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Acute toxic plant estimation in grazing sheep ingesta and feces

MARÍA SILVIA CID, TOMÁS A. LÓPEZ, CRISTINA YAGUEDDÚ, AND MIGUEL A. BRIZUELA

Cid and Brizuela are full Professors, Facultad de Ciencias Agrarias (FCA), Universidad Nacional de Mar del Plata (UNMdP), cc 276 (7620) Balcarce, Buenos Aires, Argentina, and Research Scientists, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, respectively. López is a Research Toxicologist, Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnología Agropecuaria (INTA). Yagueddú is a Teaching Assistant, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, UNMdP.

Abstract

'Romerillo' (*Baccharis coridifolia* DC), 'duraznillo negro' (*Cestrum parqui* L'Hérit.), and 'sunchillo' (*Wedelia glauca* (Ort.) Hoff.) are highly toxic species producing important economic losses of livestock in Argentina. This study assessed the accuracy and precision in the estimation of the percentage and the mass of these species in the ingesta and feces of sheep experimentally poisoned. This study also evaluated whether the quantified percentage and the calculated mass of each toxic species in the rumen+reticulum, the easiest region to sample, are good estimates of their relative consumption. Results indicate that if species fragment density is quantified, and the percentages of non recognized fragments of the toxic species in their in vitro digestion residues are accounted for (attributing some proportion of the unidentified fragment pool to the target species), estimations are accurate, but their precision differ among species. For a 3 sheep sample, the average mass estimated by microhistological analysis represented 92.3 ± 5.8 (romerillo), 96.5 ± 17.3 (duraznillo negro), and 92.0 ± 12.5% (sunchillo) ($P < 0.10$) of the actual amount of each species consumed. The percentages of the toxic species in the total ingesta plus feces produced since the intoxication did not differ ($P > 0.05$) from those in the rumen+reticulum. For the evaluated species, the microhistological analysis of the rumen+reticulum not only confirmed the ingestion of the toxic species, but also adequately estimated the percentage in which they were ingested.

Resumen

Romerillo' (*Baccharis coridifolia* DC), 'duraznillo negro' (*Cestrum parqui* L'Hérit.), y 'sunchillo' (*Wedelia glauca* (Ort.) Hoff.) son especies de alta toxicidad que causan importantes pérdidas económicas en la producción ganadera de Argentina. Este estudio evalúa la exactitud y la precisión de la estimación por microanálisis del porcentaje y de la masa de cada una de estas especies presente en la ingesta y en las heces de ovejas intoxicadas experimentalmente. Además, analiza si los porcentajes de las especies tóxicas cuantificados en el rumen+reticulum, la región más fácil de muestrear, son buenos estimadores de su consumo relativo. Los resultados indican que si la cuantificación se realiza registrando la densidad de los fragmentos de las diferentes especies, y si los porcentajes de las especies tóxicas son corregidos de acuerdo al porcentaje de fragmentos no reconocidos en los residuos de digestiones in vitro, las estimaciones son exactas, y que su precisión varía según la especie tóxica. Con una muestra de tres animales, los porcentajes estimados por microanálisis representaron 92.3 ± 5.8 (romerillo), 96.5 ± 17.3 (duraznillo negro), y 92.0 ± 12.5% (sunchillo) ($P < 0.10$) de la cantidad suministrada en la intoxicación. Los porcentajes de las especies tóxicas en la ingesta y heces no difirieron ($P > 0.05$) de los de rumen+retículo. Para las especies evaluadas, el microanálisis del rumen+reticulo no sólo permite confirmar su ingestión, sino que además estima adecuadamente el porcentaje en el que fueron ingeridas.

Key Words: Microhistological technique, accuracy, precision, *Baccharis coridifolia, Cestrum parqui, Wedelia glauca*

The heterogeneity of ecological conditions in the Argentinean Pampas favors the growth of many valuable plant species in terms of their use as forages. These different conditions also provide optimal opportunities for the growth of a large number of poisonous plants, which yearly cause the death or severe sickness of all kinds of livestock through acute or chronic poisoning. Three of these species are romerillo (*Baccharis coridifolia* DC), duraznillo negro (*Cestrum parqui* L'Hérit.), and sunchillo (*Wedelia glauca* (Ort.) Hoff.). These species have different growth forms; duraznillo negro and romerillo are shrubs, and sunchillo is a forb. Duraznillo negro and sunchillo are usually found in areas of disturbed and fertile soils; romerillo growths in hillsides, where soils are shallow. Methods for the detection of the toxic substances in the remains of dead animals are far beyond the analytical instrumentation availability of local diagnostic laboratories. Therefore, other diagnostic methods are necessary.

In sheep fed 'annual ryegrass' (*Lolium multiflorum* Lam.)– oat (*Avena sativa* L.) hay and orally administered duraznillo negro, sunchillo or romerillo in water suspensions, microhistological analysis of the epidermal fragments in the ingesta shows that distribution of these toxic species fragments is not uniform in the digestive tract (Yagueddú et al. 1998). Therefore the percentage of a toxic species estimated in 1 of the regions of the digestive tract is not necessarily a good estimate of the percentage in

This study was carried out at the Diet Botanical Composition Laboratory at the Unidad Integrada Balcarce (FCA, UNMdP – EEA Balcarce, INTA). The authors are grateful to Marina Dosanto and María Salomón (INTA) for their technical assistance, and to Dr. M. Ralphs for his insightful comments and suggestions on drafts of this manuscript. This study was financially supported by the Agencia Nacional de Promoción Científica y Tecnológica de Argentina, CONICET and the UNMdP.

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which it was ingested. Moreover, in our field conditions, vegetation heterogeneity makes fragment recognition of the toxic species substantially more difficult than in the simpler experimental situation of the toxic species fed with a basal hay diet.

When a farmer or a veterinarian brings to the Diet Botanical Composition Laboratory a sample from the digestive content of an animal found dead in the field, he does not only want to know which toxic species the animal ate, but also the amount ingested, or at least the percentage in which it was consumed. Accordingly, the objectives of this study were: (1) to assess the accuracy and precision of microhistologial estimations of the actual amounts of the 3 mentioned poisonous species present in the ingesta of experimentally intoxicated sheep grazing a multispecies vegetation, and (2) to determine if the quantified percentages of the toxic species in the rumen+reticulum and in the feces, the most common sampling points, are good estimators of their consumption.

Materials and Methods

The study was conducted at the Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnología Agropecuaria, Balcarce, Buenos Aires province, Argentina (37°45'S, 58°18'W), and consisted of 2 trials. In trial 1 we experimentally poisoned 3 sheep with each toxic species, estimated the dried weight of each digestive tract region and that of the feces, and sampled their contents for microhistological quantification of the percentages of the different species in the diet. In trial 2 each toxic species was evaluated for the percentage of recognizable fragments before and after digestion. The percentage of recognizable fragments after digestion was used as a correction factor to account for the unrecognizable fragments. Finally, with the information obtained from both trials, we calculated the mass of the toxic species in the ingesta and feces, and evaluated the accuracy and precision of the microhistological estimations.

Trial 1 (Intoxication Trial):

In November 1996, nine Corriedale ewes grazed during 8 days in a 2 ha paddock of a naturalized pasture made up by 23 species, dominated by 'tall wheatgrass' (*Thinopyrum ponticum* (Podp.) Barkw. & D.R. Dewey), ´pasto sedilla´ (*Vulpia dertonensis* (All.) Gola), and perennial and annual ryegrass (*Lolium perenne* L. and *L. multiflorum* Lam., respectively). There were 10 other minor grasses in the pasture. Although there were 8 species of forbs (which represented approximately 10% of the total biomass), only ´oreja de ratón´ (*Dichondra mycrocalyx* (Hall.) Fabris) represented more than 2% of the available forage. The cool season grasses were in bloom. Biomass availability was estimated by cutting ten, 0.10 m^2 randomly distributed frames.

Plants of the 3 mentioned poisonous species were collected from different farms where cases of intoxication had previously occurred. The collected plant material consisted of young shoots and fully expanded leaves. At the time of harvest, romerillo plants were at the vegetative stage, and those of duraznillo negro and sunchillo were in bloom. We estimated the dry matter percentage of each toxic species (24 hours, 60° C) to calculate the amount of fresh plant material to be administered to the experimental animals. The dry matter percentages were 19.6, 20.0 and 27.0% for sunchillo, duraznillo negro and romerillo, respectively

Each toxic species was randomly assigned to a group of 3 sheep. The average animal weight of each group was: 44.8 ± 5.0, 42.0 ± 2.6 and 47.3 ± 1.5 kg for duraznillo negro, romerillo and sunchillo, respectively. Sheep were brought in from the pasture, housed in separate pens, and dosed via esophageal tube as described by Yagueddú et al. (1998). We intoxicated the sheep with the lethal doses of fresh sunchillo (1.5 g DM kg^{-1} LW; Platonow and López 1978), and romerillo (1.0 g DM kg^{-1} LW; T. López and G. Pinilla, unpublished data). However, due to the large volume of plant material required for the duraznillo negro lethal dose (10.0 g DM kg^{-1} LW; López et al. 1978), one fourth of the lethal dose was used. Administered doses were: duraznillo negro 112.2 ± 13.1, romerillo 42.0 ± 2.6, and sunchillo 71.0 ± 2.3 g DM. After dosing, water was offered ad libitum. Twenty-four hours later all sheep poisoned with romerillo and sunchillo had died, and those poisoned with duraznillo negro showed the signs of poisoning usually produced by non-lethal doses of this species. These sheep were sacrificed by electric shock.

Upon necropsy the entire content of the digestive tract regions (except rumen+reticulum), and the total amount of feces collected in each pen were individually processed for microhistological quantification as described by Yagueddú et al. (1998). Because of its large size, the manipulation of the rumen+reticulum content is cumbersome, so the quantification of the botanical composition of this region was performed on a sample composed of 15 randomly taken sub-samples of 50 g DM each. The remaining mass of the rumen+reticulum was also dried and weighed, to establish the total net weight of the digestive content of this region. Samples were individually washed with tap water over a 200 mesh screen to remove soluble endogenous substances which can coagulate when samples are dried, dried (60° C, 24 hours), weighed, and ground to pass a 1 mm screen (16 mesh) for microhistological species fragment quantification according to Sparks and Malechek (1968). Samples prepared for microhistological quantification were individually soaked 30 to 60 seconds in full strength household bleach to clear the material as suggested by Holechek et al. (1982), and washed over a 200 mesh sieve to remove the bleach and very small fragments. Small aliquots of each sample were evenly spread and mounted on 5 microscope slides using gelatine:glycerine (1:7). One of the basic assumptions of the microhistological technique outlined by Sparks and Malechek (1968) is that a 1 to 1 relationship exists between relative density of identifiable fragments ground to a uniform size and percentage of dry weight of the species in the samples. Accordingly, fragment density of the different species was counted in 100 microscopic fields as an estimation of its relative dry weight. The density of unidentifiable grasses and forbs was also recorded. In our study all the determinations were made by a 15 year experienced observer, who regularly checks her accuracy with hand compounded mixes, as recommended by Holechek and Gross (1982).

Trial 2 (Digestion Trial):

Epidermis is the only vegetal tissue whose fragments have taxonomic value and are not degraded by digestion. However, not all the epidermal fragments have cytological features that allow their identification to species level. Furthermore, digestion can reduce the percentage of the identifiable fragments (Leislie et al. 1983, Samuel and Howard 1983, Holechek and Valdez 1985). We needed an accurate estimation of the number of fragments of the toxic species in the ingesta and in the feces (recognized plus non-recognized). We also wanted to know the percentage of non-recognized fragments in the digestion residues of the toxic species that could be attributed to the lack of cytological features with taxonomic value or to the diges-

tion effect. So, we analyzed the percentage of recognizable fragments before and after in vitro digestion in three, 200 g DM samples of each toxic species collected simultaneously with the plant material used in the intoxication trial. Samples were dried for 24 hours at 60° C, ground in a Willey mill with a 1 mm sieve screen, and divided in 2 subsamples for analysis of the percentage of recognizable fragments before and after digestion. In vitro digestibility was determined by a modified Tilley and Terry (1963) procedure, reducing incubation time with rumen microbes from 48 to 24 hours, and omitting the incubation with pepsin. We stopped incubation at 24 hours because the poisonous species considered in this study cause death within this period. Rumen inoculum was obtained from a steer fed on a lucerne hay maintenance diet. After 24 hours of microbial digestion, the subsample residues were recovered by filtration, and were dried. Finally, digested and undigested samples were soaked in domestic bleach to remove plant pigments. Five slides were prepared for each sample, the numbers of recognizable and unrecognizable fragments in 20 microscope fields per slide were registered, and results were expressed as percentage of recognizable fragments before and after digestion.

Differences among species in digestibility, and in percentages of fragments recognized before and after digestion, as well as the effect of digestion on each species fragment recognition (estimated by the difference in the percentages of fragment recognized before and after digestion), were analyzed by ANOVA. Differences among means were determined by the Tukey test.

Calculation of the toxic species mass

The mass of each toxic species in the ingesta and in the feces of each sheep was estimated by accounting for both the percentage of recognizable fragments and the effect of digestion on them in 5 steps. In step 1, we calculated the corrected number of fragments (recognizable plus non recognizable) of each toxic species in each region of the digestive tract and in the feces as follows: Corrected number of $fragments_i$ = (number of recognizable $fragments_i$ / % recognized $fragments_i$ after digestion) x 100, where i represents the different digestive regions or the feces. We corrected the number of fragments estimated by microanalysis assuming that the percentage of the toxic species we could not recognize was in the pool of fragments of forbs not identifiable to species level. In step 2, the remaining fragments in this forb pool were proportionally distributed among the non-toxic forb species of the diets according to the proportion of recognizable fragments. In a similar way, the fragments of grasses not recognized to species level were proportionally distributed into the grass species in the diet. In step 3, the corrected numbers of fragments quantified in the diet of each sheep were directly expressed as corrected percentages. In step 4, we estimated the total mass (g DM) of each toxic species in each region of the gastro-intestinal tract or in the feces(i), using the following equation: $Mass_i$ = net dry $weight_i$ x corrected $percentage_i$ / 100. Finally, in step 5, the mass of all the digestive regions and the feces was added to evaluate the total mass of the toxic species and compare it to the amount administered.

Accuracy and precision

For each toxic species, we evaluated the accuracy of the corrected microanalysis by t test analyzing whether the estimated mass in the total ingesta plus the feces differed from the administered amount, and the precision by the confidence interval (90%) around the mean of the 3 sheep. Next we determined by t-test whether the percentage and the mass of each toxic species in the rumen+reticulum differed from those in the total ingesta plus feces. Furthermore, we compared the mass in the rumen+reticulum with the administered mass. We made the same test in the feces, but only for the percentages. These tests were performed with the corrected percentages (for digestion and unrecognizable fragments) and also with the uncorrected percentages.

Results and Discussion

Forage availability of the paddock grazed by the sheep before the intoxications was 3335 kg DM ha^{-1}. All the sheep consumed varied diets, consisting of 16 to 20 species. The average percentages of the main species in the diets were ryegrass (11.6%), 'Bermudagrass' (*Cynodon dactylon* (L.) Pers.) (10.9%), tall wheatgrass (10.6%), 'tall fescue' (*Festuca arundinacea* Schreb.) (10.3%), pasto sedilla (9.6%) and 'pasto miel' (*Paspalum dilatatum* Poir) (8.2%).

Dry matter digestibility differed among species, being highest in sunchillo, intermediate in duraznillo negro and lowest in romerillo (Table 1). Digestion did not affect the recognition of the fragments of duraznillo negro and sunchillo ($P > 0.05$) but strongly reduced the recognition in romerillo ($P < 0.05$). In this species, digestion caused a separation of the epidermis from the lower tissues, increasing the proportion of the non recognized fragments.

The corrected percentages of the poisonous species in the different regions of the digestive tract ranged from 0 to 15.0% for romerillo, 13.1 to 22.7% for duraznillo negro, and 9.2 to 17.6% for sunchillo (Table 2). The highest percentages of romerillo and sunchillo were in the rumen+reticulum, and that of duraznillo negro in the omasum+abomasum. The net dry weight of the rumen+reticulum was 75.5, 83.8 and 87.5% of the total mass (digesta+feces) for the sheep intoxicated with duraznillo negro, romerillo and sunchillo, respectively. In our study, sheep intoxicated with duraznillo negro received only one fourth of the lethal dose, so they were still alive after 24 hours of the ingestion. This longer digestion time may account for the lower percentage of ingesta in the rumen+reticulum.

The average concentration of duraznillo negro and sunchillo in the feces were 17.0 $\pm$ 4.2 and 10.2 $\pm$ 5.8%, respectively. We could not find fragments of romerillo in the feces of any of the 3 sheep intoxicated with this species (Table 2). The distribution of the fragments of duraznillo negro and sunchillo throughout the digestive tract and in the feces was fairly uniform, but the fragments of romerillo tended to concentrate in the rumen+reticulum.

Table 1. Average ($\bar{x}$ $\pm$ SD) dry matter digestibility, and digestion effect on fragment recognition by microhistological analysis in 3 poisonous plant species that produce acute intoxication (n = 3).

Species	Dry Matter Digestibility	Recognizable Fragments (%)	
		Before Digestion	After Digestion
Duraznillo negro	69.4 $\pm$ 0.80b	74.3 $\pm$ 7.0bA	73.2 $\pm$ 1.5cA
Romerillo	67.5 $\pm$ 0.70c	57.5 $\pm$ 7.1aA	31.0 $\pm$ 5.0aB
Sunchillo	73.0 $\pm$ 0.84a	54.7 $\pm$ 4.5aA	51.5 $\pm$ 3.0bA

[abc]For each variable, means with different low letters differ among species ($P < 0.05$).
[AB]For each species, the average percentages of recognizable fragments with different capital letters differ before and after digestion ($P < 0.05$).

Table 2. Average ($\bar{x} \pm SD$) net dry weight (g DM) of the ingesta and the feces of sheep experimentally poisoned with 3 toxic species, the percentage of these toxic species quantified by microanalysis, their calculated DM mass (g DM), and the percentage of the toxic species administered represented by the amount estimated by microanalysis (% recovered). CI = confidence interval.

	Ingesta												
	Rumen + Reticulum	Omasum + Abomasum	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum	Feces	Total	Administered (g)	Recovered (%)	90% CI
Duraznillo negro													
Net dry weight (g)	421.5 ± 40.3	7.6 ± 5.3	0.0	6.8 ± 1.0	0.0	15.3 ± 1.8	14.0 ± 9.0	8.3 ± 2.5	84.7 ± 61.2	558.2 ± 67.9			
% toxic species													
corrected (*)	20.2 ± 2.3	22.7 ± 3.5	---	16.2 ± 3.3	---	13.1 ± 2.9	18.1 ± 4.7	17.8 ± 4.3	17.0 ± 4.2	19.4 ± 2.3+			
uncorrected	14.8 ± 1.7	16.6 ± 2.6		11.9 ± 2.4		9.6 ± 2.1	13.2 ± 3.4	13.0 ± 3.1	12.4 ± 3.1	14.2 ± 1.7			
Calculated DM mass (g) of toxic species (*)	85.1 ± 1.2	1.7 ± 0.9	---	1.1 ± 0.2	---	2.0 ± 0.4	2.5 ± 1.3	1.5 ± 0.5	14.4 ± 7.7	108.3 ± 8.3	112.2 ± 13.1	96.5 ±17.3	± 16.4%
Romerillo													
Net dry weight	250.0 ± 11.0	14.3 ± 4.5	0.0	4.2 ± 5.2	0.0	3.3 ± 4.0	8.3 ± 10.2	5.3 ± 6.8	13.0 ± 6.0	298.4 ± 13.6			
% toxic species (g)													
corrected (*)	15.0 ± 1.1	4.2 ± 3.0	---	7.2 ± 7.8	---	2.4 ± 1.2	2.4 ± 2.8	0.0	0.0	12.9 ± 1.0+			
uncorrected	4.7 ± 0.3	1.3 ± 0.9		2.2 ± 2.4		0.7 ± 0.4	0.7 ± 0.9			4.0 ± 0.3			
Calculated DM mass (g) of toxic species (*)	37.5 ± 3.0	0.6 ± 0.4	--	0.3 ± 0.3	---	0.1 ± 0.1	0.2 ± 0.2	0.0	0.0	38.7 ± 3.0	42.0 ± 2.6	92.2 ± 5.8	± 5.6%
Sunchillo													
Net dry weight (g)	341.1 ± 92.6	11.0 ± 1.1	0.0	0.0	0.0	5.6 ± 3.2	12.5 ± 5.5	3.9 ± 1.8	16.0 ± 3.3	390.0 ± 99.4			
% toxic species													
corrected (*)	17.6 ± 2.9	9.2 ± 6.5	---	--	---	11.6 ± 7.0	12.0 ± 6.1	15.1 ± 9.1	10.2 ± 5.8	16.7 ± 2.4+			
uncorrected	9.1 ± 1.5	4.7 ± 3.3				6.0 ± 3.6	6.2 ± 3.1	7.8 ± 4.7	5.3 ± 3.0	8.6 ± 1.2			
Calculated DM mass (g) of toxic species (*)	60.0 ± 11.1	1.0 ± 0.8	---	---	---	0.6 ± 0.2	1.5 ± 0.3	0.6 ± 0.5	1.6 ± 1.3	65.3 ± 10.1	71.0 ± 2.3	92.0 ± 12.5	± 11.9%

(*) Corrected by the percentage of non recognizable fragments in digestion residues.
(+) Calculated as: (total DM mass of the toxic species x 100) / (total digesta + feces dry weight) . These percentages do not differ from those in rumen+reticulums.

The calculated total mass of each toxic species in the ingesta and feces was accurate (Table 2). The total mass represented 96.5 ± 17.3, 92.2 ± 5.8 and 92.0 ± 12.5% of the amount administered of duraznillo negro, romerillo and sunchillo, respectively, and these percentages did not differ from 100% ($P > 0.05$). Romerillo mass was estimated with the highest precision. The 90% confidence intervals were narrowest for romerillo (5.6%), intermediate for sunchillo (11.9%), and broadest for duraznillo negro (16.4%).

When estimations were made correcting the percentage of recognizable fragments in the digestion residues, the estimated percentages of the toxic species in rumen+reticulum did not differ ($P > 0.05$) from those estimated in the total ingesta plus the feces. Moreover, for romerillo and sunchillo, the calculated mass of each toxic species in the rumen+reticulum did not differ from either for those calculated for the total ingesta plus feces, or the administered amounts ($P > 0.05$). However it did differ for duraznillo negro ($P < 0.05$).

When corrections were not carried out, the estimations were not good for any of the species. Percentage and mass estimations without correction for rumen+reticulum were 14.8 ± 1.7, 4.7 ± 0.3, 9.1 ± 1.5%, and 62.3 ± 0.8, 11.7 ± 0.9, 30.5 ± 5.7g for duraznillo negro, romerillo and sunchillo, respectively. Differences between corrected and uncorrected values reflect the magnitudes of the lack of fragment recognition in digestion residues. They clearly show the digesta microhistological analysis is not accurate for either the percentage nor the mass of duraznillo negro, romerillo and sunchillo, unless the effect of digestion is taken into account.

The percentages of duraznillo negro and sunchillo estimated in feces did not differ from the total when they were corrected ($P > 0.05$) but they did differ when they were not ($P < 0.05$). Correction by digestion requires that when an animal is found dead in the field, samples of the suspicious poisonous plant species should be collected and an in vitro digestion performed to quantify the percentage of fragments recognized in the digestion residues.

The distribution of the fragments of duraznillo negro and sunchillo throughout the digestive tract and in the feces was fairly uniform, but the fragments of romerillo tended to concentrate in the rumen+reticulum. Passage rate of the plant particles generally increases as particle size decreases and particle density increases. Romerillo was the species with the lowest digestibility, so its particles could have been denser than those of the other 2 species. Digestion not only affects fragment identification but also in some forb and shrub species can cause their complete destruction (Holechek and Valdez 1985). In our study the fact that the estimated mass of each toxic species did not differ from the administered suggests that digestion did not totally destroy any of the fragments or that the amount of fragments totally destroyed was minimum.

Both the accuracy and precision of the microhistological technique have been broadly evaluated in different ecological systems. Holechek et al. (1984) presented

a extensive discussion on these attributes when samples come from feces or from esophageal or ruminal fistulas. It is commonly accepted that the microanalysis only provides an appropriate qualitative description of the botanical composition of range herbivores diets (Holecheck et al. 1984, Holechek and Valdez 1985). However, our results indicate that correcting by the percentage of fragment recognition after digestion, and quantifying mass according to fragment density, the microanalysis of the rumen+reticulum content is an accurate estimate of the percentage in which the toxic species considered in this study were ingested.

Literature Cited

Holechek, J.L. and B. Gross. 1982. Training needed for quantifying simulated diets from fragmented range plants. J. Range Manage. 35:644–647.

Holechek, J. and R. Valdez. 1985. Evaluation of in vitro digestion for improving botanical estimates of mule deer fecal samples. J. Mamm. 66:574–577.

Holechek, J.L., M. Vavra, and R.D. Pieper. 1984. Methods for determining the botanical composition, similarity, and overlap of range herbivore diets. *In:* National Research Council/National Academy of Science (eds.), Developing Strategies for rangeland management. Westview Press, Boulder, Colo, USA, pp 425–471.

Holechek, J.L., B. Gross, S.M. Dabo, and T. Stephenson. 1982. Effects of sample preparation, growth stage, and observer on microhistological analysis of herbivore diets. J. Wildl. Manage. 46:502–505.

Leislie, D.M., J.R. Martin, E.E. Starkey, and R.C. Slater. 1983. Correcting for differential digestibility in microhistological analysis involving common coastal plants of the Pacific Northwest. J. Range Manage. 36:730–732.

López, T.A., R. Spinelli, and J. Villar. 1978. Effects of *Cestrum parqui* L'Hérit. dosification in sheep and cattle (In Spanish). Gaceta Veterinaria 40:642–650.

Platonow, N.S. and T.A. López. 1978. *Wedelia glauca.* Studies of its toxicity. (In Spanish). Producción Animal 6:620–625.

Samuel, M.J. and G.S. Howard. 1983. Disappearing forbs in microhistological analysis of diets. J. Range Manage. 36:132–133.

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

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104–111.

Yagueddú, C., M.S. Cid, and T.A. López. 1998. Microhistological analysis of sheep gastro-intestinal content to confirm poisonous plant ingestion. J. Range Manage. 51:655–660.