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The use of *n*-alkanes to estimate diet composition of ruminants grazing on species diverse plant communities — Effect of feeding selectivity on diet composition estimates

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Abstract

The present study aimed to evaluate the effect of feeding selectivity (FS) within a dietary group comprising plant species with similar alkane profile, on the estimates of diet composition of goats, sheep and cows grazing on vegetation composed of gorse-heathland with areas of improved pastures. Plant species collected in the field study were grouped in four dietary groups according to cluster analysis: D1 (*Lolium perenne*, *Pseudarrhenatherum longifolium* and *Agrostis capillaris*), D2 (*Calluna vulgaris*, *Erica cinerea*, *Erica umbellata*, *Erica australis*), while *Erica arborea* (D3) and *Ulex gallii* (D4) remained as individual components due to their distinct *n*-alkane profiles. The application of different levels of feeding selectivity (0, 5, 15, 30, 60, 90 and 100%) to the graminaceous species included in D1 group affected significantly ($P<0.05$) the diet composition estimates as a result of the modification of the alkane pattern of the dietary group used in the calculations. However, this effect was not so evident within all ranges of FS and when evaluated in the heather species of D2 group. When *L. perenne* and *C. vulgaris* were removed from D1 and D2 dietary groups and treated as individual components, there was a decrease in the FS effect on the diet composition estimates. The results obtained show that dietary groups formed by multivariate statistical analysis should be used carefully as they could be sensitive to feeding selectivity, with consequently large effects on the estimates of diet composition. This effect will depend on the similarity in the alkane profile of the plant species that composed the dietary group.

Keywords: Alkanes; Feeding selectivity; Diet composition; Ruminants

1. Introduction

Alkanes are saturated hydrocarbons present in the cuticular waxes of most of higher plants and their profile is specific to plant species and plant parts (Dove et al., 1996; Ferreira et al., 2005). These "fingerprints" have already been explored with success to estimate the proportions of different plant species and/or plant parts in mixtures of ten herbage species (Hoebee et al., 1998) and from faeces of different animal species fed up to five dietary components in sheep (Lewis et al., 2003, Valiente et al., 2003, Ferreira et al., 2006), goats

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(Brosh et al., 2003, Ferreira et al., 2005), cattle (Brosh et al., 2003; Ferreira et al., in press) and equines (Ferreira et al., in press) in indoor controlled studies.

In complex vegetation communities under free range grazing conditions there are a limited number of *n*-alkanes available as markers contrasting with the extremely high number of components on offer to the animals. Although the current analytical procedures allow the identification and quantification of 16 alkanes (C_{21} to C_{36}), some of them present very low concentrations both in plants and in faeces (especially those with chain length lower that 25 and higher than 33 carbon atoms) which means that they are less useful as markers due to their higher potential analytical error (Brosh et al., 2003; Dove and Mayes, 2005). Consequently, the number of alkane markers available for diet composition calculations is probably limited to 9 (C_{25} to C_{33}).

Moreover, the increase of the number of diet components will likely result in less accurate diet composition estimates since there is an increasing likelihood that different combinations of two or more components have a similar alkane pattern to that of another combination of components. This could result in similar faecal alkane pattern and could reduce the precision of diet composition estimates (Dove and Mayes, 2005). To overcome these limitations three possible options to obtain estimates of diet composition are normally used: 1) the reduction of the number of plant species to be discriminated, by grouping species with indistinguishable alkane profile into dietary groups (Martins et al., 2002); 2) by the elimination of plant species based on preliminary information (observation of the animals and or data on the vegetation cover available; Dove and Mayes, 2005); 3) increasing the discriminatory power by the combination of alkanes with other methods of estimating diet composition (combination of microhistological and alkane procedures used by Salt et al., 1994), or with other markers, such as long-chain fatty alcohols (Kelman et al., 2003; Bugalho et al., 2004; Ali et al., 2004, 2005) and long-chain fatty acids (Ali et al., 2004, 2005), providing a more specific "fingerprint" of each plant species.

On the other hand, Bugalho et al. (2002) pointed out that when expressing diet composition in terms of dietary groups, there is the possibility of animals selecting differently the plant species comprising the group, modifying its *n*-alkane contribution to the diet ingested. This selective behaviour could alter the contributions of the species within the dietary group into the *n*-alkane profile in faeces and may influence the accuracy of the diet composition estimate.

The objective of this work was to assess the effect of using different levels of feeding selectivity on a particular plant species within a dietary group, formed according with their similar alkane profile, on diet composition estimates. This study was carried out using a dataset of *n*-alkane pattern of herbaceous and browse species, and faeces of three species of ruminants (goats, sheep and cattle) grazing on natural vegetation communities composed of gorse-heathland with areas of ryegrass pastures.

2. Materials and methods

2.1. Experimental site

This study was carried out on an experimental plot (22 ha) located at the Carbayal Research Station (900–1000 m above sea level), at San Isidro's Mountain, Asturias, North-Western Spain (latitude 43° 21′, longitude −6° 53′) in June. The vegetation of the experimental plot consisted mainly of short heathers (*Erica umbellata* L., *Erica cinerea* L. and *Calluna vulgaris* L.), tall heathers (*Erica australis* L. and *Erica arborea* L.) and gorse (*Ulex gallii* Planchon). Some herbaceous natural species, such as *Pseudarrhenatherum longifolium* Rouy and *Agrostis capillaris* L., could also be found mixed with the heather species and gorse. This natural vegetation was interspersed with patches of improved pastures of perennial ryegrass (*Lolium perenne* L.).

2.2. Dataset

The dataset consisted of the *n*-alkane pattern of the main plant species present in the plot. The samples of the vegetation components (leaves of herbaceous species and green shoots of woody species) were randomly collected in different locations.

At the same time, rectal faecal samples were collected from 7 Asturiana de los Valles cows (622 kg, s.d. 17.8 kg), 42 adult non-lactating Cashmere goats (39 kg, s.d. 1.3 kg) and 42 crossbreed (Gallega*Latxa) sheep (45 kg, s.d. 1.5 kg) grazing on the experimental plot, managed in a mixed herd to have the same opportunities for diet selection.

Samples of the plant species and faeces were immediately frozen at −20 °C and then freeze-dried and milled through a 1 mm screen for *n*-alkane analysis. Alkane concentration of individual samples of the plant species and faeces was analysed in duplicate according to the method of Mayes et al. (1986) modified by Oliván and Osoro (1999).