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Source: Folia Zoologica, 64(2): 151-160

Published By: Institute of Vertebrate Biology, Czech Academy of

Sciences

URL: https://doi.org/10.25225/fozo.v64.i2.a8.2015

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

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Received 3 February 2015; Accepted 20 July 2015

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 forestdwelling 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, overexploitation, 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

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of food plants, predation risk, climate factors, human disturbance, animal nutritional requirements, gut capacity, animal feeding feedback and learning, and plant secondary metabolites (Illius & Jessop 1996, Rhind et al. 2002, Forbes 2003). Consequently, previous studies have tested animal food preferences by excluding the effects of various complex environmental factors (Rogers 1990, Lawler et al. 1999, Bergyall 2007).

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

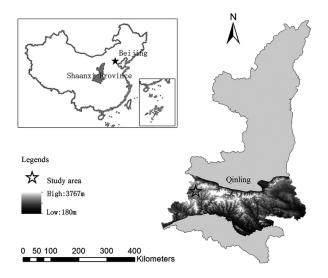


Fig. 1. Geographical location of forest musk deer's studying site.

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.

	Latin name	Family	Basis for the selection
1	Cerasus polytricha	Rosaceae	a
2	Schisandra chinensis	Magnoliaceae	a
3	Litsea pungens	Lauraceae	b
4	Kerria japonica	Rosaceae	b
5	Euonymus verrucosoides	Celastraceae	b
6	Ulmus bergmanniana	Ulmaceae	a
7	Lindera obtusiloba	Lauraceae	b
8	Lonicera fragrantissima standishii	Caprifoliaceae	b
9	Malus baccata	Rosaceae	a
0	Rhus potaninii	Anacardiaceae	a
1	Celastrus orbiculatus	Celastraceae	b
2	Acer elegantulum	Aceraceae	b
3	Acer davidii	Aceraceae	b
4	Melia azedarach	Meliaceae	a
5	Eucommia ulmoides	Eucommiaceae	a
6	Swida walteri	Cornaceae	a
7	Akebia trifoliata	Lardizabalaceae	b

Cafeteria feeding study and measurement of plant chemical characteristics

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 airdry 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.

$$\varepsilon_i = \frac{m\alpha_i - 1}{(m - 2)\alpha_i + 1}$$

where m is the number of potential dietary types, and α_i is Manly's selection index for plant species i.

$$\alpha i = \frac{r_i}{n_i} \frac{1}{\sum_{i=1}^{m} \frac{r_i}{n_j}}$$

where r_i and r_j are the proportions of plant species i and j in the diet (i and j = 1, 2, 3, ..., 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.

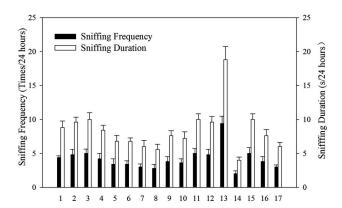


Fig. 2. The sniffing frequency and sniffing time of experimental plants by forest musk deer (Mean \pm SE). The number 1-17 represents the plants, respectively: 1-C. polytricha, 2-S. chinensis, 3-L. pungens, 4-K. japonica, 5-E. verrucosoides, 6-U. bergmanniana, 7-L. obtusiloba, 8-L. fragrantissima subsp. standishii, 9-M. baccata, 10-R. potaninii, 11-C. orbiculatus, 12-A. elegantulum, 13-A. davidii, 14-M. azedarach, 15-E. ulmoides, 16-S. walteri, 17-A. trifoliata.

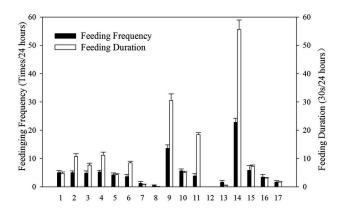


Fig. 3. The feeding frequency and feeding time of experimental plants by forest musk deer (Mean \pm SE). The number 1-17 represents the plants, for explanations see Fig. 2.

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*

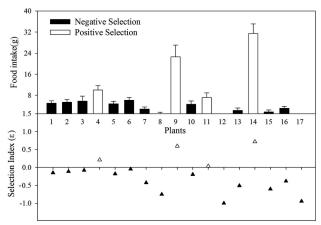


Fig. 4. The intake and selectivity index (ε) of experimental plants by forest musk deer (Mean \pm SE). The number 1-17 represents the plants, for explanations see Fig. 2.

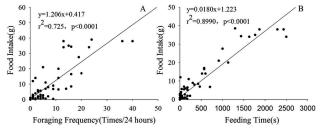


Fig. 5. The linear model of food intake and feeding frequency and feeding time of forest musk deer.

(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 ε 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).

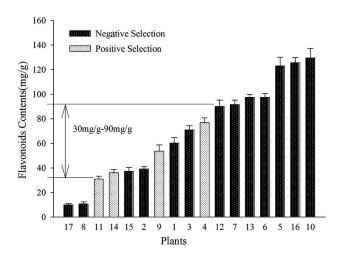


Fig. 6. The differences of flavonoids content in plants (Mean ± SE). The number 1-17 represents the plants, for explanations see Fig. 2.

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 C.

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.

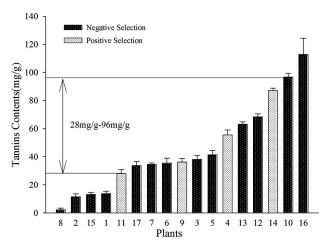


Fig. 7. The differences of tannins content in plant (Mean \pm SE). The number 1-17 represents the plants, for explanations see Fig. 2.

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 differed 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 flavonoid contents of more than 90 mg/g or less than 10 mg/g.

The average tannin content of the 17 plants was 45.40 \pm 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.*

Table 2. Basic nutritional value of focal plants.

		C-1 (1.1/						
Plant species	Initial moisture	Crude protein	Crude fat	Crude fiber	Ash content	Calories (kJ/kg)		
Cerasus polytricha	54.67	13.77	1.11	8.95	6.90	18.72		
Schisandra chinensis	80.00	8.55	2.76	11.64	8.40	16.48		
Litsea pungens	66.00	16.07	3.62	12.75	8.00	19.11		
Kerria japonica	60.00	11.92	0.27	10.53	7.80	18.67		
Euonymus verrucosoides	57.00	8.54	2.83	14.57	10.70	17.48		
Ulmus bergmanniana	60.50	15.32	7.92	8.60	8.50	18.11		
Lindera obtusiloba	60.00	16.43	4.41	12.99	5.70	19.36		
Lonicera fragrantissima	49.00	12.10	4.15	18.95	9.70	18.50		
Malus baccata	58.00	11.49	0.33	10.43	9.10	18.19		
Rhus potaninii	62.00	11.83	3.78	9.95	8.18	18.46		
Celastrus orbiculatus	64.00	12.08	7.26	15.95	11.80	16.99		
Acer elegantulum	57.00	13.00	2.89	17.18	6.20	19.42		
Acer davidii	54.67	11.52	4.08	12.73	7.50	18.32		
Melia azedarach	62.00	14.22	3.16	11.90	6.00	18.27		
Eucommia ulmoides	62.67	10.03	2.97	15.07	9.10	18.25		
Swida walteri	60.50	10.43	0.76	10.02	6.60	17.60		
Akebia trifoliata	56.00	12.15	0.93	15.26	12.30	16.16		
Mean ± SE	60.24 ± 1.58	12.32 ± 0.56	3.13 ± 0.53	12.79 ± 0.72	8.38 ± 0.47	18.12 ± 0.22		

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).

The present experiment demonstrated that food intake was not significantly correlated with sniffing

frequency or duration, particularly for several plant species such as *A. davidii*, *E. ulmoides* and *L. pungens*. Forest musk deer exhibited relatively long sniffing frequencies and durations for these species, but they ultimately fed upon them very minimally or not at all. Thus, forest musk deer can likely recognize food through their scents, which is consistent with the common hypothesis that herbivores can identify food according to the smell (Provenza & Balph 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.

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 (Nolte & Provenza 1992). Forest musk deer are small ruminants and true concentrate selectors (Hofman 1989, Kattel 1992), in that they need to consume food of high digestibility, high protein, and low fiber content (Bell 1971, Jarman 1974, Hofmann 1989, Prikhod'ko 2003, Krivoshapkin

Table 3. The mineral element content of experimental plants.

	В	55.00	33.56	32.44	34.66	31.49	45.79	27.91	34.43	25.24	22.38	19.31	62.82	23.34	15.52	29.65	16.03	27.89	31.61 ± 3.11
	Z	2.08	1.28	1.27	1.24	1.75	2.07	1.84	1.45	2.09	1.51	2.58	1.98	1.30	1.27	1.39	1.22	2.49	1.69 ± 0.11 31
Major element (g/kg) Microelement (mg/kg)	Cr	1.37	0.87	1.83	1.27	2.07	1.25	12.53	3.46	1.12	0.99	16.15	5.74	0.70	0.36	4.26	0.44	4.67	3.48 ± 1.07
	Mn	127.88	185.34	221.02	145.92	43.44	55.83	357.59	97.10	26.93	89.09	66.23	229.71	37.87	8.13	101.12	11.17	113.08	111.12 ± 22.64
	Cu	15.97	0.00	22.05	9.23	29.68	20.71	20.35	11.93	8.94	8.72	6.29	28.47	9.52	14.13	28.66	6.71	10.28	14.80 ± 2.13
	Zn	11.76	13.76	9.94	9.17	11.80	15.50	11.10	9.81	11.02	9.57	6.12	9.81	6.12	5.06	5.77	4.73	7.73	9.34 ± 0.75
	Fe	532.50	392.68	206.90	392.65	427.74	428.57	916.73	410.16	331.05	281.60	1069.84	632.24	343.95	217.30	406.75	201.53	441.52	466.69 ± 54.95
	Mg	2.49	11.52	3.44	3.31	2.61	2.45	1.91	2.26	3.47	2.01	3.80	2.65	2.07	0.91	2.31	1.30	2.95	3.03 ± 0.56
	Na	0.19	0.12	80.0	0.15	0.25	0.42	0.16	0.20	0.30	0.12	0.13	0.41	0.17	0.18	0.11	0.07	0.09	0.19 ± 0.03
	K	11.92	20.31	20.28	15.78	17.34	21.29	16.21	17.53	19.21	22.94	20.94	17.24	11.01	6.79	15.87	2.20	14.46	20.70 ± 2.27 15.96 ± 1.32
	Ca	13.88	14.23	17.29	30.73	33.26	19.24	11.30	29.78	25.32	21.70	34.99	15.47	16.00	7.09	11.90	12.67	37.03	20.70 ± 2.27
	Plant species	C. polytricha	S. chinensis	L. pungens	K. japonica	E. verrucosoides	U. bergmanniana	L. obtusiloba	L. fragrantissima	M. baccata	R. potaninii	C. orbiculatus	A. elegantulum	A. davidii	M. azedarach	E. ulmoides	S. walteri	A. trifoliata	Mean ± SE

2008). 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. Potassium is unique among the macrominerals 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

The authors would like to thank the Breeding Centre of Forest Musk Deer in Pien Tze Huang for collecting experimental plants. The research was funded by Species Salvation Program of Department of Fauna and Flora and Nature Reserve Management, State Forestry Administration of China (No. Forest musk deer 2013, 2014).

Literature

AOAC 2000: Official methods of analysis of the association of official analytical chemist, 14th ed. AOAC, Washington, D.C.

Aschbacher P.W., Kamal T.H. & Cragle R.G. 1965: Total body water estimations in dairy cattle using tritrated water. *J. Anim. Sci.* 24: 430–433.

Beale C.V. & Long S.P. 1997: Seasonal dynamics of nutrient accumulation and partitioning in the perennial C4-grasses *Miscanthus* × *giganteus* and *Spartina cynosuroides*. *Biomass Bioenergy* 12 (6): 416–428.

Bell R.H.V. 1971: A grazing ecosystem in the Serengeti. Sci. Am. 25: 86-93.

Belovsky G.E. 1986: Optimal foraging and community structure, implication for a guild of generalist grass land herbivores. *Oecologia* 70: 35–52.

Bergvall U.A. 2007: Food choice in fallow deer experimental studies of selectivity. *PhD dissertation, Stockholm University, Sweden.* http://www.diva-portal.org/smash/get/diva2:197053/FULLTEXT01.pdf

Bryant J.P. & Kuropat P.J. 1980: Selection of winter forage by subarctic browsing vertebrates, the role of plant chemistry. *Annu. Rev. Ecol. Syst.* 11: 261–285.

Bryant J.P., Provenza F.D., Pastor J., Reichardt P.B., Clausen T.P. & du Toit J.T. 1991: Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annu. Rev. Ecol. Syst.* 22: 431–446.

Chappel C.D., Sirny R.J., Whitehair C.K. & MacVicar R. 1955: Effect of mineral supplements of digestibility of a corn cob ration by sheep. *J. Anim. Sci.* 14: 153–159.

Chesson J. 1983: The estimation and analysis of preference and its relationship to foraging models. *Ecology 64: 1297–1304*.

Christian D.G. & Riche A.B. 1998: Nitrate leaching losses under *Miscanthus* grass planted on a silty clay loam soil. *Soil Use Manag.* 14: 131–135.

Christian D.G., Riche A.B. & Yates N.E. 2008: Growth, yield and mineral content of *Miscanthus* × *giganteus* grown as a biofuel for 14 successive harvests. *Ind. Crops Prod.* 28 (3): 320–327.

CITES 2010: Convention on the International Trade in Endangered Species. www.cites.org

Cooper S.M. & Owen-Smith N. 1985: Condensed tannins deter feeding by browsing ruminants a South African savanna. *Oecologia* 67: 142–146.

Demment M.W. & Van Soest P.J. 1985: A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *Am. Nat. 125: 641–672*.

Ding B.L. & Li Y.G. 1996: The forage grass poisoning and detoxification of ruminants. Foreign Veterinary-Livestock and Poultry Disease 17 (2): 7–9. (in Chinese)

Edwards J. 1983: Diet shifts in moose due to predator avoidance. *Oecologia 60: 185–189*.

Eshelman B.D. & Jenkins S.H. 1989: Food selection by Belding's ground squirrels in relation to plant nutritional features. *J. Mammal.* 70: 846–852.

Forbes J.M. 2003: The multifactorial nature of food intake control. J. Anim. Sci. 81 (Suppl. 2): 139-144.

Freeland W.J. & Janzen D.H. 1974: Strategy in herbivory by mammals, the role of plant secondary compounds. *Am. Nat. 108: 269–289*. Gao R.H., Zhang J.J. & Liang B.K. 2002: Musk deer conservation project in China. *State Forest Administration of China, Beijing, China.*

Gonzalez-V E.A., Hussey M.A. & Ortega-S J.A. 2005: Nutritive value of *Desmanthus* associated with kleingrass during the establishment year. *Rangeland Ecol. Manage*. 58: 308–314.

Green M. & Taylor R. 1986: The musk connection. New Sci. 110: 56-58.

Hofmann R.R. 1989: Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia 78: 443–457*.

Homes V. 1999: On the scent, conserving musk deer the uses of musk and Europe's role in its trade. *Traffic Europe, Brussels, Belgium*.
Hubbert F., Cheng E. & Burroughs W. 1958: The influence of potassium, sodium, rubidium, lithium, and cesium on *in vitro* cellulose digestion by rumen microorganisms with observations upon sodium and potassium influences in lamb fattening rations. *J. Anim. Sci.* 17: 576–585.

- Illius A.W. & Gordon I.J. 1990: Constraints on diet selection and foraging behaviour in mammalian herbivores. In: Hughes R.N. (ed.), Behavioural mechanisms of food selection. *Springer-Verlag, Berlin: 360–393*.
- Illius A.W. & Jessop N.S. 1996: Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci. 74: 3052–3062.
- IUCN 2012: Red List of Threatened Species. www.iucnredlist.org
- Jarman P.J. 1974: The social organization of antelope in relation to their ecology. Behaviour 48: 215–267.
- Juhnke J., Millerb J., Hall J.O., Provenza F.D. & Villalba J.J. 2012: Preference for condensed tannins by sheep in response to challenge infection with *Haemonchus contortus*. Vet. Parasitol. 188: 104–114.
- Kattel B. 1992: Ecology of the Himalayan musk deer in Sagarmatha National Park, Nepal. *PhD dissertation, Colorado State University, U.S.A.*
- Krebs C.J. 1989: Ecological methodology. Harper Collins.
- Krivoshapkin A.A. 2008: Musk deer (Moschus moschiferus Linnaeus, 1758) of the Yakutia Republic. Vestn. Yakutskogo Gos. Univ. 5: 5–9. (in Russian)
- Lawler I.R., Stapley J., Foley W.J. & Eschler B.M. 1999: Ecological example of conditioned flavor aversion in plant-herbivore interactions, effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *J. Chem. Ecol.* 25: 401–415.
- Li L.H., He L., Liu G., Liu S.Q., Liu W.H., Meng M. & Hu D.F. 2012: Discussion about relationship between biological characters and farming development of musk deer. For. Res. Manage. 2: 26–29. (in Chinese)
- Li Y.H. & Zhao L.A. 2011: Disease prevention and treatment for forest musk deer. *Modern Agric. Sci. Technol.* 17: 307–308. (in *Chinese*)
- Lisonbee L.D., Villalba J.J., Provenza F.D. & Hall J. 2009: Tannins and self-medication, implications for sustainable parasite control in herbivores. *Behav. Process.* 82: 184–189.
- Liu L. 2010: Studies on assay method of flavonoids and ultrasound-assisted extraction process in Oxytropis plants. Master Thesis, North West Agriculture and Forestry University, Yangling, Shannxi Province. (in Chinese)
- Makkar H.P.S., Bluemmei M., Borowy N.K. & Becker K. 1993: Gravimetric determination of tannins and their correlation with chemical and protein precipitation methods. *J. Sci. Food Agric.* 61: 161–165.
- Manly B.F.J., Miller P. & Cook L.M. 1972: Analysis of a selective predation experiment. Am. Nat. 106: 719-736.
- Markkola J., Niemela M. & Rytkonen S. 2003: Diet selection of lesser white-fronted geese *Anser erythropus* at a spring staging area. *Ecography 26: 705–714*.
- McDonald I.W. & Warner A.C.I. 1975: Digestion and metabolism in the ruminant. *University of New England Publishing Unit, Australia*. McKnight S.K. & Hepp G.R. 1998: Diet selectivity of gadwalls wintering in Alabama. *J. Wildlife Manage*. 62: 1533–1543.
- Meng X.X., Zhou C.Q. & Hu J.C. 2006: Musk deer farming in China. Anim. Sci. 82: 1-6. (in Chinese)
- Mohammadzadeh S., Sharriatpanahi M., Hamedi M., Amanzadeh Y., Sadat Ebrahimi S.E. & Ostad S.N. 2007: Antioxidant power of Iranian propolis extract. *Food Chem. 103: 729–733*.
- Nicholson J.W.G., Loosli J.K. & Warner R.G. 1960: Influence of mineral supplements on the growth of calves, digestibility of the rations and intra-ruminal environment. *J. Anim. Sci.* 19: 1071–1080.
- Nieva Moreno M.I., Isla M.I., Sampietro A.R. & Vattuone M.A. 2000: Comparison of the free radical-scavenging activity of propolis from several regions of Argentine. *J. Ethnopharmacol.* 71: 109–114.
- Nolte D.I. & Provenza F.D. 1992: Food preferences in lambs after exposure to flavors in solid foods. *Appl. Anim. Behav. Sci. 32:* 337–347.
- Palo R.T., Gowda J.H. & Hodar J.A. 1997: Consumption of two birch species by captive mountain hares (*Lepus timidus*) in relation to resin and phenolic content. *Gibier Faune Sauvage 14: 385–393*.
- Parry-Jones R. & Wu J.Y. 2001: Musk deer farming as a conservation tool in China. Traffic East Asia.
- Pehrson A. 1983: Digestibility and retension of food components in caged mountain hares *Lepus timidus* during the winter. *Holarctic Ecol. 6: 395–403*.
- Peng C.F. & Cao G.J. 2010: The effect of shrubs forage grass tannins on ruminant animals. *Prataculture Animal Husbandry 11: 21–23.* (in Chinese)
- Prikhod'ko V.I. 2003: Musk deer. Origin, taxonomy, ecology, behavior and communication. GEOS, Moskva: 443. (in Russian)
- Provenza F.D. 1996: Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J. Anim. Sci. 74: 2010–2020.
- Provenza F.D. & Balph D.F. 1990: Applicability of five diet selection models to various foraging challenges ruminants encounter. In: Hughes R.F. (ed.), Behavioural mechanisms of food selection. *Springer, Berlin Heidelberg: 423–460*.
- Ralphs M.H. 1997: Persistence of aversions to larkspur in native and native cattle. J. Range Manag. 50: 367–370.
- Reichlin T., Klansek E. & Hackländer K. 2006: Diet selection by hares, *Lepus europaeus* in arable land and its implications for habitat management. *Eur. J. Wildlife Res.* 52: 109–118.
- Rhind S.M., Archer Z.A. & Adam C.L. 2002: Seasonality of food intake in ruminants, recent developments in understanding. *Nutr. Res. Rev.* 15: 43–65.
- Rogers A.R. 1990: Evaluating preference in laboratory studies of diet selection. Can. J. Zool. 68: 188-190.
- Sheng H.L. & Liu Z.X. 2007: The musk deer in China. The Shanghai Scientific and Technical Publishers, Shanghai.
- Shrestha M.N. 1998: Animal welfare in the musk deer. Appl. Anim. Behav. Sci. 59 (1): 245-250.
- Tixier H., Duncan P., Scehovic J., Yant A., Gleizes M. & Lila M. 1997: Food selection by European roe deer (*Capreolus capreolus*): effects of plant chemistry, and consequences for the nutritional value of their diets. *J. Zool. Lond.* 242: 229–245.
- Verheyden-Tixier H. & Duncan P. 2000: Selection for small amounts of hydrolysable tannins by a concentrate-selecting mammalian herbivore. *J. Chem. Ecol.* 26: 351–358.

- Villalba J.J., Provenza F.D., Hall J.O. & Lisonbee L.D. 2010: Selection of tannins by sheep in response to gastrointestinal nematode infection. J. Anim. Sci. 88: 2189–2198.
- Vourc'h G., Martin J.L., Duncan P., Escarré J. & Clausen T.P. 2001: Defensive adaptations of *Thuja plicata* to ungulate browsing, a comparative study between mainland and island populations. *Oecologia 126: 84–93*.
- Vourc'h G., Vila B., Gillon D., Escarré J., Guibal F., Fritz H., Clausen T.P. & Martin J.L. 2002: Disentangling the causes of damage variation by deer browsing on young *Thuja plicata*. *Oikos 98*: 271–283.
- Ward G.M. 1966: Potassium metabolism of domestic ruminants-a review. J. Dairy Sci. 49 (3): 268-276.
- Workman C. 2010: The foraging ecology of the Delacour's langur, *Tranchypithecus delacouri* in Van Long Nature Reserve, Vietnam. *PhD dissertation, Duke University, North Carolina*.
- Yang G.M., Wang D., Zhang F.F., Tong L. & Cai B.C. 2009: Determination of total flavonoids and phenolic acids from *Oxytropis falcate*. *Pharm. Clin. Res.* 17: 376–379. (in Chinese)
- Yang Q.S., Meng X.X., Xia L. & Feng Z.J. 2003: Conservation status and causes of decline of musk deer (*Moschus* spp.) in China. *Biol. Conserv.* 109: 333–342.
- Zhang L.B. 2008: On the diet composition of musk deer in Feng county, Shanxi province. *Master Thesis, Department of Life Science, East China Normal University, Shanghai, China.*
- Zhu C.S., Wang F.R., Tang Q.S. & Li P. 2012: Research on the diagnosis, treatment, and prevention of common diseases of domestic musk deer. *Chin. J. Wildlife 33: 3–4. (in Chinese)*