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Evaluation of microhistological analysis for determining ruminant diet botanical composition

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Abstract

The accuracy of microhistological techniques for analysis of herbivore diets was evaluated with cattle, sheep, and Angora goats fed grass, forb, and shrub mixtures of known botanical compositions. Two observers performed microhistological analyses on undigested diets as offered and on feces collected. Similarity indices and chi-square tests were used to determine if differences existed among actual diets, estimated diets, and fecal samples. Botanical compositions of diets fed to all 3 animal species generally were accurately estimated by fecal analyses. In some other studies, shrubs in ruminant diets have been inaccurately estimated by the microhistological technique. However, in our study, shrubs were accurately estimated with no differences between actual and observed compositions. We attribute this to the fact that shrub materials used in our study had a high proportion of current growth relative to woody materials. Woody plant parts had lower proportions of identifiable epidermal material than leaves and young stems. In grass-forb diets, forbs sometimes were overestimated and differentiation among grasses was difficult. However, in most cases, observers could precisely estimate diets of the 3 herbivore species.

Key Words: cattle, sheep, Angora goats, food habits, feces, grasses, forbs, shrubs

In the past 50 years, the microhistological technique introduced by Baumgartner and Martin (1939) has been widely used to determine range herbivore diet botanical composition. The accuracy of this technique was tested by Sparks and Malechek (1968) and found satisfactory. They reported a nearly 1:1 correspondence between relative density of species fragments and the actual percentage composition by weight of hand-compounded diets. However, other researchers report this relationship does not occur for all forages (Holechek and Valdez 1985a, 1985b). Some factors that have a confounding effect on the accuracy of the microscopic technique include (1) differential digestibility of plant species (Stewart 1970, Slater and Jones 1971, Vavra et al. 1978, McInnis et al. 1983); (2) presence of woody materials (Holechek and Valdez 1985a, 1985b); (3) observer errors (Holechek et al. 1982); (4) procedures used in calculating the diet botanical composition (Holechek and Gross 1982b); and (5) sample preparations (Vavra and Holechek 1980, Holechek et al. 1982). The objective of our study was to evaluate the accuracy of the microhistological technique for analysis of fecal samples from cattle, sheep, and Angora goats fed several known diets containing grasses, forbs, and shrubs.

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Methods

The diets and fecal samples used in this study were obtained from various nutritional studies (Nunez-Hernandez 1987, Tembo 1987, Rafique 1988, Saiwana 1988, Arthun 1989, Boutouba 1989, Vernet 1989). Species used in the diets include blue grama (*Bouteloua gracilis* (H.B.K.) Lag.), barley straw (*Hordeum vulgare* L.), leatherweed croton (*Croton corymbulosus* Engelm.), scarlet globemallow (*Sphaeralcea coccinea* (Pursh) Rydb.), alfalfa (*Medicago sativa* L.), four-wing saltbush (*Atriplex canescens* (Pursh) J.T. Howell), juniper (*Juniperus monosperma* (Engelm.) Sarg., mountain mahogany (*Cercocarus montanus* Raf.), oak (*Quercus turbinella* Greene), and sagebrush (*Artemisia tridentata* Nutt.). The actual composition of the diets offered to the 3 species of herbivores is shown in Table 1. Diets were composited from forages ground through a 254-mm screen to minimize animal selection. Diets and fecal samples were dried and ground with a Wiley mill to pass a 1-mm screen. A composite sample of the ground fecal material was made for each diet in each experiment. Slide preparations followed the method of Sparks and Malechek (1968) as modified by Holechek (1982). The ground material for each sample was soaked in hot water for 10 minutes, followed by bleach (sodium hypochlorite) for 5 minutes to remove plant pigments. The bleached sample was rinsed with warm water until the bleaching agent smell was eliminated. Five microscope slides were prepared for each composite diet and fecal sample. Hoyer's mounting solution was used to mount cover slips on the slides. The slides were air dried for 5 to 7 days before analysis. Reference slides were made in the same manner from plant species used in compositing diets.

Two observers, trained by the procedures of Holechek and Gross (1982a), analyzed both diet and fecal samples using Nikon binocular microscopes. Samples were analyzed at 100X, although 200X magnification was sometimes used for higher resolution (Holechek and Valdez 1985a). Systematically selected fields were observed on each slide and species were recorded as being present or absent until a total of 100 frequency observations were recorded. The frequency addition procedure described by Holechek and Gross (1982b) was used to calculate the percentage composition by weight. A mean was calculated for each sample from the 2 observers' readings and was taken to represent estimates for the diet and fecal samples.

Similarity indices were calculated using Kulcyznski's formula (Oosting 1956). These indices were used to show (1) similarity between observed and actual diets (control), (2) similarity between feces and actual diets, (3) similarity between observed diets and feces, and (4) similarity between observers. A chi-square test (Steel and Torrie 1980) determined whether there were differences among each of the 4 comparisons mentioned above. Plant species were

Table 1. Percent compositions by weight of actual diets, observed diets, and feces of cattle, sheep, and Angora goats.

Diet[1]	Diet No.	Actual diet	Observed diet	Observed feces
		(%)		
CATTLE				
Barley straw	1	58	53	55
Alfalfa		42	48	45
Blue grama	2	59	55	47
Shrubs[2]		41	45	53
Blue grama	3	58	35	35
Forbs[2]		42	65	65
Blue grama	4	77	71	66
Alfalfa		23	29	35
Barley straw	5	37	34	35
Forbs[2]		63	67	66
Barley straw	6	38	38	39
Shrubs[2]		62	63	62
SHEEP				
Blue grama	7	70	56	60
Barley straw		30	45	41
Blue grama	8	58	35	39
Forbs[2]		42	65	61
Blue grama	9	59	55	51
Shrubs[2]		41	45	49
Barley straw	10	77	71	63
Alfalfa		23	29	37
Blue grama	11	58	53	57
Alfalfa		42	48	43
Barley straw	12	37	34	41
Forbs[2]		62	63	60
Barley straw	13	38	38	41
Shrubs[2]		62	63	60
Blue grama	14	29	26	36
Barley straw		58	50	48
Alfalfa		13	25	17
Blue grama	15	26	29	25
Barley straw		54	34	32
Forbs[2]		20	38	44
Blue grama	16	26	22	27
Barley straw		54	50	52
Shrubs[2]		20	28	22
ANGORA GOATS				
Barley straw	17	96	72	73
Blue grama		4	28	28
Barley straw	18	83	73	68
Blue grama		17	27	32
Barley straw	19	68	73	58
Blue grama		32	27	42
Barley straw	20	15	25	25
Blue grama		85	76	76
Blue grama	21	87	83	77
Alfalfa		13	17	23
Blue grama	22	65	70	67
Barley straw		5	6	5
Alfalfa		30	25	29
Barley straw	23	45	37	42
Blue grama		25	30	30
Four wing		30	33	28
Barley straw	24	15	18	20
Blue grama		55	52	48
Juniper		30	31	33
Barley straw	25	65	63	58
Blue grama		5	2	8
Mt. Mahogany		30	35	34
Barley straw	26	45	35	38
Blue grama		25	32	26
Winterfat		30	34	37
Barley straw	27	20	25	22
Blue grama		50	45	48
Oak		30	30	30
Barley straw	28	40	38	39
Blue grama		30	35	33
Sagebrush		30	28	29

[1]Botanical compositions of some diets do not add to 100 because of rounding off.
[2]Forbs = Leatherweed croton + scarlet globemallow (1:1).
[2]Shrubs = Fourwing saltbush + Mountain mahogany (1:1).

also segregated into grasses, forbs, and shrubs for tests of differences among forage classes. Scientific names of plants followed Mabberley (1987) and a field guide by Allred (1988).

Results and Discussion

Results from cattle (Table 2) show high average similarities (93, 92, and 98%) for actual/observed, actual/feces, and observed/feces, respectively. Observed values of diets were similar to actual values ($P>0.05$), except for the blue grama + forbs diet. Forbs (leatherweed croton, scarlet globemallow) were overestimated and blue grama was underestimated in this particular diet. Both observers in our study and in Holechek et al. (1982) found leatherweed croton scarlet globemallow easy to identify because of presence of stellate trichomes in most fields, which may explain why forbs were overestimated. Fecal compositions differed ($P<0.05$) from actual compositions in blue grama + forbs, blue grama + shrubs, and blue grama + alfalfa diets. These differences were the results of overestimating shrubs, forbs, and alfalfa in the feces, which in turn caused underestimation of blue grama. The epidermal distribution of cutin differ among the plant species (Storr 1961). This difference in the distribution of cutin has been attributed to the observed difference in digestibility of plant species. We believe digestion may have caused the shrubs, forbs, and alfalfa to break into clusters of cells and hairs or trichomes, which increased their presence relative to blue grama. However, we attribute the accuracy problem to observer error rather than differential digestibility because estimates of feces were highly (98%) similar to those of observed diets.

Results from sheep (Table 2) were similar to those from cattle. Among the diets with differences, forbs, (leatherweed croton, scarlet globemallow) and alfalfa were overestimated, while grasses (blue grama, barley straw) were correspondingly underestimated (Table 1). Forbs were overestimated in the sheep diets for the same reasons suggested for the cattle diets. Leatherweed croton, scarlet globemallow, and other species with dense stellate hairs or trichomes can be overestimated because these parts are easy to identify (Slater and Jones 1971, Sanders et al. 1980, Holechek et al. 1982). There was no consistent trend for over or underestimation of individual grass species in diets with 2 or more grass species. In some diets, blue grama and barley straw were overestimated because of the difficulty in distinguishing between the 2 species. The shape, size, and arrangement of the ordinary epidermal cells for the 2 grasses were about the same. Our observers noted that a better feature for distinguishing blue grama from barley straw was stomatal cells. Stomata cells within each species are much more constant in size and shape than ordinary epidermal cells (Storr

Table 2. Percent similarity between botanical composition of actual diets and from observed diets and feces of cattle, sheep, and Angora goats. (See Table 1 for diet compositions).

CATTLE Diet	1	2	3	4	5	6	—	—	—	—	—	—	Mean
							%						
Actual vs observed	95	95	77**	94	99	100	–	–	–	–	–	–	93
Actual vs feces	97	88**	77**	88**	100	100	–	–	–	–	–	–	92
Observed vs feces	98	92	100	95	100	100	–	–	–	–	–	–	98
SHEEP Diet	7	8	9	10	11	12	13	14	15	16	—	—	Mean
							%						
Actual vs observed	86**	77**	96	94	95	97	100	89**	80**	92	–	–	91
Actual vs feces	90*	81**	92	86**	99	96	98	100	77*	98	–	–	92
Observed vs feces	97	96	96	92	96	93	98	91	95	99	–	–	95
ANGORA GOATS Diet	17	18	19	20	21	22	23	24	25	26	27	28	Mean
							%						
Actual vs observed	76**	90**	95	91**	96	95	92	97	97	90	95	96	93
Actual vs feces	77**	85**	90*	91**	90	99	95	93	93	93	98	98	92
Observed vs feces	99	95	85**	100	94	97	95	97	94	95	97	99	96

*Significantly different ($P<0.05$) using chi-square test.
**Significantly different ($P<0.01$) using chi-square test.

1961). However, in some instances, it is impossible to separate 2 or more grass species on the basis of their epidermal features alone (Dabo et al. 1986). Sheep data showed a high average similarity (95%) between observed diets and feces.

Angora goat data were consistent with that for cattle and sheep. High average similarity indices (93, 92, and 96%) were exhibited for actual/observed diets, actual diets/feces, and observed diets/feces, respectively. Based on these data, digestion has little or no influence on proportions of identifiable plant fragments. Our results are in contrast to those reported by Slater and Jones (1971), Vavra et al. (1978), Smith and Shandruk (1979), Leslie et al. (1983) and McInnis et al. (1983). They reported reduced fecal estimates for some plant species, especially forbs because of differential digestibility. However, our findings support results from Free et al. (1970), Todd and Hansen (1973), Anthony and Smith (1974) and Dearden et al. (1975). They analyzed feces of other ruminants with considerable success. We attribute the accuracy of our results to (1) systematic training of observers (Holechek and Gross 1982a), which accounted for correct identification of the plant species in most of the diets; (2) use of bleach as a blending medium (Holechek 1982, Hinnant and Kothmann 1988) reduced pigment masking of epidermal fragments and increased the percentage of identifiable epidermal fragments; (3) use of actively growing perennial plants with a high proportion of epidermal material, which allowed for complete recovery of epidermal parts; and (4) use of the frequency addition procedure (Holechek and Gross 1982b), which provided reliable representation of dry weight composition.

Table 3. Overall comparison of actual diets, observed diets, and feces on forage class basis using chi-square test.

	Grasses	Forbs	Shrubs
CATTLE			
Actual vs observed	NS	**	NS
Actual vs feces	*	**	NS
Observed vs feces	NS	NS	NS
SHEEP			
Actual vs observed	**	**	NS
Actual vs feces	**	**	NS
Observed vs feces	NS	NS	NS
ANGORA GOATS			
Actual vs observed	**	NS	NS
Actual vs feces	**	*	NS
Observed vs feces	NS	NS	NS

*Significantly different ($P<0.05$).
**Significantly different ($P<0.01$).
NS = No significant difference ($P>0.05$).

Table 3 compares actual diets, observed diets, and feces on forage class basis. Shrubs were accurately estimated in diets of all 3 ruminants. A high proportion of stemmy materials in a browse diet is reported to cause underestimation of shrubs (Holechek and Valdez 1985a). Browse fed to cattle, sheep, and Angora goats in our study involved leaves and twigs from current growth, which, unlike old stems, have a high proportion of epidermal material. Differences in grasses and forbs reflect overestimation of leatherweed croton and scarlet globemallow, which reduced the grass estimates.

Comparison (Table 4) between the 2 observers revealed their observations were similar for all 3 ruminant species, although minor differences occurred for some diets because of problems in differentiating among grass species. In general, high precision was achieved by the 2 observers. We attribute this to the fact they were systematically trained in procedures of Holechek and Gross (1982a). Westoby et al. (1976) also stressed that accuracy of microscopic analysis of diets depends on systematic training of observers.

Based on data from 3 ruminant species (cattle, sheep, and goats) fed diverse diets, accuracy of fecal analysis is little influenced by differential digestion of plant species. Fecal estimates generally represent the diets offered. Our data substantiates that microscopic

Table 4. Percent similarity between observer 1 and 2 for observed diets and feces of cattle, sheep, and Angora goats.

CATTLE Diet	1	2	3	4	5	6							Mean
							%						
Observer 1 vs 2 (Observed)	91	90	100	98	100	95	–	–	–	–	–	–	96
Observer 1 vs 2 (Feces)	86*	92	100	91	97	95	–	–	–	–	–	–	94
SHEEP Diet	7	8	9	10	11	12	13	14	15	16	–	–	Mean
							%						
Observer 1 vs 2 (Observed)	83*	100	90	98	91	100	95	93	85	98	–	–	93
Observer 1 vs 2 (Feces)	83*	98	98	80**	90	96	93	85	84*	96	–	–	90
ANGORA GOATS Diet	17	18	19	20	21	22	23	24	25	26	27	28	Mean
							%						
Observer 1 vs 2 (Observed)	54**	78**	100	85*	94	96	90	87	100	86	86	86	87
Observer 1 vs 2 (Feces)	55**	85*	74**	89	100	95	96	85*	92	99	90	92	88

*Significantly different ($P<0.05$) using chi-square test.
**Significantly different ($P<0.01$) using chi-square test.

fecal analysis can be a useful tool to estimate ruminant diet botanical composition. Accurate estimates of the diet composition of large herbivores depend on systematic training and adequate practice by the observers. The microscope technique can only be a useful tool if observers have a 90% or more recognition level of the plant species being examined, and if they become careful not to overestimate species with stellate trichomes or hairs. Identification of such species should be based only on those trichomes attached to recognizable epidermal tissues and cell pattern and/or stomata pattern on peridermal tissues.

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