 2850 m, 3100 m) for each plant part. Lowercase letters (a, b, c) indicate differences in a given amino acid among shoots, culms, and leaves at the same altitude; uppercase letters (A, B, C) indicate differences in a given amino acid for the same plant part across different altitudes. Essential amino acids are marked with *, including threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. Non-essential amino acids include aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, tyrosine, histidine, arginine, and proline.

Discussion

Responses of *Fargesia denudata* Nutrient and Amino Acid Contents to Altitude

Altitude can affect plant physiological metabolism and organic synthesis, thereby influencing nutrient content [2]. Plant growth, development, and substance metabolism responses to temperature depend on species and environment. This study shows that altitude increase benefits crude protein accumulation in *F. denudata*, consistent with results for *Setaria italica* [25] and *Kobresia humilis* protein [26]. This is because larger diurnal temperature variations at high altitudes are suitable for crude protein synthesis [21]. However, altitude had no significant effect on crude fat, consistent with studies on forage crude fat [27] and maize crude fat [28]. The dominant factor for crude fat accumulation is day length, and the altitude gradient in this study was not large enough to cause significant differences in day length [21].

Total amino acid content in shoots and culms was highest at 3100 m, consistent with studies on *Setaria italica* amino acids [25] and *Chrysanthemum morifolium* amino acids [29]. Crude protein and amino acids are important indicators of plant cold resistance, and plants accumulate large amounts to enhance cold resistance [30]. Diao Pinchun [31] showed that leaf free amino acid content is lower than in stems and roots, while leaf crude protein content is higher at high altitudes, possibly because low temperature induces plants to produce other nitrogen-containing substances, leading to increased crude protein content [32-34].

The response of various amino acid contents in *F. denudata* shoots, culms, and leaves to altitude showed no uniform trend, indicating that altitude effects on plant physiological activities, such as nitrogen metabolism, are complex. Taking glutamic acid as an example, in the process of ammonia assimilation, differences in altitude effects on glutamic acid may result from differences in the glutamate synthase (GOGAT) pathway, glutamate dehydrogenase (GDH) pathway, and glutamine synthetase (GS) pathway, as well as differences in glutamic acid metabolism and utilization [35].

Roles of Plant Nutrients and Amino Acids in Animal Feeding

Animal feeding is primarily based on nutrient content and digestibility [14-15]. Crude protein, crude fat, and amino acid content are generally used as indicators of nutritional value, while crude fiber is an important indicator affecting digestibility [15,20]. Plant species and parts with higher crude protein, crude fat, and amino acid contents and lower crude fiber content are more preferred by animals. Xing Tingjie et al. [36] showed that cellulose content significantly affects animal feeding, while foods with high crude protein content are more preferred by *Microtus fortis*.

Amino acids are fundamental components of proteins. The higher the amino acid content, the higher the protein utilization rate by animals and the more nutrients obtained [37]. Glutamic acid can provide rich nitrogen elements for *Lactuca sativa*, promoting soluble protein synthesis in vivo [34]. Leucine not only promotes protein accumulation but also inhibits protein decomposition [38]. Essential amino acids are substances that mammals cannot synthesize or synthesize in insufficient quantities and must obtain from food [39]. The types and contents of plant nutrients affect animal feeding choices [40].

Panda food (bamboo) with high protein and amino acid content is crucial for maintaining normal metabolic functions and growth [41]. This study shows that crude protein content in *F. denudata* follows the pattern: leaf > shoot > culm, consistent with Hu Jinchu et al. [42]. Bamboo culms have high cellulose content and low protein content. Studies on *Rhinopithecus roxellana* [44] and *Trachypithecus leucocephalus* [45] show similar feeding selectivity.

This study demonstrates that along the altitude gradient, crude protein content is highest at 3100 m, while crude fiber content is lowest. Total amino acid content follows the pattern: leaf > shoot > culm, while crude fiber content in leaves is significantly lower than in culms. Bamboo shoots and leaves have relatively high protein content, and pandas feed on bamboo leaves for most of the year, rarely or never feeding on culms [43].

Plants have special strategies to cope with cold climates at high altitudes, tending to accumulate more nutrients, such as synthesizing specific proteins to resist cold [46]. This is consistent with studies on *Kobresia littledalei* [46]. High altitudes benefit the accumulation of crude protein and amino acids in *F. denudata*. Although warming will cause *F. denudata* to migrate in altitude, this migration may be beneficial in terms of crude protein and amino acids. Current evidence shows that the activity range of a considerable number of pandas has clearly moved to higher altitudes in recent decades [10], which coincides with higher nutrient and amino acid contents in *F. denudata* at high altitudes. This study provides evidence that mountain herbivores will not be endangered by extreme habitat deterioration due to global warming. However, as pandas migrate to higher altitudes, the environment at 3100 m may be harsher than at lower altitudes.

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Note: Figure translations are in progress. See original paper for figures.