Estimating Botanical Composition of Forage Samples from Fistulated Steers by a Microscope Point Method[1]

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Highlight

A microscope point method was used to develop weight prediction equations for plant species in masticated forage samples of known species weights collected at the end of two successive growing seasons. A high correlation was found in regressions of percent weight on percent points for all the masticated plant species. Two observers were consistent in their ability to estimate similar amounts of plant species in a given species mixture. With 400 microscope points, the average weight of a species was estimated within 5% of the mean at a 90% level of probability when the species constituted 30 to 60% of the sample weight.

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A problem basic to range management and animal nutrition is that of determining precisely the botanical composition of the grazing animal's diet. In recent years, rumen or esophageal fistulated cattle and sheep have been used in range diet studies (Cable and Shumway, 1966; Cook et al., 1958; Van Dyne and Torell, 1964). Botanical composition of forage samples collected by fistulated animals has been reported by several investigators (Heady and Torell, 1959; Connor et al., 1963; Van Dyne and Heady, 1965). However, only a few reports have been made concerning quantitative analyses of botanical composition.

A promising method for analyzing fistula forage samples has been described by Heady and Torell (1959), which employs a dissecting microscope with a pointer in one ocular and a movable tray with a series of fixed stops. Harker et al. (1964) used a similar method for quantitative analyses of a two-species mixture obtained from fistulated Zebu cattle. These workers obtained a reliable estimate of plant species weight with a 400-point analysis. Heady and Van Dyne (1965) also used a microscope-point method to successfully predict weights of plant species in clipped herbage samples from an annual foothill range in California.

This study was conducted to determine the number of microscope points needed for reliable estimates of plant species in masticated forage samples representing a desert grassland. A second objective was to develop equations for estimating weights

Table 1. Percent species composition by weight of orthogonal comparisons for 1964 and 1965.

Species	Species mixtures 1	2	3	4
1964				
Lehmann lovegrass (*Eragrostis lehmanniana* Nees.)	60	25	10	5
Arizona cottontop (*Trichachne californica* (Benth.)Chase)	5	10	60	25
Black grama (*Bouteloua eripoda* Torr.)	25	60	5	10
Plains bristlegrass (*Setaria macrostachya* H.D.K)	10	5	25	60
Total	100	100	100	100
1965				
Lehmann lovegrass	30	50	15	5
Plains bristlegrass	15	30	5	50
Feather fingergrass (*Chloris virgata* Swartz.)	50	5	30	15
False-mesquite (*Calliandra eriophylla* Benth.)	5	15	50	30
Total	100	100	100	100

of plant species in rumen forage samples obtained from fistulated steers grazing on the range.

Methods

Plant materials used in the study were collected at the U.S. Forest Service Santa Rita Experimental Range, near Tucson, Arizona, in conjunction with an investigation of the botanical composition of the diet of grazing steers (Galt et al., 1969). Plant species at seed stage were clipped at ground level near the end of the summer growing seasons in September of 1964 and 1965. The species were individually fed and recovered from rumen-fistulated steers by the evacuation method of Lesperance et al. (1960). The masticated plant samples were preserved by freezing at −10 C until four mixtures of the plant species were prepared for orthogonal comparisons of species. To prepare the mixtures, each sample was thawed, mixed, and then washed to remove saliva. The species were dried at 50 C for 24 hr and then weighed. Four species were combined in the proportions as shown in Table 1 to give the various mixtures. Individual species combinations were thoroughly mixed in water, dried at room temperature (20 C) and examined by a microscope point method as described by Harker et al. (1964).

A total of 1,600 points were examined on each of the four mixtures in the 1964 orthogonal comparisons. A sample constituted 400 points from examination of plant material spread uniformly over the bottom of a 45 × 15 cm tray so that four trays completed a 1,600 point analysis. The points were identified by two observers with each reading two trays, or 800 points. The analysis of variance form for observer comparisons was a hierarchal classification. Bartlett's test was made for homogeneity of tray variances (Steel and Torrie, 1960). Only 400 points were made of each sample by one observer for the 1965 mixtures.

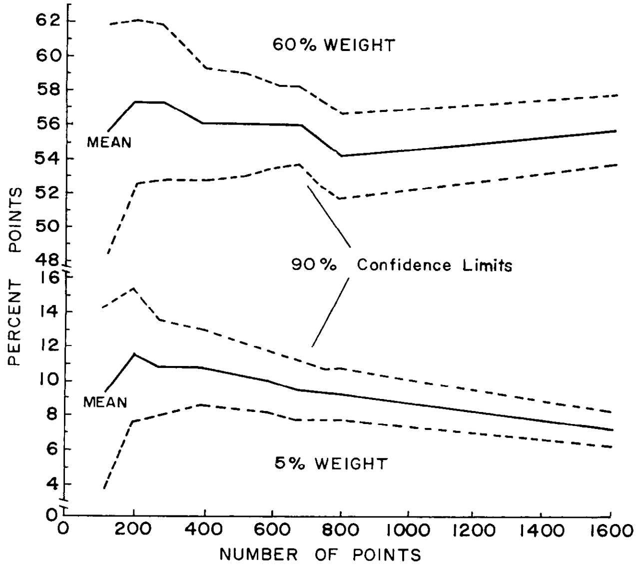

FIG. 1. Ninety percent confidence limits of the point sample means of plains bristlegrass at 5 and 60% weights of rumen samples.

The masticated plant material was examined with a binocular microscope (20 to 80 × magnification). The masticated plant fragments were identified by a key based on morphological characteristics of plant parts in known specimens. Identifying characteristics included type of pubescence, floral parts, color, surface texture, venation, size and shape of plant parts. The plant fragment at each microscope point was identified as unidentifiable or to species according to the different plant parts (leaf, stem, seedhead, etc.).

Regression equations for predicting percent weight from percent points were determined for the mixtures by linear regression of known percent weight on percent microscope points of a plant species. Percent points for a specific species were based on the total identifiable plant parts in a 400-point sample. The unidentifiable material was assumed to have the same species composition as the identifiable material.

Results and Discussion

Confidence limits were calculated for the mean hits on each species for the 1964 mixtures at different sampling intensities. Fig. 1 depicts the effect of sample size on the 90% confidence limits for means of the point samples observed on plains bristlegrass. Confidence intervals narrowed rapidly until after the first 400 points, when the change was more gradual. For plains bristlegrass at 5% weight of a species mixture with a 400 point analysis, there was a 90% probability that the true mean was between 8.5 to 13.1% points. There was a slight improvement of the estimate of the true mean with 800 points as the confidence limits narrowed to 7.7 to 10.5% points. The range was further reduced to 6.2 to 8.2% points with 1,600 points. The results indicate that 400 points may

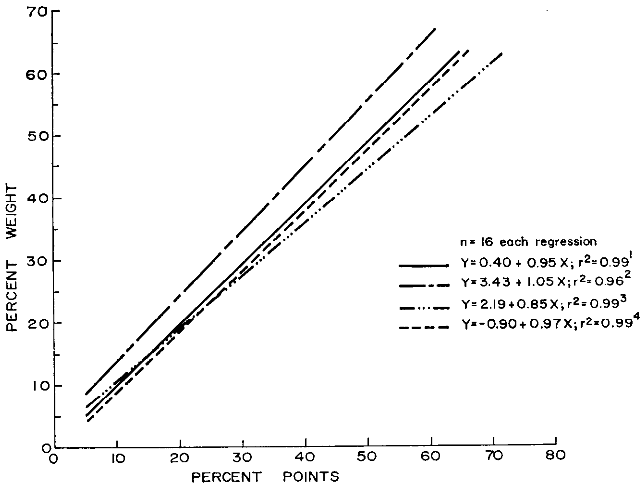

FIG. 2. Regression of percent weight on percent points for 1964 grass species of Arizona cottontop,[1] black grama,[2] Lehmann lovegrass,[3] and plains bristlegrass.[4] Each sample (n) represents a 400 point microscope point analysis.

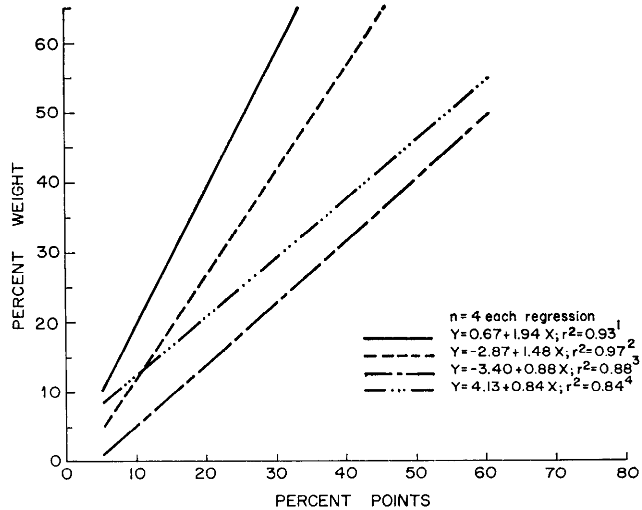

FIG. 3. Regression of percent weight on percent points for 1965 plant species of false-mesquite,[1] Lehmann lovegrass,[2] plains bristlegrass,[3] and feather fingergrass.[4] Each sample (n) represents a 400 point microscope point analysis.

give a biased estimate of the point sample means of a species at the 5% weight level as depicted by the downward trend of the species mean from 400 to 1,600 points. When plains bristlegrass comprised 60% of a species mixture, the confidence limits were 53.1 to 59.5% points with 400 points. Similarly, the range was only slightly reduced with 800 points from 52.0 to 57.0% points, and with 1,600 points 54.3 to 58.3% points. A similar pattern was observed for each species in the 1964 orthogonal comparisons. The estimate of the sample mean was improved with an increase of sampling intensity; however, the reduction was gradual for the four species from 400 to 1,600 points. A minimum time of approximately 6.5 hr was required to make a 400-point analysis of a sample. Relative efficiency ratios were computed to compare 400 with 800-point analyses. The ratio was based on the coefficient of variations and time required for each analysis (Ehrenreich, 1958). Applying the relative efficiency ratios to the four species, the 400-point samples were more efficient than the 800-point samples, since there was little difference in the coefficients of variation and analytical time was approximately doubled with 800 points.

The percent of unidentifiable material in each species mixture examined for the study ranged from 3.1 to 19.0% points. The average for all mixtures was 16.3% points. The percent of unknown material was attributed largely to plant destruction due to mastication.

Regression equations were developed for all plant species in the 1964 and 1965 mixtures to predict percent weight from percent points (Fig. 2 and 3). A high correlation was found between percent weight and percent points for all species, as shown by the coefficients of determination ($r^2 \geqq 0.84$). The grass species generally approached a 1:1 ratio of percent weight to percent points. The ratio was approximately 2:1 for false-mesquite, a half-shrub species. This difference may be a result of several factors. The density of the species or weight per point appeared to be greater for false-mesquite compared to the grass species. Another factor could have been due to some stratification of the small leaflets of false-mesquite on the sample tray.

Regression coefficients determined from the 1964 and 1965 samples were compared for plains bristlegrass and Lehmann lovegrass. There was no significant difference in the slopes of the regression of weight on points taken for plains bristlegrass between the two years. A significant difference was noted ($P < 0.05$), however, between the slopes for the two regressions of Lehmann lovegrass. A difference could be expected since growing conditions varied considerably for the two years. Available plant mositure was much greater during the summer growing season of 1964 compared with 1965. Consequently, leaf-stem ratios could have varied between the two years. Heady and Van Dyne (1965) observed differences in regression equations of percent weight on percent points of a species at different growth stages of a plant through the growing season.

The two observers were consistent in estimating similar species means for individual samples. The total number of microscope points taken by each observer for the 1964 masticated plant samples is shown in Table 2. Analyses of variance were made for each plant species at the four different percent weights. The among-tray variances for 3 of the

Table 2. Observer comparisons of total number of microscope points of plant species in 1964 orthogonal comparisons, 1,600 points per mixture.[1]

Species within mixtures	Species mixtures 1 A	1 B	2 A	2 B	3 A	3 B	4 A	4 B
	Plant hits/800 points							
Lehmann lovegrass	492	487	214	180	68	45	32	21
Arizona cottontop	43	29	65	73	393	419	196	189
Black grama	117	116	352	420	39	27	48	52
Plains bristlegrass	80	92	73	42	158	161	437	463
Unidentifiable	68	76	96	85	142	148	87	75

[1] Values for observers A and B based on two trays of 400 points each.

16 comparisons were different ($P < 0.05$), as determined by Bartlett's test for homogeneity of variance. These differences occurred once for a species at the 10% weight level and twice for species at the 5% weight of a mixture. Analyses of variance made for the remaining 13 samples detected only one case of observer differences at the 5% level of significance. These results agree with Harker et al. (1964), where no significant overall differences were detected among four observers in the way they estimated the sample weight of a species in a two-species mixture from fistulated Zebu cattle. This study supports the feasibility of training persons engaged in botanical analysis of forage samples collected by animals.

A reliable estimate was obtained for the percent weight as predicted from the 400-point analyses of the 1964 plant species mixtures (Table 3). The predicted average weight for a species at a particular percent point group varied with the species and the quantity of the species in the sample. A greater variation was observed for black grama compared to the other species. Also, the reliability of the estimate was less when a species made up a smaller portion of the mixture. The average weight was estimated within 5% of the mean at the 90% confidence level when the species comprised from 30 to 60% weight of the masticated sample mixtures. When the species comprised 20 to 30%, or 10 to 20% weight of the masticated sample mixtures, the average weight was estimated within 10% and 20% of the means, respectively. Estimates were only to within 20 to 40% of the mean when the species was 5% of the samples.

Table 3. Means and 90% confidence limits for average percent weights of plant species predicted from regressions of percent weight on percent points.

Plant species	Predicted mean weight 60	45	30	20	10	5
Arizona cottontop	±1.0	±1.0	±0.7	±0.7	±0.7	±1.0
Black grama	±2.9	±2.0	±1.5	±1.5	±1.8	±2.0
Lehmann lovegrass	±1.2	±0.9	±0.7	±0.7	±0.7	±0.9
Plains bristlegrass	±0.9	±1.2	±0.9	±0.9	±0.9	±1.2

Conclusions

Quantitative analyses for species composition of masticated forage mixtures were successfully achieved with a microscope point method. The average weight of a species mean was estimated to a high degree of reliability when the species was at least 30% weight of the masticated plant mixtures. The reliability of estimating the average weight of a species decreased with species weights less than 10% of sample mixtures. Results of the quantitative analyses indicate that 400 points may be inadequate for estimating the weight of a species at the 5% weight range.

The precision of estimating the point sample means was increased with more than 400 microscope points. The increased precision was small in relation to the coefficient of variation and the analytical time used in the analyses.

The regression equations developed from the orthogonal plant species mixtures for the different grass species were similar and approached a 1 : 1 ratio of percent weight to percent points. The ratio was approximately 2 : 1 for a half-shrub species. These results suggest that large differences in weight prediction equations may exist between different plant groups.

The consistency of the two observers to estimate similar species means in a given sample mixture indicates that trained technicians can quantitatively identify the botanical composition of masticated forage samples collected by steers.

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