TECHNICAL NOTES

Number of Fistula Samples Needed for Determination of Sheep Diet on Sagebrush-Grass Range

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Highlight: *Sheep diet was examined in the spring season on sagebrush-grass range in northeastern Idaho. Balsamroot and bluegrasses were the preferred plant species. The number of esophageal fistula samples needed for estimating the botanical and chemical components was determined. Botanical samples were more variable than chemical samples indicating a greater number of botanical samples for the same precision.*

Samples collected from esophageal fistulated sheep have been used to determine botanical and chemical components in the diet of sheep grazing on the range. The number of samples needed to estimate the diet components have been determined for relatively few vegetation types. Data collected at the U.S. Sheep Experiment Station in northeastern Idaho were used to determine optimal sample numbers on sagebrush-grass range.

In mid-May, 1968, twelve 2-year-old wethers, fitted with permanent esophageal cannulae (Cook et al., 1958), were used to collect vegetation samples representing the diet of sheep grazing sagebrush-grass range. The animals were penned each evening and turned out to graze with collection bags for about 1 hour near daybreak on 3 consecutive days. Individual samples were thoroughly mixed and split each day into a sample for chemical analysis and another for botanical analysis. During this sampling period, grazing pressure on the pasture was light (about 3 sheep days per acre).

Samples for chemical analysis were air-dried on screens and analyzed for crude protein, ash (Assoc. Offic. Agr. Chem., 1960), neutral detergent fiber, acid-detergent fiber, and acid-detergent lignin (Van Soest, 1963). Data were converted to ash-free organic matter to minimize the effect of possible saliva contamination on chemical constituents (Wallace et al., 1972).

Samples for botanical analysis were washed in a 0.073-inch sieve, rinsed with a 5% solution of acetic acid to remove saliva, ovendried at 50° C and ground in a Wiley mill with a 0.039-inch screen. Samples were analyzed at the Composition Analysis

Table 1. Botanical composition of pasture and sheep diet.[1]

Species	Production (lb/acre dry weight)	Composition (% dry weight)	
		Pasture	Diet
Grasses			
Bluebunch wheatgrass (*Agropyron spicatum*)	110.3	17.3	7.5
Indian ricegrass (*Oryzopsis hymenoides*)	9.0	1.4	2.8
Bluegrass (*Poa* spp.)[2]	19.8	3.1	18.6
Other grasses	11.3	1.8	.6
Total grasses	150.4	23.7	29.5
Forbs			
Onion (*Allium* spp.)	.1	T[3]	T
Arrowleaf balsamroot (*Balsamorhiza sagittata*)	72.1	11.3	65.6
Bastard toadflax (*Comandra umbellata*)	1.5	.2	T
Lambstongue groundsel (*Senecio integerrimus*)	.1	T	T
Other forbs	134.2	21.1	4.8
Total forbs	208.0	32.7	70.4
Shrubs			
Threetip sagebrush (*Artemisia tripartita*)	184.8	29.1	.1
Other shrubs	91.8	14.4	
Total shrubs	276.6	43.5	.1

[1] Pasture data are from 1965; diet data are from 1968.
[2] *P. secunda* and *P. nevadensis.*
[3] T = trace.

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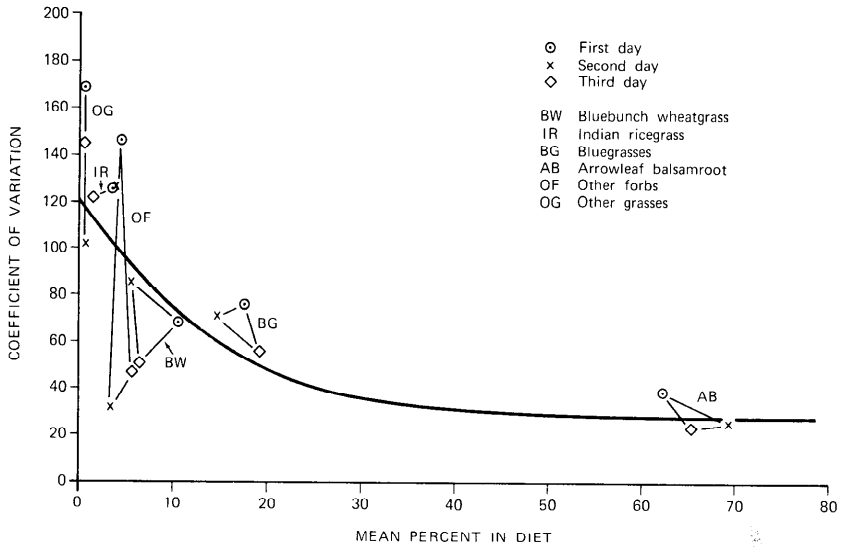

Fig. 1. *Relation between coefficient of variation and mean for major botanical constituents in a sheep's diet.*

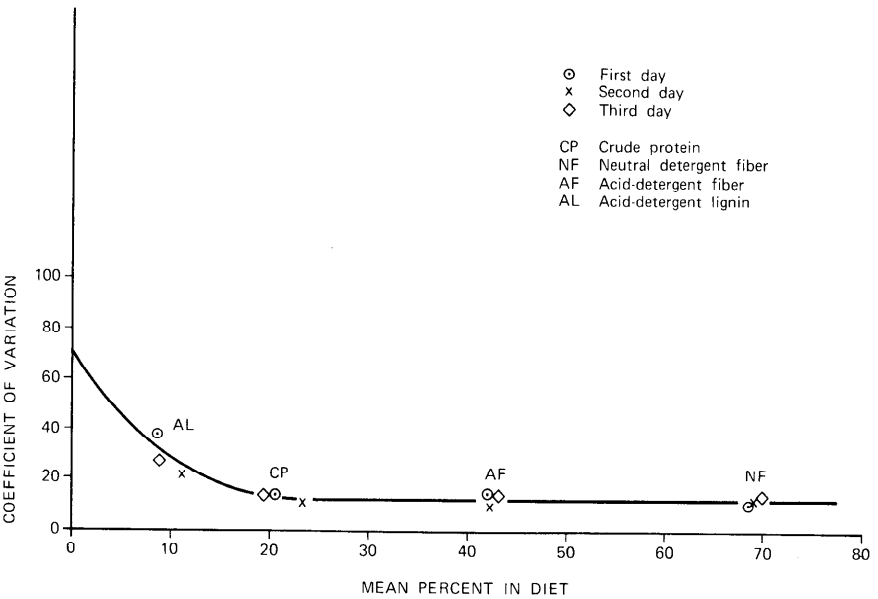

Fig. 2. *Relation between coefficient of variation and mean for nutritional constituents in a sheep's diet.*

Table 2. Means ($\bar{x}$), coefficients of variation (CV), and numbers of samples required to achieve confidence intervals of ±10% and ±20% of the sample mean at 95% and 90% probability levels.

Samples	$\bar{x}$[1]	CV[2]	0.95 level ±10%	0.95 level ±20%	0.90 level ±10%	0.90 level ±20%
Botanical						
Bluebunch wheatgrass	7.5	77	230	59	160	42
Indian ricegrass	2.8	136	625	180	505	127
Bluegrass	18.6	62	150	39	106	26
Arrowleaf balsamroot	65.6	30	37	11	26	8
Other forbs	4.8	89	306	79	216	56
Chemical						
Crude protein	21.0	15	11	5	7	4
Neutral detergent fiber	69.3	12	8	4	6	3
Acid detergent fiber	42.6	13	9	4	6	3
Acid detergent lignin	9.1	27	30	9	22	7

Laboratory, Colorado State University, by procedures reported by Sparks and Malechek (1968). One hundred fields per sample were examined with a compound microscope. Each field was examined for the presence or absence (frequency) of selected species. In the procedures reported by Sparks and Malechek (1968), average density (number per unit area) can be calculated from the species frequency in a sample by the formula $F = 100(1-e^{-D})$, where **F** is frequency, **D** is density, and **e** represents the logarithmic base 2.71828. Percent relative density was calculated for each species by the formula $RD = D/\Sigma \times 100$ where Σ is the sum of all species densities in a sample. Percent relative density was used as an estimate of percent dry weight for a species in the sample, assuming a linear relation between density and dry weight.

Using these analytical procedures for determining botanical and chemical components, the number of samples necessary for alternative degrees of precision was determined by the formula

$$n = \frac{t^2\ CV^2}{2},$$

where **t** equals the value of t at n-1 degrees of freedom, **CV** is the coefficient of variation expressed in percent, and **p** is the half-confidence interval (10% or 20% of the mean) that is desired (Snedecor, 1956).

Results and Discussion

In Table 1, a comparison of the pasture and diet composition is shown for major species. During the spring, the sheep chose more balsamroot and bluegrasses than any other species. These species contributed a far higher percentage to the diet than to the total available herbage, an indication that they were highly preferred by sheep. Shrubs contributed little to the diet during this period.

The coefficient of variation is closely proportional to the mean value of botanical and chemical components in the daily diet of the sheep (Fig. 1 and 2). The larger coefficient of variation for botanical components in contrast to that for chemical components points out the wide variety of plants that can give nutritionally similar diets (Bohman and Lesperance, 1967; Harris et al., 1967). It may also indicate that many plants in this vegetation type have about the same nutritional status in the spring when they are green and growing (Morrison, 1956).

The only significant differences

that occurred between days were in crude protein. Such differences may be related to precipitation during the first 2 days, but not on the third day, of the collections. An examination of the data indicated little linkage (or bias) between sheep and days; therefore, the daily collections of sheep samples were assumed to satisfy the assumption of independence.

The number of samples (sheep × days) necessary to predict at the 0.05 and 0.10 confidence levels within ±10% and ±20% of the mean are shown in Table 2. A large number of samples are needed to analyze the botanical constituents of the diet. In contrast, the chemical constituents can be examined with fewer samples. Plant or chemical components most abundant in a sheep's diet can be sampled with fewer samples than those that are less abundant at the same level of confidence. Our data on numbers and the coefficient of variations to be expected for the botanical and chemical diet constituents are similar to those found by Van Dyne and Heady (1965a, 1965b).

Since the coefficient of variations is proportional to the means of botanical or chemical components (Fig. 1 and 2), preliminary estimates of sample numbers needed could be calculated if the mean percent in the diet was known. For example, if a species contributed an estimated 20% to a sheep's diet, then the coefficient of variations expected would be about 47% (Fig. 1) and the number of samples needed would be about 88 to estimate within 10% of the population mean 95% of the time. Because of the sample numbers needed, a species that contributes less than 10% to the diet probably would be examined at a lower level of confidence.

In this study, partitioning the error term into sheep and day variances and reestimating it with differing numbers of sheep and days indicated that six sheep over 6 days would have given the best reduction for several plant species. Six is probably the minimum number of sheep needed to sample major botanical constituents in the diet when the cost of the experimental unit (esophageal-fistulated sheep) and the number of samples needed are considered. However, the addition of more days to obtain the required number of samples in rapid growing or drying vegetation may not be desirable because of shifts in animal preference caused by plant availability or maturity.

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Constituents of In Vitro Solution Contribute Differently to Dry Matter Digestibility of Deer Food Species

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Highlight: *This study assessed the contribution of chemical constituents used in the in vitro technique by Tilley and Terry on digestibilities of five species of plants. Apparent digestibility was lowest, 28–29%, for water alone, buffer alone, and buffer plus pepsin. Dry matter loss increased to 32–33% with either buffer + alcohol + HCl or buffer + alcohol + HCl + pepsin. Highest apparent digestibility, 44%, was reached with the addition of white-tailed deer inoculum. HCl contributed significantly to digestion while pepsin did not. Degree of digestion varied among the five species of plants tested.*

Dry matter digestibility of deer food is commonly used to estimate deer range carrying capacity. The in vitro technique of Tilley and Terry (1963) is gaining popularity over the in vivo method; however, little is known about the response of food plants to the individual components of the in vitro technique. Basically, this technique involves digesting plant material in an artificial rumen with a solution comprised of water, buffer, pepsin, alcohol, hydrochloric acid, and rumen fluid. Barnes (1966) evaluated these components by testing dry matter disappearance of grasses and legumes with the following solution combinations: buffer, buffer with pepsin; buffer with rumen fluid; and buffer, rumen fluid, and pepsin. He used a 48-hour digestion period for incubation of the sample with buffer and buffer with rumen fluid, followed by a 24-hour incubation period with pepsin. Dry matter disappearance was 33.2% with buffer alone, 41.8% with buffer and pepsin, 47.9% for buffer and rumen fluid, and 59.7% with the complete solution mixture of buffer, rumen fluid, and pepsin.

Pearson (1970), in a study similar to Barnes' (1966), evaluated three combinations of in vitro solution components for their ability to digest dry matter of grasses and shrubs from the Arizona chaparral. His data showed an average dry matter loss for the buffer alone to be 20.9%, microorganisms digested 21.9%, and the acid-pepsin accounted for 7%.

Neither Barnes (1966) nor Pearson (1970) tested dry matter disappearance of forbs, evergreen grasses, or evergreen shrubs. They did not test with water alone, nor did they separate the acid-pepsin fractions. The

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