

JOURNAL OF ANIMAL SCIENCE

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J Anim Sci 1998. 76:888-895.

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Ensiling Effects on the Ethanol Fractionation of Forages Using Gas Production

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ABSTRACT: We studied the use of gas curve subtraction to distinguish between two fractions soluble in neutral detergent solution. Samples of unfractionated (whole) forage, residue insoluble in 90% ethanol, and isolated NDF were fermented in vitro, and gas production was monitored. The gas volume associated with the ethanol solubles (A fraction) was determined as the difference between the gas from the whole forage and from the ethanol residue. The gas yield associated with the fraction insoluble in 90% ethanol but soluble in neutral detergent solution (B₁ fraction) was determined by subtracting the isolated NDF gas curve from the corresponding ethanol residue curve. This experiment included three forages (alfalfa, bromegrass, and orchardgrass) harvested at two maturities and

preserved by freeze-drying or ensiling to form a 3 × 2 × 2 factorial arrangement. Ensiling reduced the rate of gas formation from the A fraction by approximately 30% ($P < .01$). Ensiling increased ($P < .05$) the size of the A fraction (2 to 10% of DM) but did not change the volume of gas produced ($P > .05$). The gas yield from the B₁ fraction was reduced 40% ($P < .05$) by ensiling with no significant change in rate. Curve subtraction of gas production profiles may be used to obtain rate estimates for multiple neutral detergent soluble pools. The separation of the neutral detergent solubles into two pools clarified the effects caused by ensiling. Changes due to ensiling on the rate of gas produced were associated with the A fraction, and the effects on final gas volume were associated with the B₁ fraction.

Key Words: Gas, Silage, Forage, In Vitro, Carbohydrates

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J. Anim. Sci. 1998. 76:888–895

Introduction

The Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992) and Level 2 of the Beef NRC (1996) predict dietary energy and protein supplies to the animal from feed chemistry and rates of digestion and passage. The carbohydrate portion of each feed is divided into three digestible fractions: the A fraction, containing sugars, short oligosaccharides, and organic acids; the B₁ fraction, containing starch and pectin; and B₂, the digestible fiber fraction (Sniffen et al., 1992). Organic acids are treated as carbohydrates. Ruminant digestion is predicted by integrating competing digestion and passage rates.

Digestion rates for DM and NDF may be obtained using standard in vitro or in situ methods, but these methods are labor-intensive (Pell and Schofield, 1993; Cone et al., 1996). Indirect measurement of the

fermentation of the forage solubles may be obtained by gas curve subtraction (Schofield and Pell, 1995a; Stefanon et al., 1996). The gas production expected for the neutral detergent solubles (NDS) is the difference in gas produced between the unfractionated (whole) forage and its respective NDF (Schofield and Pell, 1995a).

Hall et al. (1997) estimated soluble fiber as the change in mass between a 90% ethanol extracted residue (EIR) and extraction with neutral detergent solution. The fermentation of the ethanol solubles (sugars, organic acids, short oligosaccharides, and amino acids) may be estimated by subtracting the gas curve of the EIR from that of the whole feed. The B₁ fraction is insoluble in ethanol, and its fermentation rate may be estimated by subtracting corresponding EIR and isolated NDF curves.

Ensiling reduces digestion rate and gas yield from the NDS fraction (Doane et al., 1997). The first objective of the present study was to use the gas curve subtraction approach to determine digestion rates for the A and B₁ fractions. Our second goal was to see how ensiling affects the size and digestion kinetics of the soluble carbohydrate fractions.

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Received June 16, 1997.

Accepted November 14, 1997.

Materials and Methods

The ethanol fractionation of silage was examined using three species of forage common in the north-eastern United States, alfalfa (*Medicago sativa*), bromegrass (*Bromus inermis*), and orchardgrass (*Dactylis glomerata*). Changes due to forage type, maturity, and preservation method were assessed using a $3 \times 2 \times 2$ factorial arrangement. Forages were harvested near Ithaca, New York, during 1995, on 2 d to a stubble height of 10 cm. The first cutting was taken on May 20 and the second was taken on June 7 (grasses) or June 17 (alfalfa). Because the focus of the experiment was on the analytical approach, there was no replication of the forage species by plot or by year. For this reason, no conclusions were drawn relative to species differences. The second cutting of alfalfa was harvested later to obtain a stage of maturity comparable to the later grass cuttings (full bloom and inflorescence, respectively). The grasses and the alfalfa were fully vegetative at the first cutting. As each sample was harvested, it was either freeze-dried or ensiled.

Silage Preparation

Forages were ensiled using individual silos made from polyvinyl chloride pipe (10-cm diameter) with a total volume of 4 L. Samples were chopped to a 2-cm length using a paper cutter, wilted to approximately 30% DM, inoculated at the recommended rate (1×10^8 cfu/kg; 1174 Pioneer silage inoculant, Pioneer Hi-Bred International, West Des Moines, IA), and packed to a bulk density of between .63 and .80 kg/L. When the available forage did not fill the silo completely, a piece of synthetic cloth was placed on the silage surface and the remaining volume of the silo was filled with dry soil. After 40 d, the silage was removed from the small silos and frozen (-20°C). A sample was taken for DM, pH, VFA, and ammonia determinations, and the remainder was lyophilized.

Forage Analysis

All samples were ground through a 1-mm screen in a Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA). Fiber analyses were performed as described by Van Soest et al. (1991). The permanganate lignin and ash procedures were used as described by Goering and Van Soest (1970). Soluble fiber was determined using the method of Hall et al. (1997), but no correction was made for the starch content of these forages, which was assumed to be low. The macro-Kjeldahl procedure (AOAC, 1990), modified by using boric acid in the distillation process, was used to measure N. The protein fractions were determined as described by Licitra et al. (1996). Ammonia was measured using the procedure of Chaney and Marbach (1962). Dry matter values for the silage samples were determined at 60°C .

Preparation of Ethanol Residue and Isolated NDF

Ethanol insoluble residue was prepared for fermentation by stirring .5 g of sample in 100 mL of 90% (vol/vol) ethanol for 4 h at room temperature. The sample was filtered through a $37\text{-}\mu\text{m}$ nylon mesh (Tetko®, Briarcliff Manor, NY) and rinsed three times with 90% ethanol without vacuum and once with acetone using vacuum (Hall et al., 1997). The sample then was dried at 50°C overnight to remove residual acetone.

Isolated NDF for each forage was prepared by autoclaving 150-mL serum bottles containing 500 mg of forage and 100 mL of neutral detergent solution for 1 h at 105°C (Pell and Schofield, 1993). The isolated NDF from different bottles was combined and rinsed with hot water and 100 mL of ethanol using $37\text{-}\mu\text{m}$ nylon mesh as a filter. Residual detergent was removed by soaking the isolated NDF overnight at 39°C in a solution of 1 M $(\text{NH}_4)_2\text{SO}_4$: 1 g of fiber to 100 mL of 1 M $(\text{NH}_4)_2\text{SO}_4$, with the fiber weight calculated from the NDF content. The isolated fiber was rinsed again with hot water followed by 100 mL each of ethanol and acetone and allowed to air dry.

In Vitro Gas Production

The bicarbonate-phosphate buffer of Goering and Van Soest (1970) was used for in vitro digestions. The medium was boiled to remove dissolved gases and then cooled, cysteine was added, and the pH was adjusted to 6.8 as necessary. Sodium sulfide was replaced with an equal weight of cysteine hydrochloride to protect the pressure sensors used to monitor gas volume from traces of hydrogen sulfide.

Ruminal fluid was collected approximately 4 h after feeding from a mature, nonlactating Holstein cow maintained on average-quality mixed hay in accordance with the IACUC protocol. At the outset of a fermentation, each serum bottle contained 8 mL of medium, 2 mL of ruminal fluid, and 75 mg of either whole forage (WF), EIR, or isolated NDF.

Gas production was measured every 20 min during a 48-h fermentation using a computerized monitoring system (Pell and Schofield, 1993; Schofield and Pell, 1995b). At the end of each fermentation, the pH was measured immediately after the serum bottle was opened, an aliquot was removed for VFA analysis, and NDF disappearance was determined (Pell and Schofield, 1993). All gas volumes were corrected to standard atmospheric pressure (760 mm Hg). Methane was assumed to be low (our unpublished observations), and no correction was made for the presence of water-insoluble gas.

Volatile Fatty Acid Analysis

A 1.5-mL aliquot of the medium from each fermentation was removed after measurement of pH and

centrifuged at $4,000 \times g$ for 5 min. The supernatant was removed and frozen at -20°C . After the experiment was completed, the samples were thawed and vortexed for 30 s. A $360\text{-}\mu\text{L}$ aliquot of each sample was transferred to a microcentrifuge tube containing $40\ \mu\text{L}$ of acid solution ($50\ \text{mM}\ \text{H}_2\text{SO}_4$ and $100\ \text{mM}$ quinic acid as an internal standard). After mixing and standing at room temperature for 10 min, the centrifugation was repeated and the supernatant analyzed for VFA with the HPLC method of Ehrlich et al. (1981) using a BioRad HPX-87H column ($7.8 \times 300\ \text{mm}$, Bio-Rad Laboratories, Hercules, CA) at 60°C , isocratic elution with $5\ \text{mM}\ \text{H}_2\text{SO}_4$, and UV detection at $210\ \text{nm}$. A mixture of succinic, lactic, acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids was included as a standard in all analyses. Values have been corrected to exclude the VFA added to the medium initially with ruminal fluid and silage samples. Because no succinic or lactic acid was found at the end of the fermentations, where total VFA production is mentioned, the sum of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids is implied.

Curve Subtraction

Estimation of the digestion rates for the A and B_1 fractions by curve subtraction requires that gas volumes produced by the separate preparations (EIR and NDF) be adjusted to a common basis in proportion to the content of each fraction within the whole forage. This adjustment is most easily done by using $100\ \text{mg}$ whole forage DM as the basis. Suppose, for example, that we ferment separately X_w mg of the whole forage, X_e mg of EIR, and X_n mg of NDF (all adjusted to a DM basis). Further, suppose that the fractional EIR and NDF contents of the whole forage, with no ash correction, are e and n , respectively. The gas volumes at every time point for the three samples would then be multiplied by the following factors:

$$\begin{array}{ll} \text{WF} & 100/X_w \\ \text{EIR} & e \times 100/X_e \\ \text{NDF} & n \times 100/X_n \end{array}$$

The mineral content of the forage does not contribute to gas production, and for that reason it may be useful to express results based on the OM content of the forage. For calculation of digestion rates or lag times (with units of h^{-1} and h , respectively), the basis (DM or OM) is immaterial. For comparison of gas volumes (with units of mL/mg) we must select either DM or OM as the basis. Using OM as the basis gives digestibility results that are independent of ash content. The DM basis corrections outlined above establish the relative gas volume contributions of WF, EIR, and NDF. To convert the absolute values of these relative contributions to an OM basis ($100\ \text{mg}$ forage OM), it is necessary only to correct each fraction for

the content of OM in WF and maintain the relative proportions of each fraction. Within an individual sample, the fraction of ash_{WF} (total ash) that is insoluble in NDF solution is equivalent to the ash present in the NDF preparation. A similar statement may be made for an ethanol extraction and the EIR preparation. Thus, for gas curve subtraction the determination of preparation OM is not necessary to adjust gas volumes to an OM basis. To adjust gas volumes to an OM basis, the DM factors noted above are multiplied by $1/(1 - \text{ash}_{\text{WF}})$. When comparing the gas volume produced by the EIR or NDF preparations among feeds, preparation ash content does influence the results and should be considered. The values presented in this article are expressed in proportion to the OM content of WF.

Gas associated with the A and B_1 fractions was estimated using gas curve subtraction (Schofield and Pell, 1995a). Gas produced from the ethanol soluble fraction (A fraction) was determined by difference between the average gas yields of the whole feed sample and its EIR preparation (Schofield and Pell, 1995a). Likewise, the gas volume from the soluble fiber and starch (B_1 fraction) was determined by subtracting the gas produced by a sample's isolated NDF from the gas curve of the EIR preparation. When determining the A and B_1 fractions by difference, these fractions contain amino acids and protein, which if fermented will produce some gas. When protein is fermented in the absence of carbohydrate, deamination would be maximized. The gas yield from protein is only about one-half the volume from an equivalent amount of carbohydrate (Menke and Steingass, 1988). When feed samples are fermented, protein is not the sole energy source and less gas production from protein degradation would be expected.

Rate Calculation

Kinetic analysis of the 48-h cumulative gas production was performed using the one- and two-pool logistic models (Schofield et al., 1994). A model with more parameters (the two-pool model) usually increases the fit (F statistic), and the t -values (parameter value divided by the corresponding standard error) of the individual parameters reflect changing standard errors. The two-pool logistic model was chosen if a better fit (F statistic) was combined with similar t -values compared to those obtained with the one-pool model. For cases in which low t -values ($t < 12$) were obtained from the two-pool model, the one-pool logistic model was used. The one-pool logistic model takes the form $V = V_f * [1 + \exp(2 - 4S(t - \lambda))]^{-1}$, where V is the gas volume at time t , V_f is the maximum volume at $t = \infty$, S is a rate constant called the specific rate, and λ is a constant equivalent to a lag value (Schofield et al., 1994).

The gas curves obtained by subtraction for the A and B_1 fractions approached an asymptote between 12

Table 1. Chemical composition of the experimental forages

Item	Alfalfa				Bromegrass				Orchardgrass				Effect ^b			
	Vegetative		Mature		Vegetative		Mature		Vegetative		Mature		SE	M	P	M × P
	FD ^a	S ^a	FD	S	FD	S	FD	S	FD	S	FD	S				
	— % of Whole forage DM —															
DM ^c	18.7	26.2	20.5	32.2	22.3	23.1	27.0	30.6	22.1	25.2	24.4	31.6	1.3	.002	—	—
NDF	22.8	23.0	40.2	39.0	47.8	43.6	61.1	60.4	41.1	39.3	64.6	65.1	4.2	.002	.24	.42
ADF	17.8	19.9	32.6	33.2	25.5	26.0	34.3	34.5	22.0	22.9	38.6	38.3	2.1	.001	.07	.11
CP	31.7	30.7	17.8	17.5	15.1	14.8	8.6	8.8	25.0	24.0	8.3	7.8	2.5	.001	.005	.02
NPN ^d	34.1	68.1	22.5	61.1	27.2	77.0	19.8	54.5	26.8	72.5	25.3	44.9	6.1	.10	.02	.3
solCP ^d	13.1	22.6	8.1	11.6	6.2	12.1	3.1	4.8	10.9	18.4	3.5	4.0	1.8	.003	.007	.02
NDIP ^d	1.1	1.3	1.5	1.4	2.4	.6	1.6	1.7	3.3	1.1	1.4	1.6	.2	.83	.28	.25
ADIP ^d	.4	.5	.5	.7	.2	.2	.1	.3	.2	.1	.2	.3	.05	.04	.04	.04
Lignin ^d	3.4	4.5	8.1	8.3	2.5	3.0	2.8	4.0	1.9	2.1	5.5	5.5	.6	.007	.15	.80
Ash	7.5	8.0	8.0	7.7	6.6	7.0	6.0	6.1	6.6	6.5	5.0	5.2	.3	.05	.49	.49
Lactate ^d	—	10.0	—	8.2	—	12.7	—	7.3	—	10.6	—	8.0	.8	.001	—	—
EIR ^e	76.8	54.9	80.7	68.0	76.9	61.6	80.6	75.5	75.1	57.9	82.4	78.9	2.7	.004	.003	.02
EIR ash	5.3	4.4	5.3	3.9	3.7	3.1	3.4	2.6	3.5	2.6	2.6	2.1	.3	.08	.02	.75
EIR OM	71.5	50.6	75.3	64.0	73.2	58.5	77.2	72.9	71.6	55.4	79.8	76.8	2.7	.003	.002	.01
EIR CP	24.8	11.6	14.1	8.0	11.7	8.6	6.4	5.8	19.4	7.9	6.2	4.6	1.8	.03	.03	.10
A fraction ^f	14.1	22.4	13.0	18.8	16.9	28.3	14.6	18.1	16.2	22.1	13.1	14.8	1.3	.03	.02	.10
B ₁ fraction ^f	25.0	17.2	22.5	18.4	16.0	6.9	11.3	8.3	14.4	9.2	10.4	8.7	1.7	.07	.007	.04

^aFD = freeze-dried; S = silage.

^bM = maturity; P = preservation method; M × P = interaction between maturity and preservation method.

^cDM = % as-fed.

^dNPN = nonprotein N by tungstic acid precipitation (% total N); solCP = buffer soluble CP; NDIP = neutral detergent insoluble protein; ADIP = acid detergent insoluble protein; lignin = permanganate lignin; lactate was only determined in the silages.

^eEIR = residue insoluble in 90% ethanol.

^fA fraction = nonprotein OM soluble in 90% ethanol; B₁ fraction = carbohydrates insoluble in ethanol but soluble in neutral detergent solution.

and 24 h, indicating that these fractions had been depleted. However, the computerized system records values for the entire 48-h fermentation. Beyond the early fermentation of these fractions, the changes in gas volume are related to microbial turnover, changes in the rate of NDF disappearance, and some nonadditivity of the curve subtraction approach (Cone et al., 1996; Stefanon et al., 1996). To remove the data unrelated to the feed fermentation, the gas curves for the A and B₁ fractions were truncated for curve fitting after the curves initially stabilized. All curves were fitted using Table Curve (version 2.0; Jandel Scientific, San Rafael, CA).

Statistical Analyses

A randomized complete block design was used for the in vitro fermentations. The in vitro fermentations of all samples were repeated on 3 d approximately 1 wk apart. With the blocking factor as day of fermentation or ruminal fluid used for inoculation, treatment main effects and interactions were tested on the combination of treatment × block interaction and residual error. The experiment contained a 3 × 2 × 2 factorial arrangement of forage sample (alfalfa, bromegrass, or orchardgrass), maturity (vegetative or mature), and preservation method (freeze-dried or ensiled) in three blocks. Planned comparisons among the forages were estimated using orthogonal contrasts.

Results were deemed significant at $P < .05$ unless otherwise stated. All statistical analyses were performed using the GLM procedure of SAS (1985).

Results

The chemical composition of the forages in this experiment is presented in Table 1. The DM content of the vegetative forages was lower than expected (Table 1). However, the silages in this experiment were well-preserved, with a final pH of 4.0 or less except for the first cutting of alfalfa (pH = 4.5). Lactate made up between 7 and 12 % of DM and ammonia was low (< 1% DM). Only trace amounts of butyrate were detected in any of these silages. The difference in DM content of the silages was much lower than the changes in the chemical composition associated with increasing maturity.

As expected, as maturity increased the fiber and lignin contents increased and protein decreased. Ensiling significantly increased the amount of OM soluble in 90% ethanol (decreased EIR). When the soluble OM was adjusted for the soluble protein content, the amount of soluble carbohydrate and organic acids in the A fraction still increased with ensiling. The amount of increase in the A fraction due to ensiling was smaller in the more mature forages than in those that were younger. This result can be

Table 2. Least squares means of the digestion end products from the whole forage samples

Class ^a	Gas ^b , mL	NDFD ^c	C ₂ ^d , mM	C ₃ ^d , mM	C ₄ ^d , mM	C ₅ ^d , mM	VFA, mM	A:P ^d
Sample fraction ^e								
Whole forage	25.8 ^x	.72 ^x	24.3 ^x	14.5 ^x	4.4 ^x	2.0 ^x	45.3 ^y	1.68 ^z
Ethanol residue	25.9 ^x	.71 ^y	23.2 ^y	12.3 ^z	3.8 ^y	1.5 ^{xy}	40.8 ^x	1.92 ^x
NDF residue	28.5 ^y	.72 ^x	23.7 ^y	13.6 ^y	2.9 ^z	.9 ^y	41.1 ^x	1.75 ^y
SE	.2	.002	.4	.2	.2	.3	.7	.02
Maturity								
Vegetative	26.3 ^x	.84 ^x	25.3 ^x	15.4 ^x	5.1 ^x	2.3	48.1 ^x	1.64 ^y
Mature	25.2 ^y	.62 ^y	23.3 ^y	13.6 ^y	3.8 ^y	1.8	42.4 ^y	1.72 ^x
SE	.2	.004	.6	.3	.2	.6	1.1	.02
Preservation method								
Freeze-dried	27.3 ^x	.74 ^x	24.9	14.3	4.4	1.8	45.4	1.74 ^x
Silage	24.2 ^y	.71 ^y	23.6	14.7	4.5	2.3	45.2	1.62 ^y
SE	.2	.004	.6	.3	.2	.6	1.1	.02

^aClass variables for the treatment main effects in the factorial arrangement.

^bGas volume per 100 mg sample OM.

^cFraction NDF digested.

^dC₂ = acetate; C₃ = propionate; C₄ = butyrate; C₅ = valerate; A:P = acetate to propionate ratio.

^eEthanol residue = residue remaining after extraction with 90% ethanol; NDF residue = isolated NDF preparation.

^{x,y,z}Class means in a column lacking a common superscript letter differ ($P < .05$). There was no significant interaction between maturity and preservation method for these variables.

attributed to the higher fiber content, the percentage of DM of the silage, and the smaller initial A fraction. The proportion of B₁ fraction was decreased by ensiling.

Fermentation Summary

Digestibility of the NDF was unaffected by the ethanol extraction or isolation of the NDF (Table 2). However, when equal weights were compared (100 mg OM), the isolated NDF preparation produced more gas than the intact forage or ethanol residue. When adjusted to an OM basis, the whole forage samples produced more total VFA and had a lower acetate to propionate (A:P) ratio than the EIR or NDF preparations. Because acetate production is associated with the release of CO₂ from both microbial metabolism and the reaction of acid with the bicarbonate buffer, the increased A:P ratio from the isolated NDF would account for much of the difference in gas production. The digestibility of NDF in the whole forage samples was reduced by ensiling, as was final total gas volume (about 10%). Increasing maturity also reduced final gas volumes and NDF digestion.

The fermentation of the silages produced similar quantities of VFA compared with the freeze-dried samples but with a lower A:P ratio (Table 2). The VFA production of the more mature samples was decreased and the A:P ratio elevated, as would be expected given the higher fiber content. There was no interaction between maturity and preservation for the fermentation end products.

Curve Subtraction

Although the volumes associated with the A fraction were not significantly different due to preserva-

tion method, the A fraction accounted for more of the total gas produced in the ensiled forage than in the freeze-dried samples (Table 3). The rate of digestion of the ethanol solubles was significantly reduced in the ensiled forages (approximately 30% or .04 h⁻¹), as seen in Figure 1 (Panel A) for the vegetative alfalfa cutting. In addition to a lower rate, the A fraction of the ensiled forages also had an increased lag time.

Ensiling reduced the size of the B₁ fraction (soluble in neutral detergent solution, insoluble in ethanol) about 2 mL (about 30%), but the digestion rate was similar (Figure 1, Panel B; Table 3). In these forages the average rate of fermentation for the B₁ fraction exceeded the rate estimated for the more soluble A fraction.

The greatest change in gas volume occurred in the B₁ fraction (changes in the A or NDF fractions were not significant; Table 3). The reduced contribution to the total gas produced from the B₁ fraction in the ensiled forages increased the relative proportion of gas arising from the A and NDF fractions. The silages had a smaller total gas yield, with more gas arising from the NDF and A fractions and a slower overall rate of gas production compared with the freeze-dried forages.

Discussion

The addition of a new feed to models such as the CNCPS (Sniffen et al., 1992) and Level 2 of the Beef NRC (1996) requires a comprehensive chemical analysis and a digestion rate for three distinct carbohydrate fractions. The determination of these digestion rates is time-consuming (Pell and Schofield, 1993; Cone et al., 1996), which limits the number of samples that can be analyzed. The curve subtraction

Table 3. Average^a ensiling effects on the parameter estimates from the logistic models for the forage fractions as determined by curve subtraction

Preservation method	% Total volume ^b	Gas ^c , mL	% Fast ^c	FSR ^c	SSR ^c	Lag ^c , h
A fraction						
Freeze-dried	24.7 ^y	6.6	100	.118 ^x	ND	.2 ^y
Ensiled	26.7 ^x	6.3	100	.074 ^y	ND	1.6 ^x
SE	.6	.2		.004	ND	.1
B ₁ fraction						
Freeze-dried	21.9 ^x	5.8 ^x	100	.139	ND	.7
Ensiled	15.6 ^y	3.5 ^y	100	.142	ND	.8
SE	.9	.2		.008	ND	.2
NDF fraction						
Freeze-dried	53.4 ^y	14.5	46.0	.137	.036	6.4
Ensiled	57.7 ^x	13.8	43.6	.150	.037	6.6
SE	1.3	.3	1.1	.005	.001	.2
Whole forage						
Freeze-dried	—	27.4 ^x	52.6	.107	.033	1.6 ^y
Ensiled	—	24.4 ^y	52.4	.103	.034	3.4 ^x
SE	—	.2	.9	.003	.001	.1

^aLeast squares means for the main effect of preservation method.

^bFraction of gas volume in the specified forage fraction as a proportion of the sum of the gas volumes from the A, B₁, and NDF fractions.

^cAbbreviations for the kinetic parameters from the one-pool and two-pool logistic models (Schofield et al., 1994): Gas = gas volume adjusted to represent the proportion of mass represented in the digestion of 100 mg forage OM; % Fast = the percentage of the total gas in rapid kinetic pool (100 indicates the one-pool logistic model was applied); FSR = the specific rate for the rapid fraction; SSR = the specific rate for the slower kinetic fraction.

^{x,y}Means within a fraction lacking a common superscript letter differ ($P < .05$).

approach of Schofield and Pell (1995a) can provide estimates of digestion rates for dynamic models while reducing the labor investment, although the fermentation of three separate forage fractions is still required.

The curve subtraction technique is based on the assumption that the preparation of the forage fractions (EIR and isolated NDF) does not alter the fermentation of the insoluble carbohydrates (Schofield and Pell, 1995a). Soluble sugars may alter the fermentation of the associated fiber components by changing both the rate and extent (Piwonka and Firkins, 1996). Removal of the solubles during extraction eliminates the possibility of interactions that may occur during the fermentation of the whole forage. The effects of soluble carbohydrate and fiber interactions can be assessed by comparing the NDF digestibility of the intact forage with those of the EIR and NDF residues (Giner-Chavez, 1996). The similar NDF digestibilities of the whole forages and the insoluble residues suggest little interaction affecting the extent of fiber digestion.

A second way to identify unusual results is to monitor the fermentation end products of the EIR and NDF residues. Fermentation of the whole forage OM produced more VFA compared with the EIR and NDF preparations and yielded a lower A:P ratio. This reflects the increased proportion of indigestible fiber in the EIR and NDF fractions relative to total OM. The higher A:P ratio of the EIR fraction is interesting because pectic substances are in that fraction. Hall et al. (1998) also observed an elevated A:P ratio from

the fermentation of EIR preparations for several feeds. However, the VFA profile may be altered by technique (medium or pH), microbial population, or the type of substrate present.

Doane et al. (1997) showed that the gas yield and digestion rate from the NDS fraction of silages were lower than those from fresh forage. In the present experiments we have divided the NDS fraction into the ethanol-soluble A fraction and the B₁ fraction. The gas volume associated with the A fraction was unchanged, but there was a 30% decrease in digestion rate. The size of the B₁ fraction was reduced 40% on average by ensiling without a significant change in the rate of fermentation. Thus, the observation of decreased gas volume in the NDS of ensiled forages (Doane et al., 1997) is related to changes in the B₁ fraction, and a decreased rate was observed only in the A fraction.

During ensiling, soluble sugars in the A fraction are converted largely to lactate. At the same time, microbial activity and higher acidity cleave some of the side chains from the pectic substances and hemicellulose (Pettersson, 1988) that were originally in the B₁ and NDF fractions. These side chains may be further fermented in the A fraction to yield organic acids. This would explain the increased mass of the A fraction in the ensiled forage as material removed from the B₁ and NDF accumulated within the A fraction. However, the amount of gas produced from the A fraction of the ensiled forages did not increase with the mass of the A fraction, perhaps because

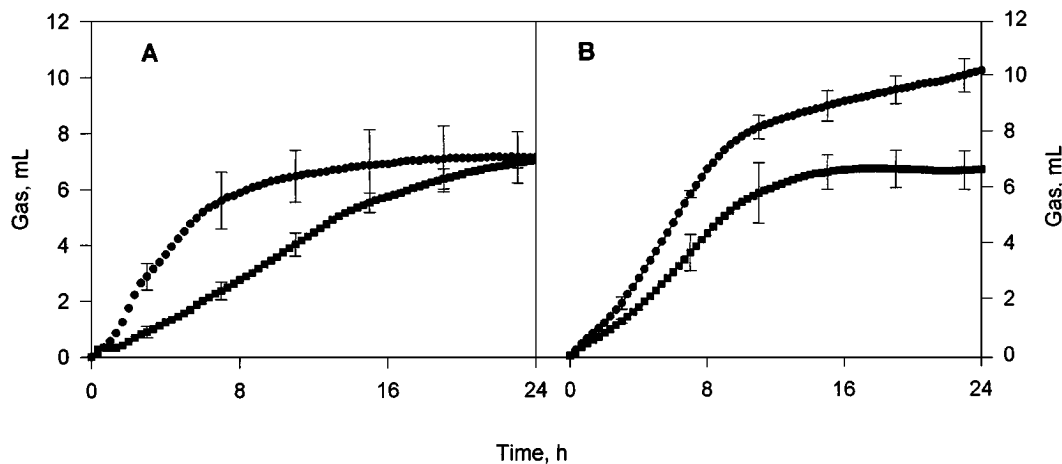


Figure 1. Gas production for the A (panel A) and B₁ (panel B) fractions of the vegetative alfalfa when freeze-dried (●) or ensiled (■). The first cutting date was May 20. Gas production is expressed based on the content of each fraction in 100 mg of whole forage OM.

lactate does not provide the same amount of energy for bacterial growth as the sugars that were originally present (Russell and Van Soest, 1984; Jaakkola and Huhtanen, 1992). The fermentation of lactate also produces propionate and could explain the lower A:P ratio observed for the ensiled forages. The decreased gas volume associated with the B₁ fraction in the ensiled material reflects the removal of material during the ensiling process and follows the changes in mass reported in Table 1. The relatively high fermentation rates of the B₁ fraction ($.14 \text{ h}^{-1}$) agree with those of Hall et al. (1998) and indicate that in these forages the B₁ fraction fermented as rapidly as the A fraction. The decreased NDF digestibility is also due to some fermentation of hemicellulose during ensiling (indicated by a tendency toward increased ADF content), which is reflected in the reduced gas production from the isolated NDF.

Summary

Curve subtraction, using a 90% EIR and isolated NDF, was used to separate the NDS fraction into two soluble pools that had distinct digestion rates. Ensiling reduced the rate of fermentation associated with the ethanol-soluble material but had less effect on the final gas volume. The size of the B₁ fraction was reduced during ensiling, but the fermentation rate was not changed.

Implications

Using the empirical relationship between acid detergent fiber content and digestibility as a basis, the energy content of forages is often predicted from the acid detergent fiber content. An implicit assumption of this approach is that the energy yield of the nonfiber fraction is constant. The results of this study indicate

that this assumption is not valid. An approach that can be used to evaluate the energy contribution of the nonfiber fractions more accurately is presented.

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