Comparison of Four Methods to Estimate the Dissolved Nitrogen Fraction in Range Plants

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Abstract

Aboveground biomass of four range species was collected at several phenological stages and total nitrogen was determined. The dissolvable nitrogen fraction within these samples was estimated utilizing four techniques: (1) in vivo nylon bag digestion in a rumen-fistulated *Bison bison;* (2) in vitro Tilley and Terry plus pepsin; (3) neutral detergent fiber; and (4) laboratory detergent fiber. Total nitrogen concentration in all plant species studied was highest during early growth and decreased with advancing maturity. A similar amount of nitrogen was removed from dead or dormant plant materials using any technique, but significantly more nitrogen was removed from green succulent material utilizing the nylon bag technique than was removed with the three laboratory assays. The amount of nitrogen removed from plant foliage was highly correlated among techniques. Equations were developed to predict nitrogen losses with the nylon bag technique using dissolved nitrogen values obtained from any of the other three techniques.

Growth and health of livestock have been associated with digestible protein in range forage (Cook et al. 1977). Conventional feeding trials, conducted with animals maintained in chambers where rates of ingestion and egestion are accurately measured, have been universally accepted as the most accurate method for determining the digestibility of forages. However, feeding trials are laborious, expensive, and limited to the study of the digestibility of only a single plant species or mixture at a time.

Both wild and domestic herbivores usually select plants or parts of plants that contain more digestible protein than the average available (Swift 1948; Cable and Shumway 1966; Dietz 1970; Bedell 1971; Wallace, et al. 1972). Plant protein content normally decreases with phenological maturation, while the fiber fraction (complex carbohydrates) increases. This change usually is accompanied by a decrease in daily food intake of herbivores (Van Soest and Moore 1965; Nagy et al. 1969).

The digestion indices of feeds are correlated for cattle using the conventional digestion trials and the in vivo nylon bag technique (Gallinger and Kercher 1964; Chenost et al. 1971; Quinton 1972; Ishizaki et al. 1976). Quinton (1972) found nitrogen loss from nylon bags in a rumen was similar to that lost with conventional feeding tests.

The digestion coefficients determined by use of the in vitro Tilley and Terry technique usually correlate with those from conventional digestion trials (Tilley and Terry 1963; Pearson 1970; Ruggiero and Whelan 1976; Urness et al. 1977; Milchunas et al. 1978). However, Taylor et al. (1960) Urness et al. (1977) reported that the Tilley and Terry technique yielded dry matter digestion indices that were significantly lower than those of conventional trials. Van Dyne (1962) found good agreement between the Tilley and Terry and nylon bag procedures.

The neutral detergent fiber (NDF) procedure for determining cell wall constituents is commonly used for estimating total fiber fraction in forages (Goering and Van Soest 1970). Presumably this assay separates dry matter of plant materials into the dissolvable cell contents, which are 98% digestible, and the cell wall constituents, which are primarily structural carbohydrates that are incompletely available and vary in digestibility. Since the majority of the digestible nutrients received by a ruminant are derived from the cellular protoplasm, the NDF fraction appears to be a good indicator of forage quality in many instances.

The purpose of this study was to compare nitrogen losses from forage samples of four shortgrass prairie species utilizing in vivo (nylon bag), in vitro (Tilley and Terry), and detergent separation techniques.

Methods

Plant materials utilized in this study were obtained from the U.S. Dep. Agr. Central Plains Experimental Range approximately 57 km north of Greeley in northeastern Colorado. The vegetation of the study area is typical shortgrass prairie with an approximate elevation of 1650 m. Average annual precipitation varies from 25 to 38 cm, with 80% occurring from May through September (Jameson 1969).

Aboveground plant materials were collected in the middle of the months of March, May, June, July, and August, 1977. Forage was clipped to simulate grazing by a large herbivore, like a bison *(Bison bison).* Plant species chosen were western wheatgrass *(Agropyron smithii),* common Russianthistle *(Salsola kali),* scarlet globemallow *(Sphaeralcea coccinea),* and fringed sagewort *(Artemisia frigida).* These plants have been reported as important range forage plants (Peden et al. 1974; Vavra et al. 1978; Flinders and Hansen 1972; Schwartz and Nagy 1976; Hansen and Gold 1977) on the study area.

The clipped plant materials were saved and dried in paper sacks at 55° C in a forced air drying oven until no additional moisture loses were recorded. The oven-dried samples were ground in a wiley mill over a 1-mm screen.

The procedures used to estimate the dissolved nitrogen (N) fraction from the plant materials were: the in vivo microdigestion nylon bag (Quinton 1972), in vitro microdigestion Tilley and Terry with pepsin (Tilley and Terry 1963; Pearson 1970), NDF (Goering and Van Soest 1970), and laboratory detergent fiber (LDF) techniques. Nitrogen fractions were determined using one standard microkjeldahl method (AOAC 1970).

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[1]"Dissolved nitrogen fraction" is designated as nitrogen losses from any of the four techniques.

Four duplicate samples of each plant materials for each date of collection were analyzed for total N before treatment. Four duplicates of each sample were then subjected to each of the four separation techniques and each fibrous residue was analyzed for N. The difference between initial N content and N content of the residue represented nitrogen lost as a result of separation technique.

In the nylon bag procedure 2-g samples of each plant material were placed inside bags made of fine mesh (47 threads/cm) nylon and suspended for 48 hours in the rumen of a fistulated bison. The bison was a resident of a native range pasture adjacent to the study area. Standard procedures (Van Dyne 1968; Quinton 1972) were followed and, after removal from the bison's rumen, the samples were washed alternately in cold and hot tap water until no color in the wash water was evident. The in vitro procedure was conducted using rumen fluid obtained from the fistulated bison. Laboratory detergent fiber in plant materials was determined using the same apparatus and procedure as for the NDF technique. However, a powdered detergent (alconox) was used in the LDF procedure. This detergent is commonly used in research laboratories for washing glassware and is inexpensive compared to NDF solution. The NDF solution was made according to specifications of Goering and Van Soest (1970) and the LDF solution was 8 g dry alconox powder dissolved in 1 liter of deionized-distilled water. The pH of the NDF solution was 7.0 and that of the LDF solution was 9.5.

Analysis of variance was conducted to determine if significant ($P<0.05$) differences existed among species and techniques. Significant means were separated using Tukey's Honest Significant Difference Test (HSD) and T-test procedures (Snedecor and Cochran 1967). Linear regression was used to study the degree of association among different techniques.

Results

More nitrogen was removed from green plant materials (those collected in early summer) with the nylon bag technique than with the in vitro, NDF, or LDF techniques (Table 1). The quantity of nitrogen dissolved with the nylon bag procedure from dead plant materials collected in March was usually similar to that removed by the other three laboratory techniques. However, more N was removed from the western wheatgrass samples collected in March by NDF and LDF techniques than was removed by the nylon bag or in vitro techniques (Table 1). More N was removed from western wheatgrass samples collected in May and June utilizing the nylon bag technique than using any of the other techniques. A similar amount of N was removed from western wheatgrass collected in July and August utilizing any of the four technique, except that the NDF technique resulted in removal of significantly more nitrogen in August samples than did the nylon bag technique.

Use of the nylon bag technique resulted in significantly more N being removed from scarlet globemallow samples from each collection than did any of the other techniques (Table 1). The nylon bag bag technique also resulted in significantly more N removal from common Russianthistle samples collected from May through August. All four techniques removed a similar fraction of N from fringed sagewort samples collected in March. However, more, N from fringed sagewort samples collected in May and June was removed with the nylon bag technique than with the in vitro or LDF treatments. In addition, more N was removed utilizing the nylon bag technique than all other techniques when fringed sagewort was sampled in July and August.

Discussion

The dissolved nitrogen fraction removed by the nylon bag technique was generally similar to that removed by the in vitro, NDF, or LDF techniques when total N in the plant materials was low. Possible reasons for this could be: (1) total nitrogen or dissolvable nitrogen was in such low concentration that the sensitivity of the technique was inadequate to distinguish among differences, or (2) the small fraction of dissolvable nitrogen could be removed equally well by detergent treatments or by microbial activity.

Presumably the sensitivity of all four techniques for estimating small quantities of dissolvable N is poor. An accurate estimation of N digestibility, however, does not require high precision since a small fraction of a percentage difference in dissolvable N will probably not affect animal performance.

Dissolved nitrogen and cell contents were positively correlated and there was a high similarity among all four techniques when the cell content percentage was low (Fig. 1). High cell wall percentages (such as found in plant materials collected in March) apparently affected and partially inhibited microbial breakdown of plant cells

Table 1. Average nitrogen (g/100 g DW) before treatments and the amount of nitrogen removed by one of four separation techniques. Samples were the aboveground parts of four range plant species obtained on five dates in 1977 from shortgrass prairie in northeastern Colorado.

Plant species	Date collected	Before treatments	Separation technique[1]			
			NB	TT	NDF	LDF
Western wheatgrass	March	1.01a[2]	0.59a[3]	0.54a	0.79b	0.71b
	May	3.80b	3.57d	3.00c	2.65b	2.25a
	June	2.22c	1.90b	1.66a	1.75a	1.61a
	July	1.47d	1.10ab	1.01a	1.19b	1.09ab
	August	1.23ad	0.83a	0.87ab	0.98b	0.88ab
Scarlet globemallow	March	1.21a	1.01d	0.57a	0.91c	0.71b
	May	2.77b	2.59d	1.91b	2.12c	1.73a
	June	2.39b	2.19d	1.71b	1.92c	1.56a
	July	2.42b	2.19c	1.68b	1.72b	1.45a
	August	1.94c	1.72b	1.22a	1.14a	1.15a
Common Russianthistle	March	0.86a	0.47ab	0.41a	0.59b	0.53b
	May	3.08b	2.88c	2.13ab	2.25b	2.03a
	June	2.87b	2.88c	2.02a	2.16a	2.32b
	July	2.87b	2.61b	2.24a	2.14a	2.19a
	August	2.21c	1.86b	1.41a	1.27a	1.45a
Fringed sagewort	March	1.39a	0.90a	0.81a	1.09a	1.01a
	May	3.35b	3.00c	2.25a	2.87c	2.45a
	June	2.18c	1.76b	1.28a	1.68b	1.33a
	July	1.61ad	1.17b	0.87a	0.91a	0.79a
	August	1.82d	1.36b	1.01a	1.09a	1.08a

[1]In vivo nylon bag technique (NB), in vitro Tilley and Terry technique (TT), neutral detergent fiber technique (NDF), and laboratory detergent fiber technique (LDF).
[2]Means for a given plant species for different months followed by the same letter are not significantly different ($p<0.05$).
[3]Means for separation techniques within a month followed by the same letter are not significantly different ($p<0.05$).

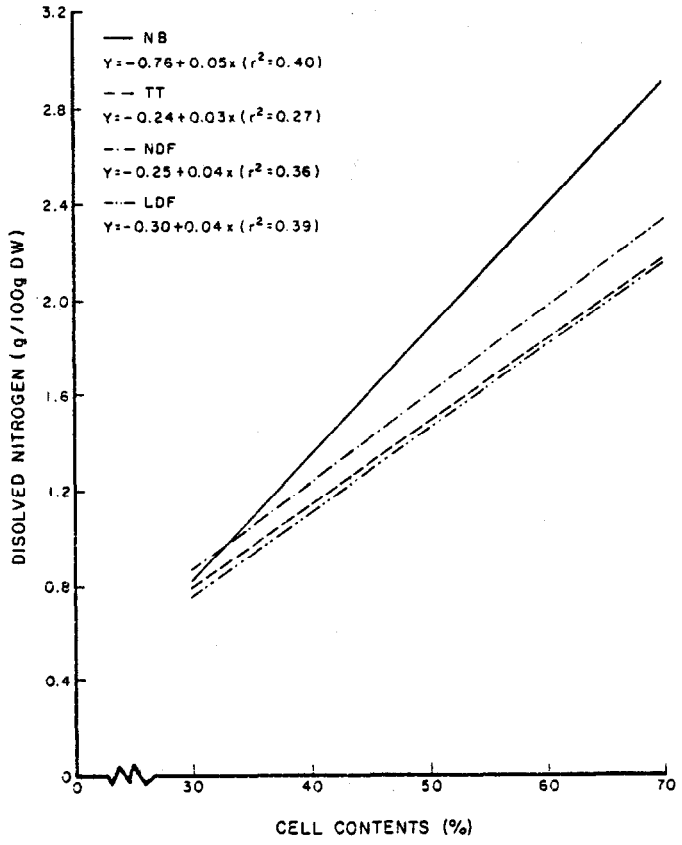

Fig. 1. *Linear regressions for grams (g) of nitrogen lost per 100 g dry weight of plant sample with percentage cell contents for the nylon bag technique (NB), in vitro Tilley and Terry technique (TT), neutral detergent fiber techique (NDF), and laboratory detergent fiber technique (LDF). Plant species included* Agropyron smithii, Sphaeralcea coccinea, Salsola kali *and* Artemisia frigida *obtained on five dates during 1977 from shortgrass prairie in northeastern Colorado representing new growth to old mature forage.*

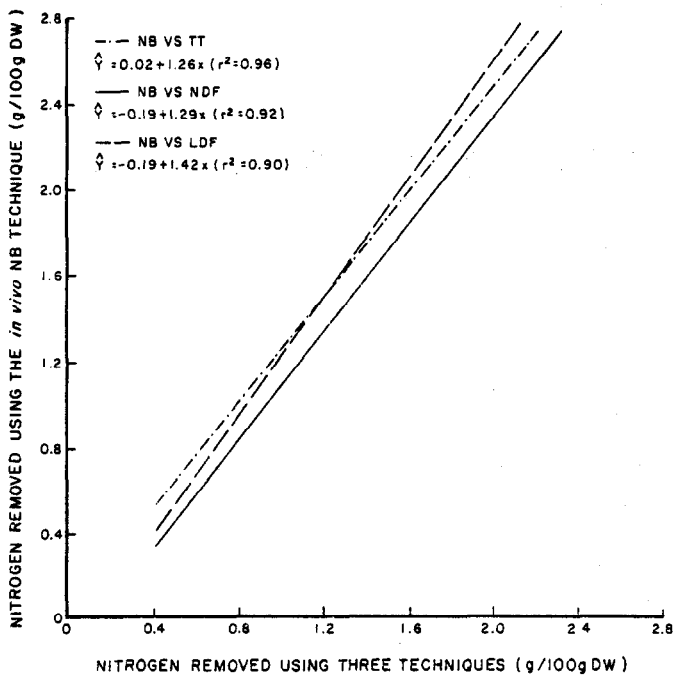

Fig. 2. *Linear regressions of gram (g) of nitrogen lost per 100 g dry weight of plant sample for the nylon bag technique (NB) versus the vitro Tilley and Terry technique (TT), NB versus the neutral detergent fiber technique (NDF), and NB versus the laboratory detergent fiber technique (LDF). Plant species included* Agropyron smithii, Sphaeralcea coccinea, Salsola kali *and* Artemisia frigida *obtained on five dates during 1977 from shortgrass prairie in northeastern Colorado representing new growth to old mature forage.*

in the nylon bag and in vitro techniques. In addition, some of the N in the cell contents was probably not dissolved because of this surrounding lignified structure. Detergents were not selective for phenological stage in the breakdown of the cell wall structure. Separation of cell wall and cell contents is a standard procedure (Goering and Van Soest 1970) and the dissolved N fraction originates primarily from cell contents.

Plant samples with relatively high amounts of protoplasm (such as materials collected in May and June) apparently can be efficiently diffused of dissolvable materials by microbial activity in the nylon bag technique (Fig. 1). Microorganisms are presumably able to dissolve most N found in cell contents plus some of the N fraction found in cell walls. Detergent treatments appear to remove N from the cell protoplasm and, as expected, were not effective in dissolving N within the cell wall.

The N contained in cell walls is such a small percentage of total N (5 to 10%) (Van Soest 1966) that it alone does not appear to cause the large discrepancy found between the nylon bag technique and detergent technqiues during periods of high total N. Detergent treatments possibly were leaving some of the dissolved N fraction remaining with the cell walls. Fonnesbeck (1976) found some dissolved N remained after treating high protein forages with the neutral detergent solution.

Results of this study suggest that detergent techniques can be used to accurately estimate the dissolved N fraction (apparent digestible N) of an in vivo trial when forages are fibrous and low in crude protein. However, plant materials high in protein concentration during active growth can be more accurately measured for dissolvable N using an in vivo digestion trial. However, nitrogen removed by the nylon bag technique was found to be highly correlated ($r \geq .97$) with the loss of N from all plant materials subjected in the in vitro, NDF, or LDF techniques. Predictive equations could, therefore, be used to estimate N removed by the nylon bag technique from dissolved N levels obtained by any of the three laboratory techniques (Fig. 2).

A similar amount of dry matter was removed from plant materials when the LDF technique was employed as when the NDF technique was used ($p < 0.05$). Van Soest and Wine (1967) indicated that separation of cell walls from cell contents beyond the pH range of 4 to 8 caused portions of the cell wall to become dissolvable. However, the laboratory detergent solution with a pH of 9.5 was no more active in removal of N from the four plant species examined than was the neutral detergent solution of the NDF technique.

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