Technical Report No. 261

ANALYSIS OF THE DISCERNIBILITY OF PLANT SPECIES DURING DIGESTION

Boyd L. Dearden, Richard M. Hansen, and R. Kirk Steinhorst

Department of Range Science
Colorado State University
Fort Collins, Colorado

Department of Range Science
Colorado State University
Fort Collins, Colorado

Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado

GRASSLAND BIOME

U.S. International Biological Program

June 1974

TABLE OF CONTENTS

ABSTRACT

A "double-sample" technique was used to study plants in the following pairs of samples: (1) the percent dry weight of plants in mixtures vs the percent relative density (RD) of microscopically discerned fragments of monocots and dicots on microscope slides, (2) the percent dry weights of plants in mixtures vs percent relative density of the microscopically discerned fragments of plant species on microscope slides, (3) the percent relative density of undigested plant fragments microscopically discerned from jackrabbit stomachs (ingesta) vs the percent relative density of fragments of each plant species in the caecums and colons of jackrabbits, and (4) the percent relative density of microscopically discerned fragments of plant species in the undigested ingesta of jackrabbits vs the percent relative density of plant species in the ingesta residues which were digested in nylon bags for 12, 48, and 60 hours in the rumens of fistulated cattle and bison.

The predictive equations, Y = (c) X, for the hand-compounded mixtures vs monocots and dicots and hand-compounded mixtures vs groups of species each showed a high correlation between the estimated percent relative density (X) and the actual percent dry weight (Y). This relationship was not strictly 1:1, but little difference in a corrected or uncorrected mean estimate of dry weight was observed. Correction factors "c" were calculated to determine the technician's degree of over or underestimation of each plant species. It appeared that trained technicians could usually record the frequency of plant species fragments in undigested mixtures containing plants that have a distinct cellular

pattern, but the dry weights of a few species are over or underestimated because they have epidermal cells that are either easy or difficult for a technician to identify.

Relative percent densities of discerned plant fragments were calculated for species of plants from 867 stomachs, 173 caecums, and 224 colons of black-tailed and white-tailed jackrabbits. The RD's of discerned plant fragments for 180 nylon bag residues were determined for the 18 monthly composited ingesta samples from both species of hares after microdigestion in ruminal fistulated bison and cattle. The relationship between the ingesta RD (Y) and the estimated RD (X) is expressed as: $Y = (c)\,X$, where "c" is the correction factor used to determine the degree of over or underestimation caused by digestion for each plant species.

The degrees to which the discernibility of the major plant species were affected by digestion in the two species of hares were tested for similarity. No significant differences were observed. Additionally, no differences were observed in the degrees to which the discernibility of the major plant species were affected by microdigestion in the rumens of fistulated bison and cattle. The changes in plant fragment discernibility caused by digestion were similar for all the comparable digested residues from caecums, colons, and nylon bags.

The degree of over or underestimation was calculated for each of the 23 most common plants occurring in the digested residues. Digestion caused one species of grass to be overestimated and three species to be underestimated. Digestion caused eight

species of dicots to be underestimated and none to be overestimated. The estimated percentages of eleven species of plants (6 grasses and 5 dicots) were not changed by digestion.

The discernibility of fragments of plants examined by a microscopic technique is discussed in relation to the qualitative and quantitative assessment of the different plant species. Suggestions are given for improvement and extension of the technique.

INTRODUCTION

The appraisal and description of samples from the ingesta of herbivores grazing on native plants has been successfully accomplished by the use of various quantitative expressions such as listing of the species of each plant, the percentage frequency of each type of food, and estimates of each food mass by weight or volume. Several forms of "point analysis" have been used to provide estimates of volume, weight, and botanical composition of food samples. The identification and quantification of plant fragments by microscopic examination of diet samples has been the most accurate of the commonly used techniques reported in the literature (Kelso 1934; Baumgartner and Martin 1939; Dusi 1949; Martin 1955; Adams 1957; Croker 1959; Heady and Torell 1959; Davies 1959; Brusven and Mulkern 1960; Hercus 1960; Lesperance et al. 1960; Ward 1960; Hegg 1961; Adams et al. 1962; Williams 1962; Ward and Keith 1962; Ridley et al. 1963; Myers and Vaughan 1965; Van Dyne and Heady 1965; Bear and Hansen 1966; Kiley 1966; Stewart 1967; Chamrod and Box 1968; Hansen and Ueckert 1970; Thetford et al. 1971; Ueckert and Hansen 1971; Laycock et al. 1972; Malechek and Leinweber 1972; Peden 1972; Todd and Hansen 1973; Flinders and Hansen 1972; Hansen et al. 1973; Hansen and Martin 1973).

Attempts to accurately quantify the amount of each plant in a herbivore's diet by microscopic methods have indicated that each investigator's quantification technique has certain weaknesses (Norris 1943; Cole 1956; Heady and Torrell 1959; Cole and Wilkins 1958; Hercus 1960; Dirschl 1962; Dirschl 1963; Bergerud and Russell

1964; Van Dyne and Heady 1965; Galt et al. 1966; Grenet 1966; Malechek 1966; Scotter 1967; Stewart 1967; Hansen et al. 1973).

Adams (1957) and Adams et al. (1962) reported a procedure to estimate the weight of food eaten by snowshoe hares by counting the number of recognized items in the feces. Valid correction factors for the difference in counts between food items can be obtained by feeding penned hares known weights of foods and then counting recognized items in the feces of the hares.

A "double-sample" technique has been used to obtain estimates of differences between the dry weights of forbs and grasses in hand-compounded mixtures and their estimated dryweights by a microscopic technique (Sparks and Malechek 1968). This procedure has recently been used to compare microscopic estimates of the abundance of plant species in "pairs" of esophageal and fecal samples from cattle, bison, sheep, and bighorns (Free et al. 1970; Hansen et al. 1973; Todd and Hansen 1973).

Several publications report that highly digestible plants, when they occur along with highly indigestible plants in the digestive tract residues of herbivores, may not be discerned by microscopic analysis as frequently as they would occur in comparable esophageal samples or in hand-compounded mixtures (Regal 1960; Grenet 1966; Stewart 1967; Casebeer and Koss 1970; Free et al. 1970). Hansen et al. (1973) suggested that the degree of digestion influenced the mean weight loss per plant fragment to a greater magnitude than decreasing total numbers of fragments. By refined preparation techniques many otherwise unobserved plant fragments can be detected in fecal material (Williams 1969).

The "double-sample" procedure was used to study plants in the following "pairs" of samples:

1) The percent dry weights of plants in mixtures vs percent relative density of microscopically discerned fragments of monocots and dicots on microscope slides.
2) The percent dry weights of plants in mixtures vs percent relative density of the microscopically discerned fragments of plant species on microscope slides.
3) The percent relative density of undigested plant fragments microscopically discerned in jackrabbit stomachs (ingesta) vs the percent relative density of fragments of each plant species in the caecums and colons of jackrabbits.
4) The percent relative density of microscopically discerned fragments of plant species in the undigested ingesta of jackrabbits vs the percent relative density of plant species of the ingesta residues which were digested in nylon bags for 12, 48, and 60 hours in the rumens of fistulated cattle and bison.

The objectives of this study are to describe and evaluate the variations observed in the microscopic discernibility of plant species in relation to the dry weight of plants in mixtures as well as in relation to changes in discernibility caused by digestion.

The success of the quantitative determinations of animal food habits depends upon an exact knowledge of the botanical

content of the diet. The relationship between food items ingested (Y) and the microscopic estimation (X) is expressed as: $Y = (c) X$. Emphasis must be placed on the determination of the correction term (c) if estimated proportions (X) are to relate to ingesta (Y). Estimates of the correction term have generally been limited in scope and intensity. The influences of climate and weather, fluctuating food availability, seasonal and annual changes, animal competition, population pressures, environmental manipulation, and factors influencing choice or selection among food items by animals of different sex, age, and condition must be understood to obtain precise estimates of "c". This study considers only a few of these associations, and correction terms have been calculated for technician's over or underestimation and the affects of digestion on the identification of plant species.

Additionally, no one has attempted until now to determine whether or not the discernibility of plants in mixtures of plants digested *in vivo* using nylon bags is similar to that which occurs during normal digestion. If the microscopic discernibility of plants in mixtures in the nylon bags changes as do plants in normal herbivore digestion then it seems possible to correct for "differences" caused by digestion more efficiently by this microdigestion technique than by using conventional feeding trials.

MATERIALS AND PROCEDURES

Preparation of Microscope Slides

Microscope slides of reference plants, hand-compounded mixtures, rumen samples, digestive tract residues, and nylon bag residues were prepared as described by Sparks and Malechek (1968), Ward (1969) and Flinders and Hansen (1972).

Fields were systematically located on each microscope slide and were viewed at 100 magnifications for identifiable fragments. Either 10 or 20 fields were examined per microscope slide. The total number of fields examined per sample is stated in the text. Within the groups of samples compared, approximately the same number of fields were examined. Microscope slides were prepared with the number of identifiable fragments per field varying from one to five and averaging approximately three discernible fragments per microscope field.

Procedure for Identification

Several microscope technicians were trained to correctly identify all species of plants used in each sample examined. Technicians were trained to identify and quantify discerned plant fragments with the use of practice slides prepared from samples of known dry weight composition. Technicians were "challenged" to quantify discerned fragments on microscope slides with the names and amounts of each plant species in the hand-compounded mixtures unknown to them. Each fragment in a sample was identified if its observed characteristics matched the leaf, stem, flower, seed, or other plant part of the same material on a reference slide.

Mixtures of plants were hand-compounded to simulate the relative proportions in which they might be expected to occur in the diets of herbivores. The plants used were collected primarily in the growing season and consisted of the aboveground parts fed upon by herbivores.

Scheme to Obtain Correction Factors

Correction factors (c) were calculated for the hand-compounded mixtures, digestive tract residues, and nylon bag residues. These factors can be used to determine the technician's degree of over or underestimation of each species contained in a known mixture of plants and to determine the differences between the estimated digesta RD (X) and the ingesta RD (Y) when examined by the microscopic technique. The relationship between the original (Y) and the estimated (X) percentages is expressed as:

$$Y = (c)\ X.$$

The correction factor "c" is obtained by dividing the original percent (Y) by the percentage which was estimated (X). An index ratio of 1.0 for a species indicates that there was no over or underestimation; a value larger than 1.0 indicates a greater original percentage than the estimate; and a value less than 1.0 indicates a lesser original percentage than the estimate. The higher the "c" value for a plant the lower is its relative discernibility, and the smaller the "c" value the higher is its relative discernibility.

Quantification

The relative percent density (RD) of recognized plant fragments in each of the samples was estimated by observing fields located systematically on each of the slides. The occurrence of each recognized plant species in each field was recorded. Average percent frequency was computed for all plant species present in the samples. The relative percent density (RD), calculated as the number of recognized fragments of a species and expressed as a percentage of the total number of fragments of all species, was the value used in all comparisons. The relationship of percent frequency per field to density of discerned fragments per field can be determined by the formula:

$$F = 100\ (1 - e^{-D}).$$

For a given percent frequency (F), a mean density (D) of discerned particles of a species per microscope field can be determined. The density of particles per field may be converted to relative percent density (RD) by:

$$RD = \frac{\text{Density of discerned fragments for a species}}{\Sigma \text{ of densities of discerned fragments for all species}} \times 100.$$

The RD is a better estimate of the amount of a species in a mixture than is percent frequency. Sparks and Malechek (1968) obtained predictive equations showing a high correlation between

the relative number of fragments counted and the actual percent dry weights for forbs versus grasses in 15 hand-compounded mixtures. The relationship was approximately 1:1.

Sparks and Malechek (1968) reported that the RD of discerned fragments could be accurately calculated by "counting" fragments or by "frequency conversion". The time required for a technician to "count" and record discerned fragments requires more work than recording frequency per microscope field (ibid).

The relative numbers of discernible fragments of plant species in two caribou rumen samples were determined by "counting" and by "frequency conversion", and the number of non-identifiable fragments was counted for 400 microscope fields per sample. The purpose of these observations was to study the relationships between the numbers of discerned and unidentifiable fragments and to illustrate the similarity between RD's determined by counting discerned fragments and by estimating discerned fragments by the frequency conversion technique.

The numbers of unidentifiable fragments in the two caribou rumens were 36% and 42% respectively (Tables 1 and 2). The RD's of discerned fragments of plant species determined by counting or by the frequency conversion method were not significantly different ($p = .95$). The counting technique required 16 hours of technician time, while the "conversion technique" required only 4 hours.

Table 1. Comparison of methods to determine relative percent density of discerned plant fragments for caribou rumen sample #1.

ITEM	Counting method with unidentifiable fragments	Counting methods without unidentifiable fragments	Frequency conversion method
Cladonia	46.56	72.90	76.21
Stereocaulon	0.22	0.34	0.18
Peltigera aphthosa	2.49	3.90	2.71
Unidentified moss	9.15	14.33	12.19
Unknown moss 1	0.27	0.40	0.45
Unknown forb 1	1.39	2.18	0.91
Unknown forb 2	2.01	3.15	5.01
Betula nana	0.11	0.17	0.27
Equisetum fluviatile	0.07	0.11	0.00
Vaccinium vitis-idaea	0.07	0.11	0.18
Salix	0.22	0.34	0.45
Festuca altaica	0.33	0.52	0.18
Hierochloe alpina	0.33	0.52	0.36
Pinaceae	0.66	1.03	0.91
Unidentifiable fragments	36.13		

Table 2. Comparison of methods to determine relative percent density of discerned plant fragments for caribou rumen sample #2.

ITEM	Counting method with unidentifiable fragments	Counting methods without unidentifiable fragments	Frequency conversion method
Cladonia	41.21	71.21	69.61
Stereocaulon	0.04	0.07	0.58
Peltigera apththosa	1.91	3.31	2.36
Unidentified moss	8.81	15.22	17.02
Unknown moss 1	0.15	0.26	0.24
Unknown forb 1	0.57	0.99	1.75
Unknown forb 2	2.57	4.43	3.88
Betula nana	0.11	0.20	0.08
Equisetum fluviatile	0.00	0.00	0.08
Salix	0.04	0.07	0.16
Festuca altaica	0.84	1.46	1.75
Hierochloe alpina	0.15	0.26	0.58
Pinaceae	1.38	2.38	1.67
Carex aquatilis	0.08	0.13	0.24
Unidentifiable fragments	42.13		

QUANTIFICATION OF MONOCOTS AND DICOTS

Twenty-four samples were hand-compounded to contain known dry weight percentages (Y) of: (1) grasses and grasslikes (monocots) and (2) forbs or shrubs (dicots). These mixtures contained forage species usually having distinctly different cellular patterns, and they contained two, three, and five species each. There were 10 species of monocots and 15 species of dicots in the mixtures. One-hundred microscope fields were examined for each of the 24 mixtures studied. Five different technicians were used to quantify discerned fragments in different mixtures.

The relationship between the estimated percent dry weight (X) and actual percent dry weight (Y) was compared using regression equations. Plant species were considered in three groups for the regression analysis: monocots (grass and grasslikes), dicots (forbs and shrubs), and monocots + dicots. The regression values are as follows:

		r^2	Sy.x	S_{b1}
Monocots	Y = .59 + .95 X	.96	4.16	.03
Dicots	Y = 3.83 + .94 X	.97	4.08	.03
Monocots + Dicots	Y = 1.94 + .94 X	.96	4.31	.02 .

Means, variances, and degrees of over or underestimation (c) of the estimated (X) and actual (Y) percent dry weight are:

	n	$\bar{x}$	$\bar{y}$	S_x^2	S_y^2	S_x	S_y	c
Monocots	48	34.64	33.54	507.25	475.89	3.25	3.14	.97
Dicots	25	29.50	31.60	699.25	635.50	5.29	5.04	1.07
Monocots + Dicots	73	32.88	32.87	570.23	523.34	2.80	2.68	1.00

The best linear regression fit is for the dicot group ($r^2 = .97$), although, the variances for this group are greater than for either of the other two groups (Figures 1, 2, 3).

Examination of the group means shows an overall overestimation (c = .97) of the actual percent dry weight for monocots and an overall underestimation (c = 1.07) for the dicots. The differences between these means are not significant as is shown by the following two sample t-tests ($H_o: \mu_y = \mu_x$):

	t	Significance Level
Monocots	.23	.82
Dicots	-.29	.78

Testing the hypothesis to show that the slopes of the lines each equal one, $H_o: \beta_1 = 1$, resulted in the following:

	t	Significance Level
Monocots	-1.81	.08
Dicots	-1.86	.07
Monocots + Dicots	-2.77	<.01

Thus, only the regression equation for the monocots and dicots has a slope significantly different from one.

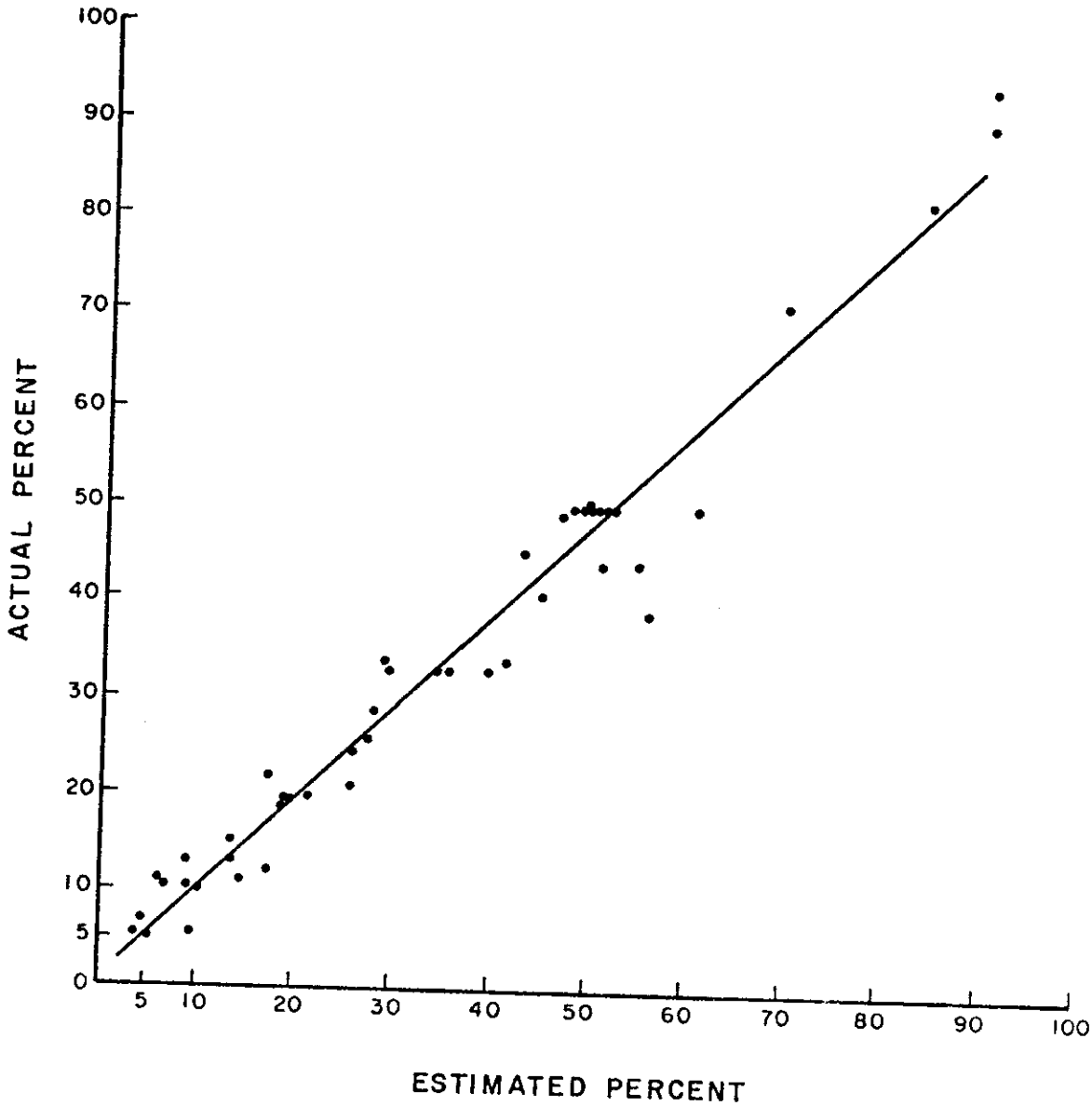

FIGURE 1. The estimated percent dry weight and the actual percent dry weight of monocot plants in plant mixtures examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

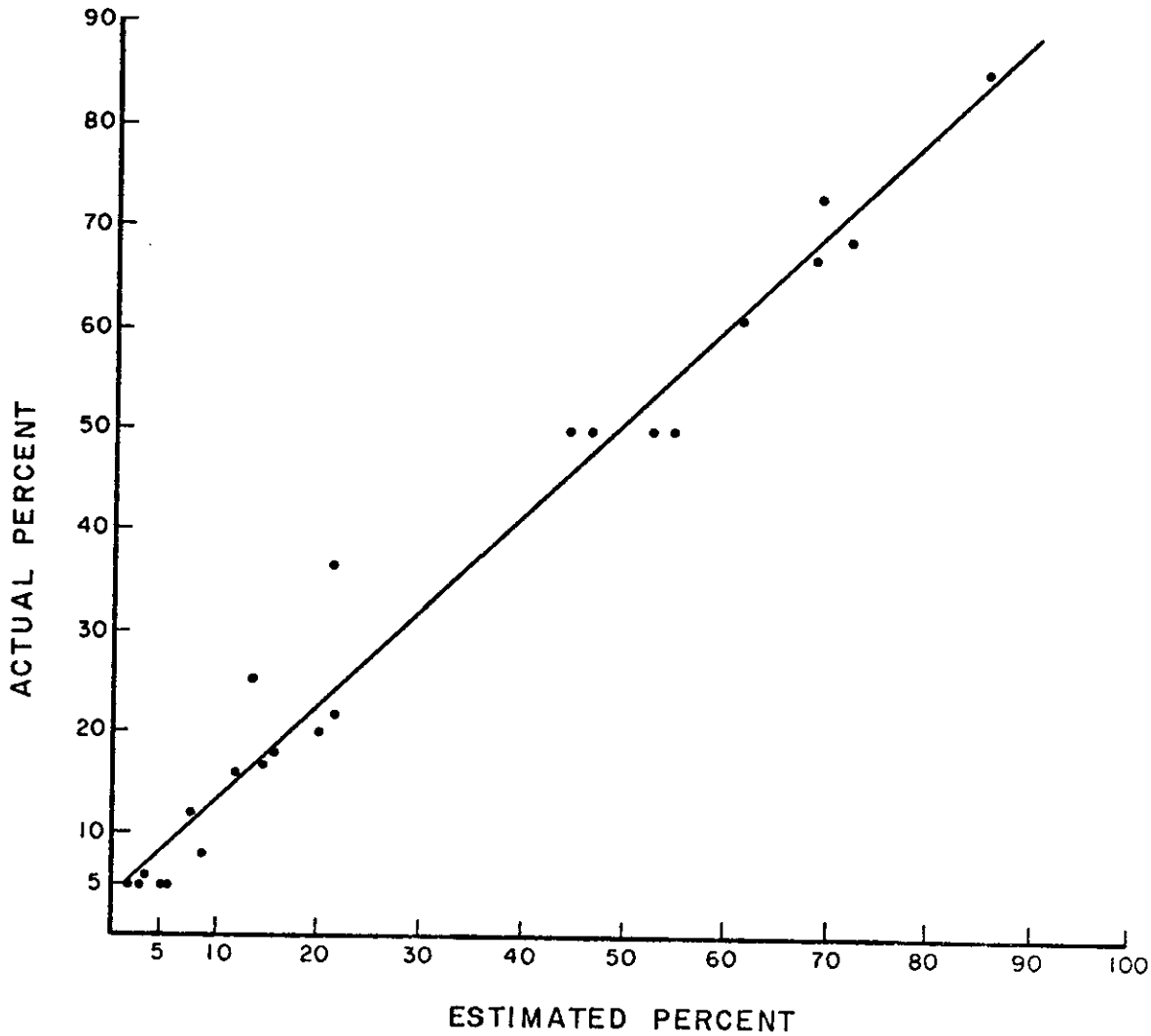

FIGURE 2. The estimated percent dry weight and the actual percent dry weight of dicot plants in plant mixtures examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

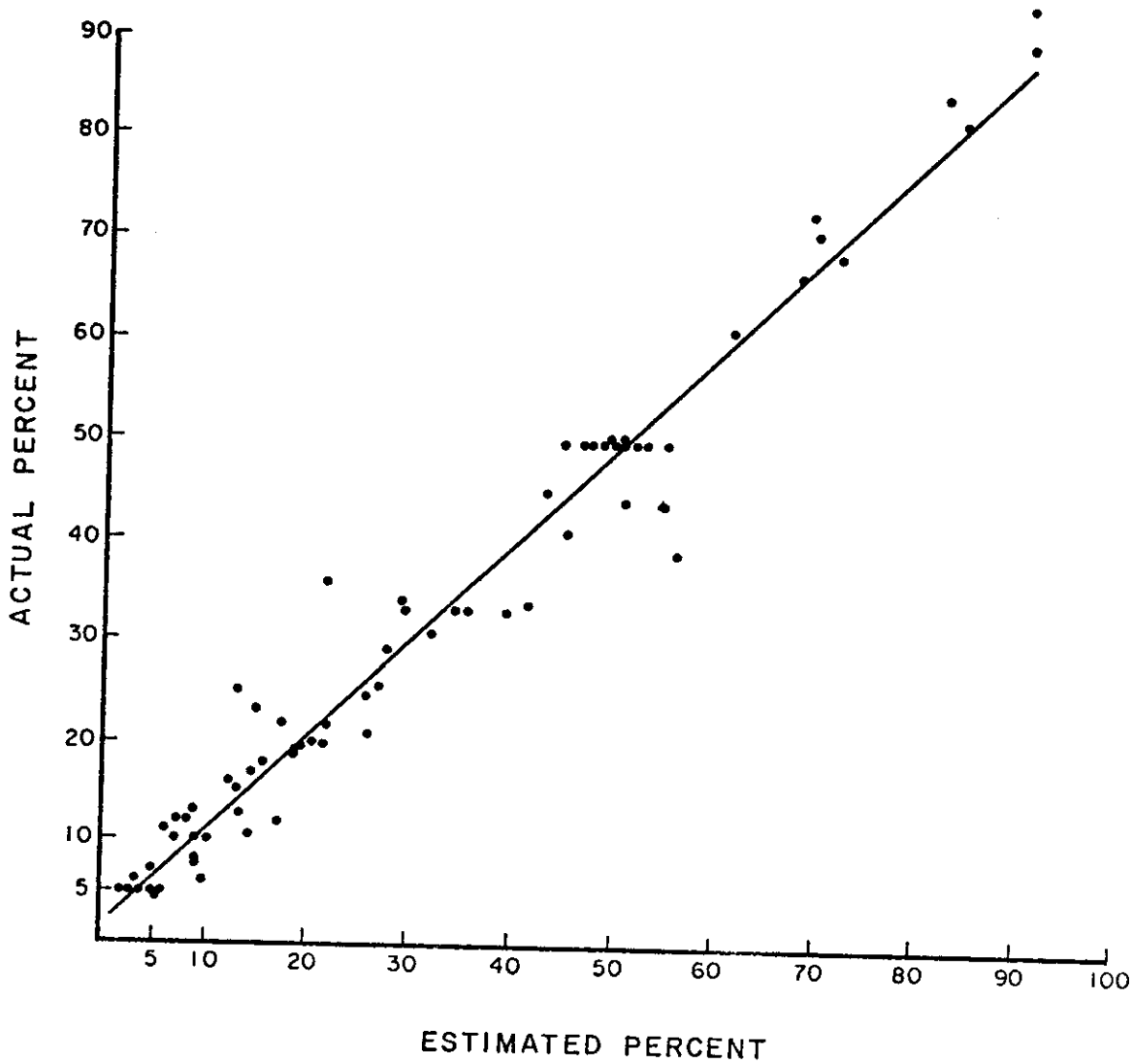

FIGURE 3. The estimated percent dry weight and the actual percent dry weight of monocots and dicots in plant mixtures examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

The hypothesis that the intercepts each equal zero, $H_o: \beta_o = 0$ was tested.

	t	Significance Level
Monocots	.54	.60
Dicots	3.10	<.01
Monocots + Dicots	2.24	.03

Only the equation for the monocots has an intercept not significantly different from zero.

The regression coefficient b_1 gives the amount of change that occurs in the actual percent (Y) for a unit increase in the estimated percent (X). Testing the regression coefficients b_1 (slope of the line) for the monocots and dicots establishes that the amount of change in the dependent variable for a unit increase in the independent variable is the same for both groups. The hypothesis was Ho: b_1 monocot = b_1 dicot, and the following was observed:

	t	Significance Level
Monocot vs. Dicot	.24	.81

The change in the actual percent (Y) for a unit change in estimated percent (X) is the same for both the monocots and dicots, and there is no difference in the slope of the lines for either group.

The RD values for simple mixtures showed an occasional "outlier" species of plant (Figures 1, 2, and 3). This outlier deviated from the hypothesis enough to suggest that its RD value might need to be multiplied by a correction factor to obtain an acceptable estimate of its percent dry weight.

QUANTIFICATION BY SPECIES IN SIMPLE MIXTURES

Five hand-compounded mixtures containing eight species of plants were used to observe variation between species of plants for over or underestimating the percent dry weights. The mixtures contained known dry weight (Y) percentages of: (1) grasses and grasslikes and (2) forbs or shrubs. There were three species of grasses or grasslikes and five species of non-grasses (forbs or shrubs) in the mixtures. The discerned fragments were recorded for 200 microscope fields for each mixture.

Examination of the mean RD (X) of each species showed a significant overestimation of the actual percentage dry weight (Y) for the grasses Poa and Oryzopsis hymenoides, while the forbs and shrubs species of Alluim, Comandra umbellata, and Senecio integerrimus were each significantly underestimated. Agropyron spicatum and Artemisia tridentata were the only grass and dicot respectively, which were not significantly over or underestimated (Table 3).

Table 3. Comparison of the estimated percentage relative density of discerned plant fragments and the actual percentage dry weight in hand-compounded mixtures. Degrees of over or underestimation (c) are also shown. The samples were examined by a technician at 100 x magnifications and fragments discerned were recorded for 200 fields/sample.

Plant species	Percent relative density and percent dry weights in mixtures												
	Mixture 1		Mixture 2		Mixture 3		Mixture 4		Mixture 5		Mean		Degree of over or under-estimation (c)
	RD	WT	RD	WT	RD	WT	RD	WT	RD	WT	RD	WT	
Agroypron spicatum	14.10	20.00	11.26	14.00	16.49	10.00	19.27	15.00	15.91	18.00	15.41	15.40	.99
Poa	29.45	20.00	35.53	14.00	33.86	15.00	27.47	10.00	30.32	10.00	31.32	13.80	.44
Oryzopsis hymenoides	12.90	10.00	17.45	15.00	20.13	10.00	12.62	10.00	4.72	5.00	13.56	10.00	.73
Balsomorhiza sagittata	15.15	10.00	16.17	21.00	20.77	10.00	22.46	10.00	30.32	20.00	20.97	14.20	.67
Alluim	4.09	5.00	1.00	5.00	0.00	10.00	.36	5.00	1.50	5.00	1.39	6.00	4.31
Comandra umbellata	8.27	20.00	8.46	16.00	1.90	10.00	2.57	10.00	5.14	15.00	5.27	14.20	2.69
Senecio integerrimus	1.89	5.00	2.27	10.00	.75	10.00	1.08	10.00	1.13	7.00	1.42	8.40	5.91
Artemisia tridentata	6.54	10.00	3.27	5.00	2.68	5.00	1.82	10.00	1.89	5.00	3.24	7.00	2.16

QUANTIFICATION OF SPECIES AND BY GROUPS OF SPECIES

Eight hand-compounded mixtures of 19 species of plants, each containing from five to twelve species, were used to observe variation between species and functional categories of plants for over or underestimation of the percent dry weights.

The hand-compounded mixtures were prepared with each sample containing at least one lichen, one moss, one sedge, one grass, and one forb or shrub. The actual percentages of dry weight used were chosen within a range that might be encountered in the diets of a large herbivore such as a caribou.

Each of the eight mixtures was examined by three different technicians. Regression estimates were made on ten slide averages (200 microscope fields) of three laboratory technicians (600 fields total). The regression equations were generally better when the data was treated by species rather than by functional groups. Analyses were both by species as well as by functional groups. These analyses consisted of: (1) testing for differences between means (Y vs X) and (2) testing the regression equation (Table 4).

The estimated (X) and actual (Y) percents are significantly different from each other for the Calamagrostis canadensis, Pleurozium schrieberi, Vaccinium vitis-idaea, Cetraria islandica, and Peltigera apthosa plant species (Table 5). These differences are also present in the grass, moss, and shrub functional groups. There can be no subjective comparisons made between the

Table 4. Summary of regression analysis showing the number of observations, the regression equation, means, variances, and degree of over or underestimation of plants by species and groups of species.

	n	Y = a + bX	$\bar{X}$	$\bar{Y}$	r^2	s_x^2	s_y^2	$s_{y.x}$	s_{b_1}	c
Calamagrostis canadensis	12	Y = 4.00 + 1.12X	7.64	12.52	.85	17.98	26.13	2.04	.14	1.64
Hierchloe alpina	12	Y = 3.76 + .88X	9.90	12.50	.21	6.97	26.42	4.81	.55	1.26
Festuca altaica	6	Y = 4.47 + .82X	8.49	11.43	.55	8.00	9.78	2.35	.37	1.35
Carex aquatilis	12	Y = 3.29 + .66X	14.05	12.49	.92	57.95	27.04	1.55	.06	.89
Carex bigelowii	5	Y = 8.00 + .72X	6.63	12.80	.41	34.02	43.20	5.81	.50	1.93
Eriophorum angustifolium	12	Y = 3.94 + .95X	8.99	12.50	.73	21.42	26.42	2.78	.18	1.39
Eriophorum brachyantherum	9	Y = 9.89 + .62X	15.07	19.17	.08	14.14	65.98	8.32	.78	1.27
Polytrichum juniperinum	12	Y = .86 + .84X	15.97	12.51	.68	25.28	26.09	3.03	.18	..78
Pleurozium schrieberi	18	Y = 3.92 + .29X	31.31	12.99	.62	223.40	30.29	3.50	.06	.41
Betula nana	12	Y = 4.62 + .85X	9.26	12.51	.84	30.64	26.58	2.19	.12	1.35
Salix pulchra	6	Y = 5.39 + 1.09X	7.74	13.81	.98	50.12	60.13	1.08	.07	1.78
Dryas octopetala	12	Y = 4.38 + .79X	10.24	12.49	.80	34.01	26.63	2.42	.12	1.20
Vaccinium vitis-idaea	5	Y = 5.18 + .94X	6.04	10.86	.43	4.78	9.82	2.73	.62	1.80
Epilobium angustifolium	6	Y = 8.28 + .63X	9.10	14.00	.18	19.70	43.20	6.65	.67	1.54
Equisetum fluviatile	6	Y = 5.63 + .57X	10.17	11.44	.75	22.82	9.85	1.74	.16	1.12
Cladonia rangiferina	18	Y = 2.08 + .77X	13.01	12.15	.75	25.37	20.28	2.33	.11	.93
Stereocaulon grande	12	Y = 5.71 + .76X	8.87	12.49	.69	31.36	26.63	3.02	.16	1.41
Cetraria islandica	6	Y = 1.58 + 2.02X	7.70	14.00	.75	7.94	43.13	3.65	.58	1.83
Peltigera apthosa	6	Y = 4.57 + .34X	20.00	11.42	.58	48.30	9.75	2.26	.14	.57
Grasses	30	Y = 4.17 + .93X	8.71	12.29	.47	11.91	21.81	3.45	.18	1.41
Sedges	38	Y = 6.32 + .67X	11.72	14.11	.42	40.74	42.97	5.06	.13	1.20
Mosses	30	Y = 6.75 + .24X	25.18	12.80	.41	198.97	27.71	4.10	.05	.51
Shrubs	35	Y = 5.01 + .84X	8.88	12.49	.79	30.97	27.91	2.47	.08	1.41
Forbs	12	Y = 7.42 + .55X	9.63	12.72	.23	19.64	25.91	4.69	.32	1.30
Lichens	42	Y = 7.66 + .39X	12.07	12.41	.28	41.18	22.54	4.07	.10	1.03

Table 5. Calculated t-values, testing for differences between actual and estimated means.

	t	Significance Level
Calamagrostis canadensis	-2.54	.03
Hierchloe alpina	-1.56	.15
Festuca altaica	-1.71	.14
Carex aquatilis	0.59	.57
Carex bigelowii	-1.57	.18
Eriophorum angustifolium	-1.76	.11
Eriophorum brachyantherum	-1.37	.20
Polytrichum juniperinum	1.66	.12
Pleurozium schrieberi	4.88	<.01
Betula nana	-1.49	.16
Salix pulchra	-1.11	.31
Dryas octopetala	-1.00	.34
Vaccinium vitis-idaea	-2.82	.04
Epilobium angustifolium	-1.51	.10
Equisetum fluviatile	-0.54	.61
Cladonia rangiferina	0.54	.60
Stereocaulon grande	-1.65	.13
Cetraria islandica	-2.16	.07
Peltigera apthosa	2.76	.03
Grasses	-3.38	<.01
Sedges	-1.61	.12
Mosses	4.50	<.01
Shrubs	-2.78	<.01
Forbs	-1.59	.14
Lichens	0.27	.79

significances of the functional groups and the species in those groups. There is no difference between the means for the lichens as a group, but Cetraria islandica and Peltigera apthosa show significant differences between means. A significant difference between means should not be used as a criterion for judgements of the respective regression equations. It is merely a statement of similarity between the means.

The c-values showed underestimation of grasses, sedges, shrubs, forbs, and lichens while mosses were overestimated. This is in contrast to the c-values obtained for simple mixtures where grasses were overestimated and forbs and shrubs underestimated. Underestimation of grasses, sedges, shrubs, forbs, and lichens is a reflection of the high degree of overestimation of the mosses. The high degree of overestimation occurred because mosses fragmented more than did the other plants,and even the tiny sized moss fragments are easy to identify.

The proportions of discerned fragments of plant species by microscopic analysis appear to depend, in part, upon the composition of plants in the mixtures.

Testing the Regression Line

Testing the hypothesis that the slopes of the lines each equal one, H_o: $\beta_1 = 1$, resulted in the t-values presented in Table 6.

There again can be no subjective comparison between the significance of the functional groups and the species of the functional groups. None of the shrub species (Betula nana, Salix pulchra, Dryas octopetala, Vaccinium vitis-idaea) showed a slope significantly different from one. However, as a whole the shrub group did show a slope significantly different from one. Likewise, the forb group as a whole displays a slope not different from one, while Equisetum fluviatile did display a slope different from one.

The species with slopes significantly different from one are: Carex aquatilis, Pleurozuim schrieberi, Equisetum fluviatile, Cladonia rangiferina, and Peltigera apthosa, and the functional groups with slopes significantly different from one are: sedges, mosses, shrubs, and lichens.

The difference between the actual (Y) and estimated (X) means (Table 5) and a slope equal to one (Table 6) should not necessarily yield similar results when viewed simultaneously. An equation with a slope equal to one may in fact have independent and dependent variable means significantly different from each other due to a large Y-intercept value. Likewise, independent and dependent variable means which are not significantly different do not insure a 45° slope.

Table 6. Calculated t-values testing for a slope (β_1) equal to 1 by species of plants and groups of species.

	t	Significance Level
Calamagrostis canadensis	0.79	.44
Hierchloe alpina	-0.21	.84
Festuca altaica	0.49	.64
Carex aquatilis	-5.66	<.01
Carex bigelowii	-0.55	.60
Eriophorum angustifolium	-0.26	.80
Eriophorum brachyantherum	-0.49	.64
Polytrichum juniperinum	-0.89	.39
Pleurozium schrieberi	-12.46	<.01
Betula nana	-1.24	.24
Salix pulchra	1.28	.25
Dryas octopetala	-1.66	.12
Vaccinium vitis-idaea	-0.10	.93
Epilobium angustifolium	-0.55	.60
Equisetum fluviatile	-2.63	.04
Cladonia rangiferina	-2.02	.06
Stereocaulon grande	-1.45	.17
Cetraria islandica	1.76	.13
Peltigera apthosa	-4.54	<.01
Grasses	-0.37	.72
Sedges	-2.57	.01
Mosses	-14.07	<.01
Shrubs	-2.07	.05
Forbs	-1.41	.18
Lichens	-6.12	<.01

Testing Intercepts Each Equal Zero

The resulting t-values from the testing of the hypothesis. that the intercepts each equal zero, $H_o:\beta_o = 0$, are listed in Table 7.

Simultaneous comparisons of the functional group categories with the species of the categories showed that at least one species in each category displayed an intercept significantly different from zero. Also, each category displayed an intercept significantly different from zero. The individual species which showed an intercept significantly different from zero are: Calamagrostis canadensis, Carex aquatilis, Eriophorum angustifolium, Pleurosium schrieberi, Betula nana, Salix pulchra, Dryas octopetala, Equisetum fluviatile, and Sterocaulon grande.

The "ideal" plant species has a 1:1 relationship between the estimated percent (X) and the actual percent (Y) dry weight. The plant species for which the slopes were not different from one while the intercepts were not different from zero are: Hierchloe alpine, Festuca altaica, Carex bigelowii, Eriophorum brachyantherum, Polytrichum juniperinum, Vaccinium vitis-idaea, Epilobium angustifolium, and Cetraria islandica.

This does not mean the function Y = X is the proper relationship between the two variables; it is merely stating that the regression estimates of β_o and β_1 are not significantly different from zero and one respectively (Table 8).

Six of the eight species previously mentioned, having slopes not different from one and intercepts not different from zero,

Table 7. Calculated t-values testing for the y - intercept (β_o) equal to 0 by species of plants and groups of species.

	t	Significance Level
Calamagrostis canadensis	3.18	<.01
Hierchloe alpina	0.67	.52
Festuca altaica	1.36	.22
Carex aquatilis	3.39	<.01
Carex bigelowii	1.90	.12
Eriophorum angustifolium	2.17	.05
Eriophorum brachyantherum	0.82	.44
Polytrichum juniperinum	-0.28	.78
Pleurozium schrieberi	2.00	.06
Betula nana	3.62	<.01
Salix pulchra	7.82	<.01
Dryas octopetala	3.00	.01
Vaccinium vitis-idaea	1.31	.25
Epilobium angustifolium	1.24	.26
Equisetum fluviatile	3.13	.02
Cladonia rangiferina	1.34	.20
Stereocaulon grande	3.39	<.01
Cetraria islandica	-0.34	.75
Peltigera apthosa	1.50	.19
Grasses	2.40	.02
Sedges	3.64	<.01
Mosses	4.28	<.01
Shrubs	6.31	<.01
Forbs	2.21	.05
Lichens	5.67	<.01

Table 8. Summary of t-tests about the means, about the slopes, and about the intercepts for species of plants and groups of species.

	Significance Levels		
	Ho: $\mu_a = \mu_e$	Ho: $\beta_1 = 1$	Ho: $\beta_0 = 0$
Calamagrostis canadensis	.03	.44	<.01
Hierchloe alpina	.15	.84	.52
Festuca altaica	.14	.64	.22
Carex aquatilis	.57	<.01	<.01
Carex bigelowii	.18	.60	.12
Eriophorum angustifolium	.11	.80	.05
Eriophorum brachyantherum	.20	.64	.44
Polytrichum juniperinum	.12	.39	.78
Pleurozium schrieberi	<.01	<.01	.06
Betula nana	.16	.24	<.01
Salix pulchra	.31	.25	<.01
Dryas octopetala	.34	.12	.01
Vaccinium vitis-idaea	.04	.93	.25
Epilobium angustifolium	.10	.60	.26
Equisetum fluviatile	.61	.04	.02
Cladonia rangiferina	.60	.06	.20
Stereocaulon grande	.13	.17	<.01
Cetraria islandica	.07	.13	.75
Peltigera apthosa	.03	<.01	.19
Grasses	<.01	.72	.02
Sedges	.12	.01	<.01
Mosses	<.01	<.01	<.01
Shrubs	<.01	.05	<.01
Forbs	.14	.18	.05
Lichens	.79	<.01	<.01

also, have actual (Y) and estimated percent (X) means not significantly different from each other. They are: Hierchloe alpina, Festuca altaica, Carex bigelowii, Eriophorum brachyantherum, Polytrichum juniperinum, and Epilobium angustifolium. Graphs of the original data points (10 slide means) and the predictive equations are shown in Figures 4 through 9.

This relationship, however, is not strictly 1:1 as indicated by the t-tests about the slopes and intercepts. One would observe little difference in a corrected or uncorrected mean estimate of dry weight. It appears that trained technicians can accurately discern the frequency of fragments in undigested mixtures containing plants that have distinct cellular patterns.

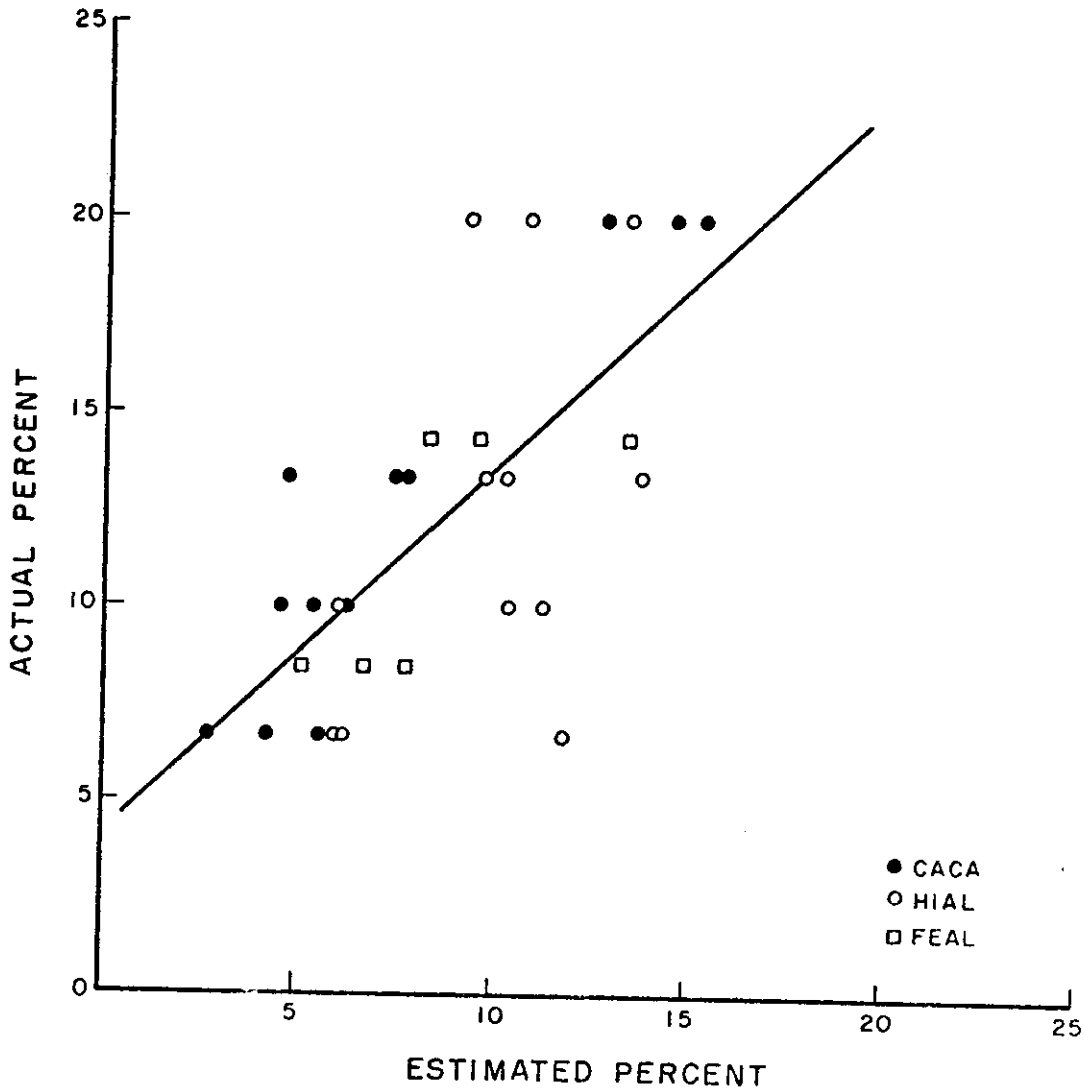

FIGURE 4. The estimated dry weight and actual dry weight of Calamagrostis canadensis (CACA), Heirochloe alpina (HIAL), and Festuca altaica (FEAL) in mixtures of plants examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

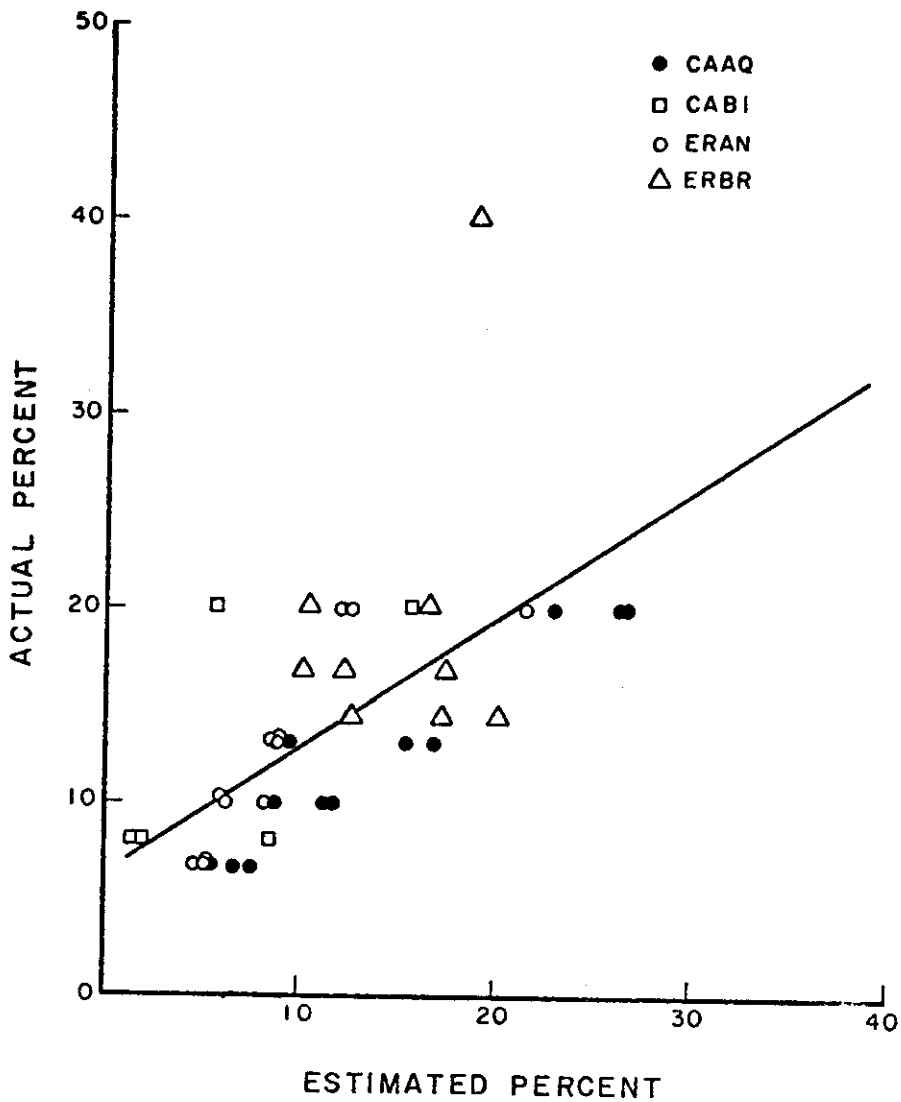

FIGURE 5. The estimated dry weight and actual dry weight of Carex aquatilis (CAAQ), Carex bigelowii (CABI), Eriophorum angustifolium (ERAN), and Eriophorum brachyantherum (ERBR) in mixtures of plants examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

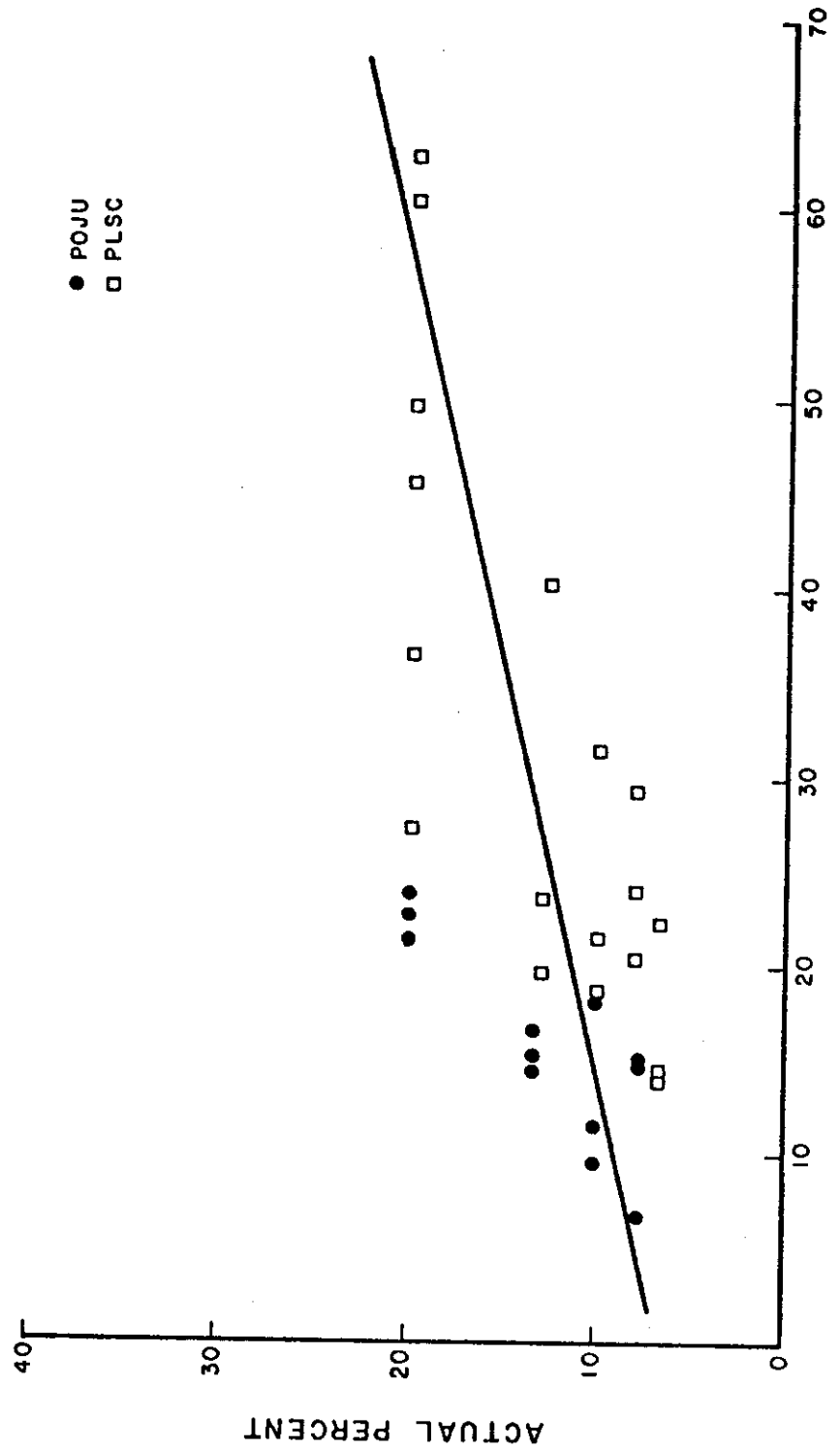

FIGURE 6. The estimated dry weight and actual dry weight of Polytrichum juniperinum (POJU) and Pleurozium schrieberi (PLSC) in mixtures of plants examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

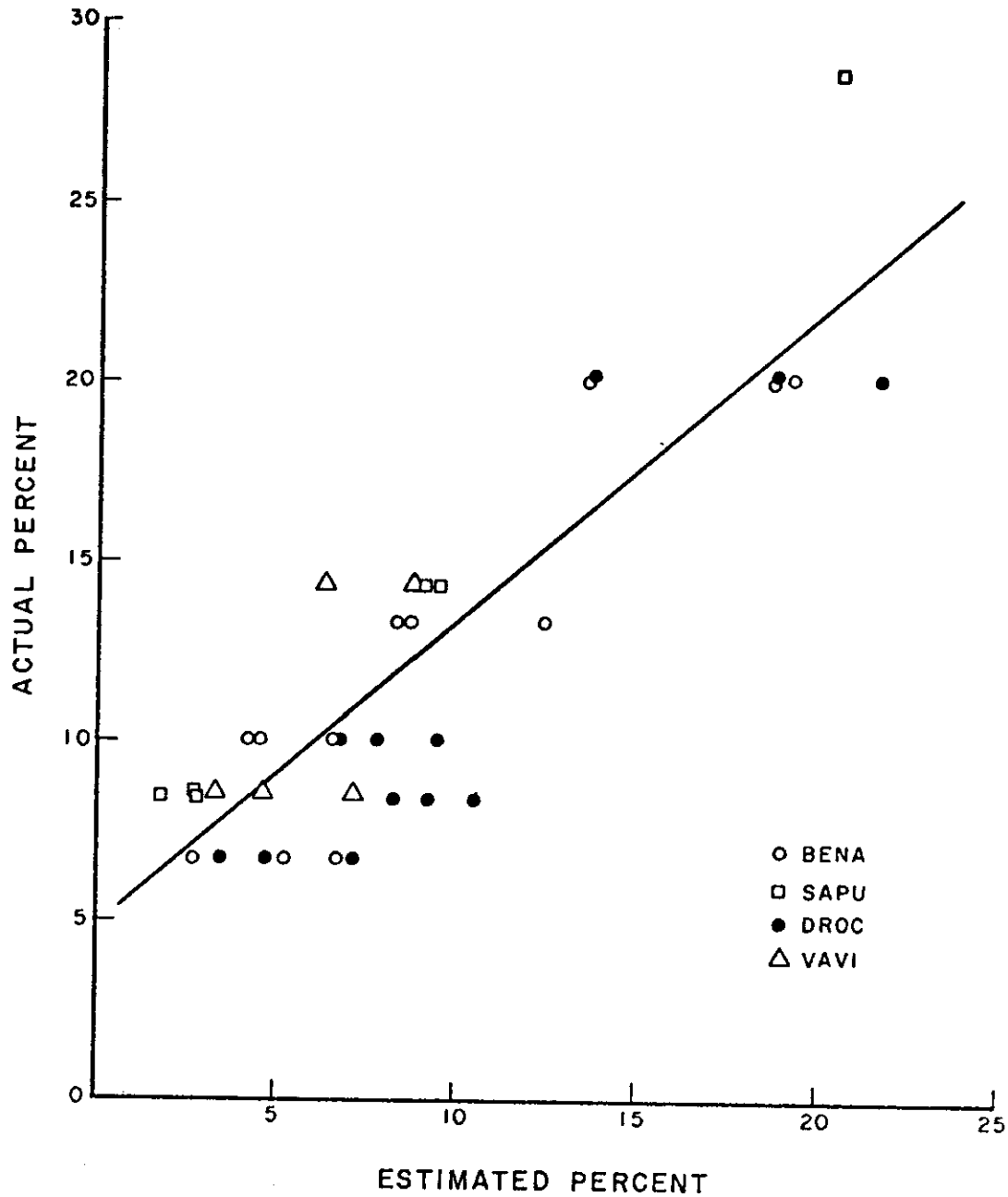

FIGURE 7. The estimated dry weight and actual dry weight of Betula nana (BENA), Salix pulchra (SAPU), Dryas octopetala (DROC) and Vaccinium vitis-idaea (VAVI) in mixtures of plants examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

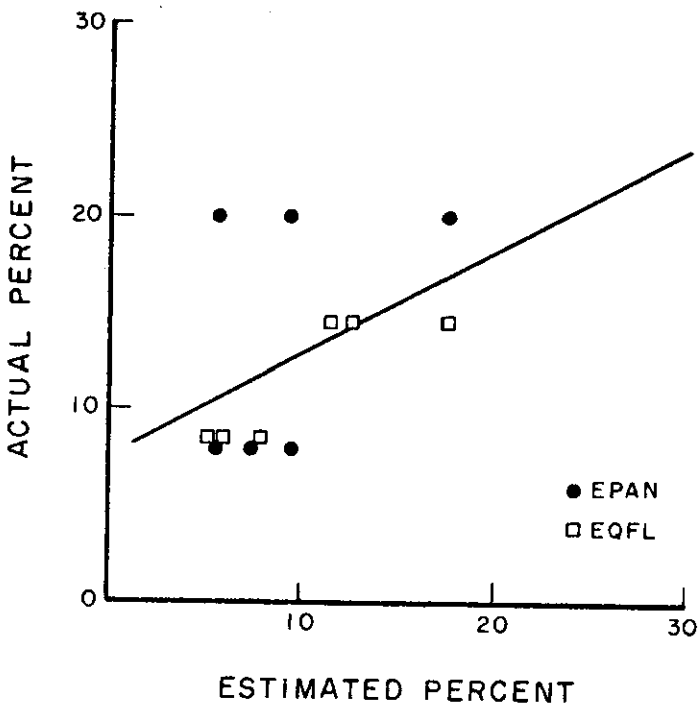

FIGURE 8. The estimated dry weight and actual dry weight of Epilobium angustifolium (EPAN) and Equisetum fluviatile (EQFL) in mixtures of plants examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

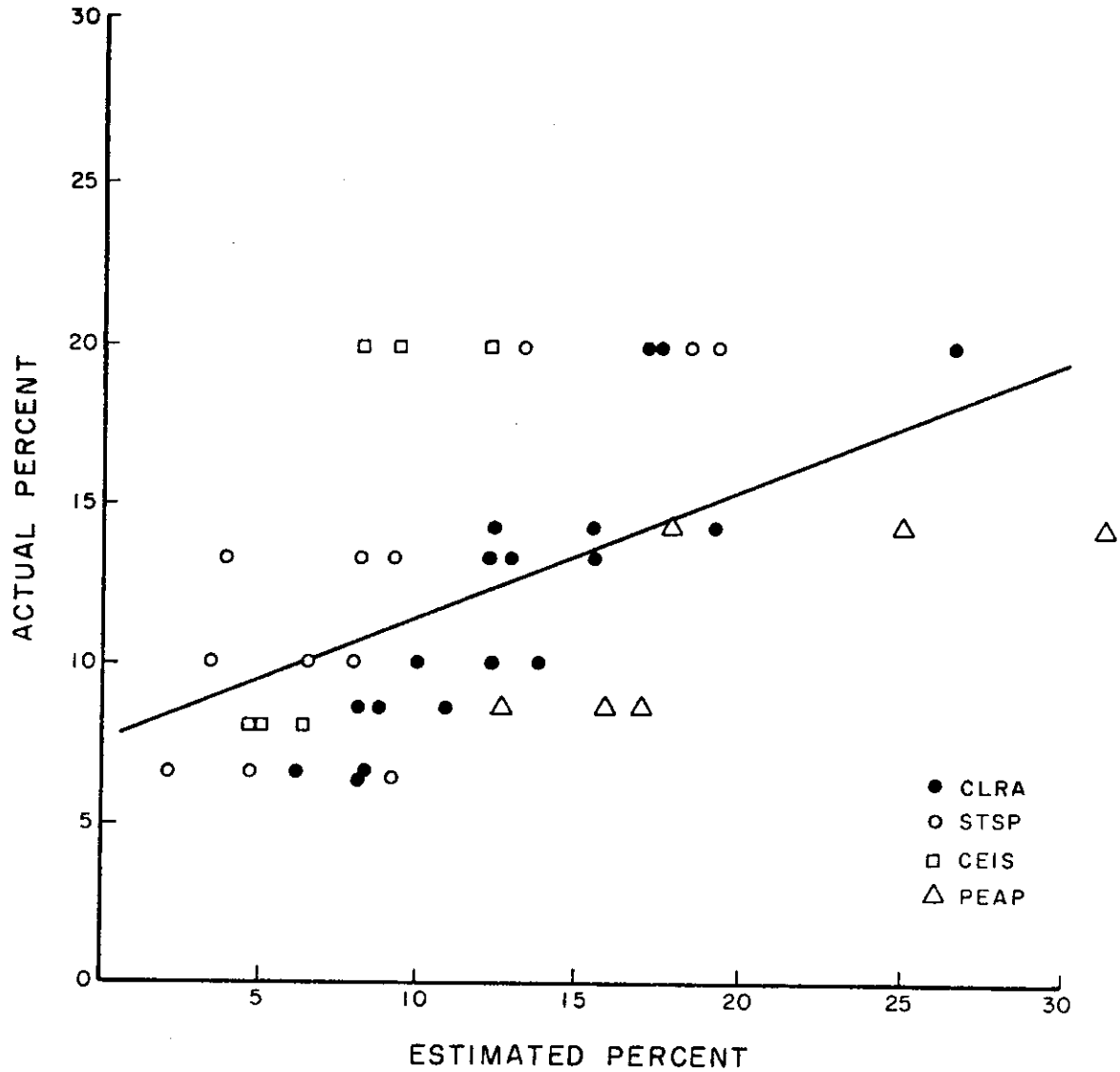

FIGURE 9. The estimated dry weight and actual dry weight of Cladonia rangeriferina (CLRA), Stereocaulon grande (STSP), Cetraria islandica (CEIS), and Peltigera apthosa (PEAP) in mixtures of plants examined by a microscopic technique for the discerned plant fragments on microscope slides. The solid line is the best linear fit.

EFFECTS OF DIGESTION ON DISCERNIBILITY

Digestive tract residues of jackrabbits were used to study changes in the discernibility of plant fragments caused by digestion. It was assumed that the "ingesta" (stomach contents) were relatively non-digested, the caecum contents were residues from the stomach, and the colon contents were residues from the caecum. Monthly composited samples of each species of jackrabbits' ingesta were additionally treated by a microdigestion technique in the rumens of bison for 48 hours and in the rumens of cattle for 12, 48, and 60 hours (Figure 10). Some unpublished observations (R. M. Hansen, personal communication) suggest that the ingesta residues in the nylon bags after digestion might be used to simulate the effects of herbivore digestion on plant fragment discernibility.

The stomachs, caecums and colons of approximately 25 black-tailed (Lepus californicus) and 25 white-tailed jackrabbits (Lepus townsendii) were collected each month during April, May, July, and September of 1970. Only the stomachs and colons were collected during December 1969. Also, the stomach contents were collected from both species of hares for October and December 1968, February, April, May, June, July, August, September, and October of 1969, and February, June, and August of 1970 (Figure 11). The stomachs, caecums, and colons were removed and chilled within a few minutes after the animal was killed to stop enzymatic activity (See Flinders and Hansen 1972 for details on numbers of animals collected and how the samples were treated).

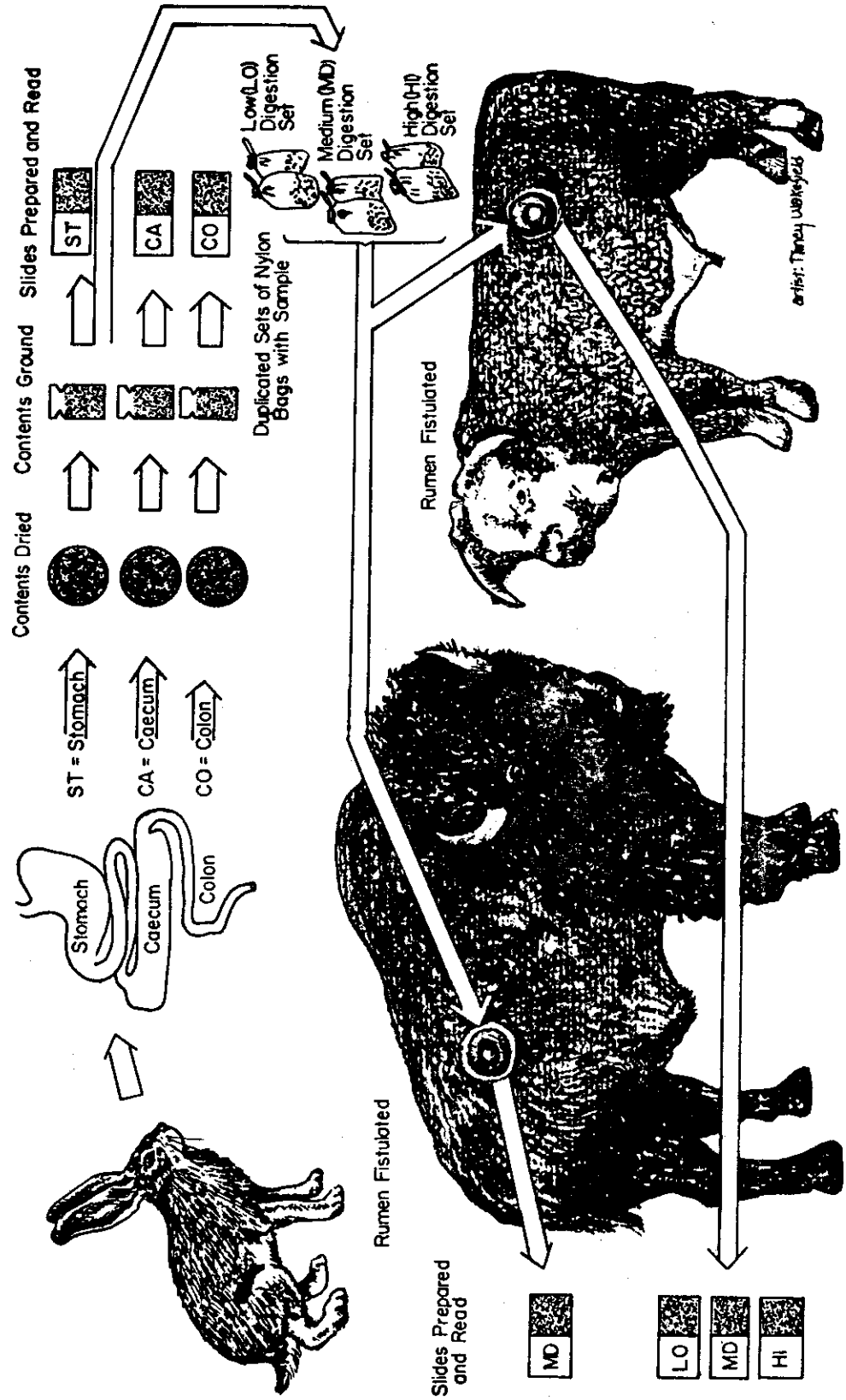

FIGURE 10. The flow and treatments of the stomach, caecum, and colon samples taken from jackrabbits.

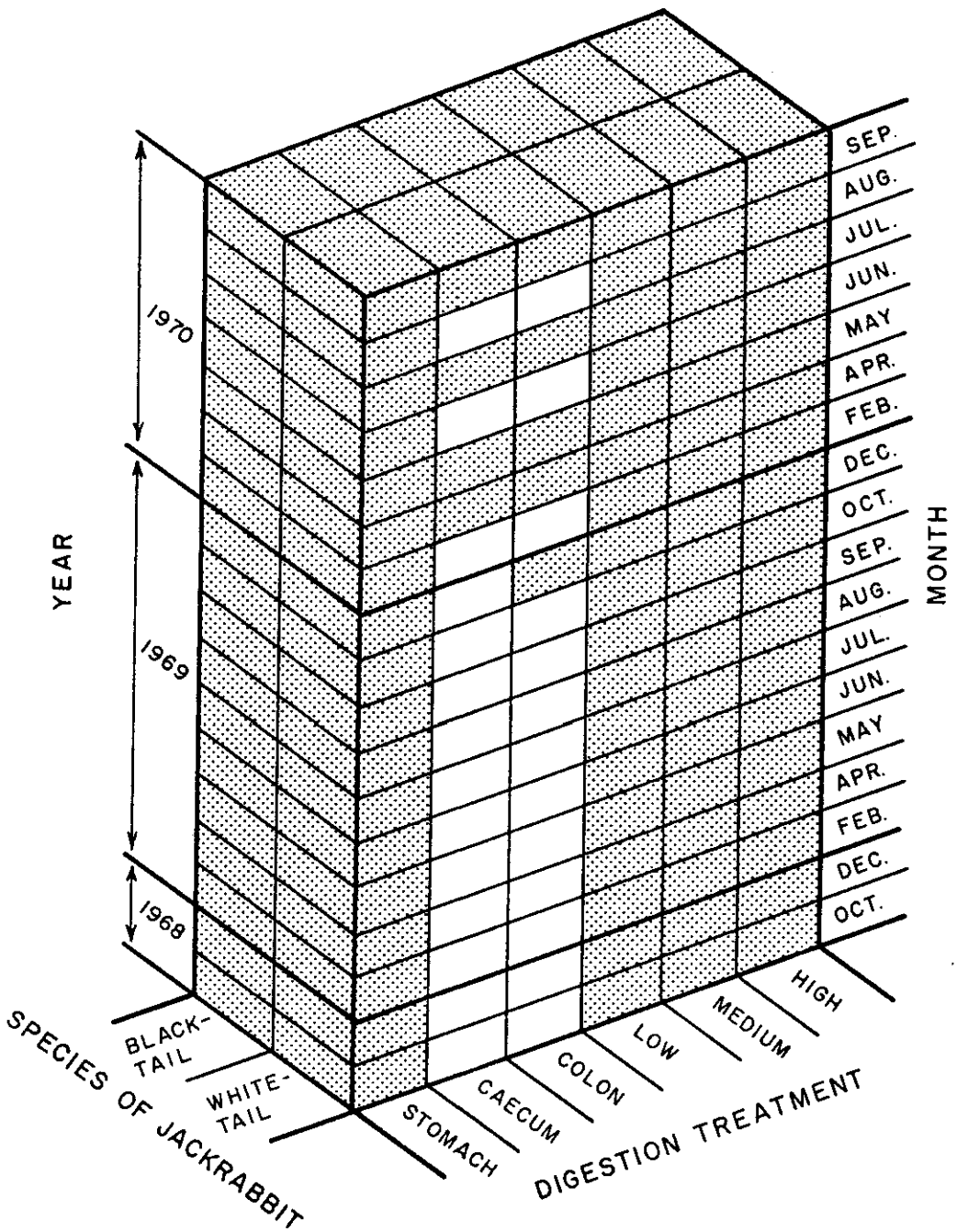

FIGURE 11. A pictorial diagram showing the samples available (shaded areas) from both black-tailed and white-tailed jackrabbits over a 18 month period and the treatments of these samples.

The relative percent densities (RD's) of discerned plant fragments were calculated for 867 stomachs, 173 caecums, and 224 colons of jackrabbits. The discerned fragments were recorded for approximately 500 microscopic fields in each sample from the stomach, caecum, or colon.

The RD's of discerned plant fragments in nylon bag residues were determined for 18 monthly composited samples each for black-tailed and white-tailed jackrabbits. One hundred and twenty fields were microscopically examined in each monthly sample for discerned fragments.

The mean number of plant species recorded per stomach of individuals of each species of hare varied with the season (Flinders and Hansen 1972). Some 71 plant species were identified in the 18 monthly diets of black-tailed jackrabbits and 67 plant species in the diets of white-tailed jackrabbits.

RD's in Ingesta vs RD's in Caecum and Colon

The RD's of major forage plants found in the ingesta (Y) and the caecum and colon (X's) of black-tailed and white-tailed jackrabbits were compared for similarity. The major species were those that made up 5% or more of the 18 monthly diets of each species of hare. Those ingested by black-tailed jackrabbits were: crested wheatgrass (Agropyron cristatum), western wheatgrass (Agropyron smithii), fringed sagewort (Artemisia frigida), smooth bromegrass (Bromus inermis), sun sedge (Carex heliophilia), summer cypress (Kochia scoparia), alfalfa (Medicago sativa), and winter wheat (Triticum aestivum). The major plant species eaten by white-tailed jackrabbits were: crested wheatgrass, western wheatgrass, fringed sagewort, vetches (Astragalus sp. and Oxytropis sp.), sun sedge, summer cypress, alfalfa, and winter wheat.

Wilcoxson's Signed-rank test, which focuses on the order or ranking of the differences of RD values and not on their numerical values, was used to test botanical compositions for differences between the major plants of the ingesta and digesta residues. The numerical value may change depending upon: (1) the effect of digestion upon the plant species, and (2) the number and types of plants consumed. Rank-order procedures were used to determine the magnitude of difference in RD's for paired botanical compositions of the major plants. The greater the magnitude of difference caused by digestion in the RD's of paired plant species, the higher the calculated values (rankings) used in the comparisons become.

No significant difference (average p = .82, range .74 to .99) occurred between the RD's of the major plant species found in the stomach contents and those of the caecums of black-tailed jackrabbits during April, May, July, and September of 1970. Similar results were obtained (average p= .56, range .26 to .75) when the RD's of the ingesta (Y) were compared to those of the colon (X) in black-tailed jackrabbits during December 1969 and April, May, July, and September of 1970.

Comparison of the major plant species of white-tailed jackrabbits ingesta (Y) vs caecum (X), and ingesta (Y) vs colon (X), for the same months as used in the black-tailed jackrabbit comparisons, showed no significant difference in RD's (average p= .74, range .60 to .92 and average p = .72, range .23 to .99, respectively).

Indices of botanical dissimilarity coefficients (BDC) between the ingesta and colons (Table 9) were used to estimate whether digestion in black-tailed and white-tailed jackrabbits caused similar differences in the discernibility for the major plant species. The index is defined as:

$$BDC = \frac{\Sigma(f_{1i} - f_{2i})^2}{n},$$

where f_i is the relative percent density (RD) of plant species i encountered in the diet sampling process conducted for plant species presence in black-tailed jackrabbits. The subscripts 1 and 2 represent paired species of plants in the stomach and colon which are being compared for the amount of dissimilarity. It is

Table 9. Mean indices of discerned plant fragment dissimilarity (BDC) of plant composition in 4 monthly ingesta samples and 4 monthly colon samples are compared for similarity in discernibility between black-tailed (BT) and white-tailed (WT) jackrabbits.

Comparison	Month and Year	Mean (BDC) Value	Significance level that these means are the same
BT stomach vs. BT colon	April 1970	26.74	.22
WT stomach vs. WT colon	April 1970	15.24	
BT stomach vs. BT colon	May 1970	234.47	.43
WT stomach vs. WT colon	May 1970	265.51	
BT stomach vs. BT colon	July 1970	154.64	.19
WT stomach vs. WT colon	July 1970	83.73	
BT stomach vs. BT colon	September 1970	143.26	.01
WT stomach vs. WT colon	September 1970	28.07	

desirable to know if this coefficient differs significantly from another dissimilarity value for the differences squared between the stomach and colon of the white-tailed jackrabbits. The test of BDC differences is made by forming a ratio of the larger variance to a smaller variance which follows the F-distribution with n_1 and n_2 degrees of freedom. Therefore,

$$F_{n_1,n_2} = \frac{\text{Larger } BDC_i}{\text{Smaller } BDC_i},$$ where i denotes the species of

jackrabbit. Ingesta-colon paired samples for each species of jackrabbit that represents the same selectivity of the total ingested forage occurs infrequently because each hare's diet was usually different from month to month. In this study, the "best" ingesta-colon pairs for each hare were those collected in April, May, and July of 1970 while the "poorest" was in September 1970.

The diet selection for each hare was similar in April, May, and July of 1970, and there were no statistically significant differences between the digestive effects of the black-tailed and white-tailed jackrabbits. In September 1970 the two species of jackrabbits selected different diets. Therefore, the differences in the magnitude of discernibility for the identical plant species between these two hares were significant.

When the array of plant species in each hare's diet was similar there was a strong indication that plant fragment discernibility was affected to the same extent by digestion

irrespective of the species of jackrabbit. Since no significant differences between the ingesta RD's and digesta RD's were observed for the major plant species,it was assumed that all species of plants are likely to be influenced the same way by digestion in both species of hares. Therefore, it was assumed for statistical analyses that "species of jackrabbits" did not need to be tested separately.

Digestion in Nylon Bags

The nylon bag digestion technique is a simple and rapid method of testing the digestibility of a large number of forage samples. The ruminal environment is little changed by the inclusion of such materials, and the complexities of duplicating ruminal conditions are avoided (Sims 1967; Van Dyne 1962; Van Keuren and Heinemann 1962; and Quinton 1972). This technique was used to evaluate the effect of digestion upon the discernibility of plant fragments.

Microdigestion trials in ruminal fistulated cattle and bison were conducted over various lengths of time to provide information on the effects of digestion upon plant fragment discernibility. Theoretically, increasing the amount of digestion might decrease relative plant fragment discernibility for plants whose structural characteristics are obliterated through digestion.

The RD of plant species found in the residues of each species of hare's monthly composited stomach samples after 48 hours of digestion in nylon bags in both rumen fistulated cattle and bison were compared for similarity. The RD's were similar, indicating that digestion by cattle and bison influenced discernibility of fragments of plants to a similar degree. Therefore, the RD's in the residues from the 48 hours of microdigestion in both cattle and bison were used as similar treatments in statistical comparisons.

Black-tailed monthly composited stomach samples

Digestor comparison	Average Significance Level for 18 months	Range of Significant Level
Cow vs. Bison 1	.48	.08 to .91
Cow vs. Bison 2	.46	.08 to 1.0

White-tailed monthly composited stomach samples

Digestor comparison	Average Significance Level for 18 months	Range of Significant Level
Cow vs. Bison 1	.56	.06 to 1.0
Cow vs. Bison 2	.58	.07 to 1.0

There was a high degree of similarity between the relative percent density of fragments of plant species discerned in the ingesta (Y) and the nylon bag residues after 12, 48, and 60 hours of microdigestion. The probability values (p) are .60, .60, and .66 respectively.

The comparison of the RD's of plant species found in the caecum and colon with those in the residues from nylon bags (NB) showed no significant differences, as is shown below:

	Significance Level	
Hours of nylon bag digestion	Caecum vs NB	Colon vs NB
12	.44	.62
48	.52	.54
60	.78	.72

Seasonal Variation

The discernibility of plant fragments after digestion may be related to the stage of growth of the plant species at the time they are eaten. Plants in an early growth stage are always more digestible than the same plant is in a mature or dormant stage. In ecosystems, with only short seasons conducive to plant growth, different plant species on the same dates are more similar in digestion coefficients than each species of plant is at different "phenological" dates (Cook and Harris 1968). Regal (1960) reported that grasses with thick cell walls are less digestible than are grasses with thin cell walls. Hardison et al. (1957) found that the bottom portion of alfalfa was not as digestible as the top portion, and as growth advanced, digestibility of the bases decreased at a rate of 0.6 percent per day, but digestibility of the top portion remained unchanged. Since the jackrabbits fed primarily upon the tender growing parts of plants (Flinders and Hansen 1972), it is likely that there were only small seasonal differences in the digestibility within a given species of plant ingested by the hares. Hence, the digestibility effect upon discernibility of fragments of a plant species would have only a small, if any, seasonal effect for the plants eaten by jackrabbits.

Probability values calculated from the comparisons of the RD's of the ingesta to those of the treatments (i.e. caecum, colon, nylon bag 12, 48, and 60 hours) were assumed to represent mean botanical similarity. The higher the probability value, the more similar were the ingesta and residues of treatments for RD's

of plant fragments. The probability values for each ingesta (Y) vs treatment (X) for the two year period were combined over months and were tested by the Coefficient of Concordance test statistic to establish that treatment rankings within months are unrelated to each other. The treatment rankings were found to be related since the probability (p) of them being unrelated was .05. Plotting the mean rank of treatment similarity within months indicates consistently low rank-order values for treatments in February and May (Figure 12).

The factors contributing to the low numerical ranks were the array of plants ingested in those months. The diet composition for February and May are shown as follows:

		Black-tail	White-tail
February 1969	Grass	7.5%	5.0%
	Forbs	92.3%	95.0%
February 1970	Grass	61.4%	90.1%
	Forbs	38.6%	6.1%
May 1969	Grass	75.7%	82.3%
	Forbs	23.1%	17.3%
May 1970	Grass	80.5%	78.6%
	Forbs	18.2%	19.2%

It was observed in the non-digested plant samples that the percentage of grasses were usually overestimated and the percentage of forbs were usually underestimated. The diet selected in any one of the above months consisted predominately of either grasses or forbs. The botanical composition of these diets deviated significantly from the average botanical compositions,

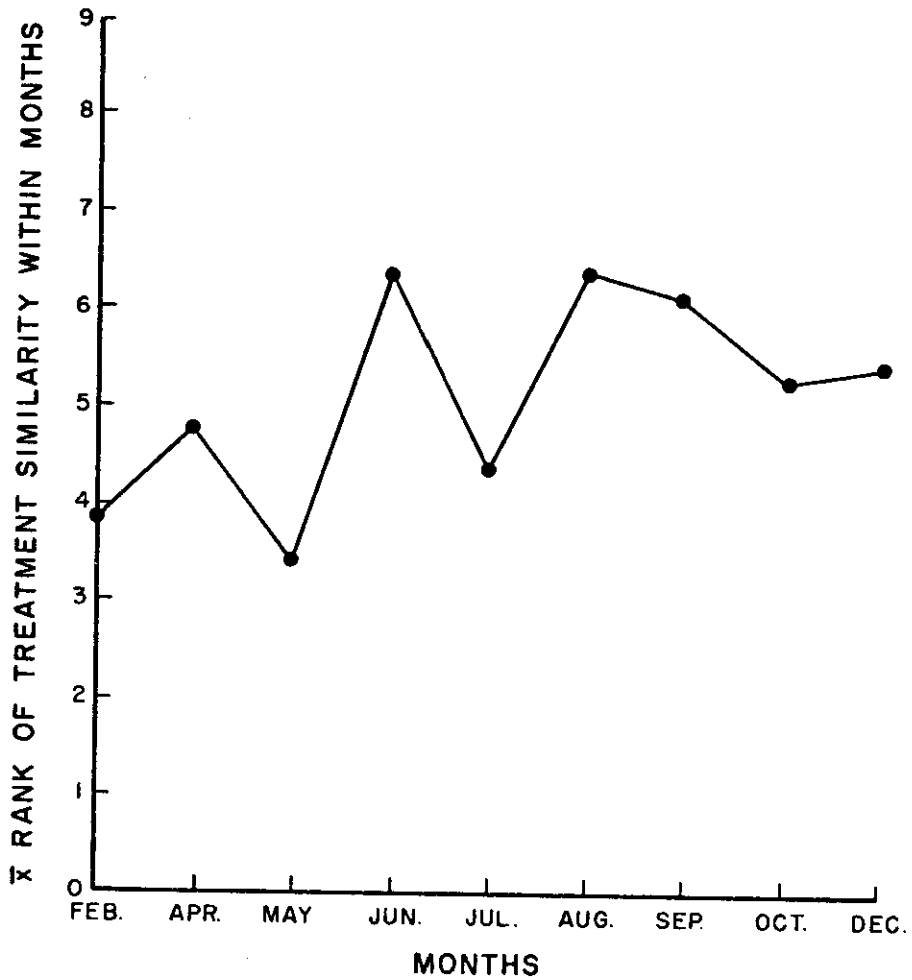

FIGURE 12. The mean rank of treatment similarity rankings within months combined over two years is an index of the divergence of the actual agreement shown in the data from the maximum possible (perfect) agreement. If there is perfect agreement the mean ranks would be: 1, 2, 3, ···, 9, though not necessarily in that order. If there had been no agreement among the k sets of rankings, then the various means would be approximately equal. The months of February and May showed some agreement while all others showed no agreement in ranks.

and the RD's of the plants found in the digested residues were either highly overestimated or underestimated. The Wilcoxon Signed-rank test showed that for February and May the ingesta vs treatment RD's were not significantly different, but the probability values were low. When the probability values were ranked in the Coefficient of Concordance test, their ranks were always low, and the means of the sums of the ranks were the lowest. The February and May rankings were unrelated, but the array of plants selected in the diets were different according to the Coefficient of Concordance test. Therefore, it is assumed that there is no seasonal pattern in similarity rankings, and the months are unrelated to each other.

Correction Terms

Correction factors (c) to equate differences between the RD's observed in digested residues (X) and the jackrabbits ingesta (Y) of plant species were calculated for 23 plant species. There were no significant differences (using a pairing design test) between the c-values calculated for ingesta vs caecum or colon and those for ingesta vs nylon bags after 12, 48, or 60 hours of microdigestion. It is assumed that there were no differences between hares, digestors, treatments, and seasons. Therefore, correction terms were calculated for each species of plant combined over all "digestion treatments".

The degrees of over or underestimation (c) were calculated by the formula:

$$\text{Degree of Estimation} = \frac{\text{Original (ingesta) relative percent density (Y)}}{\text{Estimated relative percent density (X)}}$$

Equating the original RD's (Y) with the estimated RD's (X) can be represented best by assigning a numerical rating to each. The numerical rating is obtained by dividing the actual percent by the percentage which was estimated. An index ratio of 1.0 for a species indicates estimated RD's in proportion to the original RD's; a larger figure indicates a greater original RD in proportion to that which was estimated, and a smaller figure indicates a lesser original RD in proportion to that which was estimated. A high "c-value" indicates a relative decrease in discernibility, while a smaller value indicates a relative increase in discernibility.

Overestimation caused by digestion in a number of grasses or grasslikes may be a reflection of the underestimation of other species (Table 10). Underestimation indicates that the species concerned has been affected to a greater degree by digestion than others, and therefore, the identifiable characteristics were decreased by digestion. In normal mixtures some grasses and grasslikes tended to be overestimated while others were underestimated (Table 10). The digested forb and shrub fragments were consistently underestimated if they occur with a normal array of grasses. Although the over or underestimation of species of grasses varies, the overall mean "c" value is 1.41 which indicates an underestimation. The mean "c" value calculated without the cultivated grasses that tend to grow in the cool season (crested wheatgrass, Agropyron cristatum; oats, Avena sativa; needle and thread, Stipa comata; and winter wheat, Triticum aestivum), is .92 for the remaining native grasses. The small amounts of overestimation caused by digestion in most grasses as well as overestimation of most grasses in hand-compounded, non-digested mixtures may be acceptable for practical purposes, but some species are underestimated to a similar degree. The forbs and shrubs groups (Table 10) are consistently underestimated (mean "c" = 2.40).

Eleven of the 23 plant species were underestimated because of digestion,and their "c" values differed significantly (using a pairing design t-test) from 1.0. The grasses significantly underestimated were Avena sativa, Stipa comata, and Triticum aestivum while vetches (Astragulus sp. and Oxytropsis sp.),

Table 10. Summarization of each plant species comprising more than 1% of the total diets showing the mean degree of estimation, its standard deviation, the type of plant, and its growth characteristic.

Name	n	$\bar{x}$	S. D.			
GRASSES AND GRASSLIKES						
Agropyron cristatum	68	1.21	1.16	Perennial	Cool	Cultivated
Agropyron smithii	92	1.05	.83	Perennial	Cool	Native
Avena sativa	13	2.22	1.99	Annual	Cool	Cultivated
Bouteloua gracilis	71	.76	.94	Perennial	Warm	Native
Bromus inermis	41	1.28	2.11	Perennial	Cool	Cultivated
Carex heliophila	87	.80	.97	Perennial	Cool	Native
Festuca octoflora	10	.89	1.41	Annual	Cool	Native
Sporobolus cryptandrus	70	.74	..92	Perennial	Warm	Native
Stipa comata	24	2.69	5.16	Perennial	Cool	Native
Triticum aestivum	61	2.49	2.83	Annual	Cool	Cultivated
OVERALL MEAN = 1.41* S. D. = .76						
FORBS AND SHRUBS						
Artemisia frigida	45	1.00	1.32	Perennial	Warm	Native
Astragalus sp. & Oxytropsis sp.	59	4.49	4.84	Perennial	Cool	Native
Atriplex canescens	40	1.67	2.87	Perennial	Warm	Native
Chrysothamnus nauseosus	40	2.04	2.22	Perennial	Warm	Native
Helianthus annus	20	3.46	3.05	Annual	Warm	Native
Kochia scoparia	92	2.90	3.31	Annual	Warm	Introduced
Medicago sativa	68	3.87	4.91	Perennial	Warm	Cultivated
Opuntia polyacantha	50	1.11	2.03	Perennial	Warm	Native
Psoralea tenuiflora	15	1.43	1.36	Perennial	Warm	Native
Sophora sericea	44	1.30	1.52	Perennial	Warm	
Sphaeralcea coccinea	63	1.72	2.04	Perennial	Warm	Native
Chrysopsis villosa	18	2.60	3.19	Perennial	Warm	Native
Mirabilis linearis	20	3.56	2.35	Perennial	Warm	Native
OVERALL MEAN = 2.40 S.D. = 1.16						

*Overall mean without Stipa comata, Agropyron cristatum, Avena sativa, and Triticum aestivum was .92 with a S.D. of .21.

Chrysothamnus nauseous, Helianthus annus, Kochia scoparia, Medicago sativa, Sphaeralcea cocinea, Chrysopsis villosa and Mirabilis linearis were significantly underestimated. One grass, Sporobolus cryptandrus, was significantly overestimated.

DISCUSSION

Qualitative Assessment

Evidence from other studies on the dry weight quantification for botanical composition in diets using recognizable plant fragments in the digestive tracts of livestock and wild herbivores has been consistently questioned. Martin (1955) was able to recognize, in the feces and stomachs of sheep (Ovis aries), only sixteen of the forty species which were present in the pasture and which he suspected were all being ingested. Without proof, he concluded that many species were completely digested or reduced to such small fragments as to be unidentifiable. No evidence was presented to show that the sheep whose feces he sampled had recently ingested any of the unrecorded species for fragments to be present in the feces. Croker (1959) could not identify in sheep feces one of about twenty-five grass species which were present in the pasture. The species she could not find had a thin cuticle which disintegrated in vitro, and she suspected it had been completely digested. It was not certain that the grass concerned had, in fact, been ingested recently. In a study by Hercus (1960), it was found that for every herbage species ingested some recognizable cuticle or epidermis was found in the feces of sheep, and it was concluded that feces analysis can be used to investigate the botanical composition of the diet of any herbivorous animal. Storr (1961) states that in annual plants only the cuticle, which bears an outline of epidermal cells and is identifiable, survives

maceration *in vitro* and digestion. The entire epidermis survives since cutin is deposited in all of the cell walls in perennials. He found that all of the limited number of dicotyledons ingested were recognizable in the feces. Todd and Hansen (1973) could find no significant differences between the plant fragments found in the rumens and those in the colons of bighorn sheep (*Ovis canadensis canadensis*). They concluded that the relative number of plant fragments of each kind of plant in their samples remained similar while passing through the digestive process. They suggested that digestion reduces the mean weight of fragments rather than eliminating the whole fragment. Using paired fistula samples and fecal samples of cows, bison, and sheep, Hansen et al. (1973) determined that the degree of dietary overlap between herbivores, areas, seasons, and grazing intensity is approximately the same for discerned plant fragments in the feces as it is in the paired esophageal samples. Plants having a "low frequency" are less likely to be discovered when subsampling, and these are the components which are frequently not discerned by microscopic analyses and are underestimated when fecal sampling has been done by the microscopic technique.

In this study, the major food plants were as easily identified in the caecum, colon, and residues of nylon bag digestion trials as in the ingesta. Examination of digestive tract contents provided estimates of major foods ingested by the animals and indicated the general proportions in which these food items were ingested. This study also showed that technicians can identify some species of plants more easily and more frequently than others.

This causes some fragments of plants (digested or undigested) to be over or underestimated when percentages are used to describe a diet.

Microscopic analysis provides estimates of what the animal has eaten and allows the examination of the digestive tract to be a useful and acceptable means of estimating botanical composition. Analysis of the ingesta, caecum, or colon is a direct and reliable means of approach to identify food items and to determine the presence or absence of particular species in the diet.

The in vivo digestion trials showed that digestion over varying periods of time had no large effect upon the ability of technicians to identify plant fragments. Digestion affects the mean loss in dry weight to a greater degree than losses in discernibility.

Quantitative Assessment

Since certain plants are digested more quickly and more thoroughly than others, it has been assumed that highly digestible plants are more likely to lose their identifiable characteristics than are less digestible plants. This difficulty was recognized by early food-habit workers (McAtee 1912), but it remained unassessed until now. In 1940, Davison compared food items found in the crops and gizzards of bobwhite quail and concluded that the crop was the only part of the alimentary canal in which food items remained in the same proportions as in the food ingested. Jensen and Korschgen (1947) found that even in bird crops, proportions of foods differed appreciably from those ingested.

The controlled feeding study of sheep by Norris (1943) is frequently cited to show the limitations of stomach content analysis as a quantitative estimate of forage consumed. Differential digestion was apparent, and percentages of food items found by his technique in the stomach was a poor estimate of the dry weights of foods consumed. Only the larger and easily observed plants were identified, and small fragments were not identified. Bergerud and Russell (1964) compared rumen contents of four sacrificed caribou which were fed known rations from 30 minutes to 72 hours before death. Their technique revealed that some food items were not observed in the rumen, and they suggested (without proof) that digestion rates varied between plant groups and to some extent between species within groups. Storr (1961) fed quokkas a finely chopped mixture of four perennial herbs for 5 days.

On the 6th day, analysis of the feed and feces by area measurement showed the proportions of each food plant were similar in both. The differences were clearly not significant, and there were no differences in digestibility among the plants concerned.

Limitations imposed by effects of digestion on identification, although not directly investigated, have been recognized by other workers (Errington 1932; Hartley 1948; Smith 1952; Cole 1956; Jensen 1958; Davison and Hamor 1960; Edwards and Ritchey 1960; Regal 1960; Brown 1961; Storr 1961; Martin and Korschgen 1963; Anderson et al. 1965; Scotter 1967; and Todd and Hansen 1973).

In this study the experiments fall into two groups: those in which there was quantification of mixtures of non-digested plants and those in which there was quantification after digestion. In the former group, technicians tend to correctly estimate the dry weights in simple hand-compounded mixtures of plants when the species have distinctive cellular characteristics. There is little over or underestimation by species, and the degrees of over or underestimation is non-significant for monocot or dicot mixes. In hand-compounded mixtures made to simulate a herbivore's diet technicians over or underestimated approximately 50% of the plant species in the mixtures. The magnitude of over or underestimation varies from 0.4 X to 4.0 X with some species having a 1:1 estimation. Digestion changed the identifiable characteristics of at least half of the plant species which were studied. The changes caused by digestion caused a 0.7 X to 4.0 X over or underestimation of what the RD value was prior to digestion. It is assumed that plant species which were overestimated in

hand-compounded mixtures (non-digested) are probably the same species which digestion caused to be overestimated. Likewise, plants underestimated in non-digested hand-compounded mixtures are assumed to be the same ones which digestion caused to be further underestimated. In general, plant species with thin cell walls are highly digestible and are those which are underestimated in non-digested or digested mixtures. Notable exceptions to this are some of the species of mosses.

Correction factors were calculated to accurately account for differences between the original RD (Y) and estimated RD (X) values with regard to digestion and were required for approximately half of the species (Figures 13 and 14).

Regal (1960) showed that the considerable differences in pasture grass digestibility were related to the content of indigestible tissues in the various species of grasses. Cultivated species of grass containted less than 15% ballast (indigestible tissue) in their leaf-blades, while coarse weed-grasses contained 35-57%. In this study four of the five grasses that decreased the most in discernibility were cultivated plants (winter wheat, Triticum aestivum; oats, Avena sativa; smooth bromegrass, Bromus inermis; and crested wheat, Agropyron cristatum). Needle and thread (Stipa comata) apparently behaved in a similar manner and at the time of ingestion probably contained only a small percentage of indigestible tissue. The remaining grasses and grasslikes, all of which are native species and mostly perennials, showed only small changes in discernibility after digestion with the exception of Sporobolus cryptandrus which was significantly overestimated.

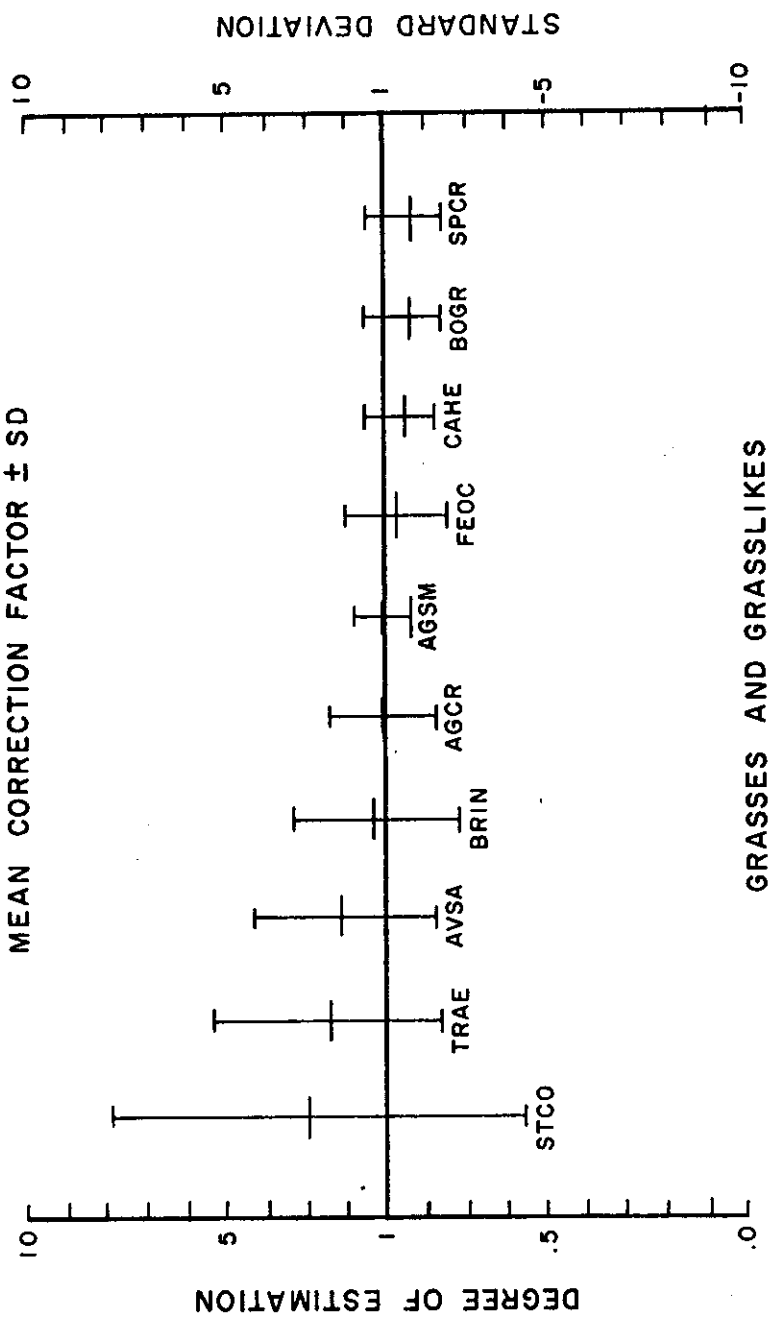

FIGURE 13. Mean c-values and standard deviation necessary to correct estimated relative percent densities of digested grasses and grasslikes to actual relative percent densities before digestion using the microscopic technique. (STCO = Stipa comata, TRAE = Triticum aestivum, AVSA = Avena sativa, BRIN = Bromus inermis, AGCR = Agropyron cristatum, AGSM = Agropyron smithii, FEOC = Festuca octoflora, CAHE = Carex heliophila, BOGR = Bouteloua gracilis, and SPCR = Sporobolus cryptandrus).

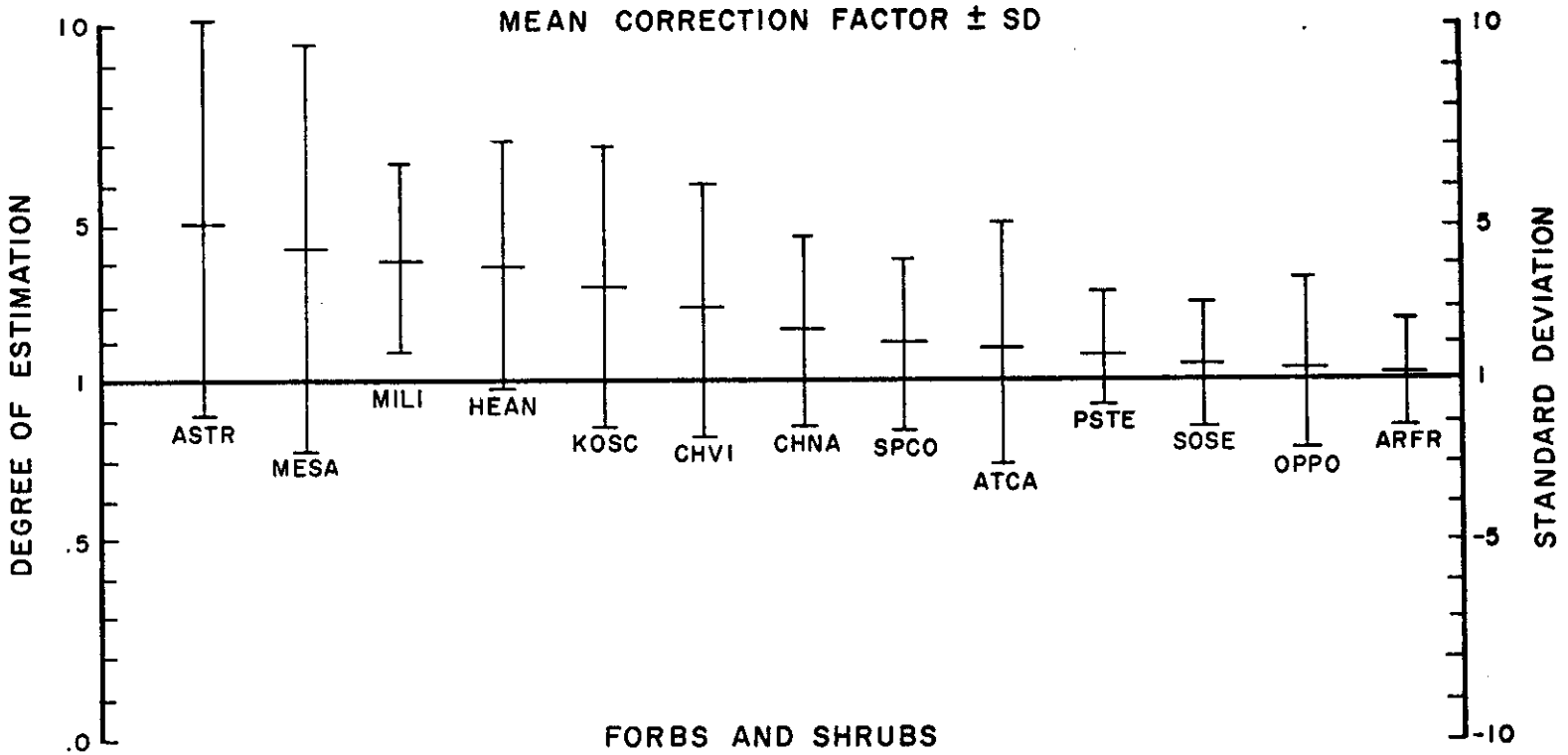

FIGURE 14. Mean c-values and standard deviation necessary to correct estimated relative percent densities of digested forbs and shrubs to actual relative percent densities before digestion using the microscopic technique. (ASTR = Astragalus sp. and Oxytropsis sp., MESA = Medicago sativa, MILI = Mirabilis linearis, HEAN = Helianthus annus, KOSC = Kochia scoparia, CHVI = Chrysopsis villosa, CHNA = Chrysothamnus nauseosus, SPCO = Sphaeralcea coccinea, ATCA = Atiplex canescens, PSTE = Psoralea tenuiflora, SOSE = Sophora sericea, OPPO = Opuntia polyacantha, and ARFR = Artemisia frigida).

Taylor (1972) reported western wheat grass (Agropyron smithii) to be slightly more digestible than blue grama grass (Bouteloua gracilis). This same trend is seen in terms of discernibility since the c-value for western wheat grass is slightly higher (indicating less discernibility) than the one for blue grama grass.

Storr (1961) showed that in some annual plants only the cuticle of the epidermis survives digestion and the entire epidermis of some perennial species survives maceration and digestion. He concluded that the observed differences in digestibility between annuals and perennials are associated with the distribution rather than the thickness of cutin. In annuals only the outerwall of epidermal cells is covered by a small layer of cutin, while in perennials cutin is deposited on all cell walls. The cuticle, in effect, extends down between cells and completely surrounds them. This may explain in part why oats (Avena sativa) and winter wheat (Triticum aestivum) decreased in discernibility. Six weeks fescue (Festuca octoflora), the only other annual grass, was found only in small quantities.

Digestion consistently decreased discernibility in forbs and shrubs. Some were less discernible than others and this was probably due to their epidermal cell wall thickness rather than the ability of the technician to identify them. Taylor (1972) calculated the digestibility of a number of forbs and shrubs, and a direct correlation between his digestibility values and the c-values were observed. The forbs and shrubs that were reported by Taylor to be highly digestible were the same ones that showed the highest decrease in discernibility.

When the diet is constant, perennial grasses forming more than 1% of the diet by dry weight can be accurately discerned in the caecum, colon, or nylon bag residues the same after digestion as before digestion. The evidence obtained on annuals, which accounts for 1% or more of the diet, suggests that they too can be identified in any sample after digestion the same as before digestion.

Difference in Digestors

It might be expected that digestors having a degree of similarity would digest plants the same. Information obtained in this study showed that the three digestors, jackrabbits, cattle, and bison, digested each plant species to a similar degree of discernibility. However, this may not always be the case. When cattle and bison were used as digestors for some plants common in reindeer diets, they could not digest some of the plants to the same extent as reindeer (R. M. Hansen, unpublished).

Not only may different plants be digested to different extents by any one animal, but different animals may digest the same plant species to different extents. Differences between animals may be less important than differences between plants, a view supported by Ivins (1960) who states that the digestibility of herbage is influenced only to a slight extent by animal characteristics and is primarily determined by herbage characteristics.

CONCLUSIONS

The microscopic analysis of epidermal tissue is a method for identifying and quantifying plant fragments. This technique may be used to determine the botanical composition and the quantity contributed by each species present in crushed, hand-clipped, macerated, and digested plant material.

Technicians identify some species of plants more easily and more frequently than others. Preparations of known dry weight compositions may be used to train technicians to consistently identify and quantify plant fragments. Some fragments of plants (undigested or digested) are over or underestimated by technicians when percentages are used to describe a diet. However, the majority of the plant species in undigested mixtures are estimated in a 1:1 relationship to their known proportions. Digestion changes the estimated percentages of about 50% of the plant species.

Normal digestion in two species of hares influenced identification of the major plant species to the same degree. The estimated quantities of plant species from residues of nylon bags after 48 hours of microdigestion in ruminal fistulated bison and cattle were not significantly different. Differences between bison and cattle as digestors were apparently less important than were differences between plants. The effects of digestion by hares on plant fragment discernibility can be accurately simulated by nylon bag microdigestion in ruminal fistulated bison and cattle.

The microscopic method used in this study could be applied to a wide range of studies. For example, live-trapped animals may be returned to the study area after a sample of its feces has been collected, and this method does not necessitate the death of the animal when one only wishes to study diets. Feces can serve as dietary samples where concurrent population studies are necessary and in research on livestock, rare and endangered species, or for animals which are protected in sanctuaries. This method is also valuable when studying animals which are hard to capture or shoot, but whose feces are easily identifiable and procured.

The accuracy of the estimation of the proportions of food items ingested is influenced directly by many complex and interrelated factors. The relationship between the ingesta (Y) and the estimated (X) is expressed as: $Y = (c) X$. Emphasis must be placed on the determination of the correction term (c) if estimated proportions (X) are to accurately relate to ingesta (Y). Estimates of the correction term have generally been limited in scope and intensity. Current investigators should attempt to obtain more precise estimates of "c" and then use the estimator (X) as comparable to the ingesta (Y).

This study has attempted to establish correction terms for technician's over or underestimation (c_1) and the affects of digestion on plant species (c_2). Correction terms are needed for such parameters as:

(1) Technician variability (c_3)

(2) Seasonal and phenological stages of plants (c_4)

(3) Physiological state of the animal (c_5)

(4) Species of the digestor (c_6)

(5) Specifics such as stress, changing environmental conditions, fluctuating food availability, animal competition, and population pressure, etc. (c_n).

These associations, which make up the differences between the estimated consumption (X) and the ingesta (Y), have only begun to be understood. Once these parameters have been defined, the correction term "c" will have the most meaning when calculated as:

$$c = \frac{c_1 + c_2 + c_3 + c_4 + c_5 + c_6 + \ldots + c_n}{n} .$$

A method for obtaining correction values for the proportions of food plants ingested by wild herbivores may be obtained by experimentation. Plants could be hand plucked or clipped to provide duplication of vegetation foraged upon by wild animals. The plant species could be ground to a uniform size, and microdigested in ruminal fistulated animals having similar digestive characteristics. Since the dry weight (Y) of each food item placed in the nylon bags for microdigestion is known, and the estimated proportion (X) of each plant species after microdigestion is known, as well as the proportions estimated by counts of epidermal fragments in the feces of the wild herbivore, correction terms may be calculated to estimate, from the RD's of feces, the dry weights ingested by the foraging wild herbivore.

Greater depth of interpretation and a more critical examination of relationships can improve the value of this technique, which has both historic and current applications.

ACKNOWLEDGEMENTS

This study was supported, in part, by the Colorado State University Experiment Station Project 1069, Alaska Game and Fish Department and National Science Foundation grants GB-7824, GB-13096, and GB-31862X.

LITERATURE CITED

Adams, L. 1957. A way to analyze herbivore food habits by fecal examinations. Trans. N. Am. Wildl. Conf. 22:152-159.

Adams, L., W. C. O'Regan, and D. J. Dunaway. 1962. Analysis of forage consumption by fecal examination. J. Wildlife Manage. 26:108-111.

Anderson, A. E., W. A. Snyder, and G. W. Brown. 1965. Stomach content analyses related to condition in mule deer, Guadalupe Mountains, N. Mex. J. Wildlife Manage. 29:352-366.

Baumgartner, L. L., and A. C. Martin. 1939. Plant histology as an aid in squirrel food-habit studies. J. Wildlife Manage. 3:266-268.

Bear, G. D., and R. M. Hansen. 1966. Food habits, growth and reproduction of white-tailed jackrabbits in southern Colorado. Colo. State Univ. Agr. Exp. Sta. Tech. Bull. No. 90. 59 p.

Bergerud, A. T., and L. Russell. 1964. Evaluation of rumen food analysis for Newfoundland caribou. J. Wildlife Manage. 28:809-814.

Brown, E. R. 1961. The black-tailed deer in western Washington. Wash. State Game Dep. Biol. Bull. No. 13. 124 p.

Brusven, M. A., and G. M. Mulkern. 1960. The use of epidermal characteristics for the identification of plants recovered in fragmentary condition from crops of grasshoppers. North Dakota Agr. Exp. Stat. Res. Rep. No. 3. 11 p.

Casebeer, R. L., and G. G. Koss. 1970. Food habits of wildebeest, zebra, hartebeest, and cattle in Kenya Masailand. E. Africa Wildlife J. 8:25-36.

Chamrod, A. D., and T. W. Box. 1968. Food habits of white-tailed deer in south Texas. J. Range Manage. 21:158-164.

Cole, G. F. 1956. The pronghorn antelope. Its range use and food habits in central Montana with special reference to alfalfa. Mont. Fish and Game Dep. and Mont. State Coll. Agr. Exp. Sta. Bull. 516. 63 p.

Cole, G. F., and B. T. Wilkins. 1958. The pronghorn antelope: Its range use and food habits in central Montana with special reference to wheat. Mont. Fish and Game Dep. Tech. Bull. 2. 39 p.

Cook, C. W., and L. E. Harris. 1968. Nutritive value of seasonal ranges. Utah State Univ. Agr. Exp. Sta. Bull. 472. 55 p.

Croker, B. H. 1959. A method of estimating the botanical composition of the diet of sheep. New Zealand J. Agr. Res. 2:72-85.

Davies, I. 1959. The use of epidermal characteristics for the identification of grasses in the leafy stage. Brit. Grassl. Soc. J. 14:7-16.

Davison, V. E. 1940. A field method of analyzing game bird foods. J. Wildlife Manage. 4:105-116.

Davison, V. E., and W. H. Hamor. 1960. A system for classifying plant foods of birds. J. Wildlife Manage. 24:307-313.

Dirschl, H. J. 1962. Sieve mesh size related to analysis of angelope rumen contents. J. Wildlife Manage. 26:327-328.

Dirschl, H. J. 1963. Food habits of the pronghorn in Saskatchewan. J. Wildlife Manage. 27:81-93.

Dusi, J. L. 1949. Methods for the determination of food habits by plant microtechniques and histology and their application to cottontail rabbit food habits. J. Wildlife Manage. 13:295-298.

Edwards, R. Y., and R. W. Ritchey. 1960. Foods of caribou in Wells Gray Park, British Columbia. Canadian Field-Natur. 74:3-7.

Errington, P. L. 1932. Techniques of raptor food habit study. Condor 34:75-86.

Flinders, J. T., and R. M. Hansen. 1972. Diets and habitats of jackrabbits in northeastern Colorado. Range Sci. Dep. Sci. Ser. No. 12. Colorado State Univ., Fort Collins. 29 p.

Free, J. C., R. M. Hansen, and P. L. Sims. 1970. Estimating the dry weights of food plants in feces of herbivores. J. Range Manage. 23:300-302.

Galt, H. D., Theurer, Brent, Ehrenreich, and others. 1966. Botanical composition of the diet of steers grazing a desert grassland range. Amer. Soc. Anim. Sci. West. Sect. Proc. 17:397-401.

Grenet, E. 1966. Les particules vegetales des feces de mouton. Ann. Zootech. 15:303-312.

Hansen, R. M., and D. N. Ueckert. 1970. Dietary similarity of some primary consumers. Ecology 51:640-648.

Hansen, R. M., and P. F. Martin. 1973. Ungulate diets in lower Grand Canyon. In press in J. Range Manage.

Hansen, R. M., D. G. Peden, and R. W. Rice. 1973. Discerned fragments in feces indicates diet overlap. J. Range Manage. 26:103-105.

Hardison, W. A., W. N. Linkous, and C. Y. Ward. 1957. Digestibility of the top and bottom portions of the alfalfa plant, as estimated from small, randomly collected samples of feces. J. Dairy Science. XL:768-773.

Hartley, P. H. T. 1948. The assessment of food of birds. Ibis 90:361-381.

Heady, H. F., and D. T. Torell. 1959. Forage preferences exhibited by sheep with esophageal fistulas. J. Range Manage. 12:28-33.

Hegg, O. 1961. Analysen von Grosswildkot aus dem schweizerischen National Park zur ermitthing der Nahrungszusammensetzung (Analyses of big-game droppings to determine their dietary composition in the Swiss national Park.) Revue Swisse de Zoologic 68(12):156-165. (Transl. by J. J. Stransky, U.S.D.A. Forest Service, Southern Forest Exp. Sta., 9 pp., Proc. 1966).

Hercus, B. H. 1960. Plant cuticle as an aid to determining the diet of grazing animals. Eighth Int. Grassland Cong. Proc. 1960:443-447.

Ivins, J. D. 1960. Digestibility data and grassland evaluation. Proc. Eighth Int. Grassland Cong. Proc. 1960:459-461.

Jensen, P. V. 1958. Stomach contents of Danish red deer. Danish Rev. Game Biol. 3:141-144.

Jensen, G. H., and L. J. Korschgen. 1947. Contents of crops, grizzards and droppings of bobwhite quail force-fed known Kinds and quantities of seeds. J. Wildlife Manage. 11:37-43.

Kelso, L. H. 1934. Pocket gopher stomachs examination cards in the files of the Denver Wildlife Res. Center, Bureau of Sport Fisheries and Wildlife.

Kiley, M. 1966. A preliminary investigation into the feeding habits of the waterbuck by faecal analysis. East African Wildlife J. 4:153-157.

Laycock, W. A., H. Buchanan, and W. C. Krueger. 1972. Three methods of determining diet, utilization, and trampling damage on sheep ranges. J. Range Manage. 25:352-356.

Lesperance, A. L., E. H. Jensen, V. R. Bohman, and R. A. Madsen. 1960. Measuring selective grazing with fistulated steers. J. Dairy Sci. 43:1615-1622.

Malechek, J. C. 1966. Cattle diets on native and seeded ranges in ponderosa pine zone of Colorado. U.S. Forest Serv. Res. Note RM-77. 12 p.

Malechek, J. C., and C. L. Leinweber. 1972. Forage selectivity by goats on lightly and heavily grazed ranges. J. Range Manage. 25:105-111.

Martin, D. J. 1955. Features of plant cuticle: An aid to the analysis of the natural diet of grazing animals, with especial reference to Scottish hill sheep. Trans. Proc. Bot. Soc. Edinb. 36:278-288.

Martin, A. C., and L. J. Korschgen. 1963. Food-habits procedures. Pp 320-329. In: S. Mosby (Ed.), Wildlife Invest. Tech. Ed. 2. Washington, D. C. The Wildlife Soc.

McAtee, W. L. 1912. Methods of estimating the contents of bird stomachs. Auk 29:449-464.

Myers, G. T., and T. A. Vaughan. 1965. Food habits of the plains pocket gopher in eastern Colorado. J. Mammal. 45:588-598.

Norris, J. J. 1943. Botanical analyses of stomach contents as a method of determining forage consumption of range sheep. Ecology 24:244-251.

Peden, D. G. 1972. The trophic relations of Bison bison to the shortgrass plains. Ph. D. Dissertation, Colorado State Univ., Fort Collins. 134 p.

Quinton, D. A. 1972. Standardization of the nylon bag technique and its use in nutritive evaluations of northeastern Colorado range sites. Ph. D. Dissertation, Colorado State Univ., Fort Collins. 102 p.

Regal, V. 1960. The evaluation of the quality of pasture grasses by the microscopic method. Proc. Eighth Inter. Grasslands Cong. 1960:522-524.

Ridley, J. R., A. L. Lesperance, E. H. Jensen, and V. R. Bohman. 1963. Relationship of animal preference to botanical composition of irrigated pastures. J. Dairy Sci. 46:128-130.

Scotter, G. W. 1967. Effects of sieve mesh size and volumetric and gravimetric analysis of rumen content determinations. (Abstr.) Amer. Soc. Range Manage. 20th Annu. Meeting, Feb. 1967, pp. 61.

Sims, P. L. 1967. The effect of stand density on characteristics of wheatgrass. Ph. D. Dissertation. Utah State Univ., Logan. 68 p.

Smith, J. G. 1952. Food habits of mule deer in Utah. J. Wildlife Manage. 16:148-155.

Sparks, D. R., and J. C. Malechek. 1968. Estimating percentage dry weight in diets using a microscope technique. J. Range Manage. 21:264-265.

Stewart, D. R. M. 1967. Analysis of plant epidermis in faeces: a technique for studying the food preferences of grazing herbivores. J. Applied Ecol. 4:83-111.

Storr, G. M. 1961. Microscopic analysis of faeces, a technique for ascertaining the diet of herbivorous mammals. Australian J. Biol. Sci. 14:157-164.

Taylor, J. T. 1972. Nutritive value of Colorado range plants. Ph. D. Thesis, Colorado State Univ., Fort Collins. 105 p.

Thetford, F. O., R. D. Pieper, and A. B. Nelson. 1971. Botanical and chemical composition of cattle and sheep diets on pinyon-juniper grassland range. J. Range Manage. 24:425-434.

Todd, J. W., and R. M. Hansen. 1973. Plant fragments in the feces of bighorns as indicators of food habits. In press in J. Wildlife Manage.

Ueckert, D. N., and R. M. Hansen. 1971. Dietary overlap of grasshoppers on sandhill rangeland in northeastern Colorado. Oecologia 8:276-295.

Van Dyne, G. M. 1962. Micro-methods for nutritive evaluation of range forages. J. Range Manage. 15:303-314.

Van Dyne, G. M., and H. F. Heady. 1965. Botanical composition of sheep and cattle diets on a mature annual range. Hilgardia 36:465-492.

Van Keuren, R. W., and W. W. Heinemann. 1962. Study of a nylon bag technique for in vivo estimation of forage digestibility. J. Animal Sci. 21:340-345.

Ward, A. L. 1960. Mountain pocket gopher food habits in Colorado. J. Wildlife Manage. 24:89-92.

Ward, A. L. 1969. Stomach content and fecal analysis: Methods of forage identification. Rocky Mountain Forest and Range Experiment Station Miscellaneous Publ. No. 1147:146-158. Fort Collins, Colorado.

Ward, A. L., and J. O. Keith. 1962. Feeding habits of pocket gophers in mountain grasslands, Black Mesa, Colorado. Ecology 43:744-749.

Williams, O. B. 1962. A technique for studying microtine food habits. J. Mammal. 43:365-368.

Williams, O. B. 1969. An improved technique for identification of plant fragments in herbivore feces. J. Range Manage. 22:51-52.