Email: derseh7@yahoo.com

Session 3, abstract number 7905

Use of a combination of n-alkanes and their carbon isotope enrichments ($\delta^{13}C$) as diet composition markers

M. Bezabih[1,2,*], W. F. Pellikaan[1], W. H. Hendriks[1]

[1] *Animal Nutrition Group, Department of Animal Sciences, Wageningen University, Box 338, 6700 AH Wageningen, the Netherlands;* [2] *Department of Animal and Range Sciences, Hawassa University, P.O. Box 5, Hawassa,Ethiopia*

Abstract

Plant cuticular n-alkanes have been successfully used as markers to estimate diet composition and intake of grazing herbivores. However, additional markers may be required under grazing conditions with botanically diverse vegetation. This study was conducted to describe the n-alkane profiles and the carbon isotope enrichment of n-alkanes of common plant species from the Mid Rift Valley rangelands of Ethiopia, and evaluate their potential use as nutritional markers. A total of 23 plant species were collected and analyzed for long chain n-alkanes ranging from C_{27} to C_{36}, as well as their carbon isotopic ratio ($^{13}C/^{12}C$). The analysis was conducted by gas chromatography/combustion isotope ratio mass spectrometry following saponification, extraction, and purification. The dominant n-alkanes in the species were C_{31} (mean±sd, 283 ±246 mg/kg dry matter) and C_{33} (149 ±98 mg/kg dry matter). The carbon isotopic enrichment of the n-alkanes ranged from -19.37 to -37.40‰. Principal component analysis was used to examine interspecies differences based on n-alkane profiles and the carbon isotopic enrichments of individual n-alkanes. Large variability among the pasture species was observed. The first three principal components explained most of the interspecies variances. Comparison of the principal component scores using orthogonal procrustes rotation indicated that about 0.84 of the interspecies variances explained by the two types of data sets were independent of each other, suggesting that use of a combination of the two markers can improve diet composition estimations. It was concluded that, while the n-alkane profile of the pasture species remains a useful marker for use in the study region, the $\delta^{13}C$ values of n-alkanes can provide additional information in discriminating diet components of grazing animals.

Key words: n-alkanes, carbon isotope, marker, diet composition, feed intake

Introduction

In tropical production systems rangelands constitute the main sources of nutrition for domestic and wild herbivores. In Ethiopia, about 62% of the total land mass is classified as arid and semi arid (Kassahun et al., 2008), which is mainly used for livestock production based on grazing. The importance of the natural pasture for commercial livestock production has increased as a result of the current trend of increasing price of meat and other animal products. However, implementation of improved feeding and grassland management practices to maintain optimum productivity and to use the available pasture resource efficiently are difficult due to a lack of knowledge into the accurate feed and nutrient intake of many livestock species.

The use of plant wax components, mainly n-alkanes, as markers for estimation of intake and diet composition of herbivores evolved in the last two decades (Dove and Mayes, 2005; Ferreira *et al.*, 2007). Although validation experiments using n-alkanes produced good estimates with less complex vegetations, grouping of species or use of other markers in addition to n-alkanes was necessary for correct estimation of diet composition of herbivores grazing botanically diverse vegetation (Oliván *et al.*, 2007b). Stable carbon isotopic (^{13}C) composition of plants has been used to estimate the proportion of C_3 and C_4 plants in the diet of herbivores (Bennett *et al.*, 1999). However, so far, the ^{13}C of n-alkanes has not been evaluated as an additional marker together with the alkane profiles themselves. Currently, the possibility of separating organic compounds of interest prior to isotope ratio analysis using gas chromatography-combustion isotope ratio mass spectrometry (GC-CIRMS) provides an opportunity to consider the ^{13}C of n-alkanes rather than the whole organic matter. The latter would improve the reliability of isotopic techniques, as plant compounds that are stable both in herbage and in faeces can be specifically targeted for isotope analysis.

There is little information about the plant wax profiles of native pasture species in Ethiopia for application in nutritional assessments of grazing animals. The aims of the present study were: 1) to describe the n-alkane profiles of pasture species commonly available in the Mid Rift Valley rangelands of Ethiopia, 2) to determine the stable carbon isotope enrichment of individual n-alkanes for each pasture species, and 3) to evaluate the potential for using the two markers to estimate the diet composition of free-ranging herbivores.

Materials and Methods

Description of study site

The research area lies in the Mid Rift Valley region of Ethiopia extending from 7°30'N to 8°00'N and from 38°35'E to 38°45'E. The area is classified as semi-arid with an annual rainfall ranging from 500 to 700 mm per annum, and with mean daily 11.4 to 26°C. The grazing lands exhibit typical savannah woodland vegetation with a scattered population of acacia trees and broadleaved shrubs. Cattle are the dominant livestock in the area followed by goats. Natural pasture is the main source of feed, supplemented by agricultural crop residues.

Plant sampling and processing

A total of 23 commonly available pasture species were collected from the study area during the months of July and August, 2008. Whole-plant pasture species were sampled from various locations by cutting at a height of 5 cm from the ground. Biomass samples of the same species collected from different sites were pooled to a sample, and dried in a forced air oven at 60 ^{o}C for 48 h. Dried samples were ground to pass through a 1mm sieve size and analysed for n-alkane concentrations and ^{13}C enrichment of alkanes.

Chemical Analysis

The chemical analysis was conducted at the laboratory of the Animal Nutrition group of Wageningen University, the Netherlands. N-alkane extraction and analysis was carried out as described by Mayes *et al*. (1986), with modifications by Salt *et al.* (1992). Tetratriacontane (C_{34}) was used as an internal standard. The extracted samples were analyzed for n-alkanes (C_{27} to C_{36}) using a gas chromatograph fitted to a flame ionizing detector, using helium as the carrier gas. With the same alkane extracts, the carbon isotope composition of the alkanes was determined by GC-IRMS. The isotope ratio of the alkanes was calculated in terms of conventional delta values ($\delta^{13}C$) as follows:

$$\delta^{13}C = 1000\ (R_{sample} - R_{standard})/R_{standard}$$

where, R_{sample} is the abundance ratio of ^{13}C to ^{12}C in the plant sample, and $R_{standard}$ is the abundance ratio of ^{13}C to ^{12}C in the standard sample (Vienna Pee Dee Belemnite, PDB).

Data Analysis

Multivariate statistical analysis including principal component analysis (**PCA**), orthogonal procrustes rotation (OPR) and redundancy analysis (RDA) were conducted

to explore the pattern of n-alkane profiles & ^{13}C enrichments of alkanes across the species, and to examine if use of $\delta^{13}C$ together with the n-alkane profiles increases the accuracy of diet composition estimation of free-ranging herbivores. Data were analyzed using GenStat for Windows (11th edition).

Results

Alkane profiles and their carbon isotope enrichments

The n-alkane concentrations (C_{27} to C_{35}) in the pasture species collected from the grazing lands is shown in Table 1. The odd-chain alkanes were found in much higher concentrations than the even-chain alkanes. The alkane C_{36} was excluded from the results as the values for most of the species were within the range of the analytical error of the GC. The stable isotope enrichment of carbon ($\delta^{13}C$) for individual n-alkanes (Table 2) showed a wide variation, ranging from -19.37‰ to -37.40‰. Generally, the odd-chain alkanes had a higher level of ^{13}C enrichment by at least one delta unit than the subsequent even chain alkanes. The level of enrichment tended to decrease, in both even and odd chain alkanes with increasing carbon number.

Table 1 the range of n-alkane concentration, mg/kg DM, for the species studied (n=23)

	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
Minimum	14	5	35	5	33	4	20	6
Maximum	429	39	281	24	1265	24	363	210
Average	81	17	94	11	283	10	149	74
SD	91	10	61	5	246	5	99	63

Table 2 the range of $\delta^{13}C$ values (‰) of n-alkanes for the species studied (n=23)

	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
Minimum	-19.46	-20.87	-19.75	-20.56	-19.67	-20.40	-21.42	-21.49
Maximum	-32.27	-34.00	-36.61	-36.66	-37.40	-35.67	-33.77	-34.58
Average	-22.00	-24.43	-22.66	-24.53	-22.58	-25.40	-23.86	-23.95
SD	2.62	2.69	3.33	3.03	3.45	3.72	2.66	2.61

Multivariate analysis

The PCA conducted based on the n-alkane profiles showed that about 91% of the variance between species was explained by the first three principal components (PC1-

PC3). Similarly, when the analysis was based on $\delta^{13}C$ values of n-alkanes, 74% of the variance was explained by the first three principal components. The scatter plot based on n-alkanes (Figure 1) shows a good species separation. However, clustering between some of the species, like *Chloris gayana*, and *Cynodon ethiopicus* was observed. The scatter plot based on the $\delta^{13}C$ values of n-alkanes (Figure 2) showed that the species were scattered along the two axes in a different way. Comparison of the two principal components by orthogonal procrustes rotation (OPR) revealed that the residual variance remaining after fitting the two PCA scores was 84.7% (Table 3). This indicated little similarity between the two PCA scores and that the majority of the variance explained by $\delta^{13}C$ values of n-alkanes was additional to that explained by the n-alkane profile of species.

Table 3 The variance (%) in the pattern of n-alkane concentration and $\delta^{13}C$ values of n-alkanes explained by the first three principal component axes (PC1, PC2, and PC3), and the residual variance (%) remaining after comparison by Orthogonal Procrustes Rotation (OPR) of the two principal component scores

Marker	Variance Explained (%)				Residual variance (%)
	PC1	PC2	PC3	Total	remaining after OPR
n-alkanes	54.6	22.1	14.1	90.8	
δC^{13} of n-alkanes	35.7	22.3	16.0	74.0	84.7

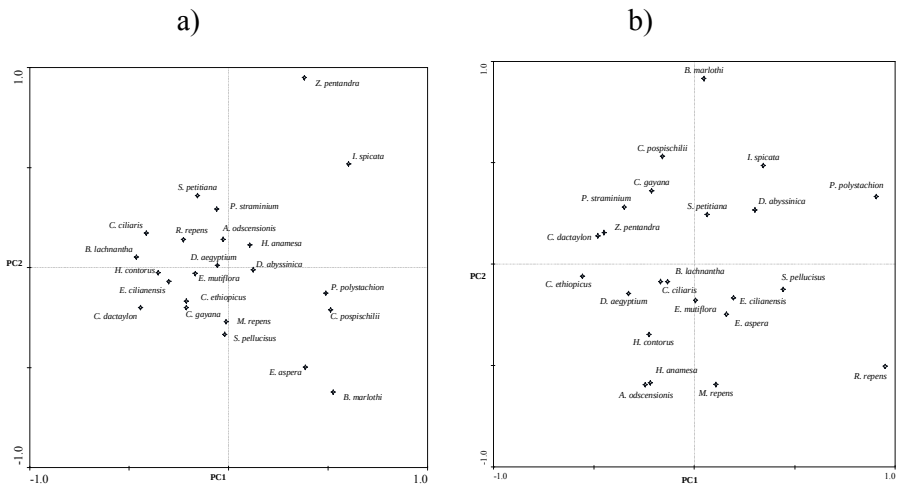

Figure 1 Scatter plot of pasture species on a two dimensional space using the first two principal components (PC1 and PC2) derived from PCA based on: a) the n-alkane concentrations, b) $\delta^{13}C$ values of the n-alkanes

Discussion

Estimation of diet composition has been achieved using least squares optimization procedures, where the faecal marker patterns are related with the potential diet component marker patters. With this procedure, in addition to the limitation that the number of diet components should be equal or less than the number of markers available, the accuracy of the estimation could decline as the botanical composition of the diet increases. In previous reports, the use of a combination of plant wax component n-alkanes, long-chain fatty alcohols, and acids have provided increased accuracy and power in the estimation of diet composition (Bugalho et al., 2004; Fraser et al., 2006; Kelman et al., 2003). The present analysis also showed that the interspecies variability in $\delta^{13}C$ values of n-alkanes could be used as an additional source of information to increase the accuracy of diet composition estimations. The increased analytical capacity to separate specific compounds prior to isotope composition analysis (Muccio and Jackson, 2009) provides enormous potential to study the isotopic ratio of not only n-alkanes but also long-chain fatty alcohols and fatty acids. The possibility of generating two different types of internal markers from a single set of compounds such as n-alkanes would be a desirable feature in terms of increasing the discriminatory power of wax components.

Acknowledgement

The assistances offered by Michel Breuer, Dick Bongers, and Jane-Martine Muijlaert during laboratory analysis; Amsalu Sisay during field sample collection and species identification; and Prof. Cajo ter Braak on the statistical analysis are highly acknowledged. This work was funded by Nuffic (The Netherlands Organisation for International Cooperation in Higher Education) and the Ministry of Education of Ethiopia.

Reference:

Bennett LL, Hammond AC, Williams MJ, Chase Jr CC and Kunkle WE 1999. Diet selection by steers using microhistological and stable carbon isotope ratio analyses. Journal of Animal Science 77, 2252-2258.

Bugalho MN, Dove H, Kelman W, Wood JT and Mayes RW 2004. Plant wax alkanes and alcohols as herbivore diet composition markers. Journal of Range Management 57, 259-268.

Dove H and Mayes RW 2005. Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. Small Ruminant Research 59, 123-139.

Ferreira LMM, Garcia U, Rodrigues MAM, Celaya R, Dias-da-Silva A and Osoro K 2007. The application of the n-alkane technique for estimating the composition of diets consumed by equines and cattle feeding on upland vegetation communities. Animal Feed Science and Technology 138, 47-60.

Fraser MD, Theobald VJ and Moorby JM 2006. Determining diet composition on complex swards using n-alkanes and long-chain fatty alcohols. Ecological Applications 16, 1901-1910.

Kassahun A, Snyman HA and Smit GN 2008. Impact of rangeland degradation on the pastoral production systems, livelihoods and perceptions of the Somali pastoralists in Eastern Ethiopia. Journal of Arid Environments 72, 1265-1281.

Kelman W, Bugalho M and Dove H 2003. Cuticular wax alkanes and alcohols used as markers to estimate diet composition of sheep (Ovis aries). Biochemical Systematics and Ecology 31, 919-927.

Mayes RW, Lamb CS and Colgrove PM 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. Journal of Agricultural Science, Cambridge 107, 161-170.

Muccio Z. and Jackson G.P. 2009. Isotope ratio mass spectrometry. Analyst 134, 213-222.

Oliván M, Ferreira LMM, Garcia U, Celaya R and Osoro K 2007. Application of n-alkanes as diet composition markers in grazing/browsing goats and sheep: effect of using different faecal recovery corrections and plant species grouping approaches. Australian Journal of Agricultural Research 58, 1013-1022.

Salt CA, Mayes RW and Elston DA 1992. Effects of season, grazing intensity and diet composition on the radiocaesium intake by sheep on re-seeded hill pasture. Journal of Applied Ecology 29, 378-387.