Botanical composition of mature ewe diets in the Kansas Flint Hills

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Transl. Anim. Sci. 2018.2:S167–S172
doi: 10.1093/tas/txy037

INTRODUCTION

Microhistological analysis of feces has several advantages over alternative techniques when ascertaining the botanical composition of herbivore diets: it does not require animal sacrifice or surgical alteration; the number of samples collected is limited only by analytical cost and time; it requires little interaction between researcher and animal; and it does not interfere with normal animal grazing habits and movements (Vavra and Holechek, 1980; Holechek et al., 1982; McInnis et al., 1983).

Sericea lespedeza (**SL**) is a noxious weed that threatens the biotic integrity of the tallgrass prairie in Kansas and Oklahoma (Eddy and Moore, 1998). Biological control using targeted grazing with sheep following traditional yearling-cattle grazing, effectively controlled vegetative propagation, and seed production by SL (Lemmon et al., 2017). Compared with beef cattle, sheep appeared to be more accepting of SL and more tolerant of its condensed-tannin content (Terrill et al., 1989; Frutos et al., 2004; Lemmon et al., 2017); however, few direct comparisons of condensed-tannin tolerance exist. In this experiment, we evaluated mature ewe selection of 17 common graminoid, forb, and shrub species previously identified as being significant components of ruminant diets in the tallgrass prairie region of the United States (Aubel et al., 2011; Preedy et al., 2013). The objectives for this experiment were to 1) characterize mature ewe diets grazing SL-infested rangeland in the Kansas Flint Hills and 2) identify patterns of discrimination by mature ewes in selection of dietary components on native tallgrass prairie.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee reviewed and approved all animal handling and animal care practices used in our experiment. All animal procedures were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010).

Our study was conducted in Woodson County, KS during the growing seasons of 2015 and 2016 on the Kansas State University Bressner Range Research Unit. Four native tallgrass pastures (30 ± 1.2 ha) infested with SL (initial basal frequency = 1.9 ± 1.39%) were grazed by mature ewes at a relatively high stocking density (0.15 ha per ewe) from 30 July to 1 October during 2015 and 2016, immediately following grazing with yearling beef cattle.

Ewes (n = 813; initial BW = 65 ± 3.1 kg) were leased from two commercial sheep operations located in western Kansas and transported via motor carrier to the research site each year (arrival date = 30 July). Ewes were weighed collectively by pasture groups before grazing began on 1 August and assigned randomly to graze one

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Received March 16, 2018.
Accepted April 14, 2018.

of four pastures. Twenty-five individual ewes were selected randomly from each pasture group at the outset of each grazing season to monitor diet composition. On 15 August and 15 September annually, all ewes in each pasture were gathered in a central corral. Individual ewes selected for diet composition analysis were sorted from the group and restrained for fecal grab sampling. Samples were placed in individual plastic containers and frozen (−20 °C) pending processing. Subsequently, individual fecal samples were dried in a forced air oven (55 °C; 96 h) and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) to a 1-mm particle size.

Ewes were weighed collectively by pasture groups at the end of the grazing season (i.e., 1 October annually). Final BW of ewes averaged 71 ± 3.6 kg. Ewes were monitored daily during the grazing period to assure they remained in assigned pastures and that fresh water was available continually. Death loss was 1.6 ± 0.22% annually and was assumed to occur through predation or disease.

Plant species composition and soil cover were assessed along two permanent transects in each pasture on 15 October ± 10.4 d in 2014 (i.e., pretreatment), 2015, and 2016 (i.e., posttreatment) using a modified step-point technique (Farney et al., 2017). Transect points (n = 100 per transect) were evaluated for bare soil, litter, or basal plant area (% of total area). Plants were identified by species; basal cover of individual species was expressed as a percentage of total basal plant area.

Approximately 59% of total basal vegetation cover on pastures used in our experiment was composed of the following forage species: big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), switchgrass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), blue grama (*Bouteloua gracilis*), side-oats grama (*Bouteloua curtipendula*), buffalo grass (*Bouteloua dactyloides*), sedges (*Carex* spp.), purple prairie clover (*Dalea purpurea*), leadplant (*Amorpha canescens*), dotted gayfeather (*Liatris punctata*), heath aster (*Symphyotrichum ericoides*), SL (*Lespedeza cuneata*), Baldwin's ironweed (*Vernonia baldwinii*), Western ragweed (*Ambrosia psilostachya*), annual broomweed (*Amphiachyris dracunculoides*), and common ragweed (*Ambrosia artemisiifolia*).

Reference standards for each above-named plant species were prepared using methods described by Holechek et al. (1982). Individual reference standards were derived by hand-clipping 10 to 20 individual plants from a homogenous stand of each plant type. Samples included vegetative stems, leaves, and flowers; fruiting culms were discarded. Samples were dried in a forced air oven (55 °C; 96 h) and then ground to a 1-mm particle size in a cyclone-style sample mill (model no.80335R, Hamilton Beach, Glen Allen, VA).

Individual fecal samples and reference standards were prepared for microhistological analysis using methods as described by Holechek et al. (1982), as adapted by Preedy et al. (2013). Approximately 1 g of individual fecal sample or reference standard was placed into a beaker and soaked overnight in 50% EtOH (v/v). After soaking, ethanol was decanted, and residue was washed with deionized H_2O over a No. 200 U.S.-standard sieve. Samples were then soaked in 0.05 M NaOH for 20 min and washed again with deionized H_2O for 5 min over a No. 200 U.S.-standard sieve.

Wet samples were placed onto microscope slides (five slides per fecal sample and three slides per reference standard) using a dissecting needle. Two to three drops of Hertwig's solution were applied to mounted samples, and slides were held over a propane flame until dry. Hoyer's solution was not used to permanently fix slide-mounted samples. The addition of Hoyer's solution and glass coverslips diminished plant fragment visibility. Slides were observed using a compound microscope (DC5-163, Thermo Fisher Scientific, Asheville, NC) at 100× magnification. The microscope was equipped with a digital camera; 20 randomly selected fields from each fecal-sample slide and each reference-standard slide were photographed and stored (Preedy et al., 2013).

Observers of microscopically photographed images were trained using methods described by Holechek and Gross (1982). Observers viewed photos of reference standards until establishing familiarity with the structural characteristics of each plant. Observers were able to view reference-standard photographs simultaneously with fecal-sample slide photographs for reference. Plant fragments were individually identified and counted within each selected slide field. The total number of occurrences of each plant species on a given slide were converted to frequency of occurrence (i.e., [total of individual species/total of all species] × 100; Holechek and Vavra, 1981). Plant fragment prevalence in slide fields was assumed to be equivalent to prevalence in fecal samples and equivalent, on a percentage basis, to botanical composition of the diets selected by mature ewes (Sparks and Malechek, 1968). Fragments not identifiable as one of the 17 range-plant species collected for use as reference standards were classified collectively as either unidentified graminoids or unidentified forbs.

Mean basal frequencies, standard deviations, minimum basal frequencies, and maximum basal frequencies of bare soil, litter, total basal vegetation, graminoids, forbs, shrubs, and individual plant species were calculated using the PROC MEANS procedure (SAS Inst. Inc., Cary, NC). Values were summarized across pastures and year of our experiment.

The percentages of bare soil, litter cover, total basal vegetation cover, graminoid basal cover, forb basal cover, shrub basal cover, and basal cover of individual plant species were analyzed as a completely randomized design using a mixed model (SAS Inst. Inc.). Class variables were year, pasture, and transect. The model contained a term for pasture only, and transect within pasture was used as a random term. Least squares means were considered different when protected by a significant *F*-test ($P \le 0.05$).

Diet composition data were analyzed as a completely randomized design using the PROC MIXED procedure of SAS (SAS Inst. Inc.). Class variables included year, period, pasture, and sheep. The model contained terms for period, pasture, and the two-way interaction. Sheep within year and pasture and period within year and pasture were considered random effects.

Pasture × period effects on diet selection patterns of mature ewes were not detected ($P \ge 0.27$; data not shown) for all 17 plant species standards, total graminoids, unidentified graminoids, total forbs, and unidentified forbs. Pasture effects on selection patterns for 16 of the 17 plant species reference standards, total graminoids, unidentified graminoids, total forbs, and unidentified forbs were not detected ($P \ge 0.08$; data not shown); however, pasture effects on selection of heath aster ($P = 0.01$) were detected. Pair-wise comparisons of pasture means for heath aster selection (1.8, 0.7, 1.2, and 0.8 ± 0.20% of mature ewe diets for pastures 1, 2, 3, and 4, respectively; data not shown) indicated atypically high ($P \le 0.03$) selection in pasture 1 compared with pastures 2, 3, and 4. The influence of that effect on the outcome of our experiment was judged to be inconsequential. Therefore, period means for selection patterns of 17 range-plant reference standards, total graminoids, unidentified graminoids, total forbs, and unidentified forbs were reported. When protected by a significant *F*-test ($P \le 0.05$), period means were separated using the method of Least Significant Difference.

Kulcyznski's Similarity Index (**KSI**; $[(2c_i)/(a_i + b_i)] \times 100$, where a_i is the % basal cover of component *i*, b_i is the % of component *i* selected by an herbivore, and c_i is the lesser of a_i *and* b_i) was used to evaluate mature ewe diet selection patterns in relation to botanical composition of pastures. For the purposes of our analysis, we assumed that KSI values ≥80% indicated little or no discrimination (i.e., selection patterns were very similar to plant availability), that KSI values between 21% and 79% indicated moderate discrimination, and that KSI values ≤20% indicated either strong preference for or avoidance of individual plant species. When KSI values were ≤20%, preference and avoidance were distinguished from one another by comparing the proportion of the specific plant in yearling-steer diets with basal cover of the specific plant on pastures.

RESULTS AND DISCUSSION

Proportions of bare soil, litter, and total basal vegetation cover were not different ($P \ge 0.85$) between pastures. Total basal vegetation cover attributable to graminoids, forbs, and shrubs were also not different ($P \ge 0.55$) between pastures (data not shown).

Proportions of total graminoids, big bluestem, little bluestem, side-oats grama switchgrass, Indian grass, blue grama, buffalo grass, sedges, unidentified graminoids, total forb and forb-like plants, purple prairie clover, leadplant, heath aster, SL, Baldwin's ironweed, western ragweed, annual broomweed, common ragweed, and unidentified forbs were not different ($P \ge 0.07$) between pastures (Table 1). Dotted gayfeather was not detected in our analysis of plant species composition.

The proportions of total graminoids and total forb and forb-like plants (i.e., all forbs plus leadplant) in the diets of grazing ewes were not different ($P = 0.67$) between sampling periods and were interpreted to indicate that mature ewe diets during late summer were not strongly dominated by either graminoids (57.4% and 58.4% of diets for mid-August and mid-September, respectively) or forbs (42.6% and 41.6% of diets for mid-August and mid-September, respectively; Table 2). Hofmann and Stewart (1972) indicated that intermediate feeders, such as sheep, should be expected to select diets that are approximately 50% grasses and 50% forbs. Our results generally support that assertion; however, graminoids made up slightly more than half of sheep diets in our experiment.

Most researchers who used fecal microhistology to describe botanical composition of sheep diets reported graminoid-to-forb proportions that were substantially different from the idealized ratios proposed by Hofmann and Stewart (1972). We concluded that environmental factors that influence the relative availabilities of graminoids, forbs,

Table 1. Basal cover of forage plants detected in the diets of mature ewes grazing native tallgrass pastures during August and September in 2015 and 2016

Item	Mean	SD	Minimum	Maximum	SEM†	*P* value‡
Total graminoids	88.7	4.25	80.0	96.0	3.29	0.75
Andropogon gerardii	12.6	5.24	5.0	22.0	4.88	0.68
Schizachyrium scoparium	6.7	5.43	*tr*	25.0	3.94	0.61
Panicum virgatum	5.2	2.95	*tr*	11.0	1.56	0.21
Sorghastrum nutans	6.9	2.93	2.0	15.0	1.65	0.20
Bouteloua gracilis	0.3	0.68	*tr*	3.0	0.44	0.72
Bouteloua curtipendula	4.1	3.67	*tr*	16.0	2.38	0.15
Bouteloua dactyloides	0.1	0.20	*tr*	1.0	0.12	0.48
Carex spp.	14.8	4.93	6.0	26.0	2.51	0.15
Unidentified graminoids	38.0	9.22	24.0	55.0	4.57	0.10
Total forb and forb-like	11.3	4.23	4.0	20.0	3.27	0.76
Dalea purpurea	0.1	0.14	*tr*	0.5	0.11	0.44
Liatris punctata¶	—	—	—	—	—	—
Amorpha canescens	0.3	0.25	*tr*	1.0	0.20	0.53
Symphyotrichum ericoides	1.2	1.19	*tr*	3.9	0.69	0.54
Lespedeza cuneata	2.8	2.31	0.2	8.4	1.59	0.33
Vernonia baldwinii	0.5	0.58	*tr*	1.9	0.57	0.46
Ambrosia psilostachya	1.6	0.97	0.2	3.6	0.45	0.07
Amphiachyris dracunculoides	1.1	2.27	*tr*	8.0	2.38	0.53
Ambrosia artemisiifolia	0.3	0.34	*tr*	1.2	0.18	0.29
Unidentified forbs	3.4	2.01	0.6	3.4	0.99	0.11

†Mixed model SEM associated with comparison of pasture main effect means.

‡Mixed model *P* value associated with pasture *F*-test.

¶Basal cover of *Liatris punctata* was below the detection limits of the plant species composition survey technique used in this experiment; however, it was detected in steer fecal material.

Table 2. Botanical composition of mature ewe diets in the Kansas Flint Hills: period effects

	Botanical composition (% of diet DM)			
Item	Mid-August	Mid-September	SEM†	*P* value‡
Total graminoids	57.4	58.4	2.13	0.67
Andropogon gerardii	11.9	9.3	1.76	0.23
Schizachyrium scoparium	20.5	20.0	1.31	0.76
Panicum virgatum	4.6	3.1	0.55	0.06
Sorghastrum nutans	5.8	5.6	1.10	0.81
Bouteloua gracilis	6.5	8.6	1.05	0.12
Bouteloua curtipendula	1.0	0.9	0.19	0.53
Bouteloua dactyloides	4.8	7.9	0.60	<0.01
Carex spp.	1.8	2.0	0.40	0.55
Unidentified graminoids	0.7	1.0	0.18	0.17
Total forb and forb-like	42.6	41.6	2.13	0.67
Dalea purpurea	12.2	12.1	1.33	0.90
Liatris punctata	2.3	2.7	0.49	0.54
Amorpha canescens	0.4	0.3	0.10	0.70
Symphyotrichum ericoides	1.0	1.2	0.13	0.22
Lespedeza cuneata	1.5	1.6	0.20	0.45
Vernonia baldwinii	11.3	11.1	1.04	0.89
Ambrosia psilostachya	5.3	4.6	0.54	0.26
Amphiachyris dracunculoides	0.2	0.1	0.08	0.19
Ambrosia artemisiifolia	7.8	7.3	1.28	0.90
Unidentified forbs	0.9	0.6	0.09	0.04

†Mixed model SEM associated with comparison of pasture main effect means.

‡Mixed model *P* value associated with pasture *F*-test.

and shrubs likely play a more significant role in diet selection by sheep than specialized anatomical or digestive features. Intermediate feeders, such as sheep, are postulated to be adaptable to diet regimens of grass-and-roughage eaters and concentrate selectors. The weight of evidence seems to indicate this hypothesis has merit.

Selection of big bluestem, little bluestem, switchgrass, Indian grass, blue grama, side-oats grama, sedges, and unidentified graminoids was not influenced ($P \geq 0.06$) by sampling period (Table 2). Conversely, ewe selection of buffalo grass nearly doubled ($P < 0.01$) between mid-August and mid-September. Selection of forbs was similarly consistent between sampling periods. Proportions of purple prairie clover, dotted gayfeather, lead plant, heath aster, SL, Baldwin's ironweed, Western ragweed, annual broomweed, and common ragweed in ewe diets did not change ($P \geq 0.19$) between mid-August and mid-September. Selection of unidentified forbs, however, decreased ($P = 0.04$) between mid-August and mid-September. Unidentified grasses and unidentified forbs were detected in only small amounts in mature ewe diets (i.e., <1% of both graminoid and forb or forb-like plant fragments). We concluded that the 17 standards that we chose for microhistological characterization of ewe diets were sufficient to allow other researchers evaluating sheep diets in the tallgrass prairie region to describe a large majority of diet components. Notably, mature ewes selected 1.5% SL in mid-August and 1.6% SL in mid-September. Lemmon et al. (2017) reported that this level of consumption was associated with significant depressions in seed production by SL and reductions in SL basal cover compared with pastures not grazed by sheep during August and September.

Kulcyznski's Similarity Index (**KSI**) was used to compare botanical composition of pastures with botanical composition of mature ewe diets to evaluate the level of discrimination mature ewes exercised in selecting diet components (Table 3). Forage plants that were consistently selected in proportion to their availability (i.e., KSI values ≥80% during both mid-August and mid-September) in native tallgrass prairie pastures used in our experiment were big bluestem, Indian grass, lead plant, and heath aster. Switchgrass appeared also to be selected in proportion to its availability (KSI = 94% and 75% in mid-August and mid-September, respectively).

In contrast, forage plants that were consistently selected in greater proportions relative to their availabilities in native tallgrass prairie pastures were blue grama, buffalo grass, purple prairie clover, dotted gayfeather, Baldwin's ironweed, and common ragweed. The only plants or plant groups that mature ewes seemed to avoid were unidentified graminoids during both collection periods and annual broomweed during mid-September only. All other forage plants or groups of forage plants were ranked as receiving moderate selection discrimination from mature ewes. Most notable was SL (KSI = 70% and 73% in mid-August and mid-September, respectively). Alipayo et al. (1992) used KSI to compare diets of known composition fed to sheep and with estimates of diet composition derived using fecal microhistology. They indicated that actual diet composition and fecal estimates of diet composition overlapped by 92%. We concluded from our experiment that mature ewes exercised notable discrimination in diet component selection.

Table 3. KSI calculations comparing basal cover of major forage plants (Table 1) with the presence of major forage plants in fecal material of mature ewes (Table 2)

	KSI[†], % similarity	
Item	Mid-August	Mid-September
Graminoids	79	79
Andropogon gerardii	97	85
Schizachyrium scoparium	49	50
Panicum virgatum	94	75
Sorghastrum nutans	91	90
Bouteloua gracilis	9	7
Bouteloua curtipendula	39	36
Bouteloua dactyloides	4	3
Carex spp.	22	24
Unidentified graminoids	4	5
Forb and forb-like	42	43
Dalea purpurea	2	2
Liatris punctata	0	0
Amorpha canescens	86	100
Symphyotrichum ericoides	91	100
Lespedeza cuneata	70	73
Vernonia baldwinii	8	9
Ambrosia psilostachya	46	52
Amphiachyris dracunculoides	31	17
Ambrosia artemisiifolia	7	8
Unidentified forbs	42	30

[†]$KSI = ([2c_i]/[a_i + b_i]) \times 100$, where a_i is the % basal cover of component i, b_i is the % of component i selected by an herbivore, and c_i is the lesser of a_i and b_i; KSI values ≥80% were interpreted to indicate little or no discrimination (i.e., selection patterns were very similar to plant availability), values between 21% and 79% were interpreted to indicate moderate discrimination, and that KSI values ≤20% indicated either strong selection or avoidance of individual plant species.

IMPLICATIONS

Small ruminant grazing may prove beneficial in reducing stands of noxious plant species, like SL, in tallgrass prairie pastures. Mature ewes selected 1.5% SL in mid-August and 1.6% SL in mid-September during this experiment. These levels of consumption were associated with significant depressions in seed production by SL and reductions in SL basal frequency compared with pastures not grazed by sheep during August and September (Lemmon et al., 2017). Biological control through targeted grazing has promised to not only assist land managers with control of noxious plant species but also create additional revenue streams. Further research is warranted to determine the dietary overlap between yearling beef steers and small ruminants in co-grazing situations.

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