A Double Sampling Technique for Estimating Dietary Composition

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Highlight: *A double sampling technique is described which has a potential to increase sampling accuracy and efficiency when estimating botanical composition of herbivore diets. When applied to wild herbivores this technique may also reduce the need for using fistulated and thus behaviorally abnormal animals.*

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Sampling for botanical composition of diets of grazing animals is difficult. The cost of maintenance and care, the short life expectancy, and the abnormal behavior of fistulated animals are among the disadvantages of dietary sampling with esophageal fistulated animals. These difficulties are accentuated with dietary sampling of wild ungulates.

The relative simplicity with which fecal samples can be collected leads to the idea that diets of wild herbivores can be estimated from them. It is known that forage species can be recognized in fecal samples by microscopic analysis of the plant cuticle (Storr, 1961, 1968; Williams, 1969). Free et al. (1970) demonstrated that percentage dry weight composition of native range plants could be determined from fecal analysis. However, Slater and Jones (1971) caution that biases can

occur in dietary estimates if fragile species such as the legume, white clover, are present in the diet. Similarly, Peden (1972) found that differential digestion rates of plant species are likely to occur in diets of bison. For clarity, differential digestion refers to the change in composition of ingesta as a result of the digestive process acting with different efficiencies on the various components of the diet. Without some correction for this bias, precise quantitative estimates from feces of dry weight composition of forage species in diets will likely be inaccurate. Given that feces can be collected from a behaviorally normal animal at a relatively low cost and given that the potential bias can be measured, the possibility exists for selecting an optimal ratio of esophageal and fecal samples such that for a given total experimental cost, the variance of the mean of the predicted dry weight composition of ungulate diets will be minimized. The description of such a method is the purpose of this paper.

Materials and Methods

During December, 1970, and March, May, June, August, and October, 1971, diets of bison were sampled using the esophageal fistula technique. For each of these particular sample periods, 2, 4, 3, 3, 4, and 4 animals were used, respectively. Each sample period consisted of four collection periods of approximately 45 minutes each. These collections were made on shortgrass range in northcentral Colorado. The extrusa from all daily collection periods within a given sample period and derived from a particular animal were mixed so that the dry weight contributions to the total from each daily collection were equal. This procedure was duplicated for each animal on an adjacent pasture having different levels of herbage biomass. Thus a total of 40 bison dietary samples was collected.

The percent dry weight composition of warm season grass (%WSG) was estimated using the microscopic cuticle analysis technique of Sparks (1967). This warm season grass classification (Sims and Singh, 1971) includes the following forage species: *Bouteloua gracilis, Aristida longiseta, Buchloe dactyloides, Sporobolus cryptandrus,* and *Muhlenbergia torreyi.* Each of these dietary samples was then subjected to a nylon bag microdigestion trial in three rumen-fistulated bison. Three to five replicates of each sample were placed in each of the three bison. The residues remaining in the nylon bags were then analyzed for %WSG using the previously described technique. For a more detailed description of the sampling and analysis techniques the reader is referred to Peden (1972).

Based on the assumptions that the calibration equation relating %WSG of digested rumen samples to that of esophageal dietary samples is linear and that most of the total digestive breakdown of cuticle is reflected in the composition of the digested rumen samples, then the following relation holds:

$$\bar{y}_r = \bar{y} + b\,(\bar{x}' - \bar{x}) \qquad (1)$$

where $\bar{y}_r$ is the %WSG predicted from the information contributed by both esophageal and fecal samples, $\bar{y}$ is the mean %WSG of the esophageal diet samples, **b** is the regression coefficient, $\bar{x}$ is the mean %WSG in the residue from the nylon bag microdigestion, and $\bar{x}'$ is the mean %WSG of any additional fecal samples.

Let **n** and **n'** be the number of esophageal fistula samples and the number of fecal samples, respectively. Following the development of Cochran (1963), the variance $V(\bar{y}_r)$ of the predicted mean will be

$$V(\bar{y}_r) = S_y^2\,(1 - p^2)\left[\frac{1}{n} + \left(\frac{1}{n} + \frac{1}{n'}\right)\frac{1}{n-3}\right] + \frac{p^2 S_y^2}{n'} \qquad (2)$$

where **p** is the correlation coefficient derived from the regression of **y** on **x** and where S_y^2 is the variance of **y**. The problem at hand now is to find the minimum $V(\bar{y}_r)$ for a given total cost **C** by determining appropriate levels of **n** and **n'** where each observation costs C_n and $C_{n'}$, respectively. This is accomplished by solving

$$n' = \frac{\sqrt[n]{(p^2)\,(S_y^2)\,(C_n)}}{\sqrt{(1 - p^2)\,(S_y^2)\,(C_{n'})}} \qquad (3)$$

for some given **n**. Note that

$$(C_n)\,(n) + (C_{n'})\,(n') = C \qquad (4)$$

Results and Discussion

The mean %WSG ($\bar{y}$) and its standard deviation of the n' = dietary samples were 69.7% and 22.4%, respectively. The mean %WSG ($\bar{x}$) and its standard deviation of the nylon bag residue were 70.8% and 23.0%, respectively. The regression of dietary %WSG (**y**) on the %WSG of the nylon bag residue (**x**) resulted in a regression coefficient (**b**) of 0.825 and a correlation coefficient (p^2) of 0.720. Given that in future n' additional fecal samples were collected, the estimated mean %WSG under double sampling would be

$$\bar{y}_r = 69.7 + 0.824\,(\bar{x}' - 70.8) \qquad (5)$$

The use of equation (5) implies that the digestive process must not totally remove the identifiable cuticle, otherwise, **b** would be undefined. The measurement $\bar{y}$ is an unbiased estimator for %WSG in the diet. Further, the double sampling procedure (the addition of the term involving **b**) provides an estimator with smaller variance than $\bar{y}$ alone. Hence, equations (1) and (5) permit more accurate estimates of dietary %WSG.

If we suppose, for example, that the total cost in man-hours (as estimated for the work of Peden, 1972) of collecting and analyzing one esophageal dietary sample is 26.5 hours (C_n), while that of a fecal sample is 8.1 hours ($C_{n'}$), then we can determine which levels of **n** and n' will minimize $V(\bar{y}_r)$ in equation (2) for a particular available total man-hour level (**C**). This is accomplished by solving equation (3), thus

$$n' = 2.9\,n \cong 3n \qquad (6)$$

Thus the optimal strategy for sampling will exist for the given p^2, C_n, and $C_{n'}$ when there are approximately three fecal samples for every esophageal one.

The foregoing discussion suggests that in diet sampling of herbivores for botanical composition, it may be advisable to adopt a double sampling procedure. The advantages may include a gain in sampling efficiency, acquisition of data from relatively normal animals, and a correction for biases associated with the effect of differential digestibility on fecal composition. The procedure described has been applied only to one dietary component, warm season grass (WSG). It may well be that different levels of **n** and n' will be obtained for other forage species. Perhaps a multivariate extension of double sampling would be appropriate whenever the entire dietary vector is to be treated as a multivariate observation (Peden, 1972).

If one can assume that differential digestion as measured in a captive fistulated herbivore is similar to that in a surgically unaltered wild one, then the possibility exists of estimating a wild herbivore's diet using captive individuals for the esophage-

al dietary sampling and relying on fecal samples alone for the wild ones. In sampling diets of free-ranging nondomesticated herbivores, costly training and care of fistulated individuals may be reduced by replacing them with readily handled domestic ones.

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