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SUMMARY – Diet selection is important for pasture ecology and animal production, and can be estimated using plant alkane concentrations provided they differ markedly between plant species or parts. For the purpose of knowing the possibility of identifying different browse pastures or their anatomical fractions as components of the diet in grazing ruminants, freeze-dried samples were ground, and n-alkanes extracted and analysed by gas chromatography. Concentrations of individual hydrocarbons were expressed as proportion of the total amount and analysed by way of stepwise discriminant analysis, which was a useful tool for classification of *a priori* defined groups (species and plant parts). The best discriminant alkanes were those with odd-chain number of carbons (except C_{21} and C_{27}) plus C_{30} and C_{32}, the two latter even at low concentrations. As conclusion, n-alkane profiles in the studied species and plant parts were different enough to allow their identification as components of the diet in grazing ruminants.

Keywords: Diet selection, n-alkanes, discriminant analysis, browse pastures, plant part, grazing.

RESUME – *"Effet de l'espèce et de la partie de la plante sur les concentrations des n-alcanes dans la cuticule de cire des arbustes des parcours de la moyenne vallée de l'Ebre (Espagne)". En écologie des pâtures et en production animale, la connaissance de la composition du régime prélevé par l'animal est importante. Elle peut être estimée à partir de la concentration des n-alcanes ayant des profils distincts dans les différentes espèces végétales ou leurs différentes parties. Pour connaître la possibilité d'identifier quelques espèces ou leurs parties comme constituants du régime des ruminants sur parcours, les contenus en n-alcanes d'échantillons lyophilisés et broyés ont été déterminés par chromatographie en phase gazeuse. La concentration de chaque alcane a été déterminée par analyse discriminante, ce qui a permis de classer les différentes espèces ou leurs parties. Les meilleurs alcanes discriminants ont été ceux ayant un nombre impair d'atomes de carbone (sauf C_{21} et C_{27}) en plus de C_{30} et C_{32}. En conclusion, les profils des n-alcanes dans les échantillons étudiés ont été suffisamment différents pour permettre leur identification comme constituants du régime des ruminants au pâturage.*

Mots-clés : *Composition du régime, n-alcanes, analyse discriminante, pâturage.*

Introduction

The nutritive value of a pasture depends mainly on its intake and the selection animals exert in consuming the different species or the various parts of a determined plant (Mayes and Dove, 2000). These parameters are difficult to estimate in grazing systems, with errors often being large due to limitations of available measurement methods. The use of plant-wax alkanes offers numerous advantages over alternative methods (Dove and Mayes, 1991; Mayes and Dove, 2000), among them the concurrent estimation of intake. However, the profiles of n-alkanes in different components of the diet have to be different enough in order to obtain accurate results (Dove *et al*., 1999), the use of discriminant analysis being a powerful tool for deciding this aspect (Valiente *et al*., 2003).

Although browse pastures are one of the main resources for grazing livestock in the Mediterranean area (Papanastasis, 1996), little is known about how they are consumed in terms of their intake and the selection of the different species. As a previous step, the investigation of n-alkane patterns of the most common plants in a determined area will provide essential information to allow further studies of these aspects.

The aims of the present work were to assess n-alkanes profiles of different species and plant parts from browse pastures in a study area representative of the Middle Ebro Valley (Spain), and to determine which were the best n-alkanes useful for identifying the different components and their proportions in the grazing ruminant diet.

Materials and methods

The present study was carried out in two adjacent areas of the Middle Ebro Valley (Spain), representative of the gypsum hills and the argillaceous valleys, which alternate in the region. Samples from the eight most common permanent species were collected in April 2002 (Table 1), annual and scarce plants not being taken into account. The former represent about 90% of the total vegetation that may be found in the study area throughout the year. At least 20 shoots of each species were sampled trying to obtain a representative set of the entire population.

Table 1. Sampling location, family, species and abbreviation used for plants analysed

Location	Family	Species	Abbreviation
Valley	Asteraceae	*Artemisia herba-alba* Asso	ARTE
Valley	Poaceae	*Lygeum spartum* (Gouan) Parl.	LYGE
Valley	Quenopodiaceae	*Salsola vermiculata* L.	SALS
Valley	Quenopodiaceae	*Suaeda vera* Forsk. Ex J.F. Gmel.	SUAE
Hill	Papilionaceae	*Ononis tridentata* L.	ONON
Hill	Cistaceae	*Helianthemum squamatum* (L.) Pers.	HELI
Hill	Cariofilaceae	*Gypsophila struthium* subsp. *hispanica* (Willk.) G. López	GYPS
Hill	Lamiaceae	*Thymus vulgaris* L.	THYM

Samples were separated by hand, immediately after collection, into stems and leaves, except *Lygeum spartum* which was separated into stems and heads. Then, samples were freeze-dried and ground through 1 mm (leaves and heads) or 0.75 mm (stems) sieves for extraction of n-alkanes.

For alkane extraction, 1.5 g each of four samples of every part of each species were weighed into 200 x 20 mm thick-walled screw-topped Pyrex test-tubes, adding the following amounts of internal standard (solution of heptane containing 1 mg/g of C_{22} and C_{34}): 25 mg for heads, 75 mg for stems and 200 mg for leaves. Then extraction of n-alkanes was carried out following the technique described by Mayes *et al*. (1986), with the modifications suggested by Oliván and Osoro (1999). Alkane analysis was carried out by on-column injection of 1 µl (0.2 µl for leaf samples) of the eluate onto a 30 m x 0.530 mm HP-1 capillary column (1.5 µm thickness) in an Agilent 6890 gas chromatograph fitted with an automatic injector and flame ionization detector. The carrier gas was helium (10 ml/min) as was the make-up gas to the detector (45 ml/min). The injector was programmed to track the oven's temperature programme as follows: 230ºC for 0.2 min and a ramp of 6ºC/min to 300ºC, maintained for further 18 min. The detector was maintained at 350ºC throughout the whole process. Peak area data were processed using an HP 3396 A integrator. 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 8 sample extracts.

Concentrations of individual alkanes were expressed as proportion of the total amount, and the ratios were arcsin transformed in order to satisfy normality. Stepwise discriminant analysis was then performed to obtain centroids for every group (species and their parts) using subsequent functions. Only discriminant functions increasing at least 10% of accumulated variability were analysed (Dove *et al*., 1996; Dove *et al*., 1999), and the centroids of each group for each function subjected to an one-way analysis of variance in order to investigate if the defined groups could be differentiated using combinations of n-alkanes, and which ones of these latter were the most useful for the purpose. The results obtained were validated using the cross-validation method (Hair *et al*., 1999). The statistical package SPSS 11.0 was used for all computations.

Results and discussion

n-alkanes content

The alkane concentration of the different species and plant parts studied in the present experiment is given in Table 2. As previously found (Dove and Mayes, 1991; 1996; Dove, 1992), odd-chain alkanes were the most abundant, C_{31} and C_{29} being predominant, whereas those with more than 34 atoms of carbon were not detectable in most samples.

Table 2. Mean concentration (mg/kg dry matter) of n-alkanes in the different species and plant parts studied in the present experiment. For abbreviations see Table 1

n-alkane	n-alkane concentration (mg/kg dry matter)							
	ARTE	LYGE	SALS	SUAE	ONON	HELI	GYPS	THYM
Stems								
C_{21}	3.80	0.96	5.50	6.01	1.18	2.41	0.43	1.85
C_{23}	5.98	12.31	n.d.	5.28	15.07	n.d.	8.42	22.24
C_{24}	n.d.	15.31	n.d.	4.74	22.01	n.d.	4.80	8.34
C_{25}	4.11	63.66	n.d.	12.13	81.56	4.91	10.68	18.27
C_{26}	2.70	30.61	5.94	5.71	46.94	2.40	6.49	1.83
C_{27}	14.10	117.66	6.86	14.05	170.08	8.23	15.60	6.84
C_{28}	5.97	41.47	3.97	1.67	27.22	1.67	5.61	3.93
C_{29}	139.39	535.37	8.10	3.75	207.49	5.52	22.93	41.11
C_{30}	12.65	32.14	n.d.	n.d.	32.69	n.d.	9.62	6.15
C_{31}	139.85	1501.09	4.49	3.04	460.16	3.88	389.88	55.56
C_{32}	4.04	13.58	n.d.	n.d.	7.03	n.d.	2.53	8.43
C_{33}	11.73	287.62	n.d.	n.d.	10.75	n.d.	5.14	82.08
C_{35}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.81
C_{36}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Leaves (heads for LYGE)								
C_{21}	1.12	2.94	1.56	2.53	2.00	3.36	0.94	1.48
C_{23}	0.41	69.51	2.90	0.93	0.25	n.d.	1.03	0.30
C_{24}	0.44	20.62	1.28	0.88	0.47	n.d.	3.17	0.31
C_{25}	0.68	437.00	2.18	2.09	0.34	0.23	3.24	0.68
C_{26}	0.56	12.38	1.24	0.65	0.16	0.33	2.36	0.93
C_{27}	8.05	333.14	5.34	5.69	1.13	0.72	4.99	4.45
C_{28}	5.62	23.63	2.02	1.00	0.87	0.31	3.53	5.08
C_{29}	106.28	544.89	7.73	7.52	15.89	1.94	19.08	68.7
C_{30}	9.66	42.45	0.82	0.71	5.16	0.35	12.67	24.07
C_{31}	43.64	1682.35	2.00	2.51	30.05	3.55	323.02	102.18
C_{32}	2.80	17.89	0.31	n.d.	1.24	0.87	4.07	37.47
C_{33}	1.91	472.58	0.23	0.19	0.90	1.30	2.20	83.10
C_{35}	1.77	4.26	n.d.	n.d.	n.d.	n.d.	n.d.	12.56
C_{36}	2.61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detected.

Total alkane contents were, in general, high, ranging from 12.95 (HELI leaves) to 3663.63 (LYGE heads) mg/kg dry matter. Average amounts in stems were higher than in leaves, whereas there were very different alkane patterns among the groups considered, a fact which was expected due to the distinct phylogenies involved (Kolattukudi, 1976). Plant age was not considered in the present study

but, although there are indications of age effect on n-alkane concentrations (Laredo *et al.*, 1991), this effect is small when compared to that of species or plant part (Dove *et al.*, 1996; Smith *et al.*, 2001).

Discriminant analysis

The centroids for every group considered, calculated from the first three discriminant functions, are shown in Table 3 and plotted in Figure 1. Significant differences between groups were found for every discriminant function, the *post hoc* Bonferroni test showing that function 1 was able to discriminate all the groups except stems of *Artemisia herba-alba* and leaves of *Salsola vermiculata*. These two groups were discriminated by function 2. The cross-validation method showed that 100% of the groups were classified correctly by the model as defined *a priori*. With respect to the information given by Figure 1, a larger distance between centroids is associated to a better differentiation of the groups. For the three combinations of functions, the closer groups were leaves of *Salsola vermiculata* and *Suaeda vera*, and leaves and stems of *Gypsophyla struthium.* For this reason, differentiation of these species and plant parts could be problematic in future studies with n-alkanes.

Table 3. Group centroids calculated from the first three discriminant functions of the model

Function	ARTE	LYGE	SALS	SUAE	ONON	HELI	GYPS	THYM
Stems								
1	-30.4	26.0	-529.0	-358.4	-186.3	-315.8	-469.2	821.9
2	65.3	-223.4	129.8	329.3	-27.2	186.7	-502.3	80.8
3	204.9	73.9	-278.8	-111.3	49.2	-440.9	123.0	183.6
Leaves (heads for LYGE)								
1	62.9	194.1	-28.5	-186.4	-56.2	503.2	-454.0	1006.1
2	261.1	-124.7	463.2	386.3	-129.5	-120.6	-597.3	-177.4
3	117.3	31.7	198.9	156.0	159.0	-305.3	41.3	-202.5

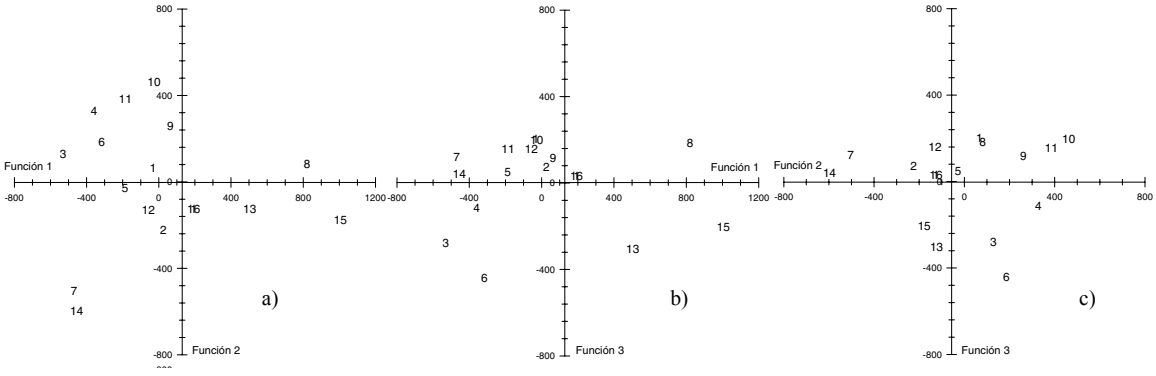

Fig. 1. Group centroids for discriminant functions 1 and 2 (a), 1 and 3 (b) and 2 and 3 (c). 1: stems of *Artemisia herba-alba*; 2: stems of *Lygeum spartum*; 3: stems of *Salsola vermiculata*; 4: stems of *Suaeda vera*; 5: stems of *Ononis tridentata*; 6: stems of *Helyanthemum squamatum*; 7: stems of *Gypsophyla struthium*; 8: stems of *Thymus vulgaris*; 9: leaves of *Artemisia herba-alba*; 10: leaves of *Salsola vermiculata*; 11: leaves of *Suaeda vera*; 12: leaves of *Ononis tridentata*; 13: leaves of *Helyanthemum squamatum*; 14: leaves of *Gypsophyla struthium*; 15: leaves of *Thymus vulgaris*; 16, heads of *Lygeum spartum*.

In general, discriminant analysis showed that n-alkane patterns of the species and plant parts studied were different enough to allow further studies involving animals, like those related with diet composition, intake or digestibility of the diet consumed. The worst separated groups are not likely to

be easily identified in such studies, and hence they should be grouped or concurrent techniques, like faecal micro histology, should be used (Smith *et al*., 2001; Dove and Mayes, 1996).

In order to know which n-alkanes were best discriminated between groups, their coefficients for the first three discriminant functions were compared (Table 4). These coefficients represent the relative contribution of each n-alkane to every discriminant function. Thus, the best discriminant alkanes were those with the highest absolute values, odd-chain ones in the present study (except C_{21} and C_{27}) plus C_{30} and C_{32} which, even at low concentrations, showed as powerful discriminants. This lack of relationship between concentration and discriminant power has also recently been pointed out by Valiente *et al*. (2003) with mixed grain:forage diets.

Table 4. Coefficients of every n-alkane for the first three discriminant functions (F) of the model

F	C_{21}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
1	75	-322	35	600	38	-105	24	224	475	-346	1773	1484	594	148
2	97	541	115	333	89	60	236	755	88	-544	-134	-77	20	83
3	237	1466	338	-175	206	57	47	714	900	655	-1012	354	127	190

Conclusions

The results of the present experiment showed that, in general, alkane patterns of the species and plant parts considered in the study area were different enough to allow studies of diet selection and intake in grazing animals, which in turn will contribute to improve the knowledge of the nutritive value of the browse pastures common in the dry steppes of the Mediterranean area.

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