Sources of Variation in Chemical Composition of Forage Ingested by Esophageal Fistulated Cattle[1]

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Highlight

Esophageal fistulated cattle were used to collect samples of grazed forage. Within-day, daily and animal variation in the lignin and nitrogen contents of the forage samples were determined in ten trials during three growing seasons. Within-day differences in the nitrogen and lignin content of forage samples were not consistent. Daily and animal variation of these chemical constituents in the diet were highest when the mean content of each was highest in the forage and when a wide variety of forage species was being consumed. Summarizing all trials, sampling forage with three animals per treatment for four consecutive days permitted the detection of differences of 10% of the mean nitrogen content at the 10% level of significance with 85% confidence. More animals would be required to make precise measurements of the lignin content of the diet.

Sampling from the esophageal fistula provides a direct method of evaluating the quality of forage consumed by grazing animals (Van Dyne and Heady, 1965; Marshall et al., 1967; Torell et al., 1967; Hoehne et al., 1967). However, variation in the chemical and botanical composition of forage samples collected via esophageal fistulae may occur within and among days and among animals because of differences in preferential grazing.

This study was conducted to estimate the magnitude of the variation in lignin and nitrogen content of forage samples collected via esophageal fistulae. The variance components of these parameters were utilized in computing the minimum number of animals and the length of trial period required to make precise estimates of the lignin and nitrogen content of the grazing animal's diet.

Experimental

Ten forage collection trials involving three sets of esophageal fistulated Hereford cattle were conducted between 1964 and 1966. A description of the fistulation procedure and type of cannulae used was reported by Hoehne (1966). A total of 376 dietary samples were collected via the esophageal fistulae as described by Streeter et al. (1968) and analyzed for Kjeldahl nitrogen (A.O.A.C., 1960) and lignin (Van Soest, 1963). Dietary samples were dried at 50 C for 72 hours. Nitrogen and lignin content was expressed on a dry matter basis. Nitrogen was used because it stimulates digestibility, partly by its stimulatory action on rumen microorganisms and partly because it possesses an intrinsically high coefficient of digestibility (Balch and Campling, 1962; Holter and Reid, 1959). Lignin was used because it contributes directly to indigestibility. It is indigestible itself, it has a binding action on the holocellulose fraction, and it limits forage consumption through slowing the rate of passage through the gut (Van Soest, 1967).

Trials 1 through 8 were conducted on a sandy site in western Nebraska characteristic of the central Great Plains. The dominant grass species were needleandthread (*Stipa comata*), prairie sandreed (*Calamovilfa longifolia*) and blue grama (*Bouteloua gracilis*). Trials 9 and 10 were conducted on a well-drained lowland flood plain in southeastern Nebraska. Big bluestem (*Andropogon gerardi*), Indiangrass (*Sorghastrum nutans*) and sand lovegrass (*Eragrostis trichodes*) were the dominant grass species.

An attempt was made to regulate stocking rates so the cattle would have ample herbage, but not so much that "spotty" grazing would result. However, proper regulation of the stocking rate was not possible in Trials 9 and 10 because of excessive growth of the herbage.

Sampling frequency, number of animals used per trial, dates of each trial and the size of each trial area are shown in Table 1. Morning samples were collected between 4:30 AM and 6:00 AM during each trial. Evening samples were collected at 6:00 PM during Trials 7, 9, and 10. Grazing time was generally limited to 25 minutes. Where forage was abundant esophageal collection bags were filled sooner, particularly if the animals had been previously confined. With the exception of Trial 7, overnight confinement was found necessary to obtain the morning samples. Mid-day confinement was not necessary to obtain evening samples except on very cool afternoons.

Paired T-tests were used to determine within-day differences in ingested forage while analysis of variance was used to determine daily and animal variation. Variance components of animals and days were employed in a statistical approach to estimate the minimum number of each variable required to detect significant differences between treatment means in future trials. The equation used to determine the minimum number of animals and days for future studies was a rearrangement of the T-test:

$$a = \frac{2\,(t)^2 s^2_{ad}}{d\,(\bar{d})^2}$$

Where a is the number of animals; d is the number of days; $\bar{d}$ is the desired difference to detect in treatment means; and s^2_{ad} is the animal × day variance component. The t^2 was replaced by two separate t's which represent a 10% level of significance with 85% probability (Snedecor and Cochran, 1967). A one-tailed test was used because one is concerned with whether one treatment is significantly greater than another. A level of 10% between treatment means was set as the difference desired to detect.

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Table 1. The number of samples collected, animals used, size of experimental area and dates of each trial.

Trial No.	Samples/ day	No. of animals	Dates	Area (hectares)
			1964	
1	one	5	July 21 thru 26	0.5
2	one	6	Sept. 2 thru 6	0.5
			1965	
3	one	5	June 9 thru 14	1.6
4	one	5	June 22 thru 27	1.3
5	one	5	July 8 thru 13	1.1
6[a]	one	4	July 28 thru Aug. 2	1.0
7[a]	two	4	July 31 thru Aug. 3	1.3
8	one	4	Aug. 24 thru 29	1.0
			1966	
9	two	9–7	June 12 thru 18	8.1
10	two	6	Oct. 7 thru 10	8.1

[a] Trials 6 and 7 were conducted on similar sites using different animals.

Results and Discussion

Within-day Variation

During the summer there was slightly but significantly ($P < .01$) more nitrogen in the samples collected in the morning than those collected in the evening (Table 2). In the fall (Trial 10) this relationship was the reverse, probably because in the morning the fasted cattle grazed to satisfy hunger. Animals selected significantly ($P < .05$) more grasses than forbs in the morning and significantly more forbs than grasses in the evening (Obioha, 1967). Similar studies by Weir and Torell (1959) and Lesperance et al. (1960) indicated that diurnal variation, when compared to animal or day variation, was relatively insignificant. Arnold et al. (1964) showed that overnight fasting induces more indiscriminate grazing and hence less selectivity in the morning.

Evening dietary samples contained more lignin than morning dietary samples in all three trials involving twice daily collections. However, these differences were small and not significant ($P > .10$) except in Trial 10 which was the only trial conducted in the fall.

Table 2. Mean nitrogen and lignin content (%) of forage samples collected via esophageal fistulae in the morning and evening.

Trial No.	Nitrogen			Lignin		
	AM	PM	P[a]	AM	PM	P[a]
7	1.81	1.76	$P < 0.01$	8.20	8.55	$P > 0.1$
9	2.92	2.63	$P < 0.01$	5.20	5.32	$P > 0.1$
10	2.02	2.26	$P < 0.01$	6.46	7.12	$P < 0.001$

[a] Probability level at which the mean difference was significant.

Table 3. Means and animal variation in the nitrogen and lignin content (%) of forage samples collected via esophageal fistulae.

Trial No.	Animals per trial	Nitrogen		Lignin	
		Mean[a]	Std. error mean	Mean[a]	Std. error mean
1	5	1.19	.042	5.76	.383
2	6	1.03***	.024	5.38	.435
3	5	2.40**	.073	5.03	.479
4	5	1.91	.056	4.71*	.333
5	5	1.58	.048	7.88*	.844
6	4	1.42	.052	13.51***	1.103
7	4	1.81	.069	8.21	.638
8	4	1.22	.050	11.96	1.496
9	6	2.93*	.112	5.25	.438
10	6	2.05	.134	6.58	.361

[a] Significance among animals, * $P < .10$; ** $P < .05$; *** $P < .01$.

Animal Variation

Animal variation in the dietary nitrogen content decreased with advance in season (Table 3). During this time the nitrogen content of the samples decreased and the number of different plant species available declined except in the fall when the animal's preference changed to forbs (Obioha, 1967).

Of the ten trials, only three (Trials 4, 5 and 6) showed a significant difference ($P < .10$) in dietary lignin content among animals within trials (Table 3). Increased variation in the lignin content of the diet at that time was attributed to the variable consumption of lambsquarters (*Chenopodium album*) seeds. The coats of these seeds were not completely digested by 72% H_2SO_4 and their presence resulted in an abnormally high artifact lignin. Animal variation in the lignin content of the dietary samples generally increased as the mean lignin content increased.

Daily Variation

Nine out of the ten trials showed significant ($P < .10$) differences in dietary nitrogen content among days (Table 4). There was a slightly higher variability in dietary nitrogen due to days than due to animals. Samples of available forage species were hand-clipped daily in Trial 10 to see if daily fluctuations in the nitrogen content of the herbage could account for the large daily variations in the consumed forage. There was no significant difference ($P > .05$) in nitrogen or lignin content among the clipped forage samples over the entire period of the trial (Obioha, 1967). This would suggest that the variation reported was due to changes in the preference of the animals for different species.

In five out of the ten trials a significant ($P < .10$) difference in daily dietary lignin content was ob-

Table 4. Means and day variation in the nitrogen and lignin content (%) of forage samples collected via esophageal fistulae.

Trial No.	Days per trial	Nitrogen		Lignin	
		Mean[a]	Std. error mean	Mean[a]	Std. error mean
1	6	1.19**	.046	5.76	.420
2	5	1.03***	.022	5.38	.397
3	6	2.40***	.080	5.03	.525
4	6	1.91***	.062	4.71***	.364
5	6	1.58**	.053	7.88	.924
6	6	1.42	.063	13.51***	1.351
7	4	1.81***	.069	8.21**	.643
8	6	1.22**	.062	11.96*	1.832
9	7	2.93*	.122	5.25*	.473
10	4	2.05**	.109	6.58	.294

[a] Significance among animals, * $P < .10$; ** $P < .05$; *** $P < .01$.

served (Table 4). Much of this variation can be attributed to the presence of variable amounts of lambsquarter seeds which were present in the dietary samples. Some of the variation can also be attributed to analytical errors which are magnified when there is a low lignin content.

Animals Per Trial and Length of Trials

Variation resulting from sampling with three animals per treatment for four consecutive days should permit the detection of mean differences of 10% in the nitrogen content of forage consumed by grazing cattle as being significant with an 85% confidence level and 10% probability (Table 5).

Use of the same number of animals and days would not provide the precision required to adequately measure the lignin content of the forage. However, one must keep in mind that a considerable portion of the observed variation in the lignin content can be attributed to factors not directly related to the selectivity of the animals, i.e., the apparent lignified seed coats of lambsquarters and the large relative analytical errors associated with forages containing low levels of lignin.

Increasing the number of days and decreasing the number of animals could be utilized to facilitate the ease of experimentation if the chemical composition of the forage does not change too rapidly. Certain environmental conditions would result in rapid plant growth and relatively rapid changes in the chemical composition and nutritive value of the plant. Under these conditions, the number of days in which samples were obtained should be kept to a minimum.

Research workers wishing to select their own probability and confidence levels in accordance with their specific experimental conditions and design could calculate the number of animals and days needed per trial by using the animal × day variance components shown in Table 6, and the formula used in this study to compute the number of animals and day combinations shown in Table 5.

Table 5. Estimates of the number of animals and days required for future studies of chemical composition of the diets of grazing cattle.[a]

Lignin			Nitrogen		
Sampling days	Animals		Sampling days	Animals	
	Avg	Range		Avg	Range
4	13	4–26	2	5	1–11
5	10	3–21	3	4	1–7
6	9	3–17	4	3	1–6
7	8	3–15	5	3	1–5

[a] 10% level of significance; 85% level of confidence.

Table 6. Animal × day variance components and the standard errors of the variance components of the percent lignin and nitrogen in the diet.[a]

Trial	Lignin		Nitrogen	
	s^2_{ad}	S.E.	s^2_{ad}	S.E.
1	.88	.22	.011	.003
2	.94	.24	.003	.001
3	1.38	.35	.032	.008
4	.66	.17	.019	.005
5	4.27	1.08	.014	.004
6	7.30	2.06	.016	.005
7	1.63	.56	.019	.007
8	13.42	3.79	.015	.004
9	1.34	.29	.088	.019
10	.52	.15	.072	.020

[a] s^2_{ad} is an animal × day variance component and S.E. is a standard error.

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