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Abstract. Diet selection by sheep grazing dryland lucerne with a high proportion of weeds was assessed in two consecutive years (2005/2006). The study was performed on 2.66 ha of pasture divided in two homogeneous paddocks subjected to a stocking rate of either 10 or 20 sheep/paddock, and grazed for 17 days. Before and after each grazing trial, an inventory was conducted to assess the frequency and the phenological state of each plant species. The amount of biomass was determined pre- and post-grazing in each paddock and for each year, and the different species identified were separated by hand, dried and weighed to calculate their contribution to the total biomass. The plants that showed the higher "selection score" were *Medicago sativa*, *Lolium rigidum*, *Eruca vesicaria* (ssp. *sativa*) and *Diplotaxis erucoides*, regardless of the stocking rate or the year of study ($P < 0.05$), whereas *Bromus rubens*, *Carduus bourgeanus* and *Onopordum acanthium* were absolutely rejected. Other species were more consumed in the paddocks with a higher stocking rate, or in the year where the initial biomass was less abundant (2005). The interactions stocking rate × species and year × species were both non significant ($P > 0.05$). The n-alkanes concentration in the consumed fraction of each eaten plant was also analysed, and the values expressed as proportion of the total alkanes subjected to a stepwise discriminant analysis. n-Alkane profiles in the eaten fraction of the consumed species were different enough to allow their identification as components of the diet.

Keywords. Grazing – Sheep – Diet selection – *Medicago sativa* – Weeds – N-alkanes – Discriminant analysis.

La sélection des mauvaises herbes par les ovins en pâturage de luzerne cultivé en sec

Résumé. *La sélection du régime a été étudiée en utilisant des ovins pâturant de la luzerne non irriguée avec une grande proportion de mauvaises herbes sur deux années consécutives (2005/2006). L'essai a été conduit sur un parcours de 2.66 ha divisé en deux parties égales supportant une charge de 10 et 20 brebis/ha pâturant pendant 17 jours. Avant et après chaque période de pâturage on a mené un inventaire des espèces végétales trouvées et de leur état phénologique. La quantité de biomasse a été déterminée avant et après le pâturage pour chaque année et chaque parcelle, et les différentes espèces identifiées ont été séparées à la main, séchées et pesées pour calculer leur contribution à la biomasse totale. Les espèces qui ont montré la meilleure "note de sélection" ont été* Medicago sativa, Lolium rigidum, Eruca vesicaria *(ssp.* sativa*) et* Diplotaxis erucoides, *indépendamment du taux d'allotement et de l'année d'étude ($P < 0,05$). D'autre part,* Bromus rubens, Carduus bourgeanus *et* Onopordum acanthium *ont été absolument refusées. D'autres espèces ont été plus consommées dans les parcelles avec un plus grand taux d'allotement ou dans les années pendant lesquelles la biomasse initiale a été moins abondante (2005). Les interactions taux d'allotement × espèce et année × espèce n'ont pas été significatives ($P > 0,05$). Les concentrations des n-alcanes dans les parties consommées de chaque espèce végétale ingérée ont été analysées par analyse discriminante, et elles ont été suffisamment différentes pour permettre leur identification comme constituants du régime des ruminants au pâturage.*

Mots-clés. *Pâturage – Ovins – Sélection du régime –* Medicago sativa *– Mauvaises herbes – N-alcanes – Analyse discriminante.*

I – Introduction

Integration of grazing sheep in the crops' cycle of the arid and semi-arid areas of the Mediterranean basin is a very interesting practice from the point of view of profitability and conservation of the environment (de Vega and Delgado, 2002). One of the proposed alternatives (Delgado, 2003) includes direct grazing of winter cereals, shrubs (*Atriplex halimus*) and lucerne (*Medicago sativa*) along the year. This latter plays an important role as source of nutrients during the last months of spring (April-May-June) and the first of autumn (October). In dryland conditions, lucerne fields are rarely pure, being the presence of wild foods rather frequent (Zaragoza and Sanz, 1978). As a consequence, animal performance is often below expected, and the knowledge of diet composition becomes essential to set up supplementation strategies. The n-alkane methodology (Mayes *et al*., 1986) is currently one of the most used for this purpose (Dove and Mayes, 2005), although its correct utilization requires the consideration of only the species, or parts of them, actually consumed by the animals (Dove *et al.*, 1996). In addition, it is needed that the n-alkane profiles of the different components of the diet are different enough to allow their identification and quantification using the faecal concentration of hydrocarbons (Dove, 1992). As the available information about those two aspects is virtually nil with reference to sheep grazing dryland lucerne characterized by a high proportion of weeds, the present experiment was set up to: (i) assess what species, and parts of them, are actually consumed by the animals, and the effect of the year of study and the stocking rate; and (ii) determine if the n-alkane profiles of the actually consumed species, or parts of them, are different enough to allow their identification and quantification.

II – Materials and methods

The study was carried out in Zuera (41° 50' N longitude; 0° 41' W latitude) during two consecutive years (2005 and 2006). The trial was performed in May, on 2.66 ha of pasture divided in two homogeneous paddocks subjected to a stocking rate of either 10 or 20 sheep/paddock, and grazed for 17 days. Thirty non pregnant and dry Ansotana ewes, with average live weight of 61 ± 2.3 (2005) and 50 ± 2.5 kg (2006) were used. Before each grazing trial an inventory was conducted indicating the phenological stage of each plant species. For this purpose, nine 1 m^2 quadrats were thrown at equidistant distances (three by width and three by length) within each paddock, indicating their position with bright coloured pegs. The different plants were identified, and their morphology (presence and spatial distribution of leaves, stems, flowers, etc.) assessed by visual observation. Another ten 0.25 m^2 quadrats were thrown diagonally, and the contents inside cut at ground level. After drying at 60°C to constant weight, the different species were separated by hand and weighed to calculate their contribution to the total biomass. Samples of each species listed were taken in the area adjacent to the 1 m^2 quadrats and kept frozen at -20°C.

After each grazing period, plants within the 1 m^2 quadrats were again visually assessed, and the parts consumed estimated by difference with the pre-grazing inventory. Then, the biomass was cut at ground level and dried at 60°C to constant weight. The different species were separated by hand and their contribution to the total dry matter (DM) calculated. The "selection score" for each plant was estimated as the proportion of the initial material which disappeared from the paddocks [(kg DM/ha before grazing – kg DM/ha after grazing)/kg DM/ha before grazing]. No correction was allowed for the growing capacity of grazed species because of the extremely low rainfall during the study periods and hence the negligible DM production assumed. "Selection scores" were subjected to an one-way analysis of variance (ANOVA) taking into account the effects of year, stocking rate and plant species, and the interactions between year and stocking rate, and between stocking rate and plant species. The PROC MIXED protocol of the SAS 8.2 statistical package was used.

Samples (n = 4) equivalent to the estimated consumed parts of each species were taken from the frozen plants referred to above, and analysed for n-alkanes concentration (Mayes *et al.*, 1986) after freeze-drying and grinding through a 1 mm screen. 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 (parts of each species) using subsequent functions. Only discriminant functions increasing at least 10% of accumulated variability were analysed (Dove *et al.*, 1996). The statistical package SPSS 14.0 was used for computation.

III – Results and discussion

Table 1 shows the species found before and after grazing in each paddock and for each year. It is remarkable that less than 35% of the initial dry biomass was in the form of lucerne (except in the paddock destined for a stocking rate of 20 sheep in 2006), whereas *Lolium rigidum* contributed much more to the forage production. No other species contributed significantly to the initial biomass, except *Eruca vesicaria* in 2005 (nearly 10% on average), and *Anacyclus clavatus* in 2006 (slightly more than 6%).

Table 1. Dry matter contribution (kg/ha) of the different plant species found in dryland lucerne paddocks before (Pre) and after (Post) grazing for 17 days. Stocking rates are per paddock (1.33 ha each)

Year	2005				2006			
Stocking rate	10 sheep		20 sheep		10 sheep		20 sheep	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Medicago sativa	77.00	27.22	207.00	13.89	252.00	138.89	459.00	141.11
Lolium rigidum	303.00	146.11	212.00	41.67	598.00	550.00	141.00	56.11
Silene vulgaris	0.00	0.56	0.00	0.00	4.00	0.56	0.00	0.00
Eruca vesicaria	60.00	11.11	54.00	6.11	34.00	17.22	8.00	1.67
Anacyclus clavatus	4.00	30.83	40.00	29.44	60.00	46.67	56.00	10.56
Diplotaxis erucoides	3.00	13.89	39.00	5.28	12.00	3.33	8.00	2.78
Euphorbia serrata (veg)†	0.00	0.00	0.00	0.00	–	–	–	–
Euphorbia serrata (flower)†	0.00	0.00	0.00	0.00	0.00	3.33	0.00	0.00
Hordeum murinum	0.00	1.39	6.00	1.67	10.00	1.39	0.00	0.00
Bromus rubens	7.00	13.89	10.00	6.39	12.00	4.17	18.00	0.56
Cichorium intybus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glaucium corniculatum	0.00	0.00	0.00	3.06	0.00	1.11	2.00	0.00
Reseda lutea	–	–	–	–	12.00	6.11	2.00	7.22
Papaver rhoeas	–	–	–	–	0.00	0.00	0.00	0.00
Onopordum acanthium	–	–	–	–	0.00	0.56	0.00	0.00
Carduus bourgeanus	–	–	–	–	0.00	0.56	0.00	0.00
Scorzonera laciniata	–	–	–	–	0.00	1.11	0.00	0.00
Unidentified material	110.00	232.78	52.00	79.17	194.00	100.00	60.00	58.89

†Veg = in vegetative stage; Flower = with flowers.
– = not found.

Biomass availability before grazing was much higher in 2006 than in 2005, all species contributing proportionally to the difference (except *Eruca vesicaria* and *Diplotaxis erucoides*, which presence in 2006 was minority). From the percentages represented by each species with respect to the total biomass pre- and post-grazing, it was clear that lucerne was preferred by the animals regardless the year or the stocking rate, whereas *Lolium rigidum* was preferred in 2005 but not in 2006. *Eruca vesicaria* was selected only when it represented a relatively important amount of the initial biomass (2005). The proportion of unidentified material was remarkable in all cases, especially after grazing.

The "selection score" for each plant, as defined in the Materials and methods section, is given in Table 2. It must be pointed out that some results did not match visual observations performed in the 1 m^2 quadrats. As such, the "selection score" for *Lolium rigidum* in the paddock subjected to a stocking rate of 10 sheep in 2006 was only 8%, despite the fact that direct observation of the plants indicated a very different situation. On the other hand, species that were absolutely rejected by the animals (*Bromus rubens*, for instance) obtained very high "selection scores" (96.91% in the paddock with 20 animals in 2006). Other minority species, such as *Hordeum murinum*, *Glaucium corniculatum* or *Reseda lutea* (Table 1) gave results highly influenced by the small number of plants present in the paddocks. In addition, negative values indicated errors associated with sampling, which was performed in different areas before and after grazing. As a consequence, only the "selection scores" for the first six species listed in Table 2 were considered in the ANOVA. Negative values were considered as missing data, as well as the surprisingly low figure obtained for *Lolium rigidum* in the paddock subjected to a stocking rate of 10 sheep in 2006.

Table 2. "Selection score" (see Materials and methods) of the different species found in a dryland lucerne field, grazed by sheep for 17 days. Stocking rates are per paddock (1.33 ha each). Species not shown had "selection scores" = 0

Year	2005		2006	
Stocking rate	10 sheep	20 sheep	10 sheep	20 sheep
Medicago sativa	64.65	93.29	44.89	69.26
Lolium rigidum	51.78	80.35	8.03	60.20
Silene vulgaris	0.00	0.00	86.11	0.00
Eruca vesicaria	81.48	88.68	49.35	79.17
Anacyclus clavatus	–670.83	26.39	22.22	81.15
Diplotaxis erucoides	–362.96	86.47	72.22	65.28
Hordeum murinum	0.00	72.22	86.11	0.00
Bromus rubens	–42.86	36.10	65.28	96.91
Glaucium corniculatum	0.00	92.06	0.00	100.00
Reseda lutea	–	–	49.07	–261.11

The results from the ANOVA (not in Table 3) showed that the plants with the higher "selection score" were *Medicago sativa*, *Lolium rigidum*, *Eruca vesicaria* (ssp. *sativa*) and *Diplotaxis erucoides*, ($P = 0.0118$), whereas *Bromus rubens*, *Carduus bourgeanus* and *Onopordum acanthium* were absolutely rejected (assessed by visual observation). The species more consumed (a higher percentage) were in the paddocks with a higher stocking rate ($P = 0.0171$), with no significant effect of the year ($P = 0.2457$), although in absolute terms the "selection scores" were higher in the year where the initial biomass was less abundant (52.83 *vs* 45.98, on average, for 2005 and 2006, respectively). The interactions stocking rate × species and year × species were both non significant ($P > 0.05$).

The n-alkane concentration in the consumed fraction of each eaten plant showed substantial changes across years, although, in general, the most abundant hydrocarbons were C_{29} and C_{31} and, to a lesser extent, C_{27} and C_{33}. n-Alkanes with a chain-length shorter than 26 or longer than 34 carbon atoms were present at very low concentrations, if any.

The centroids for every group considered, calculated from the first three discriminant functions, are shown in Table 3. A larger distance between centroids is associated to a better differentiation of the groups in their n-alkanes profile. For the three combinations of functions, the closer groups were *Lolium rigidum* and *Eruca vesicaria* (only in 2005), *Euphorbia serrata* (flowering; 2005) and *Glaucium corniculatum* (2006), *Glaucium corniculatum* (2005; 20 sheep/paddock), *Silene vulgaris* (2006; 20 sheep/paddock) and *Diplotaxis erucoides* (2006), and *Anacyclus clavatus* (2005; 20 sheep/paddock) and *Cichorium intybus* (2006; 10 sheep/paddock). In the case of these latter, the fact that both belong to the same family has already been pointed out as a reason for a similar profile of n-alkanes (Kolattukudy, 1976). The high proximity between groups may preclude differentiation of these species, and some authors have suggested the use of concurrent techniques such as microhistology (Dove and Mayes, 1996; Smith *et al*., 2001).

IV – Conclusion

Generally speaking, discriminant analysis showed that n-alkane patterns of the eaten portion of each species (estimated as pointed out before) were different enough to allow further studies of 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 used.

Table 3. Group centroids calculated from the first three discriminant functions of the model. Only the species consumed in amounts high enough to get significant samples for the n-alkane analysis were included

Year	2005			2006		
Function	**1**	**2**	**3**	**1**	**2**	**3**
Stocking rate 10 or 20 sheep/paddock						
Medicago sativa	–50.460	8.197	0.088	16.126	29.017	3.450
Lolium rigidum	–27.541	8.135	–5.579	13.659	0.137	13.717
Silene vulgaris				–8.749	–10.361	–7.185
Eruca vesicaria	–24.455	–1.397	–9.192	29.665	17.110	3.794
Anacyclus clavatus	–32.424	–2.943	17.888	37.125	–12.358	34.779
Diplotaxis erucoides	11.588	–20.291	15.902	5.833	–24.152	53.919
Euphorbia serrata (veg)†	–5.666	–55.511	–25.259			
Euphorbia serrata (flower)†	–1.680	–14.911	43.792	39.084	–6.939	–8.584
Hordeum murinum				–17.227	0.076	–39.502
Cichorium intybus				–37.517	–15.531	–6.157
Glaucium corniculatum	–2.728	–8.886	–15.658			
Reseda lutea				–29.215	74.586	30.170
Papaver rhoeas				11.236	–11.404	–39.377
Stocking rate 10 sheep/paddock						
Silene vulgaris	18.065	4.436	–6.003			
Cichorium intybus	30.870	25.943	–28.033			
Glaucium corniculatum				–1.225	–18.711	22.771
Stocking rate 20 sheep/paddock						
Silene vulgaris	11.269	4.797	–7.575			
Cichorium intybus	24.847	36.079	–20.425			
Glaucium corniculatum				–10.479	–5.117	–21.744

†Veg = in vegetative stage; Flower = with flowers.

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