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Feeding performance and preferences of captive forest musk deer while on a cafeteria diet

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Abstract. We studied feeding intake and food selection of nine captive forest musk deer (Moschus berezovskii) offered 17 species of plants in China. We also determined nutrient characteristics related to plant quality to assess their effect on food selection. Results indicated that forest musk deer exhibited positive selectivity for four species of plants (M. azedarach, M. baccata, K. japonica and C. orbiculatus) and negative selectivity for the remainder. Two plant species with the highest selectivity values accounted for 47.39% of total food intake; thus, forest musk deer exhibited the strongest preference for these species. Food intake was positively correlated with feeding frequency and duration (r = 0.764, p < 0.005; r = 0.843, p < 0.005) but was not correlated with sniffing frequency or duration. However, olfaction did play an important role in food recognition by the deer. Pearson correlation analysis (data were log10 transformed) indicated that leaf intake was positively correlated with crude protein content (r = 0.708, p = 0.001) and negatively correlated with crude fiber content (r = −0.811, p < 0.001) and ash content (r = −0.496, p = 0.043). In addition, forest musk deer preferred tannin-rich plants with high protein and low fiber. Food intake was also positively correlated with potassium content (r = 0.672, p < 0.005). Our results suggest that forest musk deer is able to positively select high quality food (high protein content) and avoid low quality food (high fiber content). However, the fact that musk deer also prefer tannin-rich food requires further research to gain deeper insight into the underlying mechanisms in the food selection of forest musk deer.

Key words: Moschus berezovskii, feeding behavior, food selection, plant components, secondary metabolites

Introduction

Musk deer (Moschus spp.) are endemic forest-dwelling small ruminants in Asia, mainly occurring in China and neighbouring countries. In the 1950s, musk deer numbered about 2.5 to 3 million, but now fewer than 100000 remain due to illegal hunting, over-exploitation, and habitat loss (Gao et al. 2002). In 2002, all species of musk deer were listed as class I protected wildlife in China. They are also listed in Appendix I of CITES (CITES 2010) and are considered endangered on the IUCN Red List (IUCN 2012). Forest musk deer (Moschus berezovskii) farming began as early as 1958 in China and has lasted for several decades. However, captive population growth has been slow and has become stagnant in the long term (Parry-Jones & Wu 2001). The general consensus among researchers is that musk deer are difficult to breed in captivity (Green & Taylor 1986, Homes 1999, Yang et al. 2003, Meng et al. 2006, Sheng & Liu 2007, Li et al. 2012), primarily due to the high incidence of disease, especially those of the digestive tract (Li & Zhao 2011, Zhu et al. 2012). Forest musk deer are concentrate selectors that mainly feed on a variety of shoots and leaves of woody plants (Sheng & Liu 2007, Zhang 2008). Captive forest musk deer are typically fed based on information from deer farms, but food items and composition vary greatly among farms and do not follow any recognized standards. Since 2002, the Musk Deer Conservation Project in China aimed to develop and implement a captive population and musk deer reintroduction program. Therefore, understanding the food preferences of forest musk deer and the factors affecting those preferences is important for developing a breeding population of musk deer.

Forage selection by herbivores is associated with many factors, including the quality and distribution...

The difficulty in maintaining a captive population has highlighted the need to determine the food preferences and improve the nutritional status of captive musk deer. Furthermore, characterizing the nutrients and secondary metabolites of the preferred food plants of captive musk deer is critical to assessing the nutritional requirements of captive deer and to facilitating the development of musk deer breeding populations.

**Material and Methods**

**Study area**

The study was conducted at the Center of Forest Musk Deer Farming and Research, located in Fengxian County (33°34′57″-34°18′21″ N, 106°24′54″-107°7′54″ E), Shaanxi Province, China, in July 2012. The center was one of the earliest artificial breeding facilities for forest musk deer in China (Fig. 1) and is located along the southern slope of the Qinling Mountains, an important natural habitat for the musk deer.

At altitudes ranging from 915 to 2739 m, the study area belongs to the middle and low mountainous region. The area experiences a monsoon mountain climate in the warm temperate zone. Average annual temperature is 11.4 °C, and mean annual precipitation is 610 mm. The local flora transitions from subtropical to warm temperate species, with dominant vegetation of broad-leaved deciduous, coniferous, and broad-leaved mixed forests. The center is located in a pristine valley with easy access to water and natural food, far from villages and roads.

**Table 1.** The experimental plants of forest musk deer’s feeding test. Explanations: a – the food plant used by keepers, b – the food plant recorded in references.

<table>
<thead>
<tr>
<th>Latin name</th>
<th>Family</th>
<th>Basis for the selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cerasus polytricha</td>
<td>Rosaceae</td>
<td>a</td>
</tr>
<tr>
<td>2 Schisandra chinensis</td>
<td>Magnoliaceae</td>
<td>a</td>
</tr>
<tr>
<td>3 Litsea pungens</td>
<td>Lauraceae</td>
<td>b</td>
</tr>
<tr>
<td>4 Kerria japonica</td>
<td>Rosaceae</td>
<td>b</td>
</tr>
<tr>
<td>5 Euonymus verrucosoides</td>
<td>Celastraceae</td>
<td>b</td>
</tr>
<tr>
<td>6 Ulmus bergmanniana</td>
<td>Ulmaceae</td>
<td>a</td>
</tr>
<tr>
<td>7 Lindera obtusiloba</td>
<td>Lauraceae</td>
<td>b</td>
</tr>
<tr>
<td>8 Lonicera fragrantissima standishii</td>
<td>Caprifoliaceae</td>
<td>b</td>
</tr>
<tr>
<td>9 Malus baccata</td>
<td>Rosaceae</td>
<td>a</td>
</tr>
<tr>
<td>10 Rhus potaninii</td>
<td>Anacardiaceae</td>
<td>a</td>
</tr>
<tr>
<td>11 Celastrus orbiculatus</td>
<td>Celastraceae</td>
<td>b</td>
</tr>
<tr>
<td>12 Acer elegantulum</td>
<td>Aceraceae</td>
<td>b</td>
</tr>
<tr>
<td>13 Acer davidii</td>
<td>Aceraceae</td>
<td>b</td>
</tr>
<tr>
<td>14 Melia azedarach</td>
<td>Meliaceae</td>
<td>a</td>
</tr>
<tr>
<td>15 Eucommia ulmoides</td>
<td>Eucommiaceae</td>
<td>a</td>
</tr>
<tr>
<td>16 Swida walteri</td>
<td>Cornaceae</td>
<td>a</td>
</tr>
<tr>
<td>17 Akebia trifoliata</td>
<td>Lardizabalaceae</td>
<td>b</td>
</tr>
</tbody>
</table>
Feeding studies were conducted in July 2012, when plants in Qinling are at peak nutritional status. Initial observations indicated that each musk deer farm provides tree leaves from two to five species and in varying amounts. Farms typically collect the same approximate amount of leaf mass to air-dry and preserve as winter feed. Based on the main plant species supplied by each farm as well as the food habits of wild musk deer and local vegetation conditions (Zhang 2008, Li et al. 2012), 17 species of edible tree leaves were collected for our feeding experiments with forest musk deer (Table 1).

The preferences of forest musk deer for a particular plant species can be assessed by comparing the proportion of the species in the diet with the proportion of the species available as food within the vegetation (Krebs 1989). Experiments were conducted in a 15 × 15 m enclosure within a larger forest musk deer enclosure. Nine adult males housed in individual pens were used for the experiment. The deer remained healthy and did not demonstrate abnormal behavior during the experimental period. Seven days before the experiment, animals were fed only mulberry leaves and artificial feed, avoiding experimental leaves. The musk deer were feeding one by one, and each individual was tested in their own yard. Fresh leaves (100 g) of each plant were placed in a bowl, and 17 bowls were placed at random in a circle. The position of each plant in the circle was recorded during the experiment. In addition, a control bowl for each plant type was positioned outside the fences to control the water loss. Feeding experiments began at 16:30 h, and all remaining leaves were collected at the same time the next day. The leaves were weighed before and after trials, and during the experimental period, monitors recorded musk deer behaviors, including sniffing frequency, sniffing duration, feeding frequency, and feeding duration during the 24-h period. Experiments were conducted twice, and the second trials were conducted three days after the first. Diet selection was determined using the electivity index “ε” (Chesson 1983). This index is often applied in diet selection studies (McKnight & Hepp 1998, Markkola et al. 2003, Reichlin et al. 2006). The electivity index ε is based on Manly’s alpha selection index (α) (Manly et al. 1972). The index ε ranges between −1 and +1, with values between −1 and 0 indicating negative selection, and values between 0 and +1 indicating positive selection.

\[
i = \frac{ma_{i} - 1}{(m-2)a_{i} + 1}
\]

where \(m\) is the number of potential dietary types, and \(a_{i}\) is Manly’s selection index for plant species \(i\).

\[
a_{i} = \frac{r_{i}}{m} \sum_{j=1}^{m} \frac{r_{j}}{n_{j}}
\]

where \(r_{i}\) and \(r_{j}\) are the proportions of plant species \(i\) and \(j\) in the diet \((i = 1, 2, 3, \ldots, m)\), \(n_{i}\) and \(n_{j}\) are the proportions of plant species \(i\) and \(j\) available, and \(m\) is the number of potential plant species.

Leaf samples were dried at 75 °C for five hours, and initial moisture in the plants was then calculated. Crude fat, total nitrogen, crude fiber, and ash contents were determined according to AOAC standards (AOAC 2000). Total lipid content was determined gravimetrically after extraction with n-hexane. Gross energy was determined using an adiabatic calorimeter (Parr, Model 1241), and 11 types of mineral element were analytically determined using the inductively coupled plasma approach. Dry matter content of plant subsamples was determined by oven-drying at 105 °C to a constant weight. All analyses were conducted in duplicate, or in triplicate whenever a difference of more than 2 % was found between two samples. Plant samples were assayed for secondary compounds, specifically flavonoids and tannins, using the NaNO₂-Al(NO₃)₃-NaOH colorimetric method (Nieva Moreno et al. 2000, Mohammadzadeh et al. 2007, Yang et al. 2009, Liu 2010) and Folin-Ciocalteu reagent colorimetric method (Makkar et al. 1993), respectively.

Data analysis

Statistical analysis was performed using IBM SPSS Statistics 20.0. Assumptions of normality were checked by examining normal probability plots and calculating a Kolmogorov-Smirnov statistic. The differences of plants intake were tested by means of Kruskal-Wallis H. The linear regression tested the relations between food intake and feeding frequency, and between food intake and feeding duration. Relation between feed intake and feeding behaviors (data were log₁₀ transformed), basic nutritional components, mineral contents and secondary metabolites of plants were tested by means of Pearson correlation analysis or Spearman correlation analysis. The differences of the secondary metabolites contents of 17 species plants were tested by means of One-way ANOVA.
analysis. Results were considered to be significant at p < 0.05. Cluster analysis was used to partition plants into different categories in terms of nutrition content. Figures were generated using GIS 10.0 and Sigmplot 12.0.

**Results**

*Feeding behavior observations*

Observations of feeding behavior indicated that forest musk deer first sniffed at the tree leave species one by one, often sniffing at certain leaves several times, and then fed selectively. The average sniffing frequency of the forest musk deer was 4.20 ± 0.40 times/24 h across all plant species, with the highest sniffing frequency for *A. davidii* and the lowest for *M. azedarach* (Fig. 2). The average sniffing duration across all plant species was 8.40 ± 0.78 s, with the longest sniffing duration for *A. davidii* and the shortest for *M. azedarach*

(Fig. 2). Pearson correlation analysis indicated that sniffing frequency was positively correlated with sniffing duration (r = 0.958, p < 0.01).

Average feeding frequency was 5.15 ± 1.33 times/24 h across all 17 species, with the highest feeding frequency for *M. azedarach* and the lowest for *A. elegantulum* (Fig. 3). Average feeding duration across all plant types was 291.59 ± 103.44 s, with the longest duration of feeding for *M. azedarach* and the shortest for *A. elegantulum* (Fig. 3).

The average leaf intake by musk deer was 6.71 ± 2.00 g across all 17 plant species, with *M. azedarach* exhibiting the highest intake and *A. elegantulum* exhibiting the lowest (Fig. 4). The electivity index values indicated that forest musk deer positively selected *M. azedarach*, *M. baccata*, *K. japonica* and *C. orbiculatus*, but negatively the other studied species (Markkola et al. 2003, Reichlin et al. 2006). The two plant species with the highest e values (*M. azedarach* and *M. baccata*) accounted for 47.39 % of the total leaf intake, while the four positively selected species of plants accounted for 62.46 % of total leaf intake. Overall, the forest musk deer exhibited significant food selection preferences among the 17 test species.
according to the feed intake (Kruskal-Wallis H test; H = 39.186, df = 16, p = 0.001).

Leaf intake was positively correlated with feeding frequency (r = 0.764, p = 0.001) and feeding duration (r = 0.843, p = 0.000), but not with sniffing frequency (r = -0.08, p > 0.05) or sniffing duration (r = -0.08, p > 0.05). Linear relationships were found between leaf intake and feeding frequency and between leaf intake and feeding duration (Fig. 5).

Correlations between selection and basic nutritional components and mineral contents of plants

The constituent and energy contents differed distinctly among experimental plant species (Table 2). Pearson correlation analysis indicated that leaf intake was positively correlated with crude protein content (r = 0.708, p = 0.001) and negatively correlated with crude fiber content (r = -0.811, p < 0.001) and ash content (r = -0.496, p = 0.043). No significant correlations were observed between leaf intake and any other basic nutritional components (p > 0.05). The plants with relatively high protein, such as U. bergmanniana, L. pungens, C. polytricha, L. obtusiloba and A. elegantulum, were not positively selected for by musk deer. Similarly, plants with relatively low fiber, such as U. bergmanniana, C. polytricha and R. potaninii, did not exhibit positive selection.

The contents of the four macronutrients and seven micronutrients differed from one another (Table 3). Pearson correlations indicated that leaf intake was positively correlated with K content (r = 0.672, p < 0.005) but was not significantly correlated with the contents of any other macro- or microelement contents.

Correlations between selection and secondary metabolites of plants

Average flavonoid content of the 17 plants was 69.44 ± 9.40 mg/g, with the highest content in R. potaninii and the lowest in A. trifoliata (Fig. 6). One-way ANOVA indicated that flavonoid content varied among the 17 species of plants (F = 821.062, p < 0.05). There is no significant correlation between leaf intake and flavonoids contents. The four positively selected plants contained flavonoids contents ranging from 30.9 to 76.0 mg/g, whereas the negatively selected plants exhibited flavonoids contents of more than 90 mg/g or less than 10 mg/g.

The average tannin content of the 17 plants was 45.40 ± 7.61 mg/g (Fig. 7), with the highest content in S. walteri and the lowest in L. fragrantissima standishii. Kruskal-Wallis H tests indicated that tannin content varied among the 17 plants (H = 49.413, df = 16, p < 0.01). Spearman correlation tests indicated that leaf intake was positively correlated with tannin content, albeit not significantly. The four positively selected plants exhibited tannin contents ranging from 28 to 87 mg/g, whereas the negatively selected plants exhibited tannin contents either more than 96 mg/g or less than 14 mg/g.

Cluster analysis was used to divide the 17 plants into three categories based on crude protein and crude fiber contents. The first category included five plants with high protein and low fiber: L. pungens, L. obtusiloba, M. azedarach, C. polytricha and U. bergmanniana. The second category included six plants with intermediate levels of protein and fiber, including K. japonica, M. baccata, R. potaninii, S. walteri, A. davidii and S. chinensis. The third category included six plants with low protein and high fiber: E. verrucosoides, E.
ulmoides, *C. orbiculatus*, *A. trifoliata*, *A. elegantulum* and *L. fragrantissima standishii*. The eight plants with the highest food intake by musk deer (food intake > 5.0 g) accounted for 82.08% of total food intake. Among these, four species belonged to the first category (*M. azedarach*, *U. bergmanniana*, *L. pungens* and *C. polytricha*), and three belonged to the second category (*M. baccata*, *K. japonica* and *S. chinensis*); only one plant species belonged to the third category (*C. orbiculatus*). Among these eight plants, Pearson correlation analysis indicated that leaf intake was positively correlated with tannin content (r = 0.781, p = 0.022).

**Discussion**

Food selection by herbivores is affected by many factors, and several hypotheses have been put forth to describe the nature of these plant-herbivore interactions. Examples include the nutrition hypothesis (Eshelman & Jenkins 1989, Nolte & Provenza 1992), plant secondary compounds hypothesis (Freeland & Janzen 1974, Bryant & Kuropat 1980), nutrient balance hypothesis (Pehrson 1983), optimal foraging theory (Edwards 1983, Belovsky 1986), and conditioned flavor aversion hypothesis (Provenza 1996, Ralph 1997). Among these eight plants, Pearson correlation analysis indicated that leaf intake was positively correlated with tannin content (r = 0.781, p = 0.022).

Generally, plants with high protein and low fiber contents are high quality food for herbivores. Many studies have demonstrated that herbivores prefer shoots and new leaves, which are typically of high quality (Demment & Van Soest 1985, Illius & Gordon 1990, Workman 2010). Such preferences are consistent with the nutrition hypothesis that herbivores can identify food according to the smell (Provenza & Ralph 1990, Provenza 1996, Ralph 1997, Vourc'h et al. 2001, 2002). However, which volatile components cause forest musk deer to ingest or reject certain plants after sniffing remains unknown, yet sniffing frequency and duration clearly function in food recognition by forest musk deer.
Forest musk deer usually prefer twigs and newly growing leaves instead of fiber-rich older leaves, and the deer maintain this preference even during seasons of food shortage. Our results indicated that food preference was significantly positively correlated with crude protein content and significantly negatively correlated with crude fiber content. Notably, some plants with relatively high protein content, such as *L. pungens*, *L. obtusiloba*, and *A. elegantulum*, and some with high K content, such as *R. potaninii*, *S. chinensis*, and *L. pungens*, did not exhibit high intake by forest musk deer. Instead, intake of major food plants (82.08% of total food) was significantly positively correlated with tannin content, indicating that tannin is an important factor affecting food preferences for forest musk deer. Similarly, roe deer (*Capreolus capreolus*) in natural habitats select tannin-rich plants (Verheyden-Tixier & Duncan 2000). Tannins are plant secondary metabolites produced to defend against herbivores (Cooper & Owen-Smith 1985, Bryant et al. 1991, Palo et al. 1997), and they often exist at high levels in twigs and leaves (Cooper & Owen-Smith 1985, Peng & Cao 2010); thus, herbivores may take in more tannins by choosing protein-rich twigs and newly growing leaves. The present experiment demonstrated that forest musk deer may prefer tannin-rich plants when their major food plants contain similar protein contents. The digestive physiology of musk deer depends to a certain extent on tannins (Tixier et al. 1997). In addition, many studies have shown that herbivorous mammals consume tannins to defend against parasitic infections (Lisonbee et al. 2009, Villalba et al. 2010, Juhnke et al. 2012). Musk deer are a relatively primitive ruminant (Shrestha 1998), and the earliest function of the ruminant stomach may have been the degradation of toxic substances from woody plants (McDonald & Warner 1975, Ding & Li 1996). The intensity of internal parasite infection of farmed forest musk deer is very high (unpublished data), which may cause deer to consume plants that are rich in tannins and protein; however, this hypothesis requires further study. If this is the case, the current food supply may not meet the digestive physiology demands of farmed forest musk deer.

The present study also demonstrated that food intake was strongly positively correlated with potassium content. This element is unique among the macronutrients required by animals, as dietary deficiencies of this element are uncommon. However, ruminants and other herbivores typically consume potassium in great excess of their dietary requirements. In addition,
ruminants appear to be well adapted to metabolize large amounts of potassium (Ward 1966). Potassium accounts for a substantial fraction of cation content in rumen fluid and is important in maintaining a desirable medium for bacterial fermentation (Hubbert et al. 1958, Nicholson et al. 1960, Aschbacher et al. 1965). A number of trials have shown that supplements containing high concentrations of potassium improve the digestibility and weight gain of ruminants (Chappell et al. 1955). Thin branches and young shoots usually contain high potassium and low fiber (Gonzalez et al. 2005, Beale & Long 1997, Christian & Riche 1998, Christian et al. 2008). Thus, selection of fresh leaves by forest musk deer may be one reason why food intake was strongly positively correlated with potassium content. The musk deer at the center have never been provided with salt blocks. Therefore, future studies should examine potassium as a dietary constituent of musk deer.

Acknowledgements

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