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N-alkane profile in some plants grazed by sheep in Chiloé Archipelago, Chile

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ABSTRACT

In this study, the pattern of n-alkanes of fifteen species collected in a naturalized grassland grazed by sheep in the Archipelago of Chiloé, Chile was analyzed. The concentrations of odd-chain n-alkanes between C_{23} and C_{31} were placed on the recommended values for obtaining reliable estimates of consumption. It was also observed that species showed marked differences in their individual patterns. The results show a promising horizon to advance in determining the composition of the diet of sheep grazing in the Archipelago of Chiloé (Chile).

Indexing terms/Keywords

Ruminants; cuticular markers; naturalized grassland; diet composition.

Academic Discipline And Sub-Disciplines

Agriculture; livestock; ruminants;

SUBJECT CLASSIFICATION

Agriculture Classification

TYPE (METHOD/APPROACH)

Intake, digestibility and diet composition estimation in grazing ruminants.

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INTRODUCTION

In grazing systems, the feeding behavior of ruminants is one determining factor of the productive performance. Selectivity and rate of consumption are two key aspects that can be modified depending on the physiological and phenological state of animals and pastures respectively. The estimation of the botanical composition of the diet and the forage intake in ruminants in extensive grazing is a key aspect in research; however, is just in these systems where it is more difficult to accurately assess consumption without affecting the feeding behavior or bothering of the animals.

Consumption assessment may be performed through pasture or animal-based methods, the latter being more accurate (Mayes et al., 1986), since they allow individual estimates. Techniques based on the use of markers (external, existing in the plants themselves) allow to quantitatively estimating the intake, digestibility and diet composition. The aerial part of the plants is covered by a waxy cuticle which protects the plant against physical and biological agents. Some of these compounds are the n-alkanes, aliphatic hydrocarbons with 21 to 36 carbon atoms (C_{21} to C_{36}). The content and relative proportion (pattern) of n-alkanes may differ markedly between plant species (Dove & Mayes, 1991, Zhang et al., 2004; Bakker & Alvarado, 2006). They are practically indigestible for most herbivorous mammals and excreted in the feces; therefore, the pattern of n-alkanes of plants and feces can be used for estimating consumption and proportions of the different species and parts plants in the diet (Mayes and Dove, 2000). There is a high literature background that supports the value of the n-alkanes as markers to estimate intake, digestibility and diet composition of grazing ruminants (reviewed *in extenso* in Dove & Mayes, 2005).

Literature has reported the concentration of n-alkanes of some species of grasses and legumes in temperate climates (Dawson et al., 2000; Mayes, 2001; Ali et al, 2005), in several species of tropical forage plants (Delgado et al., 2000) and some patagonic species in Argentina (Cesa & Bakker, 2007). However, there are no published data for the n-alkane contents in plant species from naturalized grasslands at Chiloé Archipelago (Chile). In Chiloé, sheep production is mainly carried out under extensive grazing conditions in poliyhitic naturalized grasslands that are the result of man intervention. These grasslands usually contain grasses, legumes and shrubs with very different nutritional values. Determine the total consumption and the species intake in this heterogeneous grazing situation is a key point in the nutritional management of the herd; nevertheless, the implementation of the technique requires prior characterization of the content of n-alkanes of the main species of interest associated with grazing sheep in these grasslands (Cesa & Bakker, 2007). The aim of this study was, therefore, to determine the absolute and relative concentration of n-alkanes in the most common botanical species from a naturalized grassland grazed by sheep in the archipelago of Chiloé, in order to evaluate the scope of the method of n-alkanes to estimate the intake, digestibility and diet composition.

MATERIAL AND METHODS

The work was conducted at the experimental center Butalcura Chiloe, Chile. Plant samples were taken in one hectare of naturalized grassland. Sampling was done by throwing at random 10 metal squares (1x 0.5 m) and cutting with scissors at ground level the aerial part of the plant species inside square. After the identification of the botanical species, extraction and quantification of n-alkanes were performed as proposed by Dove and Mayes (2006), with the modifications described by Keli et al. (2008) and those concerning to laboratorial conditions in our framework. Gas chromatography analyses of the purified extracts were carried out on a Shimadzu 2010 Plus Gas Chromatograph fitted with an automatic injector and a flame ionization detector, a capillary column RTX-1 (Restek) (30 m lenght x 0,53 mm i.d., 1,50 µm film thickness) and a SGE Syringe (10 µl; 4 µl inyection) with He as carrier and make up gas to the detector. Detector response factors for individual n-alkanes were determined by injecting onto the chromatograph a standard *n*-alkane mixture (C_{21}-C_{36} inclusive) after every eight sample extracts. Alkanes C_{22} and C_{34} were used as internal standards. Oven conditions were 230 °C x 0,2 minutes; 300 °C x 18 minutes at a speed of 6 ° min^{-1} . Inyector and detector temperatures were 233 °C and 350 °C respectively. Air and H flows were 420 ml min^{-1} and 42 ml min^{-1} respectively. Make up was 30 ml He min^{-1} and column flow was 10 ml min^{-1}, with a split of 1 ml min^{-1}. Heptane was used in the washing vials, thrice before and after simple inyection.

Results are given as absolute and relative concentration of each n-alkane in the sample (mg / kg DM and percentage, respectively). The total concentration of n-alkanes in each botanical species was the sum of the individual concentrations of n-alkanes (both odd and even) quantified, regardless of the concentrations of the internal standards (C_{22} and C_{34}). The relative concentration or pattern was obtained by weighting the appearance of every alkane relative to the whole in each species. A principal component analysis was performed using the XLSTAT Pro program, with the data matrix conformed by the relative content of the odd chain n-alkanes for each species.

RESULTS AND DISCUSSION

Table 1 shows the absolute (mg / kg MS) and relative (percentage of total) concentrations of n-alkanes in the plant species analyzed. Concentrations covered a range from 60 mg / kg DM to 1500 mg / kg DM; the minimum concentration recommended to be used as internal markers minimum concentration is 50 mg / kg of DM (Casson et al., 1990; Laredo et al., 1991). The species with lower concentration was *Hydrocotyle marchantioides* (65.791 mg / kg MS), while the highest was found in *Digitalis purpurea* with 1437.1 mg / kg DM. This is in the range of values found in numerous temperate and tropical forages (Dove & Mayes, 1991). The high total concentrations of n-alkanes in *D. purpurea* may be due to the high presence of flowers at the time of harvest (December).

In monocots it was observed that Juncaceae (*Juncus procerus* and *Luzula campestris*) had a low and very similar total content of n-alkanes. Compared to other species of the same genus, it was observed that the content of n-alkanes found here was similar to the *Juncus effusus* content reported by Ali et al. (2005), with higher values but similar pattern, while *Luzula sylvatica*, reported by the same authors, showed lower values , except for C_{29} that was much higher. Grasses

(*Antoxanitum*, *Holcus* and *Agrostis*) presented a moderate-low C_{29} content, which is consistent with published values for temperate grasses and wetlands around the world (Dove & Mayes, 1991; Alvarado et al, 2005), although Cesa and Bakker (2007) obtained unusual high concentrations (up to 85% of the total). In most temperate and tropical grasses, C31 and C33 are usually predominant; however, in this study, the mentioned grasses showed high values of some shorter chain n-alkanes, such as C_{23}, C_{25} and C_{27}.

Table 1. Absolute (mg / kg DM, above) and relative (% of the total, below) concentrations of n-alkanes in samples of fifteen plant species in a naturalized grassland in Chiloe Archipelago. n. d.: not detected.

	C_{21}	C_{23}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}	Total
Hydrocotyle marchantioides	2,094	2,648	7,889	n.d.	12,065	2,628	12,774	2,165	10,932	3,039	4,197	n.d.	5,36	65,791
	3,18	4,03	11,99	0	18,34	3,99	19,42	3,29	16,62	4,62	6,38	0	8,15	100
Holcus lanatus	7,911	59,355	36,602	n.d.	28,947	3,335	19,201	9,087	7,005	6,457	3,425	n.d.	6,175	187,501
	4,22	31,66	19,52	0	15,44	1,78	10,24	4,85	3,74	3,44	1,83	0	3,29	100
Juncus procerus	n.d.	4,692	7,1	n.d.	14,447	14,53	129,219	10,784	40,908	4,431	4,333	n.d.	5,455	235,899
	0	1,99	3,01	0	6,12	6,16	54,78	4,57	17,34	1,88	1,84	0	2,31	100
Luzula campestris	n.d.	4,669	33,451	3,166	140,569	5,198	39,839	0,594	10,713	2,338	1,699	n.d.	5,103	247,339
	0	1,89	13,52	1,28	56,83	2,1	16,11	0,24	4,33	0,95	0,69	0	2,06	100
Cirsium vulgare	n.d.	3,668	5,686	n.d.	19,742	3,379	92,926	4,004	115,297	3,034	17,348	n.d.	5,329	270,411
	0	1,36	2,1	0	7,3	1,25	34,36	1,48	42,64	1,12	6,42	0	1,97	100
Plantago lanceolata	n.d.	3,758	18,313	n.d.	29,594	10,285	106,181	9,212	106,568	6,187	10,665	n.d.	5,326	306,09
	0	1,23	5,98	0	9,67	3,36	34,69	3,01	34,82	2,02	3,48	0	1,74	100
Rumex acetosella	n.d.	5,626	21,707	n.d.	209,225	12,542	153,97	1,765	5,381	2,287	1,794	n.d.	5,366	419,665
	0	1,34	5,17	0	49,86	2,99	36,69	0,42	1,28	0,54	0,43	0	1,28	100
Taraxacum officinale	67,7	35,837	23,346	n.d.	44,191	5,504	72,712	7,386	136,078	9,1	44,159	8,345	5,482	459,84
	14,72	7,79	5,08	0	9,61	1,2	15,81	1,61	29,59	1,98	9,6	1,81	1,19	100
Lotus corniculatus	n.d.	4,716	29,188	n.d.	191,636	8,056	105,539	2,309	62,362	5,538	55,066	1,544	5,263	471,218
	0	1	6,19	0	40,67	1,71	22,4	0,49	13,23	1,18	11,69	0,33	1,12	100
Anthoxanithum odoratum	2,875	28,77	87,107	3,237	117,133	9,616	152,811	6,593	62,254	n.d.	11,484	n.d.	n.d.	481,88
	0,6	5,97	18,08	0,67	24,31	2	31,71	1,37	12,92	0	2,38	0	0	100
Berberis microphylla	n.d.	n.d.	8,774	n.d.	93,146	8,433	262,76	5,393	161,844	0,849	12,558	n.d.	1,651	555,407
	0	0	1,58	0	16,77	1,52	47,31	0,97	29,14	0,15	2,26	0	0,3	100
Cerastium holosteoides	5,345	4,465	10,826	n.d.	29,077	8,496	210,765	13,655	253,053	5,654	13,315	n.d.	5,804	560,454
	0,95	0,8	1,93	0	5,19	1,52	37,61	2,44	45,15	1,01	2,38	0	1,04	100
Trifolium repens	11,09	9,504	16,603	n.d.	60,467	9,183	230,69	23,635	305,539	21,632	83,24	13,073	6,101	790,759
	1,4	1,2	2,1	0	7,65	1,16	29,17	2,99	38,64	2,74	10,53	1,65	0,77	100
Agrostis capillaris	13,25	87,287	81,116	2,578	104,049	17,857	211,966	27,291	241,094	15,745	27,9	n.d.	n.d.	830,132
	1,6	10,51	9,77	0,31	12,53	2,15	25,53	3,29	29,04	1,9	3,36	0	0	100
Digitalis purpurea	1,64	2,681	7,236	n.d.	48,37	8,491	226,883	22,955	894,262	35,875	183,547	n.d.	5,187	1437,13
	0,11	0,19	0,5	0	3,37	0,59	15,79	1,6	62,23	2,5	12,77	0	0,36	100

Dicots generally presented higher total n-alkane contents, with the exception of *Hydrocotyle marchantioides*, which showed the lowest total concentration, probably due to its marshy nature. The most abundant n-alkanes in all species were C_{27}, C_{29} and C_{31}, constituting up to 56.83%, 54.8% and 62.2% of the whole, respectively, with wide variability between species. This is consistent with previous results (Malossini et al., 1990 Bugalho et al., 2004, Ferrerira et al., 2009).

To date, there is limited information on the amount and relative proportion of n-alkanes of the cuticular wax of forage species in Chile, with the exception of some data of Euphorbiaceae (Bittner et al., 2001). In addition, there is little information coming from other countries on the composition of the n-alkanes of the cuticular wax for the species analyzed in this work. Malossini et al. (1990), Kelman et al. (2003), Bugalho et al. (2004) and Ferreira et al. (2009) found in absolute concentrations generally lower for *T. repens* than those presented here.

Differences in the concentration of n-alkanes for the same species may be due to the ecological conditions of the study area or harvest time, as these factors can modify the n-alkane profile (Court et al 2005; Ferri et al, 2007). It has been reported that C_{29} is abundant in legumes (Dove and Mayes, 1991); in this study, the two legumes studied (*Trifolium* and *Lotus*) had C_{29} values of 29.17% and 22.40%. Meanwhile, the even chain n-alkanes appear at very low or undetectable levels. These results are expected and are consistent with the fact that in higher plants, even chain n-alkanes appear at low concentrations relative to odd chain n-alkanes (Dove and Mayes 1991, 1996; Dove et al, 1996).

Discrimination between species is a very relevant aspect in the consumption and diet composition estimation with the n-alkanes method, to the extent that the content of n-alkane which is used to estimate the consumption differs between species within each group to be discriminated. Figure 1 shows the principal components analysis for the study species group together with their concentration of n-alkanes.

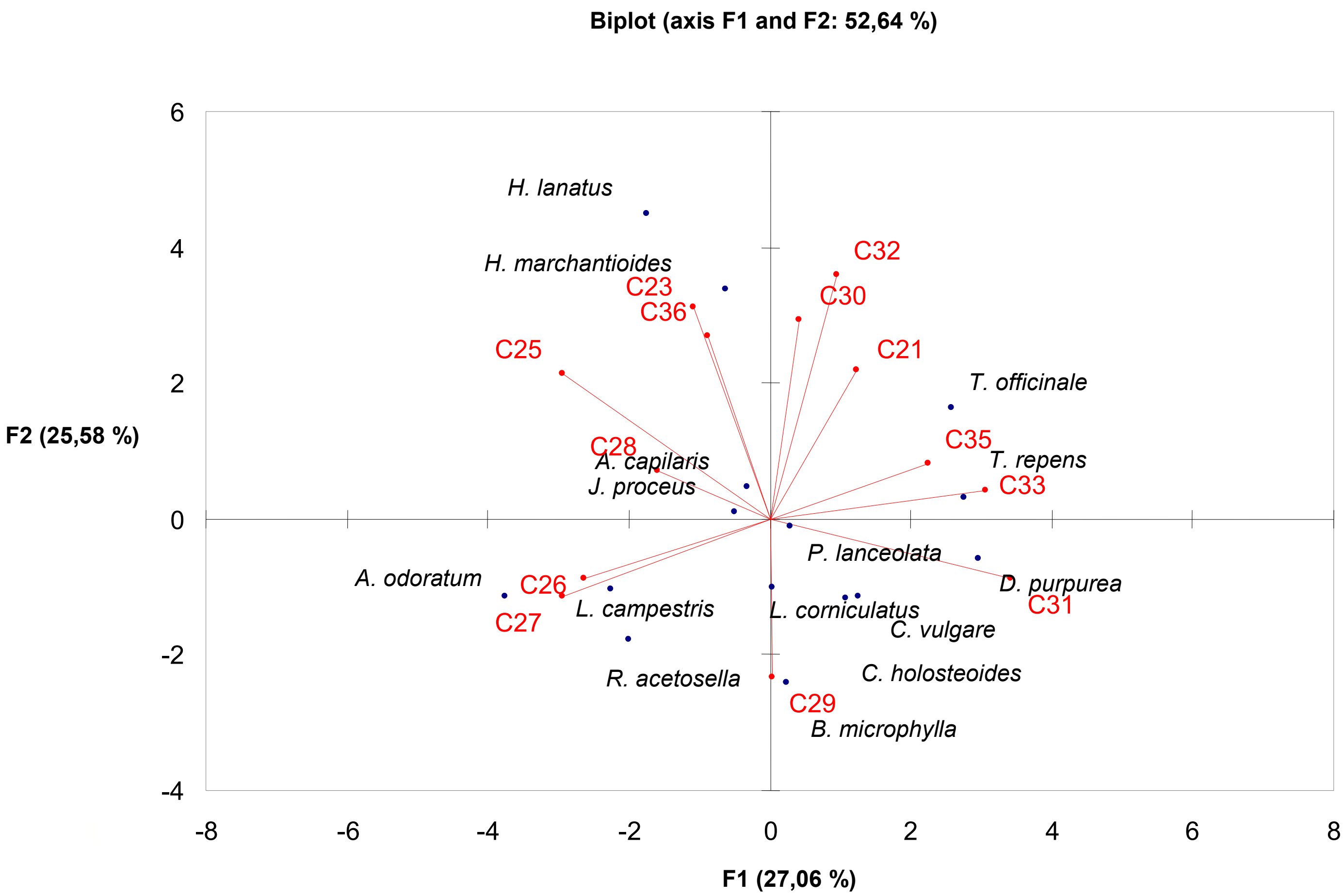

Fig 1: Variability in the plane of odd chain n-alkanes concentration in fifteen botanical species. C21 to C35: odd chain n-alkanes.

In monocotyledonous plants, a contrasting species was *Holcus lanatus*, which had higher proportions of C_{23} and C_{25} and lower amounts of longer n-alkanes. Regarding to dicotyledonous species, the most contrasting were *Berberis microphylla* and *Taraxacum officinale.* These two species are of interest, bearing in mind that authors like Oliva et al. (2012) have suggested that plants with a high total concentration and a different n-alkane pattern from the most commonly used in ruminant grazing have potential to be used as markers to estimate DMI.

Although Martínez *et al*. (2014) have recently applied the n-alkane technique to estimate the diet composition of two sheep breeds in Chiloé Archipelago, the present paper shows the first n-alkane values for different plant species in Chiloé. In general, the absolute concentrations of odd-chain alkanes between C_{23} and C_{31} were placed on the recommended values to obtain reliable estimates of consumption. The amount and composition of odd chain n-alkanes of the cuticular wax in the studied species would be suitable for the use of this material as a natural ingestion marker, since the presence of an acceptable content of n-alkanes, together with the differences in pattern between species, are necessary characteristics in the application of the n-alkane method to estimate consumption and diet composition in sheep. Although further progress in the use of this methodology and the possibility of adding other natural markers such as alcohols and long chain fatty acids is needed in order to increase the capacity of discrimination, the observed results generate a promising horizon in determining the composition of the diet of domestic ruminants grazing in the Chiloe Archipelago (Chile), and open wide possibilities for the assessment of individual food intake.

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REFERENCES

1. Ali, H.A.M., Mayes, R.W., Hector, B.L., Orskov, E.R. 2005. Assessment of *n*-alkanes, long-chain fatty alcoholsand long-chain fatty acids as diet composition markers: The concentrations of these compounds inrangeland species from Sudan. Anim. Feed Sci. and Technol. 121: 257–271.

2. Alvarado, P. I.; Bakker, M. L.; Gonda, H. L. 2005. Alcanos de láminas de hojas de álamo plateado (*Populus alba*) y su utilidad potencial para marcar suplementos. Revista Argentina de Producción Animal, v25(1), p. 48-49.

3. Bakker, M. L.; Alvarado, P. I. 2006. Alcanos de la cera cuticular de hojas de Populus alba, Populus deltoides (Salicaceae), Robinia pseudoacacia (Fabaceae), Ulmus pumila (Ulmaceae) y Fraxinus americana (Oleaceae) en Tandil, Buenos Aires, Argentina. Darviniana, v 44(1), p. 58-63.

4. Bugalho, M.N.; Dove, H.; Kelman, W.; Wood, J.T.; Mayes, R.W. 2004. Plant wax alkanes and alcohols as herbivore diet composition markers. Journal of Range Management, V 57, P. 259-268.

5. Cesa, A.; Bakker, M. 2007. Estudio inicial del contenido y patrón de alcanos en especies seleccionadas y no por ovinos en la estepa patagónica, Santa Cruz, Argentina. Reunión APPA-V Congreso Internacional de Ganadería de Doble Propósito. Revista ALPA., p. 28.

6. Dove, H.; Mayes, R. W.; Freer, M. 1996. Effect of species, plant part, and plant age on the *n*-alkane concentrations in the cuticular wax of pasture plants. Aust. J. Agric. Res. v47, p. 1333-1347.

7. Dove, H., Mayes, R. 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nat. Protoc. 1(4), 1680-1697.

8. Keli, A., Andueza, D., De Vega, A., Guada, J. 2008. Validation of the n-alkane and NIRS techniques to estimate intake, digestibility and diet composition in sheep fed mixed lucerne: ryegrass diets. Livest. Sci. 119(1), 42-54.

9. Mayes, R. W.; Dove, H. 2000. Measurement of dietary nutrient intake in free ranging mammalian herbivores. Nutr. Res. Rev. v13, p. 107-138.

10. Martinez M.E., De la Barra, R. 2014. Diet composition of two sheep breeds grazing in Chiloe Archipelago, Chile. Journal of Animal and Veterinary Advances, 13, 871-876.

11. Oliva, M.R.; Vivinetto, M.; Mogni, S.; Inza, G.; Miano, G.D. 2012. Efecto de *Populus alba* sobre la digestibilidad de mezclas de alfalfa y maíz. Revista Argentina de Producción Animal, v 32 (1), p. 121-205.

12. Zhang, Y.; Togamura, Y.; Otsuki, K. 2004. Study on the *n*-alkane patterns in some grasses and factors affecting the *n*-alkane patterns. J. Agric. Sci. (Cambridge), v142, p. 469-475.