ESTIMATING PLANT COMPOSITION OF WILD SHEEP DIETS

by

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ABSTRACT

The composition of recognized leaf fragments from the digestive tracts of wild sheep and the fecal pellets of wild sheep may be quantified by a microscope technique. The frequency of recognized plant fragments in forage fed to domestic sheep and the frequency of recognized plant fragments in the feces indicated that digestion did not greatly change the relative frequency of plant epidermal characteristics.

INTRODUCTION

A microscope identification technique for classifying fragments of leafy material eaten by herbivores that thoroughly masticate their food was described by Baumgartner and Martin (1939) and the technique was later refined by Dusi (1949). Sparks and Malechek (1968) found a quantification scheme so that they could predict a 1:1 relationship between relative density of recognized fragments and the dry weights of foodplants in hand compounded mixtures. Recent studies have shown that by using new slide preparation techniques practically all food-plants passed through a leaf-eating herbivore could be recognized in the feces (Storr 1961, Williams 1969, Free et al. 1970). Since there is little or no digestion of the epidermis that is encased in cutin there are recognizable leaf fragments in the feces even though the mass (weight) of a fragment may have been changed during digestion.

Several papers have been recently published on the potential for estimating the botanical and dry weight composition of herbivore diets from the microscopic examination of feces (Croker 1959, Dusi 1949, Free et. al. 1970, Hegg 1961, Hercus 1960, Kiley 1966, Stewart 1967, Storr 1961, 1963, 1964, 1968, Ward 1969, Williams 1969). Casebeer and Koss (1970) found the food selectivity measured from fecal and stomach-content analyses of 4 African herbivores provided similar results when the diets were almost entirely grasses. The diets of fossil sheep and goats and diets of wild sheep from Alaska, Iran and Colorado, are being determined from a microscopic examination of feces in the Composition Analysis Laboratory at Colorado State University but no one has published on the accuracy of the tecnhique for estimating dry-weight percentages of plants in the diets of sheep.

The objective of this paper is to report on the accuracy of the fecal examination technique being used at the Composition Analysis Laboratory and to publicize the value of this technique for studying diets of wild sheep.

STRATEGIES

A premise upon which this paper is based is that the digestion and fragmentation of plant leafy material in wild sheep is identical (or nearly so) to that of domestic sheep. Several unpublished observations strongly support the idea that the leafy material of one species of plant relative to leafy material of other species of plants change weight and fragment similarly during digestion in a wide variety of large-bodied herbivores. The second premise is that there is about a one-to-one relation between the relative density of recognized fragments in the forage-fed domestic sheep and the dry weights of the plants in the forage samples. The third premise is that if there is high fidelity between the species and relative percent densities of recognized fragments of plants in paired diets and feces samples of domestic sheep, then it is practical to use fecal samples from wild sheep for estimating the percentage of dry weights of plants in their diets.

MATERIALS AND METHODS

Forage samples were collected from lightly grazed blue grama rangeland in eastern Colorado in mind-June, late July, early September and mid-December to represent spring, summer, autumn and winter types of plants in diets. Columbia wethers were fed the four forage types. The sheep feces were then collected from metabolism cages.

The forage samples and the fecal samples were ground in a Wiley mill through a 0.5 mm screen. Each sample was thoroughly mixed to develop randomness for subsampling. The grinding appears to reduce any species differences for fragmentation due to chewing and digestion so that the mean size of particles of different species of plants were similar in each sample. The material used for microscope slides was washed over a 0.1 mm screen to insure mixing and to remove the small fragments. Ten microscope slides were prepared for each sample according to procedure outlined by Sparks and Malechek (1968). The material used for making slides was not stained and was only treated with clearing (Hertwig's) and mounting (Hoyer's) mediums. The four forages and four composited samples of sheep feces were then examined for the frequency of recognizable plant fragments for estimating the relative percent density (RD) of each species of plant for each kind of sample.

A reference collection was made that included each of the 20 species of plants in the forage samples. The appropriate slides of leaf, stem, flower and seed were prepared for each species. The separate parts of each plant were placed in a Waring blender with enough hot water to at least cover the blades. After two minutes at high speed, the contents of the blender were poured into a 0.1 mm mesh screen and washed. Reference slides were made directly from this material, following the same procedure as for the forage and fecal samples, but applying more material to the slides.

Each species of plant was identified in the sample when a fragment was observed that matched the material on a reference slide.

The RD of recognized fragments of plants in each of the forage and fecal samples was estimated by observing 20 systematically located fields on each of the ten slides with a compound binocular microscope at about 100 power magnification. The occurrence of each recognized species of plant in each field was recorded. Average percent frequency was computed for all plant species present in the samples. The RD, calculated as the number of recognized fragments of a species expressed as a percentage of the total number of fragments of all species (Curtis and McIntosh 1950) was calculated for each plant species.

The forage samples averaged 86, 90, 96 and 99 percent grasses and grasslikes (8 species) for the spring, summer, autumn and winter periods, respectively. There were 12 species of forbs and shrubs in the four samples whose dry weights varied from as high as 4% to only a trace.

Indices of RD's for each species of plant fragment between seasonally paired forage and fecal samples of the sheep were calculated by Kulczynski's mathematical index of similarity (Osting 1956):

$$SI = \frac{2w}{a + b}\,(100), \text{ and by}$$

a standard deviation index (Watt 1968)

$$I = \sqrt{\frac{\Sigma\,(d)^2}{n - 1}}\ .$$

In Kulczyski's index, w = the least RD of a species of plant in the paired forage-feces comparisions and $a + b$ equals the % RD in the forage plus the % RD in the feces. This index would equal 100% if the mean RD values of plant fragments in forage and feces were identical.

A low I (standard deviation index) value between paired samples can be used to indicate a tendency for the RD compositions to be similar. "I" is calculated by the sum of the squared differences in RD of each plant species compared in the pair of samples. Imagine that we superimpose, on the forage and feces samples within which fragments of plants are distributed, a grid of RD values for recognized plant fragments. The grid will contain large RD values or small RD values, whichever value is obtained from the microscope technique. The RD value of a plant in each kind of sample is calculated, and the

$$\frac{\Sigma\,(\text{RD's in forage} - \text{RD in feces})^2}{n - 1}$$

is obtained as a sum of the differences of RD's of plant species between samples being compared. The denominator, n, is the number of plant species on which the mean RD is based. Thus I is the standard deviation of RD of plant fragments about the mean RD difference and if $I = 0$ then RD characteristics of the samples are identical.

RESULTS AND DISCUSSION

The indices of similarity (SI) and standard deviation (I) both indicated that the RD's of fragments of species of plants in the food and feces of the sheep was very similar.

Table 1 - The indices of similarity and indices of standard deviations for recognized fragments of plants between the food and feces of domestic sheep during feeding tests in the laboratory.

Season	Index of Similarity	Index of Standard Deviation
spring	91%	3%
summer	89%	3%
autumn	90%	2%
winter	92%	2%
Overall mean	91%	2.5%

There were small differences in the estimated percent dry weight of the species of grasses found in the forage samples and the % RD's in fecal samples obtained from the sheep that had consumed the forage. The similarity of the % RD's in the sample pairs for the grasses is within the technique errors that might occur. Forbs and annual plants made up only a small part of the forage during the four seasons. The epidermal fragments of forbs were not found in the feces as readily as were the grass fragments. Additional work done since this research was completed suggests that if these fecal samples had been soaked in hot water before the microscope slides were made the cuticular fragments of these fragile plants would have been more easily recognized in the slides made from the sheep feces.

It is concluded that foodplants making up more than 5% of the diets of wild sheep could be satisfactorily identified and quantified by the microscope examination of 200 fields of a composite sample of feces from any season of the year. The variance between % dry weight of ingested plants and the % RD of recognized fragments in the feces is within the practical needs for research and management of wild sheep herds.

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DISCUSSION

QUESTION BY PARRY LARSON, NEW MEXICO, G & F: You determine that this method is accurate by feeding a known ration and then seeing if you could sample fecal pellets and obtain the diet in the ration. How many vegetative species were in the known diet?

REPLY BY HANSEN: About 15 to 20 species were in the diet and probably six of them were making up 95% of the diet. We fed this ration to domestic sheep, then collected the feces of the sheep and examined the feces by the microscope technique. This is one of the schemes we have used to determine how good the technique is.

Ecologists usually figure that when an index of similarity is 85 or above, your two samples are very much alike. We got values that averaged about 90 or 91 in similarity so we are confident that this is an excellent technique.

REPLY BY LARSON: It appears so. I wondered whether this is a sample with only 5 or so diet items in it, but if you have 15 or 20 species, this could be quite similar to an actual range diet.

REPLY BY HANSEN: This was an actual range diet because the material that we fed to the sheep was collected from cattle that were esophageal fistulated. The material that the cattle chewed off and dropped into a bag through their esophageal fistula is the material that we fed to these sheep.

QUESTION BY BEN ALBRICKSON, USFS, NEVADA: Dr. Hansen, could you obtain samples from the field from bedding grounds of sheep and accurately determine their diet?

REPLY BY HANSEN: Oh, yes.

REPLY BY ALBRICKSON: How old could the material be that you used?

REPLY BY HANSEN: We've examined the fecal pellets from fossil bighorn sheep which were 15,000 to 30,000 years old. We have also examined the fecal pellets of some fossil mountain goats from the Stanton Cave in Arizona. These are fecal pellets that have been preserved because they have been kept dry. We come up with some weird determinations, but it worked.

One of the problems was that we didn't have reference material from that particular age. We called one epidermal fragment a species of plant that shouldn't be in this country. The plant supposedly wasn't introduced here until 1870. We think it was just a similar plant. If we had adequate reference material from that area, we could probably know what it was.

QUESTION FROM WAYNE SANDFORT, C & F, COLORADO: Dick, I might ask you a question as relates to time on analysis. Provided basic reference material, how many samples from all the states and provinces represented here today could you analyze?

REPLY BY HANSEN: The big problem is having adequate reference material so that you don't make mistakes on what you observe under the microscope. An adequate reference collection of plants from the given area where you are going to study the diet is absolutely necessary. On the research and studies we have done in the past, we collect all the reference plants we can and we make slides of them. Then we go to the laboratory with the diets of these leaf-eating herbivores and we start trying to identify what was in the diet. You always find species of plants that we didn't collect. This might be because the life cycle of the plant only lasted for two or three weeks and we weren't in the field when it was obvious. Frequently we will find mosses, lichens or liverworts which we don't usually associate with the diet. These are interesting, but they probably don't make up too much of the diet of the large herbivores. We find just about everything they eat.

QUESTION BY GENE DECKER, CSU: Dick, concerning your food habits studies of the bighorn herd that you are going to be working on, are you going to work with fecal samples?

REPLY BY HANSEN: Yes.

REPLY BY DECKER: Over what period are you going to gather these samples, how many are you going to gather, and are you going to try to isolate the fecal samples per individual or per herd or per area? What are the mechanics of your approach?

REPLY BY HANSEN: Jeff Todd, who is a graduate student with the Cooperative Wildlife Research Unit, will actually collect the samples. It's a difficult job to convince experienced biologists that you don't need to study each fecal pellet or each fecal group to have an estimate of the diet of a herd of sheep. So what Jeff and I are going to do is take one fecal pellet from each group and put them together. They we run our microscopic analysis on the composited sample. We think this represents the diet of the bighorn sheep more closely than if we had individual sheep records. We know for a fact that one day an animal is not going to be eating the same thing he eats on the next day. If you are worried about that, you get into statistical hang-ups on what your data means.

Jeff tries to collect fecal samples in such a systematic random manner that his collection actually represents the fecal pellets of the herd of sheep. He is so conscious about this work that he gives the technicians one pellet from each pellet group and he keeps each of the pellet groups carefully separated and documented so that every one is available if there is some reason that we should have to go back and look at them individually. This can be done but it might make the workload so great that we might not get on with the job and get the work done that really needs to be done.

REPLY BY DECKER: Are you taking samples from fresh pellet groups daily, weekly or monthly?

REPLY BY HANSEN: Jeff has determined that collections at six very critical phenological stages in the year will probably represent the bighorn sheep diet the best. So over approximately a four day to a week long period he observes the sheep eating and when he sees a sheep defecate he runs over and grabs it. Maybe not immediately, but he marks it on a map so he knows which groups are fresh and which groups aren't. He collects 50 fecal pellet groups per collection period.

It's very important to get pellets that come from the bighorn sheep. There are deer feeding with the sheep and it appears almost impossible to tell the pellets of deer and bighorn sheep apart, with 100% accuracy or even 80% accuracy.

If you went into the breeding grounds of bighorn sheep to collect your pellets for comparing its diet with what is available in the area where it has been feeding, you might be just as misled as if you did the same thing in an elk study because you have to make sure that the diet you compare is being compared with the feeding area, not the bedding area, and not the defecation area. You must put these kinds of constraints into your experimental design so that the diet is actually representative of what you want to have it represent.

QUESTION BY GENE DECKER, CSU: How long does it take to analyze this material?

REPLY BY HANSEN: For example, from a place like the shortgrass prairie where my technicians are very familiar with the plant species that occur there and have examined diets and plant mixtures and even litter mixtures, I think they can go through about 50 complex samples in a week's time or less. If you run into some unknowns, it takes longer.

However, if you go into a new area where you have to learn the reference material and run down the unknowns, you might spend two or three weeks or a month learning the reference material before you can even begin. Generally, the technicians spend a day or two reviewing the reference slides before they actually read the diet slides. This constant relearning and memorizing has to be done before they can do a precise and accurate job.

QUESTION BY JERRY LIGHT, USFS, CALIFORNIA: What would you think about sending your pellet groups? This is such a big job and we haven't got much time to do it ourselves and to train ourselves.

REPLY BY HANSEN: This would be all right if we had some way to pay the technicians. Right now, we are analyzing samples from all over the grassland areas on our grassland biome study. We sometimes steal a little bit of time to look into some of these real interesting things like fossil mountain goats and fossil bighorn sheep but we are overloaded with requests for this kind of help.