Estimating Percentage Dry Weight in Diets Using a Microscopic Technique[1]

DONNIE R. SPARKS AND JOHN C. MALECHEK[2]

Graduate Research Assistants, Range Science Department, Colorado State University, Fort Collins.

Highlight

Percent composition by dry weight was accurately estimated for 15 mixtures of plants that are found in the diets of some herbivores. The mixtures were sampled by recording the frequency of occurrence of each species in 100 microscope fields using 125-power magnification, converting frequency to density, and calculating relative density as an estimate of percent composition by dry weight. Dry weight percentages were predicted directly from relative density. The microscopic technique reported in this paper would be an accurate means of determining the dry-weight composition of stomach samples, esophageal samples, rumen samples, and clipped herbage.

A rapid, reliable method for estimating plant species composition in the diets of herbivorous animals is needed. This need is emphasized by the variety of methods used for reporting diets (Norris, 1943; Torell, 1956; Reppert, 1960; Vaughan, 1967).

A problem encountered in the study of herbivore diets is the lack of a method that can be checked for accuracy. The technician needs a method that builds confidence in his own ability to estimate the composition of a diet. The procedure should be as simple as possible. Botanical analysis of the diet should accurately reflect both the plant species eaten and the amount of each that was eaten.

A microscopic technique for identification of plants eaten by herbivores that thoroughly masticate their food was described by Baumgartner and Martin (1939) and the technique was later refined by Dusi (1949). This basic technique has been employed frequently in recent years by numerous other researchers. However, no one has evaluated the accuracy of the technique for estimating dry-weight percentages of plants in the diets of herbivores.

The objective of this study was to determine if dry-weight composition of a mixture of grasses and forbs could be accurately estimated by a microscopic technique.

Methods and Materials

Samples that contained known amounts of grasses and forbs were artificially mixed. Four mixtures contained Arizona fescue (*Festuca arizonica*), mountain muhly (*Muhlenbergia montana*), Pennsylvania cinquefoil (*Potentilla pensylvanica*), and fringed sagewort (*Artemisia frigida*). Eleven mixtures included from two to four of the following species: western wheatgrass (*Agropyron smithii*), prairie sandreed (*Calamovilfa longifolia*), summercypress (*Kochia scoparia*), and alfalfa (*Medicago sativa*).

All plants used in the mixtures were actively growing when collected. The plant material was oven dried and ground over a one-mm screen to reduce all plant fragments to a uniform size. The mixtures were compounded of various combinations of species so no two samples were alike. The species and dry-weight composition of the mixtures were unknown to the authors until after the sample estimates were recorded. Mixtures were washed over a 200-mesh screen to insure mixing, to remove dirt, and to remove very small plant fragments. A small portion of the mixture was spread evenly and mounted on a microscope slide using Hertwig's Solution (Baumgartner and Martin, 1939) and Hoyer's Solution (Baker and Wharten, 1952). The slides were oven dried at 60 C. Five slides were prepared from each mixture.

Tissues of plants that were used in the mixtures were prepared and mounted on microscope slides in the same manner for study as reference material. Identification of each species in the mixtures was based on epidermal characteristics (Davies, 1959; Brusven and Mulkern, 1960; Storr, 1961). These workers found that epidermal characteristics of grasses and forbs were variable with different stages of maturity. Histological features such as size and shape of epidermal hairs, presence or absence of hairs, cell shapes, and crystals included in epidermal cells provided diagnostic characteristics for identification of forb species. Species of grasses were identified by the occurrence and position of such specialized epidermal cells as cork cells, silica cells, silico-suberose couples, and asperities. The size and shape of the guard and subsidiary cells of the stomata were also reliable diagnostic features.

The mixed samples were analyzed by examining five slide mounts of each mixture under a compound binocular microscope. Twenty locations were systematically observed on each slide. A location was considered as an area of the slide delineated by a microscope field using 125-power magnification. Only those fragments that were recognized as epidermal tissue (other than hairs) were recorded as positive evidence for the presence of a plant species at a location on the slide. Each species present for each location was recorded. Frequency percentages (number of fields that the species occurred in out of 100 locations) were tabulated for each species in the mixture. The frequency percentages were converted to particle density per field using a table developed by Fracker and Brischle (1944) and the relative density, expressed as a percentage, of each species in the mixture was calculated. The relative density of a species was used to estimate the percentage dry weight of that species in the mixture.

Regression equations that express the relationship between estimated percentage dry weight (X) and actual percentage dry weight (Y) were developed for three categories: grasses, forbs, and grass–forb combinations.

Results and Discussion

Prediction equations for grasses, forbs, and grass–forb combinations are shown in Fig. 1, 2, and 3. The ratio between estimated dry weight percentages (relative density) and actual dry weight percentages was approximately 1 : 1 for all three categories. Student's t-test showed that there was no significant difference ($P < .01$) between regression equations for grasses and forbs and that the calculated regression equations for grasses, forbs, and grass–forb combinations were not statistically different from the equation $Y = X$. Therefore, the percent composition based on dry weight of the mixtures could be predicted directly from the relative density.

For 11 of the 15 mixtures, the number of epidermal fragments of each species at a location was recorded in

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[2] Present address: Range Science Department, Texas A&M University, College Station.

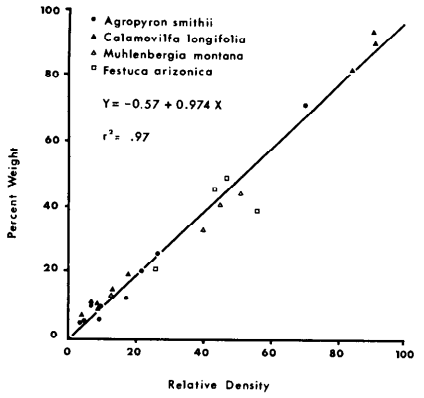

FIG. 1. Relationship of relative density to actual percent composition by weight for four grasses.

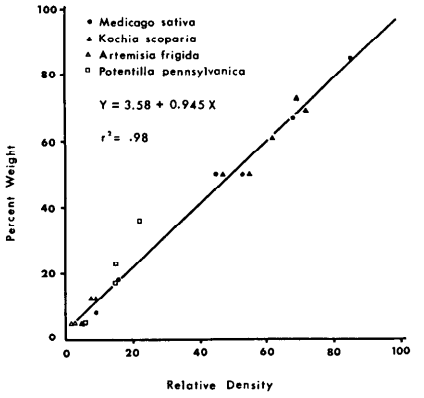

FIG. 2. Relationship of relative density to actual percent composition by weight for four forbs.

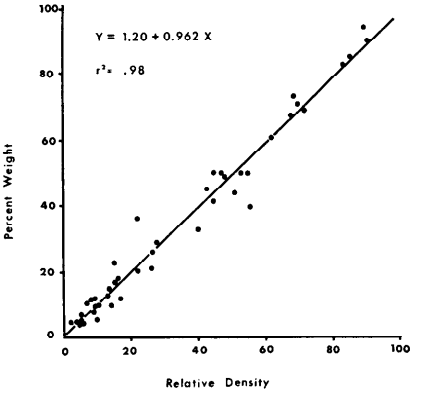

FIG. 3. Relationship of relative density to actual percent composition by weight for four grasses and four forbs.

addition to recording frequency. An estimate of the dry-weight percentages for each species was computed from the totals. A paired t-test showed no significant difference ($P < .01$) between the estimates obtained from the "particle count" technique and the "frequency conversion" technique. Either can be used with a similar degree of accuracy, but it is much easier and faster for the technician to determine if a species is present or absent than to count all recognizable fragments. The "frequency conversion" technique reported in this paper is recommended.

There are two requirements that must be met before frequency percentage can be converted to density (Curtis and McIntosh, 1950). The plant fragments must be distributed randomly over the slide, and the density of particles must be such that the most common species does not occur in more than 86% of the microscope fields. A random distribution can be attained by reducing the particles to a uniform size and thoroughly mixing them. A frequency of less than 86% for the most common species can be maintained by adjusting the amount of material mounted on the slide. This is done by trial and error until the technician becomes familiar with the material.

Storr (1961) and Heady and Van Dyne (1965) reported that weight per unit area of plant material is not consistent at different stages of maturity nor is it consistent from species to species. The 1 : 1 relationship between estimated dry-weight percentages and actual dry-weight percentages for the species used in this study may not be consistent with other species or at other stages of maturity. However, unless the plant parts in the diet being analyzed are grossly different from these in our studies, the added accuracy gained by using a prediction equation more complicated than $Y = X$ will probably not be worthwhile. If the specific gravity of a species or the phenological stage of a species is suspected of being different from that used in this study, the investigator can easily compound mixtures and develop regression equations for estimating dry-weight percentages.

The microscope technique reported in the present paper could also be used to determine the species composition of clipped herbage and the weights contributed by each species present. Thus, it should be possible to accurately estimate the yields of individual components in botanical studies.

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