

Contents lists available at ScienceDirect

Animal Nutrition



journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original research article

Estimation of herbage intake and digestibility of grazing sheep in Zhenglan Banner of Inner Mongolia by using n-alkanes



Changqing Li ^{a, b}, Alatengdalai ^b, Shuyuan Xue ^b, Atsushi Tajima ^a, Naoto Ishikawa ^{a, *}

- ^a Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8577, Japan
- ^b Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences, Hohhot 010031, China

ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form
29 October 2015
Accepted 1 November 2015
Available online 12 December 2015

Keywords: N-alkane technique Diet composition Grazing sheep Herbage intake

ABSTRACT

To reveal the seasonal dynamics of herbage intake, diet composition and digestibility and clarify the relationship of those with herbage nutrient and botanical composition of grazing sheep in Zhenglan Banner of Inner Mongolia, the n-alkane technique was used to test in sheep grazed during June, August and December. The results showed that the sheep mainly ate *Fringed sagebrush*, *Stipa krylovii* and *Carex* in proportions of 33.5, 17.9 and 21.2%, respectively, in spring. In summer, the sheep consumed cleistogenes, *Potentilla tanacetifolia, Thyme*, etc; the intake of *Fringed sagebrush*, *Carex* and *Stipa* declined. In winter, *Fringed sagebrush* accounted for 50.1% of herbage intake, and the intakes of *Cleistogenes* and *Stipa krylovii* increased to 15.3 and 18.4%, respectively. Herbage intake by the sheep in spring was 1.8 kg DM/d, and digestibility was 71.4%. Herbage intake and digestibility decreased slightly to 1.7 kg DM/d and 68.4% during the summer, respectively and decreased significantly to 1.2 kg DM/d and 36.4% in winter. There were significant correlations between diet composition and CP content in winter, diet composition and botanical composition in summer. A highly positive correlation between herbage intake and digestibility was observed in grazing sheep.

© 2015, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The malnutrition of sheep during autumn to winter season is often observed in the northern area of China because the animals are generally raised under the condition of the natural grassland throughout the year. In order to improve the performance of sheep production in this region, it is essential to know the nutritional level in sheep throughout a year. Nutrient intake depends on the plant species, herbage intake and digestibility of grazing sheep. However, it is very hard to estimate the herbage intake, diet composition and digestibility of livestock in grazing condition. Although many scientists performed plenty of research on many

* Corresponding author.

E-mail address: ishikawa.naoto.ke@alumni.tsukuba.ac.jp (N. Ishikawa).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



Production and Hosting by Elsevier on behalf of KeAi

proposed estimating methods, most methods have shown numerous limitations, such as low accuracy, complicated procedure, tedious and expensive (Wang, 1995). The n-alkane technique, on the other hand, has proven to be effective and accurate for estimating herbage intake, diet composition and digestibility of grazing sheep (Mayes et al., 1986; Dove, 1992; Mayes and Dove, 2000). In previous experiments, estimates of herbage intake using the radio C33:C32 were the most accurate among the other combination of C31:C32 (Zhang, 2002). Therefore, in the present study, the alkane pair C32:C33 with C33 as an internal marker was used to estimate herbage intake, diet composition and digestibility in grazing sheep.

It was well documented herbage intake is influenced by many factors, including the species of grass, location, growth stage and region, environmental condition, and analytical method (Laredo et al., 1991; Olivan and Osoro, 1999; Kelman et al., 2003; Dove et al., 1996). Considering the alkane recovery rate in the feces, the sampling for multiple plants and the growing period of herbage (Liu et al., 2006), only 8 species of herbage were chosen in this study. Herbage intake, diet composition and digestibility of sheep grazing on the arid grassland were determined in a recent study in Siziwang Banner, in western Inner Mongolia (Hu et al., 2014). The

results of n-alkane patterns were consistent with those reported by other researchers (Wang, 2000), proving the feasibility of detecting diet composition of grazing sheep using the n-alkane technique in Inner Mongolia. The results of Hu et al. (2014) showed that daily DM intake and DM digestibility of sheep decreased significantly from summer to winter, and a diet composition analysis indicated that *Artemisia frigida Willd* was the most dominant diet component in the arid steppe of Siziwang Banner. However, information on seasonal changes of herbage intake and digestibility in sheep is not available in Xilingol Leagure which is the center of livestock production in Inner Mongolia. Traditionally, Zhenglan Banner, located in the grassland of Xilingol, is considered to be the typical steppe (Hu and Gao, 1995). However, the herbage species are complex. There is no study that has estimated the intake and digestibility of herbage in sheep grazed in Xilingol.

Therefore, the aim of this study was to verify the seasonal changes of herbage intake, diet composition and digestibility of grazing sheep, and clarify the relationship of those with herbage nutrient and botanical composition in the grassland of Xilingol League.

2. Materials and methods

2.1. Experimental site

The experimental site, Zhenglan Banner (116.02°E, 42.25°N), located in the central of Inner Mongolia, China, is a typical steppe. The dominant species there include *Stipa krylovii*, *Fringed sagebrush*, *Leymus chinensis*, *Cleistogenes*, and *Carex*; and the major accompanying species are *Thyme*, *Potential bifurca*, *Potentilla tanacetifolia*, *Agropyron cristatum*, etc. The plants are generally 6 to 25 cm in height with average vegetation coverage of 25 to 35%.

2.2. Application of the n-alkane technique

Six grazing Mongolian one-year-old female sheep (average body weight of 45.0 ± 1.0 kg) were on pasture from June to early December on the experimental site. The sheep were free to roam all day long from summer to winter in three experimental periods: spring (from middle May to early June), summer (August) and winter (December). No supplementary feed was supplied throughout the experiment periods. In each experimental period, all sheep were given one n-alkane capsule (97% purity, Acros Organics, NJ, USA; including 60 mg C32) every 15 d from the start of experiment to the end of experiment. Digestibility and diet composition in the grazing sheep were determined using the n-alkane technique as described by Hu et al. (2014).

2.3. Collection of herbage and fecal samples

Herbage samples were collected manually. The species, positions and heights of the herbage that the sheep ate were observed for 5 min every 2 h in grazing for 5 d. Meanwhile, the herbage samples mimicking what the sheep were grazing on were collected, weighed, mixed and reserved at -20° C. Fecal samples were taken from the rectums of the sheep. Fecal sample (10 g) of each tested sheep was collected 3 times a day for 5 d in 12 time points, with an interval of 2 h. The samples were reserved in sealed bags at -20° C.

The herbage and fecal samples were analyzed for crude protein (CP), DM, Ca and P according to Association of Official Analytical Chemists (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and ether extract (EE) were determined following Van Soest (1991). Metabolizable energy concentration in the diet was calculated from apparent DM digestibility estimated using C33 alkane.

2.4. N-alkane determination and calculations

The samples were pretreated using the method proposed by Dove and Mayes (2005) and assayed using a Shimadzu GC-9A gas chromatograph.

The herbage composition was calculated using the suggested program (Eat What) proposed by Dove and Moore (1995). The dry matter intake was calculated from the pair of alkane C33 (naturally present in the herbage) and C32 alkane (dosed): DMI = D32 \times F33 /(F32 \times H33 – F33 \times H32). Where DMI is daily dry matter intake (kg/d), D32 is amount of C32 alkane dosed daily (mg/d), F33 and F32 are fecal concentrations of C33 and C32 alkanes (mg/kg DM), H33 and H32 are the herbage concentrations of C33 and C32 alkanes (mg/kg DM), respectively.

DM digestibility was calculated using C33 as an internal marker using the following formula: DMD $= 1 - (I33 \times FR33)/F33$. Where FR33 represents the faecal recovery of C33 alkane, and I33 is the dietary C33 concentration.

The recovery rates, as provided by Nigel et al. (2000) for C31, C32, C33, and C35 were 0.76, 0.87, 0.85 and 0.81, respectively.

2.5. Statistical analysis

Statistical analyses were performed using SPSS19 (SPSS Inc., Ireland). Effect of season on DM intake, DMD and diet composition was analyzed by means of one-way analysis of variance (ANOVA). Significance levels were taken at P < 0.05 to P < 0.01. Multiple comparisons between means were made using the Duncan's multiple range test.

3. Results

3.1. Alkane patterns of herbage and feces

The average n-alkane content of the herbage consumed by the sheep is shown in Table 1. As expected, the contents of C29, C31 and C33 were the highest in all herbage species. The content of C32 alkane in most species was very low. It showed that each herbage species had a specific alkane pattern. *Stipa krylovii* and *Fringed sagebrush* had the highest concentration of C31 in all seasons. *Potentilla tanacetifolia* had a higher concentration of C33 than other species. The concentration of alkanes differed in different seasons. The concentration of C31 in *Stipa krylovii* and *Fringed sagebrush* declined from spring to winter, whereas the opposite was true from spring to summer. The fecal n-alkane concentrations in different seasons are presented in Appendix Table A.

3.2. Diet composition in the grazing sheep

The diet composition in the grazing sheep was changed dramatically in different seasons (Table 2). In the present study, Fringed sagebrush was the most important dietary component for sheep in three seasons. Fringed sagebrush constituted 33.5, 28.7 and 50.1% of the sheep's diet in summer, autumn and winter, respectively. Carex was a minor component of sheep diet in three seasons. In summer, the sheep showed major preference for Fringed sagebrush, which together with Carex, Stipa krylovii and Leymus chinensis, comprised more than 90% of the diet. In summer and winter, the sheep consumed mostly Fringed sagebrush (28.7 and 50.1%), Carex (18.4 and 19.2%) and Stipa krylovii (14.8 and 15.4%). The Leymus chinensis and Potentilla tanacetifolia were consumed only in spring, whereas Potential acaulis and Thyme were consumed only in summer. In spring and summer, the botanical composition of experiment site was dominated by Fringed sagebrush, Carex, Stipa krylovii, rare and inedible herbage species. The rare and inedible

Table 1N-alkanes concentration of different herbages species of Zhenglan Banner in different seasons (mg/kg DM).

Seasons	Name	C25	C27	C29	C31	C32	C33	C35
Spring	Carex	14.51	23.22	87.91	169.77	1.56	154.71	23.65
	Cleistogenes	25.54	76.28	421.39	554.76	0.34	257.95	9.28
	Fringed sagebrush	31.31	34.33	149.45	993.94	0.39	292.73	5.43
	Leymus chinensis	37.41	94.57	165.51	232.32	0.76	116.87	23.73
	Potential bifurca	5.09	58.89	75.41	172.76	9.03	305.12	55.65
	Potentilla tanacetifolia	19.48	53.18	233.45	590.26	12.77	390.45	7.95
	Stipa krylovii	17.36	41.89	306.07	1,021.65	0.87	208.64	19.17
	Thyme	53.44	88.74	302.82	286.69	13.64	363.3	31.72
Summer	Carex	6.38	15.35	224.65	412.28	0.59	496.1	9.58
	Cleistogenes	83.28	151.03	183.78	352.92	2.58	413.31	14.93
	Fringed sagebrush	24.65	53.53	165.76	357.22	1.83	141.32	9.01
	Leymus chinensis	26.21	66.9	136.65	203.59	2.54	232.34	14.93
	Potential bifurca	16.76	49.54	129.32	116.74	2.2	282.33	8.49
	Potentilla tanacetifolia	25.7	49.28	176.8	246.09	1.55	367.64	8.65
	Stipa krylovii	24.81	76.57	172.76	552.98	1.37	201.13	4.92
	Thyme	36.16	66.37	320.99	459.5	9.2	471.4	23.84
Winter	Carex	14.54	33.87	99.62	213.24	0.55	126.96	15.66
	Cleistogenes	10.83	33.52	86.39	345.08	1.86	76.65	9.98
	Fringed sagebrush	18.95	31.05	97.38	234.15	0.78	20.78	7.49
	Leymus chinensis	7.29	22.65	165.44	254.67	0.41	113.59	9.75
	Stipa krylovii	15.43	49.17	253.23	264.47	0.65	564.31	31.28

Table 2The diet composition of grazing sheep and botanical composition in different seasons in Zhenglan Banner (%).

Seasons	Item	Fringed sagebrush	Cleistogenes	Potential acaulis	Carex	Stipa krylovii	Leymus chinensis	Potentilla tanacetifolia	Thyme	Rare and inedible herbage
Spring	Diet composition ² Botanical composition	33.5 ± 1.3 ^b 13	-	_	21.2 ± 1.1 12	17.9 ± 1.0 ^a 14	17.6 ± 1.0 9	9.8 ± 0.5 10	-	44
Summer	Diet composition Botanical composition	28.7 ± 2.1 ^b 15	10.8 ± 0.9^{b}	8.1 ± 1.0 7	18.4 ± 0.9 13	14.8 ± 1.2 ^b 10	-	-	19.2 ± 0.8	41
Winter	Diet composition Botanical composition	50.1 ± 1.4^{a} 36	15.3 ± 0.9 ^a 15	_	19.2 ± 1.2 10	15.4 ± 1.1 ^a 25	_	_	_	14

 $^{^{}a,b}$ The different letters indicate significant differences in different seasons at P < 0.05.

herbage species accounted for 44 and 41% in spring and summer, when the diversity of herbage species was higher. In winter, only *Fringed sagebrush* (36%), *Cleistogenes* (15%), *Carex* (10%) and *Stipa krylovii* (25%) were detected in the botanical composition. The botanical composition of the experiment site was scarce, and the proportion of rare and inedible herbage species was only 14% of the pasture.

3.3. Herbage intake and digestibility

It was observed that herbage intake reached 1.83 kg/d in spring, followed by 1.73 kg/d in summer and 1.21 kg/d in winter. Herbage intake did not differ in spring and summer (P > 0.05) but it was significantly different in winter (P < 0.05) (Table 3). As pasture grows, its digestibility deteriorates. The digestibility of herbage species in spring, summer and winter were 71.44, 68.39 and 36.37%, respectively. The dry weight:fresh weight ratio of grass increased from spring to winter, and it differed significantly between summer and winter. As expected, grass production was significantly low in winter (P < 0.05). Herbage digestibility in spring and summer did not show any difference, but it differed significantly in winter (P < 0.05). Herbage intake correlated significantly with digestibility, but not with dry weight:fresh weight ratio or with grass production.

The nutritional components of the pastures are presented in Appendix Table B. In the present experiment, as the season changed from summer to autumn and then to winter, CP and ME values decreased in all herbage species, while the NDF and ADF levels

increased. The CP, NDF, and ADF of pastures significantly varied during the experimental period. The present research showed a significant correlation between diet composition and the CP content of pasture during winter (Table 4). The correlation between diet composition and the proportion of grass was high for all seasons, but it was only significant for summer. There was no significant correlation between diet composition and NDF.

4. Discussion

The lack of knowledge of diet composition and nutritional status of sheep in different seasons has restricted the development of sustainable grazing systems in China. In northern china, sheep suffer from exposure to extend periods of nutrient deficiencies, in some cases, up to 7 months of the year. Therefore, it is important that grazing systems are designed to make the best use of the nutrients supplied by the native pasture during the short period of abundance during the spring and summer. The feed intake of grazing sheep is difficult to estimate. However, the discovery that different herbage species contain unique fingerprints of alkanes has revolutionized the ability for scientists to determine the intake of different pasture species by grazing animals (Malossini et al., 1990, Dove and Mayes, 1991; Hameleers and Mayes, 1998). Since the original work quoted above, the alkane technique has been widely used to assess herbage intake, DM digestibility and diet composition in grazing animals (Kelman et al., 2003; Newman et al., 1995; Zhang, 2002). The alkane recovery rates tend to increase with

¹ Botanical composition: the proportion of each grass in the steppe.

² Diet composition: the proportion of each grass in the diet. The diet composition was calculated using the suggested program (Eat What) proposed by Dove and Moore (1995).

 Table 3

 The herbage intake and digestibility in grazing sheep and characteristics of natural pasture in different seasons.

Item	DM intake, kg DM/d	DM digestibility, %	Dry weight/fresh weight, %	Grass production, g/m ²
Spring	1.8 ± 0.1^{a}	71.4 ± 1.6^{a}	47.45 ± 5.75 ^a	93.74 ± 5.4^{a}
Summer	1.7 ± 0.1^{a}	68.4 ± 1.3^{a}	59.28 ± 4.18^{b}	111.39 ± 6.0^{a}
Winter	1.2 ± 0.1^{b}	36.4 ± 1.2^{b}	63.28 ± 3.29^{b}	58.67 ± 4.0^{b}
Correlation				
DM intake, kg DM/	d	0.997*	-0.798	0.882

^{*} The star letters indicate significant differences of correlation in different seasons at P < 0.05. DM = dry matter.

Table 4The correlation between diet composition and CP, NDF or the botanical composition of Zhenglan Banner in different seasons.

Seasons	CP	NDF	Botanical composition
Spring	0.17	0.05	0.52
Summer	-0.26	0.53	0.97*
Winter	0.92*	-0.55	0.85

 $[\]mbox{CP} = \mbox{crude protein}; \mbox{NDF} = \mbox{neutral detergent fiber}.$

carbon-chain length (Sun et al., 2008). A similar trend was observed in a recent study in Inner Mongolia (Hu et al., 2014) and this study.

To our knowledge, there are no available data on the botanical composition of the diet selected by sheep grazing on the Inner Mongolia typical steppe. The results clearly demonstrated that diet components selected by sheep differed between seasons. Fringed sagebrush, Carex and Stipa krylovii were the main herbage species for all seasons, presenting 90% of the diet during the spring. Leymus chinensis and Potentilla were selected by sheep only in the spring. Thyme, Potential acaulis and Cleistogenes became the main proportion of the diet in the summer. These seasonal changes might be modulated by plant palatability, particularly plant proportion and the radio of dry weight to fresh weight. Dziba et al. (2003) and Rosa et al. (2002) thought it might be modulated by plant height and aromatic compounds present in it. However, in this study, the effect of height and flavors on diet selection were not investigated. Nevertheless, there was a significant correlation between botanical composition and CP content. The characterization of diet components of sheep grazed on an Inner Mongolian steppe was the key aim of this study. The experiment site chosen for this study would have been classified as a typical steppe. But in the present study, Fringed sagebrush was the most dominant diet component, a species normally dominant in desert steppes (Hu et al., 2014). Of course, this may reflect the fact that over the past few decades, the Inner Mongolian grasslands have been undergoing drastic changes with desertification becoming widespread even in areas like Xilingol. There are numerous reasons for it. Firstly, it was due to over grazing; and secondly, the general deterioration of the land has arisen because of population increase and mining activities.

The intake and digestibility of herbage changed depending on seasons, especially in the winter, when herbage intake decreased greatly. The results are consistent with those previously reported by Hu et al. (2014) for the Inner Mongolian desert steppe. But in the current research, herbage digestibility was generally higher than that for a typical desert steppe pastures in the spring and autumn, lower in the winter. This is probably due to the fact that sheep grazed on desert steppe pastures are adapted to dry grass in terms of their rumen functions. There was a strong correlation between herbage intake and digestibility. This is not really surprising because the crude fiber level in forage is higher in the winter and the protein and ME values are lower, so that grass is not easy digested and leaves rumen (Li et al., 2015). Accordingly,

a large number of fiber stays in the rumen, thus the sheep feel satiety and do not intake more feed. Herbage intake of grazing sheep have a certain relationship with grass production (Liu et al., 2013), but in this study, the grass production was lower and the dry weight: fresh weight ratio of grass also was lower, there were bad palatability, which lead it difficulty to intake more herbage.

Herbage species with a higher nutritional value appear to affect sheep's diet selection. With the decrease of herbage availability and nutritive value across the grazing season (spring to winter), the feed intake of sheep decreased. In addition, digestibility remained similar across different seasons. In the present study, diet composition significantly correlated with the CP content in the winter, and correlate with the proportion of grass in the summer; this indicates that sheep have the ability to select grass. Furthermore, the diet composition of grazing sheep was not affected by the NDF content of herbage, probably due to the heavy diet selection by the sheep that maintained the rumen environment within a certain physiological and microbiological range (Morand-Fehr, 2005).

In brief, during the spring all the herbage species were lush, with a lot of twigs and tender leaves, making them highly nutritious. All these contributed to the increased intake and digestibility of herbage by sheep, which did not exhibit herbage selection. In the summer, as precipitation increased, herbage growth also accelerated, leading to increased biomass and fiber level. The palatability of the pastures decreased somewhat, resulting in a slight reduction of herbage intake and digestibility. With the onset of winter, there was hardly any plant growth, ending up with coarse stems and stubbles that were rich in fiber and poor in nutrients. Under these circumstances, sheep attained a minimum of nutrition during the winter (Li and Wang, 1998). Our findings call for ways to manipulate pasture management and to promote sheep performance.

5. Conclusion

The results from the present study showed that the characteristics of alkanes in herbage are species specific within each season. This research revealed that *Fringed sagebrush* was the most dominant diet component, with different proportions in different seasons. A positive correlation between diet component and botanical composition was observed only in summer. There was a significant correlation between diet component and CP in winter. Thus, it is clear that herbage intake of sheep depends on the botanical origin and nutrient content of pasture.

Acknowledgments

The present study was supported by the Modern Agroindustry Technology Research System (CARS-39) grant from the Chinese Ministry of Agriculture. We wish to express our gratitude to the researchers who helped us with this work.

 $^{^{}a,b}$ The different letters indicate significant differences in different seasons at P < 0.05.

^{*} The star letters indicate significant differences of correlation in different seasons at P < 0.05.

Appendix

Table AN-alkanes concentrations of grazing sheep fecal in different season (mg/kg DM)

Seasons	C25	C27	C29	C31	C32	C33	C35
Spring	20.9 ± 7.3^{b}	51.7 ± 23.0^{b}	372.1 ± 50.3^{a}	480.0 ± 190.9^{a}	195.9 ± 46.6	359.4 ± 148.6^{a}	8.0 ± 3.1
Summer	26.1 ± 8.0^{b}	70.8 ± 14.9^{a}	31.7 ± 2.5^{b}	318.0 ± 42.0^{b}	186.7 ± 23.4	242.4 ± 80.4^{b}	10.9 ± 2.7
Winter	15.5 ± 8.1^{a}	40.1 ± 9.4^{b}	41.2 ± 7.0^{b}	465.1 ± 94.0^{a}	115.8 ± 10.7	119.6 ± 26.8^{c}	9.9 ± 2.6

 $^{^{}a,b,c}$ The different letters indicate significant differences in different seasons at P < 0.05.

Table BThe nutritional components of the pastures in different seasons

Seasons	Species	DM, %	CP, %	EE, %	NDF, %	ADF, %	Ca, %	P, %	ME, kJ/kg
Spring	Carex	85.05 ± 0.05	11.14 ± 1.31	6.84 ± 2.54	41.07 ± 5.34	24.89 ± 1.46	0.97 ± 0.34	0.54 ± 0.08	6.67 ± 0.28
	Fringed sagebrush	86.71 ± 0.03	12.13 ± 0.8	7.48 ± 4.6	46.11 ± 3.55	28.82 ± 0.94	1.26 ± 0.16	0.51 ± 0.05	6.99 ± 0.2
	Leymus chinensis	87.84 ± 0.85	11.22 ± 0.46	5.06 ± 0.46	47.1 ± 0.48	26.22 ± 1.91	0.99 ± 0.13	0.52 ± 0.03	7.13 ± 0.35
	Potentilla tanacetifolia	84.98 ± 0.33	11.15 ± 1.51	5.9 ± 2.76	42.57 ± 0.4	24.55 ± 0.58	0.75 ± 0.11	0.49 ± 0.06	6.75 ± 0.02
	Stipa krylovii	84.28 ± 2.96	13.96 ± 4.09	5.5 ± 2.38	54.83 ± 6.51	27.85 ± 3.68	0.9 ± 0.01	0.52 ± 0.14	6.56 ± 0.25
Summer	Carex	85.23 ± 1.04	9.41 ± 0.51	4.6 ± 0.1	47.21 ± 0.02	26.11 ± 1.85	1.36 ± 0.03	0.46 ± 0.03	7.1 ± 0.01
	Cleistogenes	88.7 ± 1.44	12.23 ± 0.86	2.94 ± 0.05	41.99 ± 2.81	31.17 ± 1.28	1.04 ± 0.16	0.5 ± 0.02	7.1 ± 0.1
	Fringed sagebrush	86.81 ± 2.17	11.47 ± 0.94	2.82 ± 0.15	55.12 ± 6.27	24.65 ± 5.11	1.45 ± 0.3	0.56 ± 0.03	7.09 ± 0.21
	Potential bifurca	81.22 ± 0.19	11.56 ± 0.33	3.43 ± 0.05	41.36 ± 2.2	22 ± 3.63	0.78 ± 0.01	0.9 ± 0.03	8.26 ± 0.64
	Stipa krylovii	87.53 ± 0.99	10.04 ± 0.37	3.5 ± 0.52	62.62 ± 0.5	32.52 ± 0.06	0.91 ± 0.02	0.38 ± 0.02	6.82 ± 0.08
	Thyme	86.48 ± 0.29	8.82 ± 0.1	5.09 ± 0.09	40.56 ± 0.27	27.9 ± 0.22	0.84 ± 0.03	0.44 ± 0.01	7.26 ± 0.05
Winter	Carex	85.28 ± 34.82	6.9 ± 3.5	3.44 ± 1.53	52.27 ± 21.74	33.09 ± 14.33	1.15 ± 0.48	0.65 ± 0.28	4.67 ± 1.91
	Cleistogenes	86.39 ± 4.16	8.42 ± 2.51	1.92 ± 0.54	61.36 ± 11.69	40.42 ± 6.49	0.99 ± 0.18	0.73 ± 0.13	3.86 ± 1.19
	Fringed sagebrush	88.26 ± 1.52	11.67 ± 0.34	2.56 ± 0.32	52.92 ± 3.27	32.14 ± 3.26	1.12 ± 0.02	0.66 ± 0.08	5.16 ± 0.15
	Stipa krylovii	91.8 ± 4.56	7.75 ± 1.37	2.57 ± 0.56	66.49 ± 5.15	34.65 ± 3.13	0.87 ± 0.03	0.47 ± 0.12	5.23 ± 0.55

DM = dry matte; CP = crude protein; NDF = neutral detergent fiber; EE = ether extract; ADF = acid detergent fiber; ME = metabolizable energy.

References

Association of Official Analytical Chemists. Official methods of analysis. 15th ed. 1990. Arlington, Virginia, USA.

Dove H. Using the n-alkanes of plant cuticular wax to estimate the species composition of herbage mixtures. Aust J Agr Res 1992;43:1711–24.

Dove H, Mayes RW. The use of plant wax alkanes as marker substances in studies of the nutrition herbivores: a review. Aust J Agr Res 1991;42:913–52.

Dove H, Mayes RW. Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. Small Rumin Res 2005;59:123—39.

Dove H, Mayes RW, Freer M. Effects of species, plant part, and plant age on the nalkane concentrations in the cuticular wax of pasture plants. Aust J Agr Res 1996;47:1333—47.

Dove H, Moore AD. Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant cuticular wax. Aust J Agr Res 1995:46:1535—44.

Dziba IE, Scogings PF, Gordon IJ, Raats JG. The feeding height preferences of two goat breeds fed Grewia occidentalis L. (Tiliacecae) in the Eastern Cape, South Africa, Small Rumin Res 2003;47:31–8.

Hameleers A, Mayes RW. The use of n-alkanes to estimate herbage intake and diet composition by dairy cows offered a perennial ryegrass/white Clover mixture. Grass Forage Sci 1998;53:164–9.

Hu HL, Liu YZ, Li YK, Lu DX, Gao M. Use of the N-alkanes to estimate intake, apparent digestibility and diet composition in sheep grazing on Stipa breviflora Desert Steppe. J Integr Agric 2014;13:1065–72.

Hu ZZ, Gao CX. Improvement of the comprehensive and sequential classification system of grasslands lindices of grassland classes and index chart. Acta Prataculturae Sin 1995:4:1–7.

Kelman W, Bugalho M, Dove H. Cuticular wax alkanes and alcohols used as markers to estimate diet composition of sheep (Ovis aries). Biochem Syst Ecol 2003;31: 919–27.

Laredo MA, Simpson GD, Minson DJ, Orpin CG. The potential for using n-alkanes in tropical forages as a marker for the determination of dry matter intake by grazing ruminants. J Agr Sci 1991;117:355—61.

Liu GH, Lin LJ, Zhang YJ, Wang SP, Han JG, Ma XZ. Study on the accuracy and precision of n-alkanes as markers for estimation of diet composition and herbage intake of sheep. Sci Agric Sin 2006;39:1472—9 (In Chinese with English abstract)

Liu GH, Zhang YJ, Wang SP, Han JG. Estimation of diet composition and herbage intake of grazing sheep at different stocking rates in Inner Mongolia typical

steppe by using n-alkanes technique. Acta Prataculturae Sin 2013;22:71–9 (In Chinese with English abstract).

Li Y, Wang MJ, Li ZY, Wang ZB, Xin HS, Zhang YJ. Effect of supplementing different rations of wet corn gluten feed on rumen degradation and nutrient digestibility in dairy cows. Chin J anamal Sci 2015;51:54–8.

Li YH, Wang SP. Grazing intake of inner mongolian fine-wool sheep and their preference for grassland forage species. Acta Prataculturae Sin 1998;7:50—3 (In Chinese with English abstract).

Mayes RW, Dove H. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. Nutr Res Rev 2000;13:107–38.

Mayes RW, Lamb CS, Colgrove PM. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J Agr Sci 1986;107:161–70.

Malossini F, Piasentier E, Bovolenta S. N-alkane content of some forages. J Sci Food Agr 1990;53:405–9.

Morand-Fehr P. Recent developments in goat nutrition and application: a review. Small Rumin Res 2005;60:25—43.

Newman JA, Thompson WA, Penning PD, Mayes RW. Least-squares estimation of diet composition from n-alkanes in herbage and faeces using matrix mathematics. Aust J Agr Res 1995;46:793–805.

Nigel RB, Martin RLS, Franz S, Michael K. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling in cows. Ann Zootech 2000;49:1–3.

Olivan M, Osoro K. Effect of temperature on alkane extraction from faeces and herbage. J Agr Sci 1999;132:305—11.

Rosa GD, Moio L, Napolitano F, Grasso F, Gubitosi L, Bordi A. Influence of flavor on goat feeding preferences. J Chem Ecol 2002;28:269–81.

Sun ZW, Zhou DW, Ferreira LMM, Zhong QZ, Lou YJ. Diet composition, herbage intake and digestibility in Inner Mongolian Cashmere goats grazing on native Leymus chinensis plant communities. Livest Sci 2008;116:146–55.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583—97.

Wang S. A review of methods use to determine botanical composition of diets for herbivores. Acta Prataculturae Sin 1995;14:8–16 (In Chinese with English abstract).

Wang SP. The dietary composition of fine wool sheep and plant diversity in Inner Mongolia steppe. Acta Ecol Sin 2000;20:952–7 (In Chinese with English abstract).

Zhang YJ. Introduction of plant wax alkane technique. In: Proceedings of China International Grassland Conferences, Beijing; 2002. p. 77–81.