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Determining Optimal Sampling Schemes to Study Red Deer Diets by Fecal Analysis

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Abstract. Studies on red deer feeding ecology have been conducted in Portugal for several years and in various locations using microhistological techniques on feces. These analyses are extremely time consuming requiring the optimization of the multistage sampling scheme used. Estimates of variance components based on samples from red deer pellets in Vila Viçosa (southern Portugal) indicate that variability among individual pellets of the same pellet group is minimal. It is concluded that using 100 plant fragments observed per pellet, a minimum number of 4 pellet groups provides a reasonable compromise between precision and cost.
Key words: red deer; microhistological analysis; sampling; diet

Sumário. Nos últimos anos têm-se vindo a realizar em Portugal diversos estudos sobre a ecologia alimentar do veado com o recurso à utilização da análise micro-histológica de fezes. Estas análises exigem enorme consumo de tempo, pelo que interessa optimizar os esquemas de amostragem a múltiplos níveis geralmente utilizados. Baseados em amostras fecais de veado em Vila Viçosa (no Sul de Portugal) as estimativas das componentes de variância indicam uma variação mínima entre os diversos elementos constituintes do mesmo grupo fecal. Conclui-se que, com a identificação de 100 fragmentos epidérmicos por cada elemento do grupo fecal, é necessário um mínimo de 4 grupos para atingir um compromisso razoável entre precisão e custo.
Palavras-chave: veado; análise micro-histológica; amostragem; dieta

Résumé. Pendant les dernières années plusieurs études ont été réalisées au Portugal sur l'écologie alimentaire du cerf à l'aide de l'analyse micro-histologique de fèce. Ces analyses exigent énormément de temps d'où l'interêt d'optimiser des schémas d'échantillonage généralement utilisés à plusieurs niveaux. Basées sur les échantillon fécaux du cerf à Vila Viçosa (dans le sud du Portugal), les estimations des composantes de variance indiquent une variation minimum parmi les divers éléments constituants du groupe fécal. On conclu que, avec l'identification de 100 fragment épidermiques pour chaque élément du groupe fécal un minimum de 4 groupes est nécessaire pour atteindre un compromis raisonnable entre la précision et le coût.
Mots clés: cerf; analyse micro-histologique; échantillonage; alimentation.

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Introduction

Diet studies from herbivores are often based on microscopic recognition of plant epidermic fragments preserved in the feces. The analyses based on microhistological techniques generally assume that:

1. Plant epidermis endure digestion processes, maintaining its microanatomic features when excreted.

2. The amount of each plant epidermis present in the feces is proportional to the ingested amount of its plant.

However, it is known that microhistological examination of fecal material has some limitations in diet evaluations. These can be caused by problems related to differential digestion of different species, resulting in differential particle size reduction or by differential detection and recognition under microscopic observation (HOLECHEK *et al.*, 1982).

In spite of these limitations, fecal analysis has been used all over the world for over 60 years to determine the diet of different herbivores (BAUMGARTNER and MARTIN, 1939; STEWART and STEWART 1970; ELLIOTT III and BARRETT, 1985; GARCIA-GONZALEZ and CUARTAS, 1989; FRASER and GORDON, 1997), and various methods to overcome the inconveniences of the technique have been developed, such as digestive correction factors to compensate underestimation of very digestible plant species (ANTHONY and SMITH, 1974; BAKER, 1986; ALIPAYO *et al.*, 1992; GARCIA-GONZALEZ and CUARTAS, 1992; BARTOLOMÉ *et al.*, 1995).

Nevertheless, the determination of the minimum number of individual pellets needed to derive a suitable dietary estimate of a given herbivore population has received little attention (GARCIA-GONZALEZ, 1992). The sampling strategy generally used involves a multistage or cluster sampling scheme, where primary sampling units are pellet groups (defecations), second-stage units are individual pellets and third-stage units are microscopic slides, where individual fields are scanned and individual plant fragments identified.

The sample sizes suggested vary from five to 15 individual pellets from different pellet groups, in studies of rabbits (CHAPUIS, 1980) and red deer (ANTHONY and SMITH, 1974).

In the next step it was often assumed to be convenient to mix feces of some individuals and take subsamples, from which microscopic slides are prepared. The number of microscopic slides prepared by sample varies from one (HOSEY, 1981) to six (STEWART, 1967).

The amount of fragment identifications per sample typically varies between 100 and 400, and it is sometimes recognized that above 200 identifications species percentages change only slightly (GARCIA-GONZALEZ, 1992).

This sampling strategy has the problem of hiding the original variation between sampling units when mixing pellets. This process gives an unbiased estimation of the average diet but underestimates the variation and provides a false idea of precision.

In this study we aim to provide a method to assess optimal sampling schemes for the estimation of red deer (*Cervus elaphus*) diets by microhistological fecal analysis.

Material and methods

Study area and deer population

The study site was a 267-hectare hunting reserve located at 38º47'N and 7º25'W, in the counties of Borba and Vila Viçosa, Alto Alentejo. This area is closed by walls built to preserve former royal hunting grounds. Elevation above sea level ranges from 290 to 430m. The climate is Mediterranean, with hot dry summers and mild winters. Vegetation is dominated by cork oak (*Quercus suber*) and holm oak (*Quercus rotundifolia*) in a park-like woodland ("montado"), with areas of dense gum cistus shrubs (*Cistus ladanifer*) and pastures. Along a few water lines there are rows of ash trees (*Fraxinus angustifolia*) and bramble thickets (*Rubus ulmifolius*).

At the time of the field work (1993) a total of 273 red deer individuals were censused: 55 males, 125 females and 93 calves (ERENA/ISA, 1997).

Reference material

Samples from all available plant species in the study area were collected during August 1993. In the laboratory and after their identification, all fresh or dried plant parts (leaves, stems, flowers and fruits) were placed separately in bottles, properly identified, and soaked in a solution of sodium hypochlorite, from 18 to 48 hours depending on the fragility of the cuticles (dependent on species and season). This process stops when almost all fragments were white. This procedure is made to make easy the observation and identification of all epidermal characteristics from all *taxon* (HANSEN *et al*., 1978; ALIPAYO *et al*., 1992).

Subsequently, each material was then filtrated through a 0.5mm sieve and washed gently with tap water until all sodium hypochlorite had been removed (MAISELS, 1988; MAIA *et al*., 1996). The plant material is then placed into each individual bottle, filled with water, with the sodium hypochlorite remain in the plant tissues acting as a preserver (BAKER, 1986).

Reference collection of epidermal tissues

In a small Petri dish with water, each epidermis were separated from the inner tissue, using two nippers. Sometimes, bistoury was used to clean the adjacent tissues. These procedures require magnifying glasses, using different magnifications depending on the structure of the tissue and degree of lignification (METCALFE and CHALK, 1965). The tissue was then mounted on a slide (75x25mm^2) in glycerine, covered with a cover slip (22x22mm^2) and sealed with nail polish. Natural colours appear by this method as they may appear in dung (ANTHONY and SMITH, 1974; DAVITT and NELSON, 1980).

Then, with a microscope with a standard size magnification (100x) and (400x), in phase contrast, and a micrometer, a detail study from each epidermal *taxon* tissue/structure was done, using the terminology proposed by METCALFE (1960), STORR (1961), METCALFE and CHALK (1965) and SCOTT and DAHL (1980).

Microphotographs from all *taxon*/ /structures were then made with the same magnification to facilitate a fast comparison between the reference collection and the fecal material (ZYZNAR and URNESS, 1969; GRENN, 1987).

Fecal sampling

In August 1993, four different fresh pellet groups were collected at the study site and preserved in the freezer separately in plastic bags. To perform the analysis, from each of these four groups, three different individual pellets were randomly selected. After defrost, each individual pellet was mixed with tap water ca. 350ml. in an electric blender, for three minutes, at a variable speed depending on the fragility of the cuticles. With this procedure, the inner tissue is separated from the epidermis or cuticle and its fragments of similar size are randomly mixed for adequate subsampling. This mixture was then put through a 53µm sieve (FITZERALD and WADDINGTON, 1979) and the residue transferred with a little tap water into a 17cm Ø Petri dish, where a solution of sodium hypochlorite was added. When the material turned white two subsamples per slide were taken from the Petri dish to be observed through the microscope. This operation was done to guarantee a minimum dimension of 1mm² of the epidermis in the sieve, minimum size which a tissue can be identified (TODD and HANSEN, 1973; FITZGERALD and WADDINGTON, 1979). From each of these pellets five microscopic slides were prepared and 10 plant fragments identified.

Quantitative information from epidermal tissues

From the Petri dish five microscope slides were examined (SPARKS and MALECHEK (1968); TOOD and HANSEN 1973; ALIPAYO, 1992) under a binocular microscope fitted with a 1mm² eyepiece, at a magnification of 100x (sometimes 400x). The first 10 fragments of epidermal tissue were identified for each slide, for a total of 50 fragments/pellet. Fragments were counted in systematic transects across a slide along alternate rows to avoid duplication (GREEN, 1987; STEWART and STEWART, 1970; BARTOLOMÉ *et al*., 1995). Identification of fecal remnants was based on comparison with photomicrographs and the individual description done for each individual structure from each *taxon* (STEWART and STEWART 1970; ALVAREZ, 1990; SHERLOCK and FAIRLEY, 1993).

Each individual slide resulted in information on the proportion (in the ten fragments) of seven specific plant groups (previously studied in the reference collection):

1. Oaks (*Quercus suber* and *Q. rotundifolia*)

2. Other tree species (*Fraxinus angustifolia* and others)

3. Gum cistus (*Cistus ladanifer*)

4. Bramble (*Rubus ulmifolius*)

5. Grasses (various species)

6. Legumes (various herbaceous species)

7. Non-identified

As the statistical validity of tests of significance after analysis of variance requires that the experimental errors are independently and normally distributed with common variance, and this is seldom the case in binomial data expressed as decimal fractions, a transformation is generally required. Therefore, the angular or inverse sine transformation (arcsin) was used, as this is especially recommended when the proportions cover a wide range of values (STEEL and TORRIE, 1980).

The sampling scheme used is a clear example of a three-stage sampling design where the population contains N first-stages units (pellet-groups), each with M second-stage units (individual pellets), each of which has K third-stage units (microscopic slides). In this case the corresponding numbers for the sample are n=4, m=3, and k=5 respectively.

If, at each stage, the fraction randomly sampled is negligible, the mean and the variance (COCHRAN, 1977) can be estimated as:

$$\equiv{y} = \frac{\sum_{i}^{n}\sum_{j}^{m}\sum_{u}^{k} y_{iju}}{n\,m\,k}$$

$$V\left(\equiv{y}\right) = \frac{S_1^2}{n} + \frac{S_2^2}{nm} + \frac{S_3^2}{nmk}$$

where S_1^2, S_2^2 and S_3^2 are the estimates of the variance components obtained from the analysis of variance (NAS-NRC, 1962) shown in Table 1.

The estimates are:

$$S_3^2 = MS3$$

$$S_2^2 = (MS2 - MS3)/k$$

$$S_1^2 = (MS1 - MS2)/(mk)$$

It can be observed that, when fractions sampled are negligible, as in our situation, the estimated variance can be computed as:

$$V\left(\equiv{y}\right) = \frac{MS1}{nmk} = \frac{\sum_{i=1}^{n}\left(={Y}_i - \equiv{Y}\right)^2}{n(n-1)}$$

Thus, the estimated variance can be computed from a knowledge of the first-stage unit means only. This estimate is conservative if the fractions sampled at each stage are not negligible.

From the above analysis it is possible to develop a system for optimal sampling, assuming that the cost function has the form:

$$C = c_1 n + c_2 nm + c_3 nmk$$

where the total cost (C) is dependent upon the time to find an additional fresh pellet group in the field (c_1), the laboratory time for preparing another individual pellet for microscopic observation (c_2), and the time for preparing and observing each microscopic slide (c_3).

Table 1 - Computation of variance among pellet groups, among pellets within groups, and among microscopic slides within pellets.

Source of variation	Degrees of freedom	Sums of squares	Mean square	Components of variance
Among pellet groups	n-1	$mk\sum_{i}^{n}\left(={Y}_i - \equiv{Y}\right)^2$	MS1	$S_3^2 + k\,S_2^2 + mk\,S_1^2$
Among pellets	n(m-1)	$k\sum_{i}^{n}\sum_{j}^{m}\left(\overline{Y}_{ij} - ={Y}_i\right)^2$	MS2	$S_3^2 + k\,S_2^2$
Within pellets	nm(k-1)	$\sum_{i}^{n}\sum_{j}^{m}\sum_{u}^{k}\left(Y_{iju} - \overline{Y}_{ij}\right)^2$	MS3	S_3^2
Total	nmk-1			

It can be seen (COCHRAN, 1977) that the optimum values for k (the number of microscopic slides to be observed per pellet) and m (the number of pellets to be analysed per pellet-group) can be approximated by:

$$k_{opt} = \sqrt{(S_3^2 / S_2^2)(c_2 / c_3)}$$

and

$$m_{opt} = \sqrt{(S_2^2 / S_1^2)(c_1 / c_2)}$$

Results and discussion

Variance components of the proportion of various food items in deer diets are shown in Table 2.

Using the average values of S_1^2 =18.63, S_2^2 =5.60 and S_3^2 = 88.11, and the observed time costs of c_1 =60 minutes, c_2 = 45 min., and c_3 = 60 min., it was possible to derive the optimal sampling scheme for a fixed cost. In this case the optimal number of pellets per group (m_{opt}) can be computed as:

$$m_{opt} = \sqrt{(5.60/18.63)(60/45)} = 0.63$$

and the optimal number of microscopic slides per pellet (k_{opt}) as:

$$k_{opt} = \sqrt{(88.11/5.60)(45/60)} = 3.44$$

These results imply that, due to the strong similarity of information provided by pellets of the same fecal group, one pellet per fecal group should be considered as the optimal strategy, and therefore m_{opt} =1.

In this case the situation would simplify to a two-stage sampling design with the corresponding equations for the variance and total cost:

$$V\left(\equiv{Y}\right) = (S_1^2 + S_2^2)/n + S_3^2/(nk) =$$

$$= (S_1^2 + S_2^2 + S_3^2/k)/n$$

and

$$C = (c_1 + c_2)\, n + c_3\, nk = (c_1 + c_2 + c_3\, k)\, n$$

Using the computed values for the variance components and the observed costs it is possible to see how combinations of n (pellet groups) and k (microscopic slides per pellet group) result in different standard errors and total costs (figure 1).

Table 2 - Variance components and frequency of red deer diet items in southern Portugal. An $\arcsin\sqrt{p}$ transformation was used

	Food items						
Variance Components	**Oaks**	**Other trees**	**Gum cistus**	**Bramble**	***Grasses***	**Legumes**	**Non identified**
Among pellet-groups S_1^2	29.3	3.9	30.5	37.6	14.1	5.1	9.9
Among individual pellets S_2^2	0.0	8.7	0.0	15.7	0.0	8.0	6.8
Within pellets-among Microscopic slides S_3^2	100.2	68.4	102.6	50.9	106.1	112.0	76.6
Frequency of the sample (%)	30.5	3.0	9.8	21.0	17.5	7.0	11.2

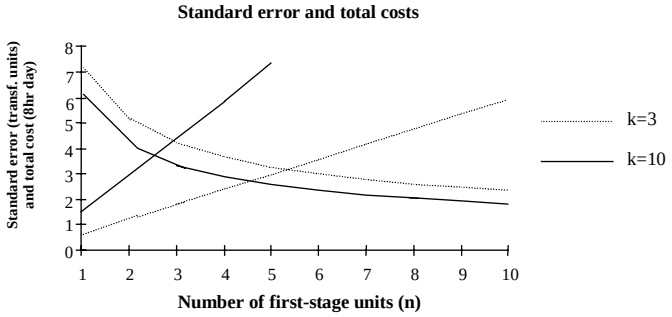

Figure 1 - Standard errors (in transformed units) and total sampling costs (in 8 hour work days) resulting from combination of n (first-stage units = pellet groups) and k (microscopic slides).

The values of k=3 and k=10 were used to show differences in the relative precision and costs associated with the sampling. The value k=3 is close to the optimal value computed and k=10 (equivalent to 100 plant fragments identified) is the minimum value used in most studies reported in the literature.

From the analysis of Figure 1 some conclusions can be drawn. For example, for a fixed total cost, such as 6(x8hour) days it would be possible to analyse about 4 pellet-groups, with 10 slides per pellet, resulting in a standard-error of about 2.8, or alternatively, 10 pellet--groups with 3 slides per pellet, resulting on a standard-error of about 2.3. Similar analyses can be tried by defining thresholds for the allowable standard error. For practical purposes, and considering the shape of the curves of Figure 1, it is suggested that a number around 5 first-stage units provides a reasonable compromise between precision and cost. This number could increase to 6 (if only 30 fragments per pellet are analysed) or decrease to 4 (in the case that 100 fragments per pellet are observed) still allowing a reasonable value of the standard error (below 3), resulting in 95% confidence intervals of around plus or minus 5-6% (after retransforming to original units), which is generally sufficiently precise for this type of studies.

From the above discussion it is apparent that some important conclusions can be drawn:

1. Microhistological analysis provide an interesting way to assess deer diets, in spite of the known limitations of the method,

2. Estimates of variance components indicate that variability among individual pellets of the same pellet--group is minimal and that no effort should be made to sample more than one pellet per group,

3. A minimum of 3 microscopic slides, with identification of 10 plant fragments each, is required, as results from the analysis of the optimal sampling, given the values for variance components and associated costs. Many authors refer the minimum value of 100 plant fragments (equivalent to 10 microscopic slides), probably because of the fact that, in most cases, the density of deer populations is much smaller than in Vila Viçosa (almost

1 deer/ha), and visibility is also smaller, resulting in much more time in searching new fresh pellet groups,

4. Using 100 plant fragments per pellet, it results that a number of 4 pellet groups provides a reasonable compromise between precision and cost. If this value could be increased to 6, the same precision could be obtained by using only 3 microscopic slides per pellet (30 plant fragments).

5. The results presented are only valid for the conditions of the study area (Vila Viçosa) during that season and year, but they can provide first guidance for similar studies in other situations. However, it should be stressed that similar analysis can always be performed in pilot surveys providing the basis for optimising these time-consuming sampling activities.

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