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The Use of Herbage N-alkanes as Markers to Estimate the Diet Composition of Yaks on the Qinghai-Tibetan Plateau

L. M. Ding[1, 2, *] and R. J. Long[2, 3]

[1] Key Laboratory of Arid and Grassland Ecology, Ministry of Education, School of Life Sciences, Lanzhou University, Lanzhou, 730000, China

ABSTRACT : The chemical components in plant cuticular wax can be used as markers to estimate the species composition of the diet of grazing animals. In this experiment, composition of the diet of yak on the Qinghai-Tibetan plateau was estimated using n-alkane analysis. During the grazing period, samples of whole plants of the species present, plus fecal samples voided by the yak, were collected, air-dried and ground prior to the extraction of cuticular wax n-alkanes. The species composition of the yak diets was estimated by relating fecal alkane contents to those of the plant species, using the 'EATWHAT' software package. The results showed that the n-alkane technique can detect the main dietary components selected by yak. The diet consumed by yak contained 33% *Kobresia humilis*, 67% *Stipa aliena* in summer pasture; 26% *Potentilla anserine*, 74% *Carex qinghaiensis* in autumn pasture; 52% *Carex qinghaiensis*, 32% *Heteropappus bowerii* and 16% *Saussurea semifasciata* in winter pasture and 5% *Carex qinghaiensis*, 95% *Achnatherum splendens* in spring pasture. The apparent selection for forbs is likely to be a reason for nutritional constraint of yak inhabiting alpine environments. (**Key Words :** Yak, Qinghai-tibetan Plateau, N-alkane, Diet Composition)

INTRODUCTION

The species composition of the herbage available to and consumed by grazing animals is an important variable in studies of their nutritional status. Our understanding of the foraging behaviour of free-ranging herbivores is often constrained by our inability to make meaningful measurements of herbage diet composition. Herbage composition of feed ingested by grazing animals is difficult to assess. Direct observation of animals, use of oesophageally fistulated animals or fecal cuticle analysis have commonly been used, but all of these methods are very tedious, time consuming and/or do not allow differentiation to the level of plant species or plant parts (Dove and Mayes, 1991). Oesophageally fistulated animals have the disadvantage of requiring surgery and might be of restricted use only.

The use of n-alkanes as fecal markers for estimating the diet composition of grazing animals has provided a significant advance in such studies and has been used successfully to estimate the proportions of plant species in the diet of free-ranging herbivores (Dove and Mayes, 1991, 1996; Bugalho et al., 1999). Because the different pasture species differ in their alkane patterns, and alkanes are largely indigestible during their passage through the herbivore's alimentary tract, the composition of a mixed herbage diet can be determined from the patterns of n-alkanes found in herbage and faeces (Dove and Mayes, 1996). The n-alkane technique has been successfully applied to estimate diet composition, intake and digestibility in sheep, red deer and steer (Dove and Mayes, 1991; Duncan and Mayes, 1999; Bugalho and Myes, 2002; Ru et al., 2002; Valiente and Delgado, 2003; Walter Kelman and Miguel Bugalho, 2003; Premaratne et al., 2005).

The experimental site used in the present study lies in an alpine area, and the species composition is complex. The yaks graze in different areas of natural grassland in the different seasons. The aim of the present study was to use the n-alkane method to estimate the diet composition selected by yak when grazing alpine pastures and to explore whether the method can provide accurate assessments of dietary selection by yak under these conditions.

* Corresponding Author: Long Ruijun. Tel: +86-931-8915650, Fax: +86-931-8915650, E-mail: longrj@lzu.edu.cn

[2] International Centre for Tibetan Plateau Ecosystem Management, Lanzhou University, Lanzhou, 730000, China.

[3] College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, China.

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MATERIALS AND METHODS

Study area

The study area was an alpine grassland on the Qinghai-Tibetan plateau, and not very far from Qinghai Lake (37°18′N, 100°16′E) at an altitude of 3,275 m. The region has an alpine climate. Mean annual precipitation is around 327.1 mm and mean annual temperature is -0.5-0.1°C. The lowest temperature in January is about -31.7°C and the highest temperature in August is about 14.5°C. During autumn, the yaks' grazing pasture lies near the bank of Qinghai Lake which abuts with their spring and winter pasture. Their summer pasture is of a swamp type and lies in Qilian mountain valley.

The experiment was conducted from July to August 2004 in summer pasture, during September 2004 in autumn pasture, from October to December 2004 in winter pasture and from March to April 2005 in spring pasture.

Field measurements and preparation of sample

Herbage mass (DM g m^{-2}) was measured by cutting all grass and forb species to ground level within fifteen randomly placed 0.50 m×0.50 m quadrat frames. Thirty herbage or forb species were chosen to measure height with a ruler in different pastures. The cover of each species was measured using 10 equal grid portions with a 1 m×1 m quadrat. Samples of the most common individual herbage species were also collected at the same time. All the samples were ground through a 1 mm screen. One sample of each species was dried in a forced draught oven at 60°C for 24 h to calculate DM, then combusted in a muffle furnace to calculate organic matter (OM). Sub-samples were air-dried before determination of acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), fat and n-alkane concentrations. The species in summer pasture were: *Kobresia huimlis*, *Stipa aliena*, *Lancea tibetica*, *Kobresia pygmaea*, *Gentiana squarrosa*, *Leontopodium leontopodioides*, *Thalictrum alpinum*, *Oxytropis deflexa*, *Saussurea superba*, *Potentilla fruticosa*, *Taraxacum brevirostre*, *Potentilla anserine*, *Poa calliopsis*, *Potentilla multifida*, *Leontopodium nanum*. The species in autumn pasture were: *Batrachium bungei*, *Potentilla anserine*, *Kobresia pygmaea*, *Setaria viridis*, *Carex qinghaiensis*, *Triglochin palustre*. The species in winter pasture were: *Carex qinghaiensis*, *Stipa cryloviі*, *Achnatherum inebrians*, *Pedicularis alaschanica*, *Heteropappus altaicus*, *Stellera chamaejasme*, *Saussurea semifasciata*, *Artemisia frigida*, *Agropyron cristatum*.

Fresh faeces were collected by following eight different yaks on different days in the same period. Visible non-faeces objects were avoided when taking fecal samples from the ground. Fecal samples were air-dried before analysis of n-alkane concentrations. One part of the fecal sample was dried in the oven at 60°C for 24 to determine DM.

N-alkane analysis

The alkane extraction procedure was carried out using a modification of the method of Mayes et al. (1986) and Dove et al. (2006). Dried and milled herbage samples (2.0 g) and fecal samples (1.0 g) were weighed along with an internal standard (tetracosane, C24 alkane), into screw-capped flasks and were heated with 1 mol L^{-1} ethanolic KOH solution (13 ml) for 48 h at 90°C. After cooling to 55°C, 3 ml water and 8 ml heptane were added to each flask and the flasks were shaken vigorously. The top, non-aqueous layer was removed and evaporated to dryness, re-dissolved in 2 ml heptane and applied to a silica-gel column (Silica gel 60, Qingdao haiyang Chemical co., Ltd., 5 ml syringe). The hydrocarbons were eluted from the columns with heptane, concentrated to about 20 ml, evaporated and re-dissolved in 1 ml heptane. The solution was transferred to a centrifuge tube for injection into a gas chromatograph.

The hydrocarbons in the purified extracts were analysed by gas chromatography on a ThermoFinnigan gas chromatograph (Trace-GC2000) fitted with a flame ionization detector. The column was a 30 m×0.53 mm inside-diameter capillary column type SPB1 with 1 μm film thickness; Lanzhou Institute of Chemical Physics, China), temperature-programmed from 210 to 295°C at 6°C min^{-1}. The carrier gas was nitrogen (30 ml min^{-1}). The injector and detector were maintained at 300°C throughout the whole process. The chromatographic data was processed using Trace GC software (Chrom-chard). Detector response factors for individual n-alkanes were determined by injecting onto the chromatograph a standard n-alkane mixture (C_{29}, C_{31}, C_{32}) after every 10 sample extracts.

Estimation of diet composition

The concentrations of the n-alkanes, from both fecal and vegetation samples, were compared iteratively within a least-squares optimization routine. The non-negative least-squares procedure was implemented using the software EATWHAT (Dove and Moore 1995).

RESULTS

Height, cover, DM and chemical composition of the herbage

The chemical composition of herbage species is presented in Table 1. Generally, the content of NDF in the species in winter and spring pasture was higher than in summer and autumn pasture. High CP contents were found in some leguminous forages, such as *Oxytropis deflexa* and *Taraxacum brevirostre*, in the summer pasture. The height, coverage and DM weight of the main species in the four

Table 1. Nutritional composition of the forages (DM basis) (mean±SD) (n = 3)

Species	ADF (%)	NDF (%)	CP (%)	OM (%)
In summer pasture				
Kobresia humilis	27.83±0.55	47.43±0.12	7.95±0.09	93.25±0.95
Stipa aliena	27.40±1.87	50.74±3.20	7.59±0.33	94.29±0.50
Lancea tibetica	25.44±0.97	39.83±7.97	9.32±0.47	94.02±1.20
Kobresia pygmaea	21.97±0.47	49.37±0.40	10.60±0.54	92.06±0.52
Gentiana squarrosa	23.23±0.40	38.86±0.55	10.64±0.59	91.23±0.57
Leontopodium leontopodioides	24.91±0.74	42.40±0.71	8.19±0.29	90.76±0.03
Thalictrum alpinum	23.40±0.48	40.57±0.16	9.67±0.29	93.43±0.74
Oxytropis deflexa	23.43±1.20	43.78±0.92	15.72±0.38	92.02±0.38
Saussurea superba	21.29±0.88	35.05±7.19	7.02±0.07	93.35±1.06
Potentilla fruticosa	23.74±0.45	40.16±0.68	7.85±0.50	91.12±1.42
Taraxacum brevirostre	20.72±0.26	42.05±0.21	14.38±1.00	91.94±0.29
Potentilla anserina	29.57±0.71	41.03±0.61	10.07±0.38	91.22±0.55
Poa calliopsis	24.40±2.36	38.88±0.19	14.04±0.31	91.97±0.07
Potentilla multifida	23.68±0.61	43.67±1.28	11.09±1.18	92.4±0.17
Leontopodium nanum	20.01±1.00	39.81±1.07	8.35±0.40	93.03±0.23
In autumn pasture				
Batrachium bungei	23.90±2.96	40.78±1.28	8.90±0.42	92.77±0.19
Potentilla anserina	27.83±1.11	46.17±1.71	9.20±0.10	93.51±0 69
Kobresia pygmaea	23.00±068	44.44±2.55	9.33±0.10	93.28±0.48
Setaria viridis	27.36±0.29	42.64±0.19	9.44±0.10	94.02±0.21
Carex qinghaiensis	26.79±0.23	48.68±1.49	10.59±0.48	91.37±0.81
Triglochin palustre	23.44±0.38	45.05±1.92	9.42±0.10	93.58±0.03
In winter pasture				
Pedicularis alaschanica	26.77±1.45	49.38±0.68	9.38±0.57	95.50±0.52
Heteropappus bowerii	27.41±0.87	43.32±0.55	9.55±0.42	93.00±0.47
Stipa crylovii	27.48±0.94	40.60±0.09	6.76±0.31	94.12±0.28
Stellera chamaejasme	27.35±1.00	58.37±0.59	6.69±0.40	94.83±0.23
Saussurea semifasciata	26.15±1.07	38.92±0.02	9.09±0.69	92.74±0.62
Artemisia frigida	28.82±0.14	38.49±1.00	9.58±0.02	92.91±0.38
Achnatherum inebrians	26.31±0.66	45.92±0.92	5.44±0.38	95.50±0.21
Agropyron cristatum	30.65±1.23	51.52±0.81	4.88±0.17	91.50±0.19
Carex qinghaiensis	29.81±0.23	50.21±0.31	8.96±0.21	93.82±0.36
In spring pasture				
Carex qinghaiensis	32.11±0.23	53.89±0 .40	5.44±0.38	94.05±0.69
Achnatherum splendens	24.70±0.02	51.27±0.83	4.07±0.02	94.83±0.29

pastures are presented in the four graphs (Table 2).

N-alkane concentration

Concentrations of C29 and C31 alkanes were highest in the herbage group, and the concentration of C32 alkane in most species was very low and almost zero. Particular alkanes differentiated individual species (Table 3). In summer pasture, *Kobresia huimlis* and *Saussurea superba* had the highest concentrations of C29 alkane; whilst *Gentiana squarrosa* and *Leontopodium leontopodioides* had the highest concentrations of C31 alkane, compared with other species (Table 3). In autumn pasture, the highest concentrations of C29 alkane were found in *Batrachium bungei*, *Setaria viridis* and *Triglochin palustre*. In the autumn, *Potentilla anserina* had a higher concentration of C31 alkane than any other species (Table 3). The mean concentration of n-alkanes was lower in winter pasture than in summer and autumn pasture. In winter, most species had the highest C31 concentration except for *Stellera chamaejasme*, *Artemisia frigida* and *Achnatherum inebrians*. *Saussurea semifasciata* had a higher concentration of C29 alkane than any other species (Table

Table 2. The height (cm), cover (%) and herbage dry matter mass (DM, g m^{-2}) of predominant species in summer, autumn, winter and spring pasture (mean±SD)

Species	Height (cm) n = 30	Cover (%) n = 10	DM (g m^{-2}) n = 15
In summer pasture:			
Kobresia humilis	3.03±0.98	53.83±8.26	44.64±5.69
Stipa aliena	7.43±1.31	8.17±2.25	5.90±2.83
Lancea tibetica	0.48±0.49	6.67±1.42	11.60±2.32
Kobresia pygmaea	1.50±0.22	32.00±8.66	29.52±4.26
Gentiana squarrosa	1.58±0.60	1.50±0.66	0.47±0.46
Leontopodium leontopodioides	3.16±0.71	6.13±1.30	5.40±1.36
Thalictrum alpinum	0.99±0.11	15.50±1.01	5.70±1.59
Oxytropis deflexa	3.96±0.76	0.83±0.00	1.30±0.08
Saussurea superba	0.87±0.22	5.83±3.79	9.19±1.43
Potentilla fruticosa	2.90±0.42	0.50±0.00	3.04±0.77
Taraxacum brevirostre	1.26±0.33	2.70±0.00	1.36±0.23
Potentilla anserina	1.35±0.38	1.00±0.00	5.40±0.58
Poa calliopsis	1.52±0.49	10.33±2.19	1.88±0.50
Potentilla multifida	1.28±0.33	25.17±6.61	6.45±1.94
Leontopodium nanum	1.00±0.21	10.67±2.21	4.08±0.97
In autumn pasture:			
Batrachium bungei	2.63±0.65	10.5±1.33	2.72±0.62
Potentilla anserina	5.03±0.98	30.4±3.10	20.13±5.38
Kobresia pygmaea	3.72±0.87	24.4±1.77	39.70±4.69
Setaria viridis	32.46±4.30	2.40±0.06	2.97±0.89
Carex qinghaiensis	9.41±1.36	28.40±4.40	38.18±6.12
Triglochin palustre	2.59±0.71	5.20±0.57	3.40±1.08
In winter pasture:			
Pedicularis alaschanica	5.61±0.85	12.46±1.90	6.01±0.46
Heteropappus bowerii	13.43±1.78	16.09±3.38	15.05±4.96
Stipa crylovii	2.48±0.76	19.69±3.95	10.44±4.14
Stellera chamaejasme	16.81±0.54	7.53±1.77	8.77±2.48
Saussurea semifasciata	5.92±3.87	28.49±6.36	7.88±1.98
Artemisia frigida	22.06±6.21	13.81±5.06	3.43±1.39
Achnatherum inebrians	14.77±2.61	8.64±2.09	5.41±0.58
Agropyron cristatum	17.55±2.90	2.16±0.25	2.01±1.05
Carex qinghaiensis	5.87±0.36	43.17±6.99	26.03±8.17
In spring pasture:			
Carex qinghaiensis	12.64±1.89	34.01±7.50	41.74±9.45
Achnatherum splendens	65.41±5.25	67.35±11.52	45.32±6.89

3). Two species were measured in spring pasture. The higher concentration of C29 was found in *Carex qinghaiensis*. However, no C31 or C32 were found in this species (Table 3).

Composition of the diet

Although fifteen species were analysed from summer pasture, the EATWHAT software showed that there were two species (*Kobresia huimlis*, *Stipa aliena*) in yak diet composition (Figure 1). *Kobresia humilis* was 33%, and *Stipa aliena* was 67% of the diet. *Potentilla anserine* (34%) and *Carex qinghaiensis* (66%) were detected in the yak diet composition in autumn pasture (Figure 2). The yak diet contained three species in winter pasture (Figure 3): *Heteropappus bowerii*, *Saussurea semifasciata*, and *Carex qinghaiensis*. The proportion of *Carex qinghainsis* was the highest (52%) and that of *Heteropappus bowerii* and *Saussurea semifasciata* was 32% and 16%, respectively. In

Table 3. The n-alkane content of species in summer, autumn and winter pasture (mg/kg DM) (mean±SD) (n = 2)

Species	C29	C31	C32
In summer pasture			
Kobresia humilis	368.5±85.3	143.5±11.8	0
Stipa aliena	334.2±23.4	649.5±27.7	0
Lancea tibetica	64.9±6.25	200.4±12.8	20.3±1.5
Kobresia pygmaea	306.1±17.1	454.7±22.1	0
Gentiana squarrosa	322.3±19.7	1,135.2±29.7	50.1±4.0
Leontopodium leontopodioides	593.7±43.1	529.7±40.3	24.9±1.6
Thalictrum alpinum	278.9±10.0	474.2±18.8	28.9±1.7
Oxytropis deflexa	160.7±9.4	157.6±8.2	0
Saussurea superba	173.5±7.4	60.01±2.91	0
Potentilla fruticosa	473.6±19.8	189.7±10.9	0
Taraxacum brevirostre	476.5±20.9	300.8±36.1	18.8±0.1
Potentilla anserina	0	210.1±14.7	24.7±1.3
Poa calliopsis	360.9±17.1	181.9±12.5	0
Potentilla multifida	347.9±15.8	169.7±10.3	0
Leontopodium nanum	693.3±47.6	962.4±53.9	36.6±3.7
In autumn pasture			
Batrachium bungei	361.1±8.2	0	252.8±10.8
Potentilla anserina	540.5±14.2	163.6±5.6	19.3±2.6
Kobresia pygmaea	125.3±5.4	52.3±5.3	0
Setaria viridis	323.9±6.1	0	0
Carex qinghaiensis	717.0±7.3	58.0±3.1	0
Triglochin palustre	281.1±6.2	0	0
In winter pasture			
Pedicularis alaschanica	223.5±11.8	507.8±6.9	62.2±5.1
Heteropappus bowerii	395.8±4.7	807.8±8.3	2.3±0.1
Stipa crylovii	367.4±8.3	737.0±7.2	16.2±1.3
Stellera chamaejasme	315.7±9.8	16.7±1.7	32.9±2.7
Saussurea semifasciata	955.6±10.0	688.5±11.8	25.7±2.5
Artemisia frigida	236.4±3.8	91.8±6.3	0
Achnatherum inebrians	100.3±4.8	5.2±0.1	0
Agropyron cristatum	134.9±7.5	231.2±6.1	0
Carex qinghaiensis	284.7±5.2	804.8±8.7	175.1±9.3
In spring pasture			
Carex qinghaiensis	603.5±16.8	0	0
Achnatherum splendens	113.1±5.2	379.7±3.3	0

spring pasture, a high proportion (95%) of *Achnatherum splendens* was found in the diet composition. The other species was *Carex qinghaiensis* with 5% proportion (Figure 4).

DISCUSSION

The results of this study indicate that the differences between species in their cuticular wax alkane levels can be exploited successfully to estimate the main species composition of mixtures of herbage. Species other than those detected by the EATWHAT software were present in the diet, but only in small quantities. These species may be detected under the conditions of a large range of alkanes analysed. If we want to measure the major diet composition under grazing conditions, the n-alkane technique and the 'EATWHAT' software are a good choice.

The botanical composition of the diet of large herbivores can sometimes be similar to the botanical composition of the top layer of sward (Hodgson, 1986). *Carex qinghaiensis* was one of the most abundant browse species in autumn and winter pasture and the proportion in

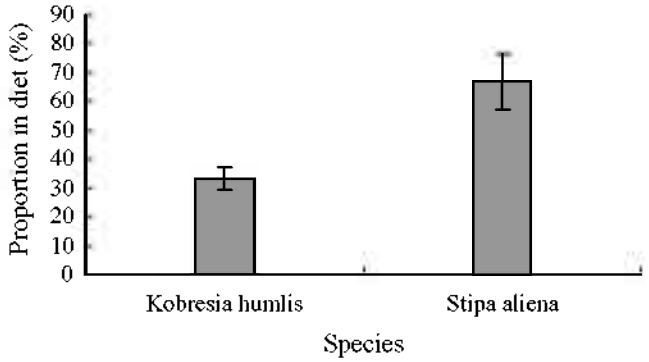

Figure 1. Composition of the diet of yak during summer pasture. Vertical line on each bar represents standard error of the mean (n = 8).

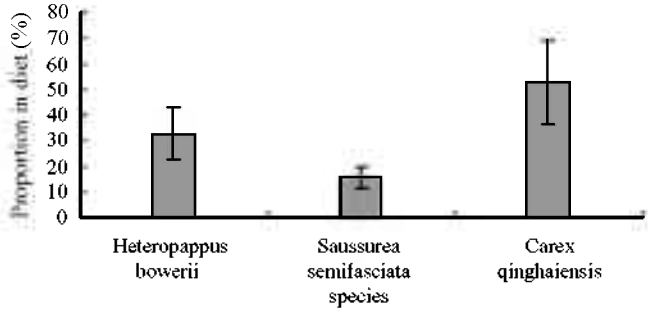

Figure 3. Composition of the diet of yak during winter pasture. Vertical line on each bar represents standard error of the mean (n = 8).

the diet of yak remained relatively constant. On the spring pasture, *Achnatherum splendens* provided the greatest cover and herbage mass of the two species present, and constituted the majority (95%) of the diet selected. The dominant species, *Kobresia humilis* was just a small proportion of the diet compared with *Stipa aliena* on summer pasture. The reason is that *Stipa aliena* has a good texture, and the height of *Kobresia humilis* is too low for yaks to graze (Table 2).

In this study we also found that yak consumed certain forbs in most circumstances, e.g. *Potentilla anserine*, *Heteropappus bowerri*, *Saussurea semifasciata*. The ingestion of forbs could be partially related to the requirements of yak for crude protein, a limiting factor in the nutrition of ruminants. Usually, concentrations of crude protein are very low in the herbage group layer, especially the senescent herbage layer (Fonseca, 1998). This is also proved by our results (Table 1). For example, in winter pasture, *Heteropappus bowerii* and *Saussurea semifasciata* contain relatively high levels of crude protein and are an important source of crude protein for yak. However, although *Artemisia frigida* also contains high levels of crude protein, the yaks rarely choose it. We found it has a strong smell which may have inhibited selection by the yak. The other forbs were not detected in the yak diet. The proportion or the height of these species is very low in the pasture. For example, the height of *Stipa crylovii* was lower than other herbage species; the herbage mass of *Pedicularis alaschanica* was lower than other species; and the cover of *Pedicularis alaschanica*, *Stellera chamaejasme*, *Achnatherum inebrians* and *Agropyron cristatum* was very low (Table 2). On the other hand, these species may contain some anti-nutritional compounds that have inhibitory effects on grazing animals. Further research remains to be investigated on this issue.

The alpine environment is very harsh, with low oxygen, low temperature and high ultraviolet radiation. In order to maintain a sustainable grazing ecosystem, effective and reasonable grazing management is indispensable. Although summer pasture is a growing season, the stocking rate has to be controlled in order to maintain good pasture, for example, to reduce the number of livestock and retain enough forage in the ground. There is a lack of high quality food provided by winter and spring pastures when most of the herbage layer becomes senescent. Yaks are likely to suffer nutritional constraints before re-growth of the

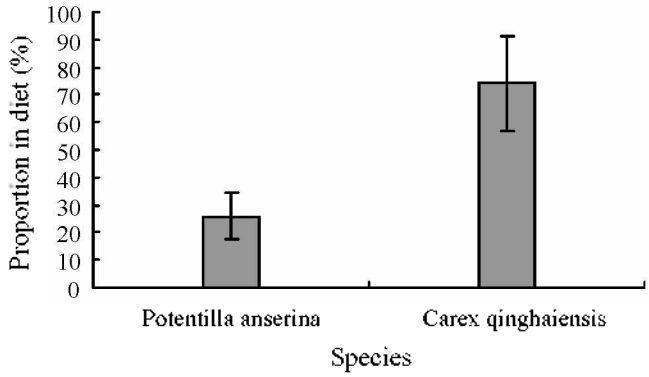

Figure 2. Composition of the diet of yak during autumn pasture. Vertical line on each bar represents standard error of the mean (n = 8).

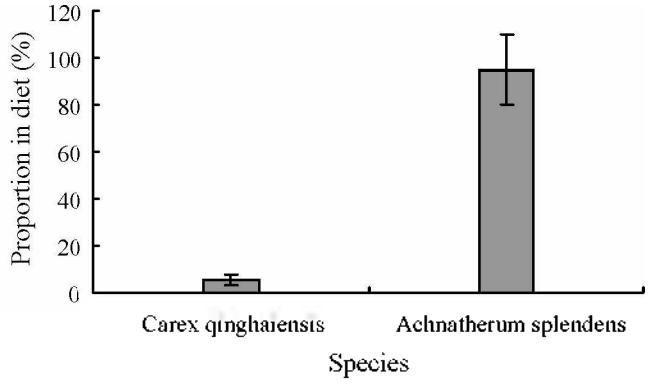

Figure 4. Composition of the diet of yak during spring pasture. Vertical line on each bar represents standard error of the mean (n = 8).

herbage layer towards the end of the spring season. Supplementary feeding at the end of the winter and in the early spring or, if economically viable, irrigation or fertilizer application to pastures, may improve the capacity of grassland by providing additional food to yak.

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