Evaluation of Different Calculation Procedures for Microhistological Analysis

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Abstract

This study evaluated 3 procedures for calculating dry weight composition of forage mixtures when microhistological analysis was used. Dividing the frequency of each species by the total frequencies of all species gave a slightly more accurate representation of dry weight composition than converting frequency to relative density or using actual density. The frequency addition procedure is much quicker than either procedure involving density.

One of the basic assumptions of the microhistological technique outlined by Sparks and Malechek (1968) is that a one to one relationship exists between relative density and percent dry weight of identifiable fragments ground to a uniform size through a 1-mm screen. Sparks and Malechek (1968) determined that in 11 different mixtures the "frequency conversion" method using the Fracker and Brischle (1944) table gave the same dry weight composition estimates as the more laborious "particle count" (density) method. The mathematical rationale for converting frequency to density in microhistological studies is discussed by Johnson (1982). Sparks and Malechek reported that frequency observations from 100 microscope fields examined at 125X gave an accurate evaluation of plant fragments on microscope slides. However, they did state that exceptions may exist. Other studies conducted by Vavra and Holechek (1980), Johnson and Pearson (1981), Holechek and Gross (1982), and Holechek et al. (1982) have confirmed that the relationship reported by Sparks and Malechek (1968) is generally true with a few exceptions. The objective of this study was to evaluate the accuracy of 3 procedures for calculating the percent by weight of hand-compounded mixtures when microhistological analysis was used.

Methods

In the summer of 1980 10 known plant mixtures were compounded using species from semidesert range in southcentral New Mexico (Table 1). Species used in the diets included tobosa grass (*Hilaria mutica*), mesa dropseed (*Sporobolus flexuosus*), black grama (*Bouteloua eriopoda*), blue grama (*Bouteloua gracilis*), green sprangletop (*Leptochloa dubia*), silver bluestem (*Bothriochloa saccharoides*), Russian thistle (*Salsola kali*), faint crown (*Aphanostephus ramosissimus*), leatherleaf croton (*Croton corymbulosus*), scarlet globemallow (*Sphaeralcea coccinea*), fourwing saltbush (*Atriplex canescens*), gray oak (*Quercus grisea*), and honey mesquite (*Prosopis glandulosa*). All plant material was dried in a forced air oven and ground through a micro-Wiley mill with a 1-mm screen. Five slides were prepared from each mixture by soaking diet material in sodium hydroxide as discussed by Holechek (1982) and mounted as discussed by Sparks and Malechek (1968). At least 20 frequency observations were recorded per slide to insure high repeatability between slides as discussed by

Holechek and Vavra (1981). Twenty microscope fields were read at 125X for each slide by 3 experienced observers trained by Holechek and Gross (1982) procedures. All identifiable particle fragments in each field were recorded. Hairs and trichomes were disregarded. All unidentifiable fragments in each field were fully observed by moving the microscope slide to allow complete examination. Because our objective was to identify the calculation procedure giving the most accurate results, observers did not use correction factors to prevent over or under evaluation of certain species as recommended by Dearden et al. (1975), Vavra and Holechek (1981), and Holechek and Gross (1982).

Three procedures were used for estimating dry weight composition of each mixture. In the first procedure the number of identifiable fragments of each species in all fields was divided by the total number of identifiable fragments of all species in all fields. This procedure is equivalent to the "particle count" method of Sparks and Malechek (1968). Procedure two involved the conversion of frequency to density with the Fracker and Brischle (1944) table as discussed by Sparks and Malechek (1968). Procedure three involved dividing the frequency of each species by the total number of frequencies for all species.

The estimated percentage of each species in each mixture was compared to the actual percentage using the standard *t*-test and the 3 observers as replications. The percent similarity between estimated and actual dry weight composition was calculated with Kulczynski's formula (Oosting 1956).

Results and Discussion

The frequency addition procedure gave slightly better overall accuracy than the actual density or frequency conversion procedures when similarity indices for the 10 mixtures were averaged (Table 1). Estimated and actual species values differed significantly ($P < .05$) in 38%, 38% and 31% of the cases for the

Table 1. Percent similarity between actual and estimated percent by weight mixture composition when actual density, frequency conversion, and frequency addition calculation procedures are used.

Mixture ¹	Actual density	Frequency conversion (Fracker and Brischle Table)	Frequency addition
1	95	95	95
2	95	95	97
3	76	80	87
4	97	94	95
5	92	90	93
6	75	82	76
7	87	90	93
8	94	97	97
9	83	70	88
10	93	94	94
X	89	89	92

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Table 2. The percent by weight composition of 10 hand-compounded mixtures when 3 different methods are used for calculation.

	Total number of identifiable fragments in 100 fields	Percent frequency	Frequency converted to density (Fracker and Brischle table)	% dry weight using actual density	% dry weight using Fracker & Brischle frequency to density conversion	% dry weight using frequency	Actual % dry weight
Mixture 1							
Tobosa grass	33	28	33	19	18	18	20
Mesa dropseed	39	32	38	22	21	21	20
Russian thistle	36	31	37	21	21	21	20
Faint crown	28	26	30	16	17	17	20
Fourwing saltbush	38	34	41	22	23	23	20
Total	174	151	179	100	100	100	100
Mixture 2							
Mesa dropseed	144	74	135	80*1	80*	72	75
Russian thistle	35	29	34	20*	20*	28	25
Total	179	103	169	100	100	100	100
Mixture 3							
Black grama	26	22	25	14*	16*	19*	30
Leatherleaf croton	127	65	105	69*	66*	58*	45
Honey mesquite	31	26	30	17*	19*	23	25
Total	184	113	160	100	100	100	100
Mixture 4							
Green sprangletop	34	28	33	20	19	22	20
Scarlet globemallow	75	55	80	43	46*	43	40
Honey mesquite	65	45	60	37	35*	35*	40
Total	174	128	173	100	100	100	100
Mixture 5							
Tobosa grass	19	19	21	10	10	10	11.1
Blue grama	22	20	22	11	11	11	11.1
Silver bluestem	21	20	22	11	11	11	11.1
Leatherleaf croton	37	33	40	19*	20*	18*	11.1
Faint crown	16	16	17	8*	8	9*	11.1
Russian thistle	18	17	19	9	9	9	11.1
Gray oak	21	19	21	11	10	11	11.1
Fourwing saltbush	20	18	20	10	10	10	11.1
Mountain mahogany	19	19	21	11	10	11	11.1
Total	193	181	203	100	99	100	99.9
Mixture 6							
Black grama	96	80	129	55*	62*	56*	80
Scarlet globemallow	29	21	24	16*	11	14*	10
Leatherleaf croton	51	43	56	29*	27*	30*	10
Total	176	144	209	100	100	100	100
Mixture 7							
Blue grama	53	45	60	26*	28*	29*	33.3
Tobosa grass	56	47	63	28*	29*	31	33.3
Leatherleaf croton	94	61	94	46*	43*	40*	33.3
Total	203	153	217	100	100	100	99.9
Mixture 8							
Blue grama	44	39	50	24	25	25	25
Silver bluestem	41	37	46	22	23	23	25
Mesa dropseed	42	38	48	23	24	24	25
Scarlet globemallow	56	43	56	31*	28	27	25
Total	183	159	200	100	100	99	100
Mixture 9							
Green sprangletop	31	25	29	15*	12*	18*	25
Leatherleaf croton	136	86	196	67*	79*	62*	50
Fourwing saltbush	35	27	32	18*	8*	20*	25
Total	202	138	247	100	99	100	100

Table 2. Continued.

	Total number of identifiable fragments in 100 fields	Percent frequency	Frequency converted to density (Fracker and Brischle table)	% dry weight using actual density	% dry weight using Fracker & Brischle frequency to density conversion	% dry weight using frequency	Actual % dry weight
Mixture 10							
Tobosa grass	31	29	34	14	14	14	15.0
Black grass	26	26	30	12	12	12	12.5
Silver bluestem	24	23	26	11	11	11	12.5
Mesa dropseed	38	38	48	17*	20	18	20.0
Green sprangletop	11	10	10	5	4	5	5.0
Russian thistle	13	13	14	6	6	6	5.0
Faint crown	8	8	8	4	3	4	5.0
Leatherleaf croton	22	20	22	10*	9*	9*	5.0
Fourwing saltbush	34	32	38	16	16	15	15.0
Honey mesquite	12	12	13	5	5	6	5.0
Total	219	211	243	100	100	100	100.0

*Observed value differs significantly ($P<.05$) from the actual value.

actual density, density conversion and frequency addition techniques, respectively (Table 2).

Most of the mixtures containing leatherleaf croton showed poor agreement between estimated and actual species values regardless of the diet calculation technique used (Table 1). This is explained by the fact that leatherleaf croton requires the use of a correction factor for accurate estimation because of excessive fragmentation.

The frequency addition procedure did not provide the most accurate evaluation of dry weight composition for all mixtures. Mixture six (Table 2) demonstrates that when an overestimated species is a minor mixture component, the density conversion procedure actually gives the most accurate results. This is because the Fracker and Brischle (1944) conversion increases the density of a species with a high frequency to a greater extent than for a species with a lower frequency. Conversely mixtures three and nine demonstrate how the Fracker and Brischle (1944) conversion can severely reduce accuracy when the overestimated species is a major mixture component. The higher accuracy of the frequency conversion procedure is explained by the fact it reduces overestimation of species with easily identifiable fragments and/or that fragment excessively during sample preparation. This source of error is magnified by the actual density procedure. The density conversion procedure either magnifies or reduces this source of error depending on whether the frequency of the overestimated species is low or high in relation to other species in the mixture.

Our results indicate that the frequency addition procedure gives equal or better accuracy than the frequency to density conversion or the actual density procedure for calculating relative percent dry weight composition. It has the additional advantage of being much quicker and more simple.

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