NUTRITION, FEEDING, AND CALVES

Observations on In Situ Degradation of Forage Cell Components in Alfalfa and Italian Ryegrass

I. ANDRIGHETTO, L. BAILONI, G. COZZI, and H. F. TOLOSA
Department of Animal Science
University of Padova
Padova, Italy

B. HARTMAN, M. HINDS, and D. SAPIENZA
Pioneer Hi-Bred International, Inc.
Des Moines, IA 50309

ABSTRACT

The rate and extent of degradation of forage feed fractions contained in alfalfa and Italian ryegrass hays were determined. Nylon bags filled with 4 g of each forage were suspended in the rumen of two cannulated cows immediately before feeding and incubated for 10 different times (0, 2, 4, 8, 12, 24, 48, 72, 120, and 168 h). The alfalfa hay, which had lower NDF, showed a lower extent, but a higher rate, of NDF degradation than the Italian ryegrass (41.1 vs. 59.8% and 4.64 vs. 2.91%/h, respectively). Alfalfa cell walls were degraded more rapidly than Italian ryegrass even though their lignin content was higher. The hemicellulose fermentation of alfalfa showed a longer lag time (13 h) and an undegradable fraction nearly twice that for Italian ryegrass (63.3 vs. 37.1%). Cellulose from alfalfa was degraded at a higher rate than NDF or ADF, indicating that cellulose may be the primary site of hydrolysis of the cell wall in the rumen. Calculations based on in situ degradability indicate that alfalfa can have a higher inclusion than Italian ryegrass in diets for dairy cows because of lower NDF and greater availability of cell contents.

(**Key words**: forage, in situ degradation, alfalfa, ryegrass)

Abbreviation key: **CC** = cell contents, **CE** = cellulose, **HE** = hemicellulose, **IRG** = Italian ryegrass.

Received October 13, 1992.
Accepted March 19, 1993.

INTRODUCTION

The quantification of cell-wall composition and the estimation of its degradability will enable nutritionists to formulate diets for lactating cows more accurately. Cell-wall composition is especially important when diets with high forage content are fed or when low or medium quality forages are available for feeding.

In individual forages or feedstuffs, the amount of cell walls and the linkages of cell-wall carbohydrates to phenolic acids are related closely to OM digestibility (13, 15, 24). Degradability of the linkages of cell-wall carbohydrates affected the availability of the cell contents (**CC**) to ruminal microorganisms (19).

Mertens (8) has shown that DMI is influenced by ruminal fill when the cows are fed high fiber diets. The DMI often is associated with NDF content because of its high correlation (7, 18, 22) and its ease of determination. Feedstuffs with a high rate of NDF degradation were correlated positively with DMI (14, 16, 22). However, feeds similar in NDF can have different DMI, which is limited by the amount of ruminal undigested NDF (8).

This study investigated the differences between in situ degradation kinetics of alfalfa and Italian ryegrass (**IRG**) hays for total DM, NDF, CC, ADF, cellulose (**CE**), and hemicellulose (**HE**).

MATERIALS AND METHODS

Two forage species, alfalfa (*Medicago sativa* L.) and IRG (*Lolium multiflorum* ssp. *italicum*) were used in the experiment. Samples of the two forages were collected near Padova, Italy (located in the northern Po Valley) and evaluated at the laboratories of Padova University.

Both forages, at harvest, were estimated to be at mid to late maturity. Samples were collected, oven-dried at 60°C for 48 h, ground (5-mm screen, Wiley mill; Thomas Scientific, Swedesboro, NJ), and prepared for in situ analysis. Ruminal degradation in situ of the feed fractions was determined by the nylon bag technique (11). Bags measuring 10 × 15 cm with a pore size of 40 μm were used. Quantities (4 ± .4 g) of each forage were placed into bags in a sample size of 13 mg/cm^2 of bag surface.

Bags were suspended in the rumen of each of two dry Holstein cows (500 kg of BW) fitted with ruminal cannulas. The cows were fed a standard ration of 6 kg/d of mixed grass hay (about 60% IRG, 30% orchardgrass, and 10% others) and 2 kg/d of a mixture of equal amounts of corn, barley, sunflower meal, and soybean meal. The forage to concentrate ratio of the diet was 75:25, and the total CP was 13% on a DM basis.

Nine incubation times (2, 4, 8, 12, 24, 48, 72, 120, and 168 h) were measured separately by placing two bags of each forage into the rumen before the morning feeding. Incubation times were measured separately during the 22-d test period to avoid prolonged contact of the ruminal environment with oxygen by repeated opening of the cannulas and to maintain a constant relationship between the initiation of each incubation period and the consumption of feed by the cows. After removal, the bags were washed immediately in cold water for 15 min and oven-dried at 60°C for 48 h. Four bags of each forage were washed without incubation in order to estimate losses from washing.

Prior to incubation, samples of the dried forages were ground (2-mm screen Wiley mill) and analyzed for proximate components (1) and fiber fractions (3). Prior to being combined, the residuals in the two bags removed from each cow at the end of each incubation time were measured for duplication of DM disappearance, combined within cows, ground (2-mm screen Wiley mill), and analyzed for total DM, NDF, ADF, acid-detergent sulfuric acid lignin, and acid-insoluble ash using the Goering and Van Soest procedure (3) to determine the amount of the different fiber fractions remaining in the bags after incubation. Residual CC in the bags was calculated by decreasing the undegraded amount of DM by the remaining quantity of NDF, residual HE by decreasing the undegraded NDF by the remaining ADF, and residual CE by decreasing the undegraded ADF by the remaining acid-detergent lignin plus acid-insoluble ash present in the bags after suspension in the rumen.

The degradation parameters of DM, NDF, CC, ADF, HE, and CE of the two forages were computed using DUD (the derivative-free iterative method) in the nonlinear regression procedure (PROC NLIN) of SAS (12). The generalized equation (9) was

$$Y = A + B\ (1 - e^{-K_B(T-JT)}),$$

where

Y = potential degradability (percentage),
A = readily degraded fraction (percentage),
B = fraction degradable at measurable rate (percentage),
K_B = degradation rate (percentage per hour),
T = time (hour), and
JT = lag phase (hour)

with the following assumptions:

T = JT when time ≤lag time, and
T = T when time >lag time.

Effective degradability values were calculated adapting the equation to the general model proposed by Van Soest et al. (20). The assumed rates of 4, 5, and 8%/h described three ruminal retention times (25, 20, and 12.5 h, respectively).

The statistical analysis of the degradation parameters was conducted by a weighted ANOVA technique described by Johnson and Milliken (5) using PROC GLM of SAS (12). The effective degradability values of the different feed fractions were calculated and compared by a model comparison technique described by Hinds and Milliken (4). The experimental design considered forage and cow to be factors and cow to be a block effect.

RESULTS AND DISCUSSION

Figure 1 and Table 1 show the differences in the composition between the two forages.

Alfalfa hay was higher in CP and CC than IRG, but the HE content of alfalfa hay was less than one-third that of IRG; ADF and CE were similar in both forages; and lignin was 50% higher in alfalfa. The lignification index (Table 1), on either an NDF or an ADF basis (19), was higher in alfalfa (17 and 20% vs. 8 and 13%, respectively).

The degradation kinetics of different feed fractions of alfalfa hay and IRG are described in Figure 2, and the corresponding effective degradability values at three ruminal passage rates are in Table 2. The readily degraded fraction and the degradation rate of alfalfa DM were almost twice that of IRG DM. These degradability advantages in alfalfa may result from the amount (43%) and ready availability of CC (Tables 1 and 2). Effective DM degradability, at a ruminal turnover rate of 5%/h, was 49% for alfalfa compared with 31% for IRG ($P < .01$). The lower DM degradation of IRG may be explained by the lower amount of the readily degradable fraction and by the lower degradation rate (Table 2).

Kinetics of NDF degradation did not show any readily degradable fraction for either forage (Figure 2 and Table 2). The potentially degradable fraction of NDF in IRG was higher

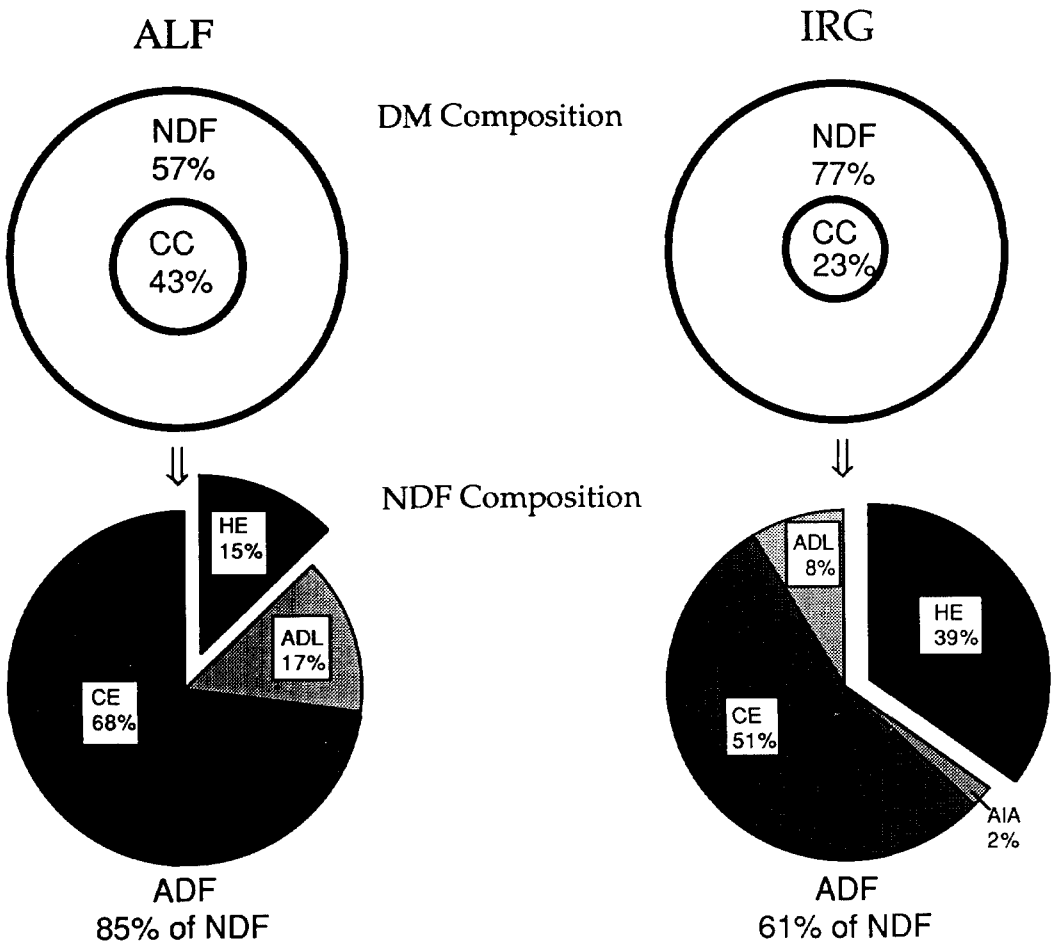

Figure 1. Comparison of feed fractions of alfalfa (ALF) and Italian ryegrass (IRG) hays. ADL = Acid-detergent lignin, CC = cell contents, CE = cellulose, HE = hemicellulose.

TABLE 1. Chemical composition of alfalfa (ALF) and Italian ryegrass (IRG) hays.

Component	ALF	IRG
DM, %	90.80	92.10
	(% DM)	
CP	16.73	7.83
Crude fiber	41.31	40.65
Ether extract	1.45	1.57
Ash	9.02	7.58
Nitrogen-free extract	31.49	42.55
NDF	56.86	76.62
Cell contents	43.14	23.38
ADF	48.29	46.97
Hemicellulose	8.57	29.65
Cellulose	38.64	39.44
Acid-detergent lignin (ADL)	9.65	6.18
Acid-insoluble ash	0	1.35
ADL/NDF[1]	16.97	8.07
ADL/ADF[2]	19.98	13.16

[1]Lignification index based on NDF.

[2]Lignification index based on ADF.

(59.8 vs. 41.1%; $P < .05$), but its degradation rate was lower (2.9 vs. 4.6%/h; $P < .05$). After 24 h in situ, NDF degradations of IRG and alfalfa were about 50 and 70% of the maximum extent, respectively, as in previous in vitro (9, 16) and in situ (10, 21) experiments. The NDF degradation pattern of the two forages agrees with the results of Varga and Hoover (21) in which NDF content of forages was correlated positively (r = .98) with the potentially degradable fraction of NDF and negatively (r = –.98) with the rate of NDF degradation.

In spite of higher NDF degradation ($P < .05$), IRG had a lower effective DM degradability at all of the assumed rates of passage ($P < .05$). The readily degradable DM appeared to result from the availability of the CC. Therefore, complete hydrolysis of cell walls may not be necessary to make CC available for digestion in the rumen. Cell walls may only have to be permeable to digestive enzymes, their substrates, or their end products.

In alfalfa, CC was available in the rumen at 8.2%/h, resulting in 81% degradation within a ruminal retention time of 20 h (5%/h, ruminal passage rate). The higher lignin content of alfalfa did not reduce the in situ disappearance of the CC, which agrees with observations of Van Soest (19). In IRG, CC was available in the rumen at 2.1%/h, resulting in 53% degradation within the same ruminal retention time. Because these results have not been corrected for contamination from the residues of microbial fermentation, the direct comparison of the rates and extents is weakened. Alfalfa hay has more CC than IRG (Table 1); therefore, the potential microbial contamination could have been diluted, and the apparent digestibility of the CC could have been increased. Phenolic acid linkages with other cell-wall components also may affect the availability of CC for degradation by the ruminal microorganisms of the two forages.

Ruminal degradation of alfalfa HE showed a lag time of 13 h and an undegradable fraction nearly twice that of IRG (63.3 vs. 37.1%; $P < .05$). The detrimental effect on HE digestibility may have been dependent on the linkages between the phenolic acid components of lignin and the uronic acids of HE (6). Lignin content of forages is related negatively to HE digestibility (19). Sullivan (17) calculated a negative correlation (r = –.83) between these two cell-wall components. However, alfalfa HE represented only a relatively small amount of the total DM (8.6%; Table 1), and its low degradability did not appear to reduce significantly the total DM disappearance of the forage. The high lignin content of this forage appeared to inhibit primarily the extent of digestion of some fiber components (23).

The HE content of IRG was 29.7% (Table 1), but, because of the low degradation rate, only 31% of this cell-wall component was available in the rumen of a dairy cow with a ruminal passage rate of 5%/h (Table 2). This result may be dependent on the monosaccharide composition of HE in grass species and particularly on the high xylose content (23). The late maturity of the plant at harvest could have had an effect on in situ degradability, considering the progressive lignification that occurs in IRG stems and leaves with maturation (6).

The kinetics of NDF, ADF, and CE degradation were similar within each forage (Table 2 and Figure 2). All three components had no readily degradable fraction, but, in IRG, the fraction available at measurable rate was higher ($P < .05$) and showed a lower ($P < .05$) degradation rate. Also, in IRG, ADF and CE had longer lag times. The similarity between

ADF and CE degradation patterns within plant species confirmed the limited contribution of lignin hydrolysates to the fermentable substrate pool in the rumen. However, ADF showed a fraction degradable at a measurable rate 8 units lower, on average, than CE, possibly because of the negative effect of lignin binding within the lignocellulose complex. Table 2 shows that alfalfa CE was degraded at a higher rate than ADF, which degraded faster than did NDF. This observation suggests that CE was the primary site of hydrolysis in the digestion process of cell-wall components of alfalfa. Regardless of the ruminal turnover rate, the effective degradabilities of ADF and CE were higher ($P < .01$) for alfalfa (Table 2). Silica, cutin content, and the crystalline state of CE may have limited the rate of penetration by ruminal microbes into the lignocellulose complex of IRG. Negative feedback also may

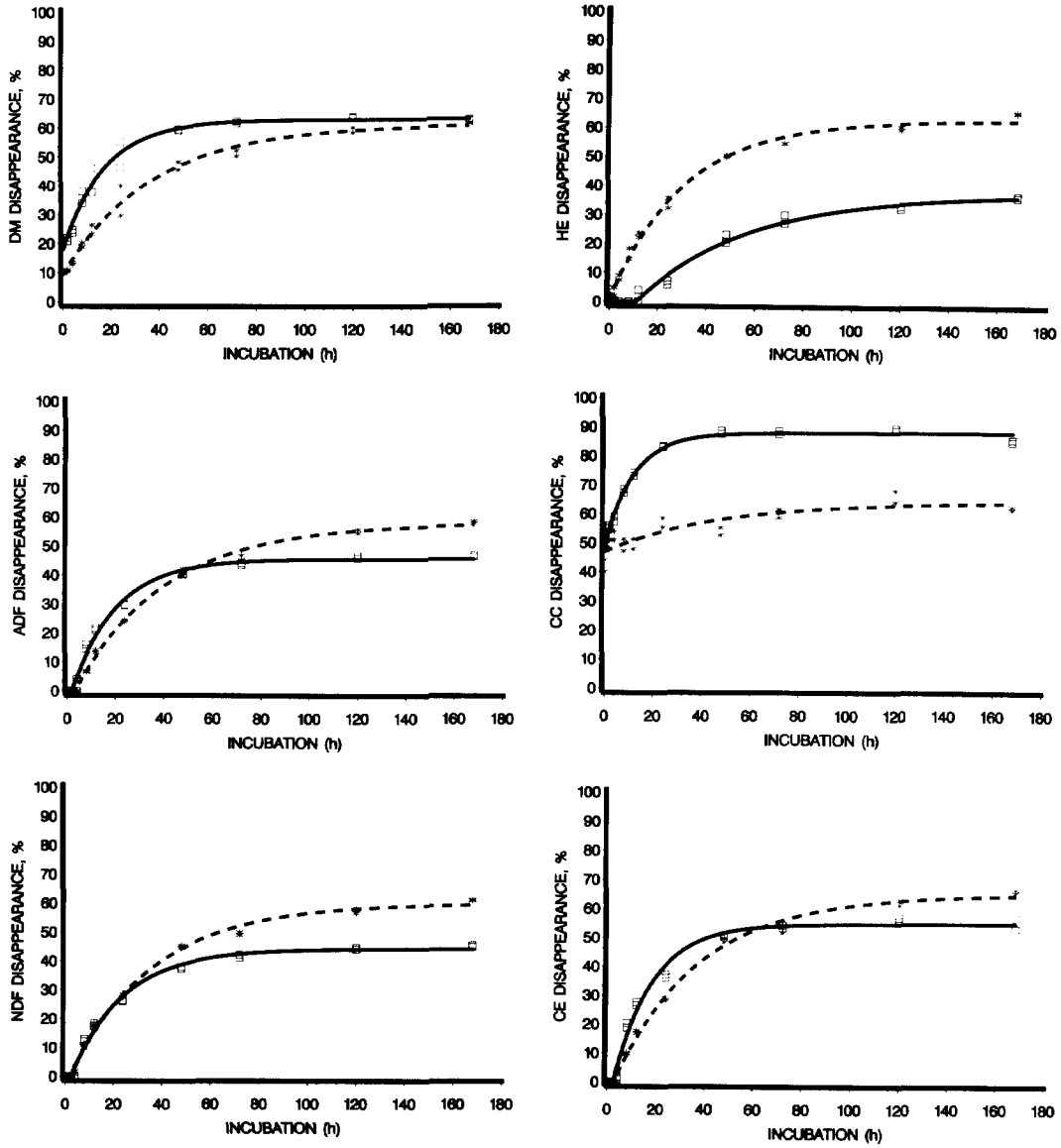

Figure 2. Comparison of in situ degradation kinetics in alfalfa (□) and Italian ryegrass (*) hays. CC = Cell contents, CE = cellulose, HE = hemicellulose.

TABLE 2. Degradable parameter estimates and effective degradability values of alfalfa (ALF) and Italian ryegrass (IRG) hays.

Component and forage type	Parameter estimates[1]					Effective degradability[2]		
	A	B	K_B	JT	Undegraded[3]	4%/h	5%/h	8%/h
DM								
ALF	17.9	45.1	5.64	0	37.0	52.1**	48.6**	40.3**
IRG	8.9	51.8	2.81	0	39.3	34.6	30.8	23.5
SE	1.3	2.8	2.82	. . .	2.1	3.4	3.4	3.1
NDF								
ALF	0	44.1*	4.64*	2.5	58.9*	28.6	24.5	15.7
IRG	0	59.8	2.91	2.0	40.2	29.2	24.4	15.1
SE	. . .	.4	.53	.4	.4	2.0	1.8	1.3
Cell contents								
ALF	48.8	39.1**	8.23	0	12.1*	83.0**	80.5**	73.5**
IRG	46.7	18.2	2.09	0	36.3	54.0	52.9	50.8
SE	2.1	.1	2.70	. . .	1.2	4.1	3.8	3.1
Hemicellulose								
ALF	0	36.5*	2.28	13.0	63.3*	9.7**	6.5**	.6**
IRG	.7	62.3	3.29	0	37.1	35.4	30.6	20.9
SE	. . .	2.1	2.57	. . .	1.5	2.3	2.1	1.7
ADF								
ALF	0	45.6*	5.45*	2.1	54.4*	32.4**	28.3**	19.0**
IRG	0	58.1	2.65	3.3	41.9	25.4	20.8	12.0
SE	. . .	.5	1.52	1.8	.5	1.9	1.7	1.3
Cellulose								
ALF	0	54.8*	6.22*	2.6	45.2*	41.3**	36.4*	24.4**
IRG	0	65.1	2.86	2.8	34.9	30.5	25.2	15.0
SE	. . .	.8	.82	.2	.8	2.5	2.4	1.9

[1]A = Readily degraded fraction (%), B = fraction degradable at measurable rate (%), K_B = degradation rate (%/h), and JT = lag phase.

[2]Effective degradability at three ruminal passage rates.

[3]Undegraded = 100 − (A + B) (%).

*$P < .05$.

**$P < .011$.

TABLE 3. Estimated ruminal availability of alfalfa (ALF) and Italian ryegrass (IRG) hays according to ruminal passage rate.

	Ruminal passage rate					
	4%/h		5%/h		8%/h	
Forage type	ALF	IRG	ALF	IRG	ALF	IRG
DM (RD[1]), g/kg of DMI	521	346	486	308	403	235
Cell contents (RD), g/kg of DMI	358	126	347	124	317	119
NDF (RD), g/kg of DMI	163	224	139	187	89	116
Cellulose (RD), g/kg of DMI	160	120	141	99	94	59
Hemicellulose (RD), g/kg of DMI	8	105	6	91	1	62
Ruminal retention time, h	25		20		13	

[1]RD = Ruminally degradable.

occur onto the ruminal microbes or their exoenzymes by the phenolic acids released during HE digestion of IRG (2).

Table 3 shows the nutritional differences that are due to the degradation kinetics of the two forages. For example, within the first 25 h of digestion in the rumen, 521 g/kg of DMI were digested in alfalfa hay compared with 346 g/kg in IRG. The composition of this digested DM was 69% CC for alfalfa and 36% for IRG. After 25 h in the rumen, 41% of the CE and 10% of the HE of alfalfa were digested compared with 31 and 35% in IRG.

CONCLUSIONS

Results of this study showed the influence of forage species on extent and rate of degradation of feed fractions in the rumen. To determine the optimal quantity and type of forage to include in balanced dairy rations, consideration should be given to in situ DM degradability, the availability of forage cell walls (NDF), and their components (ADF, HE, and CE).

Based on calculations presented in this paper, IRG of this quality is a suitable feedstuff for cows for which rate of passage approximates 4%/h. Such feeding conditions provide sufficient time for ruminal microorganisms to hydrolyze cell-wall components, which are the principal source of degradable nutrients in IRG.

The degradation kinetics of alfalfa hay showed that its lower NDF content and its high effective DM degradation can promote fast disappearance from the rumen and, consequently, less ruminal fill. The higher lignification in alfalfa than in IRG did not limit the degradation of total DM or cell components (CC and CE). All of the fiber fractions of alfalfa were degraded at a higher rate ($P < .05$) except for HE, which, however, was at a lower concentration. The principal degradable nutrients in alfalfa are CC and CE, which are available earlier than in IRG, thus making the alfalfa hay evaluated in this experiment more suitable for higher producing dairy cows (5 or 8%/h, ruminal passage rate).

For a better understanding of ruminal degradation of HE, additional studies must be conducted with both forages to explain the chemical composition of HE, its association with phenolic acids, and its changes during plant maturation. Also further measurements should be made to evaluate the relationship of forage type and quality and the degradation kinetics of their fiber components on milk production.

REFERENCES

1 Association of Official Analytical Chemists. 1980. Official Methods of Analysis. 12th ed. AOAC, Washington, DC.
2 Engels, F. M., and R. E. Brice. 1985. A barrier covering lignified cell walls of barley straw that restricts access by rumen micro-organisms. Curr. Microbiol. 12:217.
3 Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.
4 Hinds, M. A., and G. A. Milliken. 1987. Statistical methods for using nonlinear models to compare silage treatments. Biom. J. 7:825.
5 Johnson, P., and G. Milliken. 1983. Simple procedure for testing linear hypotheses about the parameters of nonlinear model using weighted least squares. Commun. Statist. Theor. Methods SC12 (2):135.
6 Jung, H. G. 1989. Forage lignins and their effects on fiber digestibility. Agron. J. 81:33.
7 Kilmer, L. H., P. J. Wangsness, E. M. Kesler, L. D. Muller, L. C. Griel, Jr., and L. F. Krabill. 1979. Voluntary intake and digestibility of legume and grass diets fed to lactating cows and growing wethers. J. Dairy Sci. 62:1272.
8 Mertens, D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. J. Anim. Sci. 64:1548.
9 Mertens, D. R., and J. R. Loften. 1980. The effects of starch on forage fiber digestion kinetics in vitro. J. Dairy Sci. 63:1437.
10 Nocek, J. E., and A. L. Grant. 1987. Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of haycrop forages preserved at different dry matter percentages. J. Anim. Sci. 64: 552.
11 Ørskov, E. R., F. D. DeB. Hovell, and F. Mould. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:195.
12 SAS/STAT™ User's Guide: Statistics, Version 6 Edition. 1985. SAS Inst., Inc., Cary, NC.
13 Seoane, J. R. 1982. Relationships between the physical and chemical characteristics of hays and their nutritive value. J. Anim. Sci. 55:422.
14 Shaver, R. D., L. D. Satter, and N. A. Jorgensen. 1988. Impact of forage fiber content on digestion and digesta passage in lactating dairy cows. J. Dairy Sci. 71:1556.
15 Smith, L. W., H. K. Goering, and C. H. Gordon. 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55:1140.
16 Smith, L. W., H. K. Goering, D. R. Waldo, and C. H. Gordon. 1971. In vitro digestion rate of forage cell wall components. J. Dairy Sci. 54:71.
17 Sullivan, J. T. 1966. Studies of the hemicellulose of

forage plants. J. Anim. Sci. 25:83.
18 Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24:834.
19 Van Soest, P. J. 1982. Nutritional Ecology of the Ruminant. O&B Books, Inc., Corvallis, OR.
20 Van Soest, P. J., C. J. Sniffen, D. R. Mertens, D. G. Fox, P. H. Robinson, and V. Krishnamoorthy. 1982. A net protein system for cattle: the rumen submodel for nitrogen. Page 265 *in* Protein Requirements for Cattle. Proc. Int. Symp. November 19–21, Oklahoma State Univ., Stillwater, OK.
21 Varga, G. A., and W. H. Hoover. 1983. Rate and extent of neutral detergent fiber degradation of feedstuffs in situ. J. Dairy Sci. 66:2109.
22 Varga, G. A., and E. C. Prigge. 1982. Influence of forage species and level of intake on ruminal turnover rates. J. Anim. Sci. 55:1498.
23 Wedig, C. L., E. H. Jaster, and K. J. Moore. 1986. Composition and digestibility of alfalfa and orchardgrass hemicellulose monosaccharide by Holstein steers. J. Dairy Sci. 69:1309.
24 Wheeler, W. E., D. A. Dinius, and J. B. Coombe. 1979. Digestibility, rate of digestion and ruminoreticulum parameters of beef steers fed low-quality roughages. J. Anim. Sci. 49:1357.