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Mineral Composition of Rumen Fistula Samples Compared to Diet

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Highlight: Forage sampling using fistulated grazing animals is a generally accepted technique to measure dietary forage quality and botanical composition, but is it a satisfactory technique to evaluate dietary mineral intake? Using a variety of diets which were fed to rumen-fistulated steers, the fistula samples had relatively larger concentrations of ash, Si, Na, P, Zn, and Co ($P<0.05$) than did diet samples. Small decreases in the Mg and Ca concentrations of the fistula sample, as well as the small increases in N, K, Mn, Fe, and Mo values, were not generally different from diet concentrations. Regression equations predicting diet-mineral concentrations of all diets, given the concentration in the fistula sample, were accompanied by errors of 8 to 37% of the true value. Smaller errors can be expected when similar diets like alfalfa hay are used throughout a given study.

The use of esophageal or rumen-fistulated animals for collecting forage samples is common in range nutrition. This procedure accommodates the animals' preference for individual plants and certain plant parts. When compared with clipped or hand-plucked forage samples, fistula collected samples often will contain more crude protein and total ash, but less soluble carbohydrates. Fistula samples are also useful in determining intake and botanical composition of the grazing diet. Researchers generally conclude that fistula samples are more representative of forage consumed by the grazing animal than are hand-plucked or clipped samples (Lesperance et al. 1974).

Fistula sampling, however, introduces certain biases because of mastication, salivary additions, and rumen epithelial secretions. Additional biases associated with esophageal fistula collections may include leaching of forage nutrients through the screen-bottomed collection bag, incomplete sample collections (Hoehn et al. 1967; and Kiesling et al. 1969), and occasional rumen bolus regurgitation.

Forage samples divided for direct chemical analysis and for ingestion by the fistulated animal and retrieved for analysis showed concentration changes in some forage quality parameters associated with ingestion (Lesperance et al. 1974). Esophageal- and rumen-fistula samples were higher in fiber and lignin, but lower in nitrogen-free extract (NFE), soluble carbohydrates, and Ca. Fiber and lignin increases and NFE decreases were at least partially attributable to sample preparation. The esophageal- and rumen-fistula samples should also have higher concentrations of ash, N, P, K, Na, and Cl than do diet samples because of saliva mineral concentration (McDougall 1948). Mineral concentrations will change because of (1) a concentrating effect when some forage solubles are lost; (2) a solubilization and loss, if saliva is removed from the fistula sample by drainage, hand-squeezing, or washing; or (3) an increase from salivary or rumen epithelial sources (Cundy and Rice 1968; and Weston and Kastelic 1967).

Mineral contamination of ingested samples is proportional to the amount of saliva excreted per unit of forage dry matter and to the mineral concentration in the saliva. Salivary secretion rates are greater when cattle graze fibrous roughages than when they are fed concentrates or succulent forages (Setia et al. 1971). Cattle consuming air-dried hay secrete about 4 liters of saliva for the first kilogram dry matter consumed (Lesperance et al. 1974). The extent of mineral enrichment of either esophageal- or rumen-fistula forage samples should be known before these samples are used in determining the mineral status of the grazed forage.

Our objective in this study was to determine the accuracy with which the dietary mineral concentration could be predicted from measuring the concentration in the rumen-fistula sample.

Methods and Procedure

We used the rumen evacuation technique (Lesperance et al. 1974) to sample several feeds fed to three rumen-fistulated yearling steers that were maintained on good quality grass hay (not analyzed) and they experienced several tests per day with a minimum of 1.5 hours between tests. Animals did not have access to drinking water during the test period. Before sampling, the entire rumen-reticulum contents were removed and the sides of the rumen cleaned by downward strokes of the hand. The rumen-reticulum was washed with water, and the water then evacuated. We used this technique because experimental animals were available and because recovery of diet samples were incomplete when using the esophageal-fistula.

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Table 1. Mineral composition of three diet groups before and after ingestion and the error associated with $\hat{Y}$ when the mineral composition of the diet (dependent variable) is regressed on the rumen sample concentration.

Element	Mineral concentration in diet sample (mg/kg)[1]			Mineral enrichment in rumen sample (%)[1]			Prediction error associated with $\hat{Y}$ of sample diet (± %)[1, 2]		
	All diets	Alfalfa hay	Grass hay	All diets	Alfalfa hay	Grass hay	All diets	Alfalfa hay	Grass hay
Ash	90,000	82,000	85,000	24**[3]	40**	19	12	2	6
Silica	27,000	28,000	42,000	12*	17*	–0.6	18	27	8
N	26,000	27,000	17,000	1	–2	1	8	6	3
Na	1,680	66	130	640**	1,400**	570**	26	8	43
K	15,900	17,000	16,100	7*	4	–2	12	7	27
Mg	2,540	2,750	2,470	–7*	–3	–21	11	3	16
Ca	16,600	16,800	12,700	–2	–1	–3	12	7	3
P	2,600	1,980	1,270	47**	92**	52*	37	4	20
Mn	29	14	57	5	15**	–13	21	5	16
Fe	270	130	160	19	30	5	25	1	23
Zn	27	15	12	22**	54*	14	26	9	17
Co	0.22	0.23	0.10	17**	30**	58*	21	9	17
Mo	2.3	1.7	2.1	3	18	–17	23	12	17

[1] n = 18, 7, and 4 for all diets, alfalfa hay, and grass hay, respectively.
[2] Sample-diet standard deviation from regression ($\hat{Y} \pm Sy{\cdot}x$) divided by mean dietary mineral concentration and expressed as percent.
[3] *, ** = Paired t-test significant at t = .05 and .01, respectively, between mean mineral levels in diet compared with those in rumen sample.

About 2 kg of the test feed were divided in half—for direct chemical analysis, and for ingestion by the test animal. After cattle consumed all the feed offered, generally within 30 to 45 minutes, the freshly ingested test sample was removed and the former rumen contents replaced in the rumen. All samples were dried at 60°C for 48 hours and ground to pass 20-mesh screens.

This study was part of a larger study by Ansotegui et al. (1971) and included 18 of 62 previously tested feeds: an alfalfa hay-barley (5:1) mix, four pelleted concentrates, two pelleted alfalfas, seven baled alfalfa hays, and four baled grass hays.

Samples were wet-washed, using an HNO_3:$HClO_4$ (3:1) mixture. Phosphorus was determined by vanadomolybdate, and the other minerals by standard atomic absorption techniques. Cobalt and Mo were determined on an atomic absorption instrument equipped with a graphite furnace. We did not determine Cu because of contamination during sample preparation. Total N was determined by the Kjeldahl procedure, and total ash by dry-ashing 1-g sample at 550°C for 4 hours. To calculate acid-insoluble residue (AIR), which we assumed to be equal to silica, the ashed sample was moistened with water, dissolved in 20-ml concentrated HCl, and dried on a hot plate. The ash was re-dissolved with 25-ml 2N HNO_3, diluted with water to 100 ml, and then filtered through Whatman No. 1 paper. The residue was thoroughly washed with water, then re-ashed for 4 hours at 550°C, moistened with water, acidified with 2-ml HCl, dried on a hot plate, and reweighed after cooling.

Statistical methods included a paired comparison t-test between feed and fistula samples and regression by least squares analysis.

Results and Discussion

Rumen-fistula samples contained significantly ($P < 0.05$) more Na, P, and Co than did the original diet sample (Table 1). Fistula samples in previous studies have contained up to 10,000 mg/kg dry matter (DM), more Na, and up to 3,200 mg/kg DM more P than measured in diet samples (Table 2). We found no previous data on increases in Co levels. The fistula samples also contained more Zn than did the diet samples. The relatively large Zn concentration increases in the rumen-fistula sample confirmed similar increases of 32 mg/kg DM reported in esophageal-fistula samples (Little 1975). Intravenously injected ^{65}Zn was found by Weston and Kastelic (1967) to enter the rumen from both salivary and rumen epithelial secretions. This was further subtantiated by Grace (1975), who measured more Zn and Co leaving the stomach of sheep than was consumed, but no changes in the quantities of Cu and Mn. The short-term sampling used in our study, together with previously reported esophageal data, indicated that salivary Zn (Little 1975) and P (Lesperance et al. 1974) contributions are appreciable.

Table 2. Literature values (g/100 g) for mineral enrichment of esophageal samples taken from cattle (unless noted otherwise). Data are actual values determined by subtracting concentration in diet from that in fistula sample.[1]

Reference	Diet	Ash	N	P	Na	K	Mg	Ca	Cl
Ansotegui et al. (1971)[2]	Variety				0.77**	0.14*	0.01*	0	
Cundy and Rice (1968)	Hay, haylage, silage	9 to 13	–0.3 to + 0.4						
Hoehne et al. (1967)	Prairie sandreed	1.7*	–0.03*	0.32**				–0.08	0.19
	Blue grama	4.6**	0.06	0.01				0	0.20
Kiesling et al. (1969)	Alfalfa and tobosa hay	3	0.7						
Langlands (1966)[3]	Chopped alfalfa	0.76*		0.12**	1.0**	–0.69**		0.05	
	Fresh alfalfa	1.29*	0.18*	0.18**	0.9**	–0.23**		0	
	Fresh phalaris	–0.24		0.11*	0.9**	–1.05		0.02	
Lesperance et al. (1974)[2]	Variety	2 to 3.6		0.18**					
Little (1972, 1975)[4]	Chopped hay	4.3	0.26	0.20			0.01	–0.02	
Scales et al. (1974)	Alfalfa, grama, wheatgrass	2.9	0.38	0.13					

[1] *, ** = Paired t-test significant at t = 0.05 and 0.01, respectively, between mean mineral levels in diet compared with those in the rumen sample.
[2] Rumen fistula.
[3] Esophageal fistulated sheep.
[4] Little also reported + 0.01% S, + 1 ppm Mn, – 0.6 ppm Mo, + 0.6 ppm Cu, + 31 ppm Zn**, and – 0.2 ppm Ti.

After feed was ingested by cattle, the Mg and Ca concentrations decreased slightly (Table 1). The decrease in Mg of approximately 200 mg/km DM in this study compared favorably with the 100-mg value reported by Ansotegui et al. (1971) for the larger group of feed samples. We detected small decreases in silica, K, Mg, Ca, Mn, and Mo levels in the ingested grass samples. Small increases or decreases have been previously reported for the Mg and Ca concentration in fistula samples as compared with diet samples (Ansotegui et al. 1971; Hoehne et al. 1967; and Little 1975). Ansotegui et al. (1971) reported rumen-fistula samples from a wide variety of feeds averaged 400 mg/kg DM more K than the original diet, which compared favorably with the 1,100 mg/kg DM more K we calculated from the data for all diets.

Table 1 shows the errors associated with predicting dietary mineral concentration when only the mineral concentrations in the rumen-fistula sample are given. The errors are greatest for the all-diet group because of the wide range in mineral concentrations as compared with the alfalfa-hay or grass-hay group. The alfalfa-hay group had the lowest errors, which suggests that the prediction error is directly related to diet diversity. Thus, the diet mineral content of homogenous forage samples might be predicted from fistula samples with less than 10% error.

In general, the errors associated with the predicted diet-mineral concentrations were greater than 10% for the all-diet and grass-hay group, but less than 12% for the alfalfa-hay samples. Langlands (1966) reported that forage N and Ca concentrations could be estimated from esophageal-sample values with less than 10% error. However, the Na, K, and ash concentrations of the diet could not be reliably estimated from esophageal-sample values. Little (1975) found that Ca, S, Cu, and Mg levels in chaffed hay diets could be predicted from esophageal samples with an error of $< \pm 9\%$. Little (1975) also reported that the predicted Mo and Mn concentrations had an error of $\pm$ 15%, whereas Zn enrichment of fistula forage samples was appreciable and highly variable.

Dietary mineral intake from ingested feeds, salt, and soil is the major source of minerals transported via the blood to body tissues, including the salivary glands. Thus, variations in the mineral intake could affect the mineral concentration in the excreted saliva (Setia et al. 1971). For example, Little (1972) and Scales et al. (1974) reported higher N concentration in fistula samples than in the diet samples when diet-N concentrations were low, but lower N concentrations than in the diet sample when diet-N concentrations were higher. Omitting all supplementary salt from diets of fistulated test animals might decrease the degree of mineral contamination of the boluses, especially Na and Cl, but such a practice may affect animal behavior. Therefore, additional research is needed on this subject.

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