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TECHNIQUES AND STRATEGIES

Review of available techniques for determining the diet of large herbivores from their faeces

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Abstract. Accurate determination of herbivore diets is normally of great interest in most ecological studies. We review two techniques of diet determination, based on fecal analysis, that had been attracted much attention because of its suitability for wild animals. The micrographic technique is based on the recognition of indigestible forage fragments, mainly epidermis and cuticles, under the microscope. The alkanes technique is based on the recognition of alkane pattern (saturated hydrocarbons of the cuticular plant wax) from herbivore faeces. The advantages of both techniques include ease of sampling and, that they do not interfere with animal behaviour or not imply its death. The main disadvantage of the micrographic technique is the possible under or overestimation of the consumed plant species frequencies because of the effect of differential digestion. The main problem of alkane technique is that, for the moment, only diets with few components can be determined. We discuss the possibilities for improving both techniques and we suggest their complementarity as a mean of increasing its accuracy.

Key-words: diet assessment, micrographic technique, alkanes, faecal analysis

Introduction

In ecosystems with great plant diversity the determination of the diet of free-range herbivores is still a problem because the methods available are not very accurate. The microhistological technique, through which the vegetal epidermis or cuticles that remain in the faeces of the animals are identified, has been used with success over the last 50 years (Dusi 1949; Croker 1959; Hercus 1960; Storr 1961; Kiley 1966; Stewart 1967; Hansen 1975; Hughes 1975; Hansen and Clark 1977; Chapuis 1980; Korfhage, Nelson and Skovlin 1980; Hosey 1981; Shank 1982; Butet 1985; Bhadresa 1986; Morgantini and Hudson 1989; Merrill; 1994). However, this technique has been used more to describe the qualitative rather than the quantitative aspect of herbivore diets.

Over the last then years several papers have been published about a new technique that uses saturated hydrocarbons (n-alkanes) of the cuticular wax of pasture species as markers to determine the diet of herbivores (Mayes *et al.* 1986; Dove *et al.* 1989; Mayes 1989; Dove and Mayes 1991; Dove 1992; Mayes *et al.* 1994). This technique determines with accuracy the quantitative aspect of the diet when it has two components (e.g. ryegrass/clover, heather/grass) but in more complex situations, it is very difficult to identify the species that constitute the diet, although potentially it could be done.

In this paper we review the limitations of these two techniques and we suggest several ways of improving the accuracy of both techniques.

Microhistological technique

The microhistological technique is based on microscopic recognition of the different plant species from the epidermic fragments preserved in faeces or stomachs. The advantages of this technique include ease of sampling and the fact that it does not interfere with animal behaviour and does not require the death of the animals (Chapuis and Lefeuvre 1980; Holechek *et al.* 1982; Hanley *et al.* 1985), which makes it a suitable technique for determining the diet of free-range herbivores.

Its accuracy has been compared to other techniques: esophageal fistula, ruminal analysis, utilization and bite-counts (Martin 1955; Tood and Hansen 1973; Anthony and Smith 1974; Dearden *et al.* 1975; Vavra *et al.* 1978; Sanders *et al.* 1980; Kessler *et al.* 1981; Gill *et al.* 1983; McInnis *et al.* 1983; Bullock 1985; Homolka and Heroldová 1992; Lewis 1994). The reviews and comparative studies indicate that this technique is as good or even better than any other for establishing the qualitative composition of the diet (Johnson and Pearson 1981; Holechek *et al.* 1982; Alipayo *et al.*1992; Mohammad *et al.* 1995).

However, this authors also point out the main disadvantage, which is that this technique relies on the acceptance of two basic principles: 1) that plant epidermis endures the digestion process maintaining its microanatomic features when excreted, and 2) that the amount of each plant epidermis present in faeces is proportional to the ingested amount of this plant. These two assumptions are not always maintained, since a differential digestion of plants may exist and the epidermis of some species might be

difficult to identify (Stewart 1967). As a result errors in the estimation of the species frequencies in the diet can occur. In general terms, it is accepted that grasses and ligneous species are overestimated, whereas forb species are underestimated (Holechek and Valdez 1985).

The microhistological technique is, however, particularly useful in comparative studies of the diet of wild herbivores (Chapuis and Lefeuvre 1980; Butet 1985; Morgantini and Hudson 1989; Merill 1994) and in studies where the plants are ranked according to their abundance and not their frequency (Vavra *et al.* 1978).

A further disadvantage lies in the fact that the method is slow and time-consuming and that the identification has to be carried out by a trained technician (Sanders *et al.* 1980; Holechek and Gross 1982; Alipayo *et al.* 1992)

How to improve the microhistological technique

To improve the quantitative aspect of this technique, digestibility tests which provide correction factors to compensate the under or overestimations due to the lack of identification of some plants, have to be carried out (Sparks and Malechek 1968). Dearden *et al.* (1975), and Leslie *et al.* (1983) based these correction factors on equations of lineal regressions. However, due to the fact that the proportion of plants found in the faeces depends not only of the proportion offered but on the over or underestimation of the other plant species, it is more convenient to carry out multivariate regressions in which all the relationships between variables are taken into account (Krzanowski 1988). In one experiment in which sheep and red deer were fed with known proportions of alfalfa, grasses and shrubs, the estimated diet of animals improved considerably after using these correction factors from multivariate regressions (Cuartas *et al.* unpublished).

Gill *et al.* (1983) point out that it is possible to find individual variations in the digestion of plant species and also that the digestibility of one species might depend on whether this plant is on its own or mixed with others. However, Holechek and Gross (1982) found regression lines very similar to those found by Sparks and Malechek (1968), although they point out the need to determine them for each specific situation, because they can be affected by local conditions (Bartolome *et al.* 1995). Therefore, the disadvantage of the correction factors is that they should be determined for each experimental situation and the test of in vivo digestibility can not always be done. One alternative is to carry out tests of in vitro digestibility, in spite of the fact that these are not as accurate as in vivo tests, because they do not take into account the effects of chewing nor effects related to the digestion (Holechek and Valdez 1985).

Another source of error of the microhistological technique, is the subjectivity of the technician who carries out the analysis (Holechek *et al.* 1982; Holechek and Gross 1982; Mohammad *et al* 1995). One way of improving this aspect of the technique is trough the automation of the identification process. Thanks to advances in the technology of image processing, it would be possible to build a data base with the images of the plant epidermis and then to create a software which would enable the computer to identify the epidermis (this method has been currently under study, Martin *et al.* unpublished).

The automation of this process would also improve the speed of analysis. In the faecal samples analysis there are two phases: 1) the sample preparation and 2) the identification. The sample preparation is done in 15 minutes as follows: faecal samples are first mixed in a mortar and then introduced in a test tube to which 3 ml of nitric acid are added. The tube is boiled for 1 minute in a water bath and then its contents are emptied into a 200 ml beaker with boiling water and kept boiling for another 4 minutes. After that, the contents of the beaker are passed through 1 and 0.2 mm sieves and the intermediate fraction is gathered. This is rinsed with tap water and then stored in tubes with acetic acid and formol solution (Stewart 1967; Anthony and Smith 1974; Garcia-Gonzalez 1984). Far its analysis, a fraction is placed on a slide with a drop of Hoyer liquid and all the epidermis fragments found are identified under the microscope from a reference collection (Garcia-Gonzalez 1983; Aldezabal and Garcia-Gonzalez 1992). As the identification process is quite slow, the number of samples done per day is at most two with 400 identifications each, depending on the diversity of the sample.

Alkane technique

The alkanes are saturated hydrocarbons of the cuticular wax of plants. The alkane technique is based on the utilization of these components as markers. Assuming that each plant species has a different proportion of odd-chain alkanes (C23-C25), the botanic composition of the herbivores' diet can be determined from the alkane pattern of plants that have been ingested and of the animals' faeces.

This technique was first described 10 years ago (Mayes *et al.* 1986) and it has been shown to be effective for diets containing two species (e.g. ryegrass/clover, heather/grass) (Dove and Mayes 1991). Some advantages of this technique are:

1- That the differential digestibility of plants does not affect its identification because this technique is based on the alkane concentration of plants, however, since there is an incomplete recovery of faecal alkanes, some corrections have to be made.

2- This technique allows the determination of the intake, and the botanic composition of diets.

3- The number of samples analyzed per day is approximately 6, what is slightly superior than that of the microhistological technique (2 samples), without automation. The alkane analysis are carried out in 3 phases: 1) sample preparation and extraction of alkanes 2) gas chromatographic analysis 3) calculation and statistical analysis. Usually the samples are analyzed in groups of 24 and 4 days are required to complete the whole process which gives a mean of 6 samples per day (once the samples have been freeze-dried).

The disadvantages of this technique are:

1- The number of plants that can be differentiated in a diet is limited because the proportion of plants are calculated solving simultaneous equations and the number of alkanes should equal the number of species to obtain a solution. The equations are as follows (Dove 1992):

$$a_i W + b_i X + c_i Y + d_i Z = e_i$$

where W to Z are the amounts of the (up to) four pasture species in the mixture and a to e, are the concentration of alkane i in the four species and their mixture respectively. The amounts of each species in the mixture are then expressed as frequencies. The alkanes that can be used to estimate the composition of the mixture of four species are C25, C27, C29 and C31.

It must also be borne in mind that some alkanes are more useful than others , therefore, those alkanes which differ more between species should be a greater consideration in the equations. It can also happen that one species with low levels of alkane compared to others in the mixture, might be given a low sensibility estimation (Dove and Mayes 1991).

2- Corrections must be made for the incomplete faecal recovery of individual alkanes. The individual alkanes are not recovered completely, but a recovery factor can be determined dosing a group of grazing animals with a mixture of even-chain alkanes (e.g. C24, C28, C32, C36). Assuming that the recovery of alkanes dosed is similar to the recovery of odd-chain alkanes of similar length of the plants, the recovery factors are calculated by interpolation of the faecal recoveries of the dosed alkanes versus their carbon-chain length (see Dove and Mayes 1991 for more details).

3- The plants that constitute the diet have to be identified prior to analysis. The alkane technique is based on the comparison of alkane patterns of faeces with the alkane pattern of ingested plants. Therefore, it is essential to know the identity of plants ingested. Mayes *et al.* (1994) have used the direct observation technique to gather this information.

How to improve the alkane technique

Mayes *et al* (1994) suggest two ways of improving this technique to obtain accurate estimations of diets:

1) Considering, in addition to alkanes, the use of other plant components as markers, such as: long-chain alcohols, b-diketones or alkenes,

2) Using the microhistological technique to identify the plants that make up the diet.

In addition, providing that the alkane technique gives an accurate estimate of the proportions of grass, forbs and shrubs of diets, the values obtained for each one of these groups could be used to correct the values of the microhistological technique. and recalculate all the specific components of each group, assuming that the species which belong to each one of these groups have a similar digestibility (Holechek and Gross 1982).

As a conclusion it can be said that both the microhistological and alkane technique should be improved to increase accuracy in the estimation of diet composition of large herbivores, and that the combination of the two techniques, alkane with help of microhistological technique or vice-versa, would give a complete description of herbivore diet, i.e. the identity of plants ingested, its proportions and their intake.

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