Folia Zool. – 51(1): 11–15 (2002)

Diet estimation by faeces analysis: sampling optimisation for the European hare

Krisztián KATONA[1] and Vilmos ALTBÄCKER[2]

[1] *St.Stephen University, Department of Wildlife Biology and Management, Páter K. u. 1, H-2103 Gödöllő, Hungary; e-mail: katonak@ns.vvt.gau.hu*

[2] *Eötvös Loránd University, Department of Ethology, Jávorka u. 14, H-2131 Göd, Hungary; e-mail: altbac@ludens.elte.hu*

Received 20 June 2001; Accepted 30 October 2001

A b s t r a c t . We investigated how the sampling process of microhistological faeces analysis could be optimised for an accurate estimation. Spring diet composition of European hare (*Lepus europaeus* Pallas, 1778) was determined in a juniper shrubland at Bugac, Hungary. Both inter and intraobserver reliability was high permitting us to separate the components of variance due to the methodological steps in the faeces analysis. Estimates varied depending on the number of independent droppings, pellets/individual, subsamples/pellet and epidermis/subsample. The variance was much higher among than within the independent pellet groups. The cumulative frequency estimate stabilised at around 100 epidermis fragments per pellet. We conclude that the most critical steps of the sampling procedure are the collection of independent droppings and the identification of a sufficient number of epidermis fragments. We propose to collect at least 10 independent droppings, one pellet/individual, and analyse 100 epidermis fragments as an optimum for estimating the relative frequency of forage classes reliably. The importance of the individual variability in the diet should be emphasised.

Key words: microhistology, faecal pellet, accuracy, minimum sample size, *Lepus europaeus*

Introduction

Feeding habits of mammals are in the centre of interest of population biology (L o d é 1996) and ecology (M á t r a i et al. 1998). Several methods have already been involved in the investigation of food composition (H o l e c h e k et al. 1982). The most widely used indirect technique for determining diet composition of herbivores is the microhistological identification of epidermis fragments in the stomach content or faecal pellet (B a u m g a r t n e r 1939, D u s i 1949).

The effect of sample size on the estimated diet composition has already been proved (H a n s o n & G r a y b i l l 1956). The greater the diet diversity of a species and the smaller the similarity of individuals, the larger the required minimum sample size is (K o v á c s & T ö r ö k 1997). Data on individual variability in the diet of herbivorous mammals, however, are very scarce (M á t r a i & K a b a i 1989, H o m o l k a & H e r o l d o v á 1992). Microhistological faeces analysis method includes multiple successive sampling from the individuals, pellets and epidermis fragments. Sampling size, therefore, could affect the estimate in all consecutive sampling steps. The lack of the information on the precision of the faecal sampling has already been emphasised (H o l e c h e k et al. 1982). The applied minimum sample size could vary according to study species, as for deer (A n t h o n y & S m i t h 1974), hare (H o m o l k a 1987a) and rabbit (C h a p u i s 1980). Sample size,

however, could also differ from study to study as the characteristics and size of study area change (Hanson & Graybill 1956, Homolka 1987b).

Problems to design a valid diet composition analysis derive from the difficulties of the determination of the sample sizes required. To optimise the sampling process, a general knowledge on the variances of different sampling levels should be established. For this comparison a single basic sample of faecal pellets is needed, which has never been used by earlier investigators studying the minimum sample sizes on consecutive sampling levels. The aim of our investigation was to assess a valid sampling strategy to obtain precise results. In order to optimise faecal sampling, therefore, we compared the variances observed at the consecutive steps of faeces analysis in European hare (*Lepus europaeus* Pallas, 1778).

Materials and Methods

The study area (1200 by 1400 m, 168 ha) was situated in the Bugac Juniper Forest core area in Kiskunság National Park, central Hungary (46°38' N; 19°40' E). This protected area has shrubland vegetation (for a detailed description of it see Kertész et al. (1993) and Kalapos (1989)).

Fresh faecal pellets of European hare were collected on a single day, 22 April, 1998. Altogether 10 independent droppings, each containing five pellets, were collected. (Faeces of European hare is called droppings, which could include only one or several faecal pellets). Sampling points were separated by at least 200 m intervals ($\bar{x} \pm SD = 578 \pm 272$ m). Spring diet composition of hares was determined by microhistological faeces analysis. Proportion of diet components was estimated in each pellet by the number of fragments for a particular forage class relative to the total number of fragments. The distinguished forage classes were monocotyledons (grasses), dicotyledons (forbs and browses), gymnosperms (evergreens), barks and seeds.

For microhistological analysis the following preparation procedures were conducted (Mátrai et al. 1989). A subsample mixed from five parts of 0.002 g was taken out from each pellet into test tubes. From five pellets of different droppings a subsample of one part of 0.01 g was taken parallely out into five other test tubes. After boiling in 20 % nitric acid solution for 1.5 minutes epidermis fragments were dispersed on microscopical slides into a mixture of 0.1 ml of 87 % glycerine and 0.05 ml of 0.1 % Toluidine-Blue. All fragments found on the slides were identified under 160X magnification. We had the following investigations.

A) One pellet from each droppings was chosen and 200 epidermis fragments of it were identified. Just the minimum required number of fragments (100) were then categorised from the remaining pellets. Minimum sample sizes for different sampling units were determined by rarefaction analyses (Kovács & Török 1997) and by saturation curves of the proportion of forage categories.

B) Intra and interobserver reliability were determined by analysing one pellet from each droppings two times. Statistical analysis of observer reliabilities was performed by Spearman-correlation (Caro et al. 1980).

C) Diet composition estimated from the same pellets by subsamples including one bigger or five smaller parts were compared by Wilcoxon matched-pairs signed rank test.

D) Determining diet composition of all five pellets of five droppings variances within and among droppings were tested by Kruskal-Wallis one-way analyses of variance by ranks.

To quantify diet overlaps between different samples Renkonen's proportional similarity index was always calculated (H u r l b e r t 1978):

$$S_{is} = \Sigma min(P_{1,i} ; P_{2,i})$$

where $P_{1,i}$ is the proportion of prey category i in one individual, $P_{2,i}$ in the other individual. Similarity and diversity indices and rarefaction curves were calculated using the software NICHE (S c h l u t e r , D., University of British Columbia, Vancouver).

Results

There was no significant negative correlation between the distance of sampling points of droppings and their diet similarity (Spearman-correlation: N=45, r_s=0.24, p=0.12) supporting the independence of droppings.

A) Rarefaction analyses and saturation curves indicated that a sample size of 100 epidermis fragments (Fig. 1), 10 independent droppings and one pellet of a single droppings were surely sufficient for obtaining unbiased estimates of diet composition.

B) Intra and interobserver reliability were measured to be high (Spearman-correlation: N=10, r_s >0.8 in all cases for monocots, dicots and gymnosperms; r_s >0.6 for seeds and barks).

C) Diet composition estimated from either a single 0.01 g subsample or from a mixture of five 0.002 g subsamples seemed to be identical (Wilcoxon matched-pairs test: N=5, p>0.38 for all forage classes).

D) Based on the analysis of all five pellets of five droppings, diet composition of pellets significantly differed among droppings (Kruskal-Wallis test: df=4, p<0.05 for all forage classes) (Fig. 2), but within droppings they were identical (Kruskal-Wallis test: df=4, p>0.7 for all forage classes).

In the course of sampling procedure similarity was the lowest, therefore variance was the highest among independent droppings as shown in Table 1.

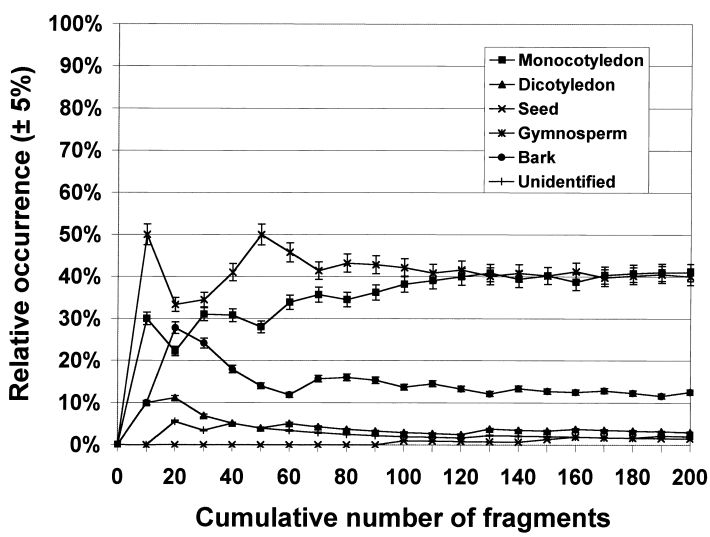

Fig. 1. Stabilisation curves of epidermis fragments identifying to broad forage classes. Figure shows typical curves to determine an optimum sample size of the epidermis fragments based on the analysis of 200 fragments to broad forage classes in a single pellet. Relative occurrence was given by the number of fragments for a particular forage category relative to the total number of fragments. Confidence interval of five percent is indicated.

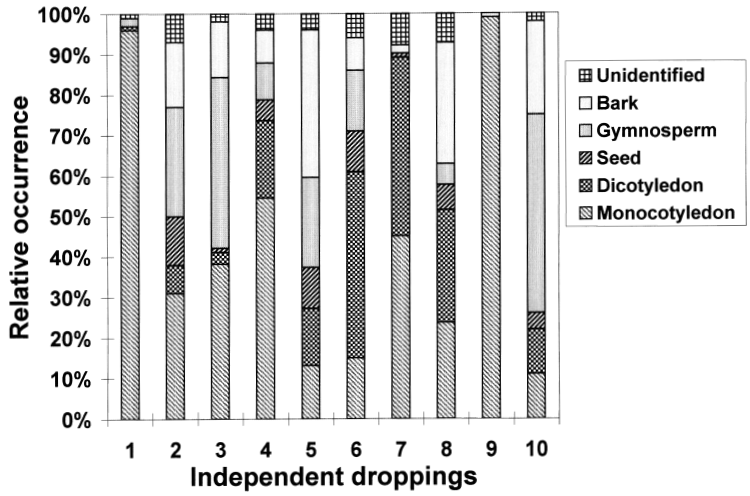

Fig. 2. Variability in the diet composition among independent hare droppings. Diet composition of a single pellet from each droppings is provided after identifying 200 epidermis fragments per pellet to broad forage classes. Relative occurrence was given by the number of fragments for a particular forage category relative to the total number of fragments.

Table 1. Variability in the sampling procedure of microhistological faeces analysis. Sample size (N), value of Renkonen's proportional similarity index (Sis) and statistical significance of differences for the given sampling factors are shown.

Sampling variability	Sample size (N)	Proportional similarity (Sis)	Significance
Among droppings	10	0.51	$p<0.05$
Within droppings	5	0.86	NS
Between subsamples	5	0.90	NS
Interobserver reliability	10	0.91	NS
Observer1 reliability	10	0.92	NS
Observer2 reliability	10	0.94	NS

Discussion

Our results suggest that sampling procedure could significantly affect the result of microhistological analysis. In our study, the largest contribution to overall variance was revealed among droppings, indicating considerable individual differences among the hares. Mátrai & Kabai (1989) and Homolka & Heroldová (1992) have already proved considerable individual differences in deer species. We have also found a low similarity among independent droppings of hares, but cumulative composition analysis indicated that 10 droppings gave a reliable estimate. This is very similar to the minimum sample size suggested by Homolka (1987a) for hares and by Chapuis (1980) for rabbit colonies inhabiting a well-defined smaller habitat. This individuality of diet composition casts doubt on the validity of many diet analyses done from a faecal pellet mixture. These investigations might reveal the pressure on the vegetation by the given herbivore population rather than the actual food choice of the individuals. Similarity of the pellet compositions was much higher within than among droppings, similarly to Chapuis (1980), suggesting the importance to collect more droppings and only one pellet/individual.

Number of identified fragments should be the other main factor of sampling design as H o m o l k a (1987a) and C h a p u i s (1980) have already pointed out. There is no consensus in the literature on the minimum sample size, but it often seems to be larger than necessary. In rabbits in our study area M á t r a i et al. (1998) identified 100-600 fragments in different seasons. C h a p u i s (1980) applied a sample size of 400 fragments, but estimated 200 to be sufficient.

In summary, the different steps in the sampling procedure influence the accuracy in different ways. The two major factors in faecal sampling optimisation are the sample size of droppings and epidermis fragments. Estimation of diet composition is less sensitive to the observer reliabilities and to within-droppings and within-pellet variance. We are confident that our directives will help to design future faecal analyses to gain accurate and biologically interpretable results with optimal effort and without underestimating the variety of dietary habits of animals.

A c k n o w l e d g e m e n t s

We are indebted to K. M á t r a i for her help in applying microhistological techniques and to K. N a g y for her participation in the interobserver reliability study. Two anonymous referees made constructive comments on the manuscript, while L. S á r i revised the final English version. Kiskunság National Park permitted us to work in the protected study area. The work was funded by the Hungarian Science Foundation (T 017468 to V. A l t b ä c k e r).

L I T E R A T U R E

ANTHONY, R. G. & SMITH, N. S., 1974: Comparison of rumen and faecal analysis to describe deer diets. *J. Wildl. Manage., 38(3): 535–540.*

BAUMGARTNER, L L. & MARTIN, A. C., 1939: Plant histology as an aid in squirrel food-habits studies. *J. Wildlife Manage., 3:266–268.*

CARO, T. M., ROPER, R., YOUNG, M. & DANK, G. R., 1980: Inter-observer reliability. *Behaviour, 69 (3–4): 303–315.*

CHAPUIS, J. L., 1980: Méthode d'étude du régime alimentaire du lapin de garenne, *Oryctolagus cuniculus (L.)* par l'analyse micrographique des fèces. *Rev. Ecol.- Terre Vie, 34: 159–198 (in French, with a summary in English).*

DUSI, J. L., 1949: Methods for the determination of food habits by plant microtechniques and histology and their application to cottontail rabbit food habits. *J. Wildlife Manage., 13(3): 295–298.*

HANSON, W. R. & GRAYBILL, F., 1956: Sample size in food-habits analyses. *J. Wildlife Manage., 20(1): 64–68.*

HOLECHEK, J. L., VAVRA, M. & PIEPER, R. D., 1982: Botanical composition determination of range herbivore diets: a review. *J. Range Manage., 35(3): 309–315.*

HOMOLKA, M., 1987a: Problems associated with investigations to the diet of the European hare. *Folia Zool., 36(3): 193–202*

HOMOLKA, M., 1987b: The diet of brown hare (*Lepus europaeus*) in central Bohemia. *Folia Zool., 36(2): 103–110.*

HOMOLKA, M. & HEROLDOVÁ, M., 1992: Similarity of the results of stomach and faecal contents analyses in studies of the ungulate diet. *Folia Zool., 41(3): 193–208.*

HURLBERT, S. H., 1978: The measurement of niche overlap and some relatives. *Ecology, 59(1): 67–77.*

KALAPOS, T., 1989: Drought adaptive plant strategies in a semiarid sandy grassland. *Abstracta Botanica, 13: 1–25.*

KERTÉSZ, M., SZABÓ, J. & ALTBÄCKER, V., 1993: The Bugac Rabbit Project. Part I: Description of the study site and vegetation map. *Abstracta Botanica, 17: 187–196.*

KOVÁCS, T. & TÖRÖK, J., 1997: Determination of minimum sample size to estimate diet diversity in anuran species. *Herpetol. J., 7: 43–47.*

LODÉ, T., 1996: Polecat predation on frogs and toads at breeding sites in western France. *Ethol. Ecol. Evol., 8: 115–124.*

MÁTRAI, K. & KABAI, P., 1989: Winter plant selection by red and roe deer in a forest habitat in Hungary. *Acta Theriol., 34(15): 227–234.*

MÁTRAI, K., ALTBÄCKER, V. & HAHN, I., 1998: Seasonal diet of rabbits and their browsing effect on juniper in Bugac Juniper Forest (Hungary). *Acta Theriol., 43(1): 107–112.*