J. Range Manage.
54: 239–242 May 2001

Comparative rumen and fecal diet microhistological determinations of European mouflon

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Abstract

The population of European mouflon (*Ovis musimon* Pallas) established on an island of the sub-Antarctic Kerguelen archipelago is characterized by a demographic cycle. Every 2–5 years, there is a massive winter mortality due to food shortage. A good knowledge of food resources utilization appeared essential to understand the population growth dynamics. We investigated the validity of the microhistological analysis of feces by a comparative analysis of 30 paired rumen and fecal samples collected in winter. Sixteen and 17 food items were identified respectively in rumen and fecal samples. Most fragments could be accurately determined because plant diversity was low. Both methods gave similar results. Though quantitative differences appeared between methods for some items, the same 4 major food constituents were identified in relatively close proportions in both rumen and fecal samples. There is a risk of slight overestimation of annual meadow-grass (*Poa annua* L.) and mosses in feces, and of *Azorella selago* Hook. f. in the rumen.

Key Words: diet analysis, rumen, feces, *Ovis musimon*.

Résumé

La population de mouflon (*Ovis musimon* Pallas), établie dans une île de l'archipel de Kerguelen, présente une évolution cyclique de sa démographie. Tous les 2 à 5 ans, une mortalité massive des individus intervient par manque de nourriture. L'analyse de l'utilisation des ressources trophiques est essentiel pour expliquer la dynamique de la population. Dans ce but, la validité de la méthode basée sur l'analyse micrographique des fèces a été testée par la comparaison des contenus des panses et des fèces de 30 mouflons prélevés en hiver. Seize des 17 items consommés ont été trouvés respectivement dans les rumens et dans les fèces. La plupart des fragments peuvent être précisément identifiés en raison de la faible diversité en espèces végétales disponibles. Les deux méthodes donnent des résultats similaires, les 4 principales espèces consommées ayant été identifiées dans des proportions très proches dans les panses et les fèces. Cependant des différences quantitatives apparaissent pour quelques items dont la graminée *Poa annua* L. et les mousses, surestimées dans les fèces et *Azorella selago* Hook. f., surestimé dans les panses.

The population of European mouflon (*Ovis musimon* Pallas) present on Ile Haute (6.5 km^2; 49°24'S 69°56'E), in the sub-Antarctic Kerguelen archipelago, originated from a pair introduced in 1957 (Lésel 1967). Since the end of the 1970's, the population has followed a cyclical demographic pattern during which population size fluctuated between approximately 250–300 and 700 individuals, with periodic die-offs due to undernutrition occurring every 2–5 years (Boussès et al. 1992, Réale 1996). The analysis of food resources utilization is thus central to understanding the population dynamics. It was however necessary to develop a non-invasive method to avoid disturbances of the population dynamics. The population diet was studied during 5 years using the fecal microhistological analysis. This method is limited in the analysis of complex diets (e.g. Stewart 1967, Scotcher 1979, Holechek and Gross 1982a, McInnis et al. 1983, Putman 1984), which often preclude identification to the species level. Moreover, the representation of diets obtained from feces and stomach content analyses can differ significantly (Anthony and Smith 1974, Hanley et al. 1985, Holisova et al. 1986, Lewis 1994). In this paper we examine the hypothesis that the fecal microhistological analysis is representative of the diet on Ile Haute by comparing it with rumen contents.

Authors wish to thank D. Discours for the microhistological analysis of rumen and feces contents. Thanks to G. Stirling for correcting the English. The research was supported by the Institut Français pour la Recherche et la Technologie Polaires (Pr n°276) and the Centre National de la Recherche Scientifique (UMR 6553, Université de Rennes I).

Manuscript accepted 29 Aug. 2000.

Materials and Methods

The island landscape is a treeless area dominated by rocky and bare soils (68% of the area) while swards and meadows represent only 26% and peat bogs 6%. The number of native vascular plant species is limited to 31 in the Kerguelen archipelago (Greene and Walton 1975), of which only 10 are common on Ile Haute. These species include 3 grasses, *Agrostis magellanica* Lam., *Deschampsia antarctica* Desv. and *Festuca contracta* T. Kirk., a rush *Juncus scheuchzerioides* Gaud., some forbs *Azorella selago* Hook. f. (abundant), *Cotula plumosa* Hook. f., *Galium antarcticum* Hook. f., *Ranunculus biternatus* Smith, *R. pseudotrullifolius* Skottsberg, a dwarf shrub *Acaena magellanica* Vahl. and a fern *Blechnum penna-marina* Kuhn. Mosses are abundant in wetland areas. Some alien grasses (annual meadow-grass, *Poa annua* L., mainly) and forbs (*Cerastium fontanum* Baumg., *C. glomeratum* Thuill., *Sagina procumbens* L., *Taraxacum officinale* Wiggers) increased the carrying capacity of

the island (Chapuis et al. 1994). On the seashore, 3 abundant algae species are available at low tide, *Ulva lactuca* L., *Durvillea antarctica* (Cham.) Har. and *Macrocystis pyrifera* L.

For the purpose of this study, 30 mouflon ewes were shot on the island during the austral winter of 1994, from 21 July to 21 September. The rumen content was thoroughly mixed and one liter was collected, frozen in the field and then oven-dried at 65°C in the laboratory. Approximately 20 fecal pellets were obtained per mouflon and preserved in a 5% formalin solution. Biases due to differential plant fragmentation in rumen and fecal samples were overcome by dry-milling the rumen samples through a 2 mm screen (Sparks and Malechek 1968). After a 30 second maceration in sodium hypochlorite (Abbas 1988), rumen or fecal samples were washed through a 0.2 mm mesh sieve to eliminate small unidentifiable fragments (Sparks and Malechek 1968). No stain was used and subsamples were spread out on slides under 22 mm^2 cover slips. For each rumen and fecal sample, 4 microscope slides were prepared and plant fragments were counted in 25 microscope fields per slide at 100 magnification. An extensive photomicrographic reference collection of epidermal tissue from Ile Haute plant species was prepared and used to identify plant fragments. Isolated trichomes were not considered. The mean number of fragments examined per rumen and fecal sample was 506 ± 58 (n= 60). Most epidermal fragments were identified to a species level. There were a few species with very similar epidermis (*Deschampsia antarctica-Agrostis magellanica*) and others we could only identified at a genus level (e.g. *Cerastium* spp., *Ranunculus* spp.). All the analysis were performed by the same trained observer, who practiced the technique for several years and previously determined the diet of various herbivores implanted in the Kerguelen archipelago.

We used the similarity index of Sørensen (1948) for qualitative comparison and the Schoener's Index (Schoener 1968) was applied to each individual mouflon's samples for quantitative comparison between rumen and fecal contents. Difference between rumen and fecal contents was tested using the Wilcoxon test (non parametric method for paired data, Sokal and Rohlf 1981). The relationships between data obtained from rumen and fecal materials were determined by least square linear regression (arcsin transformed) for food items with a normal distribution, and by Spearman' rank correlation when the data were non-normal.

Sørensen's Index	a: number of food items common to rumen and fecal contents
$QS = 2a/b+c$	b: number of food items presents only in the feces
	c: number of food items presents only in the rumen
Schoener's Index	p_i: proportion of item i in the rumen
$PS = 1 - 0.5 \sum \lvert p_i - q_i \rvert$	q_i: proportion of item i in the feces

Results and Discussion

Sixteen food items were identified in rumen samples and 17 in fecal samples (Table 1). The difference was due to the infrequent observation (1 to 3 fragments), of *Cotula plumosa* in only 6 fecal samples. All plant species identified in the rumen contents were also identified in the fecal samples. Thus, the list of plants consumed by the mouflons was slightly more complete in fecal than ruminal samples, a trend observed in many other studies (e.g. Owaga 1977, Hanley et al. 1985, Wallage-Drees et al. 1986) though some opposite results were also obtained (Smith and Shandruk 1979). As a result, the fecal analysis appeared to be appropriate for a qualitative study of the mouflon diet on Ile Haute. Moreover, the paired analysis of rumen and fecal contents of mouflons showed that the mean number of food items observed in the feces (12.1 ± 2.0) was significantly greater than in the rumen contents (10.1 ± 2.2; z = -3.98, P < 0.001). This result is usually attributed to the fact that fecal samples correspond to longer feeding periods than stomach contents (Anthony and Smith 1974, Wydeven and Dahlgren 1982, Lewis 1994). Consequently, and apart from the fact that animal sacrifice is not required, a major advantage of the fecal approach consists in the fewer sample sizes required to obtain a fixed level of qualitative precision. The mean Sørensen's Index (QS) between rumen and fecal samples was 0.77 ± 0.10. This index reached 0.87 ± 0.09 when we considered only food items representing more than 1% of the plant fragments, and 0.98 ± 0.05 when the representation level was elevated to 2%. This rapid increase of the QS index indicate that both methods were almost equivalent with regard to the major diet constituents.

The Schoener's Index (PS = 80.8 ± 6.7, range from 66.3 to 93.4) indicates both methods gave very similar results. In particular the 4 major items identified were the same in rumen and fecal contents: annual meadow-grass, *Azorella selago*, *Juncus scheuchzerioides*, mosses (Table 1). Together, they represented more than 87% of the plant fragments identified in the feces and more than 91% of those found in the rumens. These dominant items were detected in all the fecal samples and in most rumen samples. The frequency of occurrence in fecal and rumen samples ranged from 7 to 97% (Table 1).

The paired comparison of the relative abundance of each item showed significative differences between rumen and fecal contents for 12 food items (Wilcoxon tests, Table 1). Most of these food items were more abundant in feces, especially annual meadow-grass (13.1% in feces versus 5.9% in rumens) and mosses (5.8% versus 2.8%). Exceptions were *Azorella selago* (61.1% versus 75.2%) and algae (0.4% versus 1.2%). The greater proportion of *Azorella selago* in rumen could be due to the particularly high fiber content of this plant, that increases the retention time in the rumen (Gaare et al. 1977, Owaga 1977). Nevertheless, *Azorella selago* remained by far the most abundant species by both methods, while the magnitude of differences between rumen and fecal samples was relatively small for the annual meadow-grass (6% and 13% respectively). Todd and Hansen (1973) suggest small statistical differences might be of little biological significance. Generally, grasses and mosses are over-represented in fecal materials, while forbs are under-represented (Batzli and Pitelka 1971, Neal et al. 1973, Anthony and Smith 1974, Vavra et al. 1978, Hanley et al. 1985, Wallage-Drees et al. 1986, Lewis 1994). The over-estimation of grasses is commonly attributed to the resistance of epidermis to digestive processes (Neal et al. 1973, Bartolome et al. 1995). The over-representation of mosses is linked to the fact that they fragment more than other plants during the digestive process and have very microscopically discernible epidermis. Consequently, minute fragments which are easily identified (Dearden et al. 1975) may be over-represented.

The relative abundance of food items in rumen and fecal samples were highly correlated (Spearman, Rho = 0.81), especially for the 4 most highly consumed food items (Table 1): *Azorella selago* (r = 0.82), annual meadow-grass (r = 0.73), *Juncus scheuchzerioides* (r = 0.87), Bryophytes (r

Table 1. Relative abundance (mean ± SE), percent frequency of occurrence (% Freq.) and correlation coefficients (simple correlation when appropriate or Spearman' rank correlation) of food items in 30 rumen and fecal samples from mouflon ewes collected in winter 1994 on Ile Haute, Kerguelen archipelago. Differences between relative abundance in feces and rumen were tested using Wilcoxon tests.

Plant species/taxon	Feces analysis (n=30)			Rumen analysis (n=30)			Wilcoxon test		Simple correlation	Spearman' rank correlation	
	Mean	±SE	% Freq.	Mean	±SE	% Freq.	Z	P	(R)	Rho	P
Monocotyledons											
Deschampsia/Agrostis	2.15	2.10	90.0	2.16	2.07	96.7	0.39	NS	0.81	***	
Festuca contracta	0.57	1.03	63.3	0.69	1.06	53.3	0.75	NS	0.70	***	
Poa annua	13.14	8.75	100.0	5.89	4.85	100.0	4.68	***	0.73	0.73	***
Unidentified Graminaea	0.27	0.25	66.7	0.41	0.46	73.3	1.72	NS	0.10	NS	
Juncus scheuchzerioides	7.65	6.39	100.0	7.24	6.53	100.0	1.06	NS	0.87	0.88	***
Dicotyledons											
Acaena magellanica	0.02	0.06	13.3	0.01	0.05	6.7	0.41	NS	0.25	NS	
Azorella selago	61.14	14.36	100.0	75.17	10.64	100.0	4.64	***	0.82	0.82	***
Cerastium spp.	1.67	1.83	80.0	0.20	0.28	46.7	4.32	***	0.50	**	
Cotula plumosa	0.06	0.13	20.0	—	—	—	2.21	*	-		
Galium antarcticum	0.57	0.73	63.3	0.21	0.40	30.0	3.76	***	0.70	***	
Ranunculus spp.	0.22	0.47	40.0	0.04	0.09	20.0	1.99	*	0.10	NS	
Sagina procumbens	1.24	1.12	86.7	0.09	0.13	36.7	4.37	***	0.05	NS	
Taraxacum officinale	1.37	1.13	93.3	1.03	1.31	66.7	1.69	NS	0.50	**	
Unidentified Dicotyledons	1.57	1.28	90.0		1.00	1.12	76.7	2.68	**	0.63	***
Pteridophytes											
Blechnum penna-marina	1.12	1.26	83.3	0.32	0.67	50.0	3.98	***	0.54	**	
Bryophytes	5.79	7.68	100.0	2.84	4.18	93.3	3.84	***	0.82	0.80	***
Algae	0.36	0.78	33.3	0.95	1.20	63.3	2.17	*	-0.49	**	
Unknown	1.10	0.91	93.3	1.75	1.06	100.0	2.97	**	0.10	NS	

*, **, *** Significant at the 0.05, 0.01 and 0.001 levels respectively. NS: non significant.

= 0.82). Among other items, a high correlation existed for *Festuca contracta*, *Deschampsia/Agrostis* and *Galium antarcticum* (Spearman' rank correlation, Table 1). The only exceptions were 3 plant species which had a very low representation (*Acaena magellanica*, *Ranunculus* spp., *Sagina procumbens*) as well as the undetermined fragments.

Conclusion

An accurate comparison between rumen and fecal methods requires 1) identical treatments of rumen and fecal samples by trained technicians (Westoby et al. 1976, Holechek and Gross 1982b); 2) correct samples size; 3) and the determination of most plant fragments. In our study, fecal and rumen samples were prepared similarly and examined by the same experienced person (point 1). Point 2 was also guaranteed by counting more plant fragments (approximately 500) per sample than the methods recommended. These usually range from 200 to 300 per sample (Chapuis 1980, Abbas 1988). Accurate determination of plant fragments was also obtained: 86.8% of the fragments observed were determined to a species level in feces and 90.6% in rumen samples. Most remaining fragments were determined to a genera or placed in broader categories (algae, bryophytes…). The proportion of totally unidentified fragments was only 1.1 ± 0.9% in the feces and 1.8 ± 1.1% in the rumen. This result indicates that the micrographic method represents a significant improvement compared to the macroscopic volumetric method of rumen examination, which often result in a high proportion (up to 95%) of undetermined material (Edwards and Ritcey 1960, Bergerud and Russell 1964, Kessler et al. 1981). More importantly, the undetermined fraction is also reduced compared to many micrographic studies of herbivore diet (Zyznar and Urness 1969, Batzli and Pitelka 1971, Neal et al. 1973, Kessler et al. 1981, Homolka and Heroldova 1992, Lewis 1994). These results were due to the low diversity of food resources which limits misidentification of epidermal fragments.

It appears that both methods gave quite similar results on Ile Haute. All plant species identified in rumen were observed in fecal pellets, validating the fecal microhistological analysis from a qualitative viewpoint. Quantitative results from both methods were also very similar, particularly in regard to the major constituents of the diet. The microhistological analysis of feces seems thus applicable to a long term monitoring of diet variations of the mouflon population. In interpreting these results, we must however be aware that mosses and annual meadow-grass are probably slightly overestimated while *Azorella selago* is underestimated by fecal studies compared to rumen analysis. The method will allow us to determine the relative temporal variations of species one to another, and thus to focus on important seasonal trends in the mouflons' diet.

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