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Technical Note:
Physical factors that influence fecal analysis estimates of herbivore diets

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Abstract

Microhistological analysis of epidermal fragments in feces is often used to estimate the diet of herbivores but is not generally accepted as a consistently reliable method. Gross errors arise, especially when diets are composed of herbage components with widely different morphological and structural characteristics. The present study investigated the possibility of using such physical characteristics to improve the reliability of the method.

Over a 7 day period, 4 rumen-fistulated beef cows were given a fixed diet composed of a shrub, a grass, and a forb component. On the last 2 days, samples of rumen content and feces were taken for analysis of epidermal fragment. Forbs were under-estimated, grasses over-estimated, and shrubs correctly estimated. Correction factors to estimate true diet composition were defined as the biomass represented by the specific epidermal fragments (epidermal weight index) and the degree of degradation to which the epidermis is subjected in the digestion process (epidermal erodibility factor). These factors account for characteristic physical features of the different dietary components and were measured directly or were derived from the calibration experiment. The utility of such factors depends on accurate determination of the component variables and may be overshadowed by sampling error and observer bias in the microhistological identification of epidermal fragments.

Key Words: microhistological analysis, epidermis, diet composition, *Acacia cyanophylla, Chloris guayana, Vicia sativa*

Histological analysis of epidermal fragments of plants in fecal samples of herbivores was introduced by Baumgartner and Martin in 1939 to identify the botanical composition of forage consumed by herbivores. This method, with various modifications, has been used widely in studies with both wild and domestic animals (e.g. Hercus 1960, Steward 1967, Sparks and Malachek 1968, Chapuis 1980, Vavra and Holechek 1980, Garcia-Gonzalez 1984). From the beginning, the limitations of the method for accurate quantitative determination of species composition of ingested forage have been recognized (Holechek et al. 1982). As a rule, the digestion process has little effect on the epidermis of perennial plants especially when the cuticle surrounds the epidermal cells (Storr 1961) but differences in specific leaf weight, or more precisely, the relationship between recognizable epidermal area of a feed item and its biomass, can influence the estimate of biomass represented by the epidermal fragment (Gill et al. 1983). In young growing tissues and in annual plants, particularly forbs, the epidermal cells can be eroded by mastication and digestion to the extent that many fragments become unrecognizable (e.g. Hercus 1960, Stewart 1967, Vavra et al. 1978, Brazle and Harbers 1977, Brazle et al. 1979, Spencer and Akin 1980).

Consequently, whenever herbivore diet is composed of components with widely differing physical characteristics it has been necessary to check the relationship between the botanical composition of ingested feed and the results of the fecal analysis. This has often revealed large errors in estimates of diet composition (Hercus 1960, Stewart 1967, Vavra et al. 1978, Smith and Shandruck 1979, McInnis et al. 1983, Gill et al. 1983). Dearden et al. (1975) derived empirical correction factors for species in a hand-compounded diet fed to reindeers. Alipayo et al (1992) achieved good precision over a large range of composited diets given to cattle, sheep, and Angora goats without using any correction factors. They ascribe their precision to a number of factors including the "use of actively growing perennial plants with a high proportion of epidermal material" and good training of technicians. But even in this study, there are instances of large deviations between actual diet and fecal analysis estimates. Gill et al. (1983) found gross mis-estimations and concluded that no correction factors could improve the reliability of the method. However, their criticism of the method suggests that a large part of the error in the estimate was due to 2 factors: (a) differences in the relationship between epidermal fragment frequency and associated plant biomass; and (b) differences in the degradation (or erosion) of epidermal tissue during mastication and digestion. The data of

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Alipayo et al. (1992) show that differential mechanical degradation of the epidermis may account for part of the errors in diet estimates: there was greater similarity between fecal analysis and microhistological analysis of feed samples ground in a Wiley mill to pass a 1 mm sieve than between fecal analysis and actual diet fed.

The following study was undertaken to obtain a preliminary estimate of the range of values that one can expect in the above-mentioned 2 factors. The first factor is an epidermal weight index that can be calculated from direct measurements of the biomass and the epidermal area of the forage items. The second factor, the epidermal erodibility, can be derived from the vivo calibration experiments. The present experiment was planned to include a grass, a forb, and a shrub component so as to obtain estimates of the 2 factors in representatives of these major forage categories.

Materials and Methods

Feed and Experimental Animals

A forage mixture was prepared in which the fractions of the components on a dry weight basis were 16% shrub twigs (*Acacia cyanophylla* Lindley), 32% freshly cut grass (*Chloris guayana* Kunth) and 52% forb hay (*Vicia sativa* L.). This diet was fed during 7 consecutive days to 4 rumen-fistulated 3 to 8 year-old Simford cows with liveweight between 349 to 474 kg. The animals were each fed 8.0 kg day^{-1} of the forage mixture (5.3 kg dry weight), an amount that they ate without leaving residues.

Sampling and Slide Preparation

On each of the last 2 days, after the last remains of any previous diet were evacuated (Hercus 1960, Stewart 1967), feces samples were taken from each of the 4 animals, and on the last day, a sample was taken of rumen contents. Two sub-samples of the wet feces and rumen contents were then lightly ground in a mortar to separate out the epidermal fragments. In addition, 2 sub-samples of the feed mixture were ground by hand in a mortar to obtain fragments with similar dimensions to those of the ingested material. From each sub-sample, 10g were placed in a test-tube with 5 ml of concentrated HNO_3 (Crocker 1959, Stewart 1967, Chapuis 1980, Garcia-Gonzalez 1984). HNO_3 was preferred to NaOH (Gross et al. 1983, Holechek and Gross 1980, Vavra and Holechek 1980) because it digests non-epidermal tissue more completely (R. Garcia-Gonzalez, personal communication). The test-tubes were placed for 1 min. in a bath of boiling water and the samples were then diluted with 200 ml of water. This suspension was then passed through 1.00 mm and 0.25 mm filters (Sparks and Malachek 1968, Garcia-Gonzalez 1984). The 0.25 to 1.00 mm fraction was dispersed in 50% aqueous solution of glycerin. Samples of the suspension were spread on glass microscope slides at a density that precluded any significant overlapping of fragments, and left to dry overnight. Cover-slips (24 × 60mm) were then fixed to the slides with DPX microhistological varnish. Two slides were prepared from each sub-sample.

Fragment Identification

Each slide was examined under an optical microscope at 100× magnification by a highly experienced observer. Three traverses were scanned, each one 2 mm wide and 60 mm long with 3 mm between traverses. All fragments in each traverse were identified and counted. The data from both slides of each sub-sample were pooled and converted to percentages. The percentage values for each sub-sample were subjected to the arcsin (angular) transformation (Sokal and Rohlf 1969) before statistical analysis. Significant differences were determined by means of the Fisher Protected LSD method (Fisher 1949).

Epidermal Weight Index

Ten samples of each of the 3 different feed components were laid out and fixed on foolscap paper sheets with leaves fully opened. These sheets were then xeroxed to obtain an estimate of the total surface area of the samples (Fig. 1). The *Vicia* hay samples were first moistened and unfolded to obtain an accurate estimate of their surface areas. The silhouettes were then cut out, oven dried at 80°C, weighed and calibrated against 10 × 10 cm squares of the same xerox paper to give area in cm^2. The forage samples were similarly dried and weighed to give the epidermal weight index as g cm^{-2}.

Results

The mean species composition of the rumen samples (as determined by microhistological analysis) was very similar to that of the diet (Table 1). The Kulczynski similarity index between the species composition of the forage fed and the rumen and feces samples was as high as the values presented by Alipayo et al. (1992) (Table 2). The similarity index for the feces estimates were lower than those for the rumen estimates. There were signif-

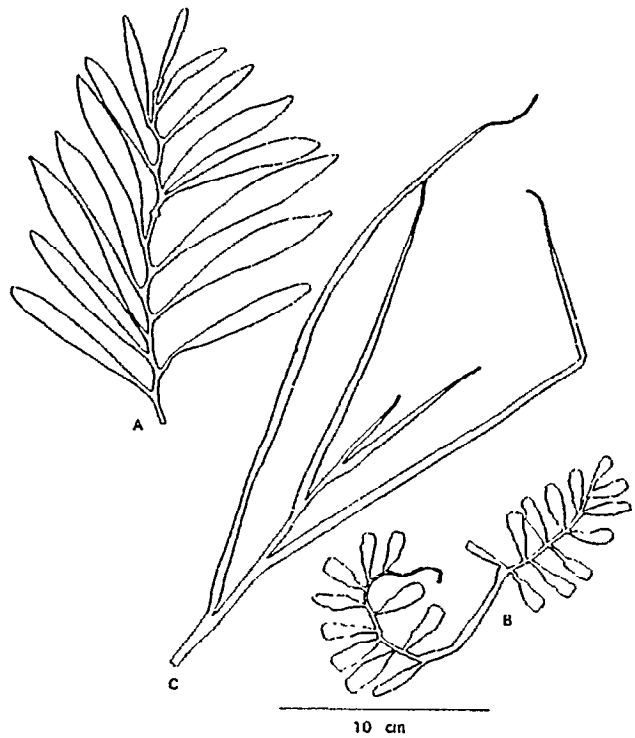

Fig. 1. Silhouettes of the components of the feed items used in the experiment: A, *Acacia cyanophylla*; B, *Vicia sativa*; C, *Chloris gayana*.

Table 1. Species composition of forage fed to beef cows calculated from dry weight (D.W.) compared to species composition of forage, rumen content, and feces determined by microhistological analysis (M.A.) of epidermal fragments.

Component	Forage		Rumen[1]	Feces[2]
	D.W.	M.A.	M.A.	M.A.
	---------- % ----------			
Acacia	16	17	14±1,2	18±0,8
Chloris	32	32	34±1,8	36±1,8
Vicia	52	50	52±1,8	46±1,2

[1]Values are means ± s.e.(n=8).
[2]Values are means ± s.e. (n=16).

icant differences between cows in both the rumen (p=0.01) and feces (p=0.05) estimates (determined by ANOVA). The lower significance of the differences in the fecal samples vs. the rumen samples would suggest that intestinal mixing tended to homogenize the ingesta. The species composition of the fragments in the feces of cow 1 was very similar to that of the forage, but that of cow 3 was quite different (Table 3). Differences in age and weight of the cows were not related to the differences in species composition of the epidermal fragments in the feces of the individual cows.

The differences between sampling days and the interaction day × cow were not significant. The non-significant day effect and the significant between cow effect suggest that it is more important to sample more animals than more days and that fecal samples of individual animals, even when fed the identical diet, are not necessarily representative of the diet of the herd.

The mean overall values (Table 3) show significant differences between the diet fed and the species composition of the epidermal fragments in the feces. The shrub component was not significantly different, but the grass component was over-estimated and the forb component was under-estimated.

Discussion

The under-estimation of forbs in fecal analysis has been reported many times in the past (Holechek and Gross 1982) and is assumed to be caused by the greater loss of forb epidermis as a consequence of maceration and digestion. However, there have also been reports of over-estimation of forbs (e.g. Gill et al. 1983, Alipayo et al. 1992). Over-estimation of the grass component in relation to the shrub component could be caused by the fact that the epidermal weight index of the grass leaves was lower than the shrub leaves (Table 4) so that for the average epidermal fragment, the grass would be over-estimated in relation to the shrub. This relationship could be modified as a result of differences between

Table 2. Kulczynski similarity index (I)[1] between diet composition calculated from dry weight (D.W.) and offered forage, rumen content, and feces, determined by microhistological analysis (M.A.).

	Forage D.W.	Forage M.A.	Rumen M.A.
Forage M.A.	98		
Rumen M.A.	98	96	
Feces M.A.	94	95	94

[1]I=100 × 2w/(a+b), where w is the sum of the lowest values for each pair of species in 2 compared samples; a and b are the sums of the 2 species in the same samples (Oosting, 1958, p. 77).

Table 3. Species composition of epidermal fragments in feces sampled from 4 cows on each of 2 consecutive days.

	Species			Kulczynski index[1]
	Acacia	*Chloris*	*Vicia*	
	---------- % ----------			
Cow 1	17	32 a[2]	51 c	99
Cow 2	18	37 ab	45 ab	93
Cow 3	19	40 b	41 a	89
Cow 4	17	37 ab	46 bc	94
Day 1	16	38	46	94
Day 2	19	35	46	94
Overall mean	18	36	46	94
Diet composition	16	32	52	
Difference	+1,8	+4,3	-6,2	
LSD (p=.05)	2,3	2,3	2,6	

[1]Similarity with actual diet composition.
[2]Different letters indicate significant differences between cows and within component (p=0.05) by Fisher's protected LSD method. Significant differences were calculated from data subjected to arcsin (angular) transformation.

the diet items in their sensitivity to epidermal erosion. Instead of using the empirical type of correction factor proposed by Dearden et al. (1975) and by Gill et al (1983), it may be more efficient to use factors based on the mechanism that causes the deviation between the fecal analysis and the composition of the ingested forage. These factors are mass of the feed item in relation to its epidermal area and erodibility of the epidermis by mastication and digestion. The fraction of species i in the ingested feed, C_i, could then be estimated as follows:

$$C_i = \frac{F_i h_i/(1-b_i)}{\sum_i (F_i \cdot h_i/(1-b_i))} \quad (1)$$

where,

F_i is the measured fraction of epidermal fragments of species i in the fecal sample.

h_i is the epidermal weight index of species *i* (= weight of feed item/epidermal surface area)

b_i is the epidermal erodibility of species *i* (non-erodible to completely erodible represented by O to 1)

The epidermal weight index can be determined relatively easily and should include the non-leaf material that is part of the feed item (see 'Material and Methods'). The epidermal erodibility can be derived from experimental data by assuming an arbitrary epidermal erodibility factor for a reference component, b*, and calculating the value for the other diet components, b_j, by rearrangement of terms in a variation of expression (1):

$$\frac{k^*}{k_j} = \frac{F^* h^*/(1-b^*)}{F_j h_j/(1-b_j)} \quad (2)$$

$$b_j = 1 - \frac{(1-b^*)\, k^* F_j h_j}{k_j F^* h^*} \quad (3)$$

where,

k^*, k_j are the (known) fractions of the reference and the dependent components in the diet respectively;

F^*, F_j are the (known) fractions of the epidermal fragments in the feces of the reference and the dependent component, respectively;

h^*, h_j are the (known) epidermal weight indices of the references and the dependent component respectively.

If we take the shrub as the reference component and assume b^* = 0.1, the erodibility factors for the other components in the pre-

sent experiment are as given in Table 4. The 3 forage categories are very clearly separated, both with regard to the epidermal weight index, h, and the epidermal erodibility index, b. Such large recognizable differences between forage items are a necessary condition for a robust correction procedure.

Potential sources of error in the determination of the measured correction variables should be taken into account. Worst case combinations of the SD's in the determination of the epidermal weight index, h, (Table 4) can lead to deviations of 10–25% in the values of the erodibility index, b, and eventually to lower similarity with actual diet than the uncorrected fecal analysis data. Sample size should be large enough to reduce this source of error to a minimum. In some cases, other sources of error, particularly observer bias in identification of the epidermal fragments (Holechek and Gross 1982, Alipayo et al. 1992), and inadequate sampling of feces may be more important sources of error than differences in feed component characteristics.

Table 4. Measured variables (k, actual diet composition; F, diet composition estimated by fecal analysis; h, epidermal weight index) and the calculated erodibility index, b, for the species used in the experiment.

Species (j)	k	F	h	b
	(%)	(%)	(mg/cm^2)[1]	
Acacia	16	18	19,2±1,08	0,10
Chloris	32	36	11,1±2,00	0,48
Vicia	52	46	6,2±1,67	0,77

[1]Mean ± S.D.

Conclusions

The large between-species variation of both the epidermal weight index and the epidermal erodibility index can explain the contradictions in the conclusions of various reports on the reliability of fecal analysis results. Differences in the physical characteristics between species within each forage category (shrubs, grass, forbs), as well as different ratios between the components in the diet can lead to over, under or accurate estimates of the species in the diet when based on uncorrected fecal analysis. It can therefore be concluded that while it is unlikely that there is any universal correction factor for any species, it is possible to define factors that explicitly take into account those forage item characteristics that cause the deviations between diet estimates by fecal analysis and actual diet ingested in any specific case. Such factors could go a long way to improve the reliability of the method, especially where the constituent variables can be determined with a high degree of precision.

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