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The Effect of Preservation Method on the Neutral Detergent Soluble Fraction of Forages

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ABSTRACT: Fermentation of neutral detergent solubles (NDS) was assessed using a $3 \times 3 \times 3$ factorial arrangement. Three forage species (alfalfa, bromegrass, and orchardgrass) were collected at three maturities and preserved either by freeze drying, oven drying at 50°C, or by ensiling. Each feed sample and its isolated NDF were fermented in vitro and gas production was monitored. Gas yield from NDS was determined as the difference between gas from the unfractionated forage and from its respective NDF. The forages ranged from 23 (immature alfalfa) to 68% NDF (mature orchardgrass). The silages were well fermented with a final pH of ≤ 4.5 . Increasing maturity

decreased the final gas volume but did not change the rate of gas production from the NDS fraction. There was little difference in gas production between freeze-dried and oven-dried forage samples. Ensiling decreased gas yield from the unfractionated forage. The rate of gas production from the NDS fraction of the ensiled forages decreased an average of $.05 \text{ h}^{-1}$ compared with the freeze-dried sample. Gas yield from the NDS fraction decreased (from the freeze-dried sample) between 7 and 36% upon ensiling. The curve subtraction approach can be used to evaluate the effects of ensiling on the neutral detergent-soluble fraction of forages.

Key Words: Gas, Silage, Preservation, Carbohydrates, Digestibility

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Introduction

Ensiled forage is commonly fed to ruminants throughout the world, but prediction of the feed value of silage remains problematic (Van Soest, 1994). Ensiling a forage does not change DM digestibility but does convert sugars to volatile acids or ethanol (McDonald, 1981). Because the volatile acids are not fermented in the rumen, the ATP equivalents from ruminal fermentation of the soluble material may be less than 50% of that in the original forage (Van Soest and Allen, 1993).

Use of a single assay to measure the soluble carbohydrate fraction of silage is difficult because of the variety of sugars and organic acids present (Van Soest, 1994). Schofield and Pell (1995a) presented an indirect method for analysis of the neutral detergent soluble (NDS) fraction using curve subtraction. This approach was validated by Stefanon et al. (1996) using water-extracted forages. The NDS contribution may be calculated as the difference in gas produced between the unfractionated sample and its respective

ND fiber, assuming the fiber is unaltered by the extraction method. Curve subtraction permits measurement of the size and digestion kinetics of the soluble fraction without requiring a battery of analyses to describe the broad spectrum of forage materials.

The objectives of this experiment were to explore whether the curve subtraction technique of Schofield and Pell (1995a) could assess differences in the NDS fraction of fresh and ensiled forage and to measure differences in digestibility and digestion kinetics due to preservation method.

Materials and Methods

The effects of forage type, forage maturity, and preservation method were assessed using a $3 \times 3 \times 3$ factorial arrangement. The three forages studied were alfalfa (*Medicago sativa*), bromegrass (*Bromus inermis*), and orchardgrass (*Dactylis glomerata*). Beginning in May 1995 in Ithaca, NY, forages were harvested at approximately weekly intervals, three times (early, middle, and late maturity), to a stubble height of 10 cm. The harvest schedule included vegetative and reproductive growth of the grasses, and alfalfa approached midbloom on the final cutting date

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(June 17). We used three methods, freeze drying, drying in a forced-air oven at 50°C, and ensiling, to preserve the harvested forages.

Silage Preparation

Forages were ensiled using silos made from polyvinyl chloride (PVC) 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 between .63 and .80 kg/L. When the available forage did not fill the silo, 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 freeze-dried.

Forage Analysis

All samples were ground through a 1-mm screen using a Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA). Fiber and ash analyses were performed as described by Van Soest et al. (1991). The macro-Kjeldahl procedure (AOAC, 1990), modified by using boric acid in the distillation process, was used to measure nitrogen. 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 silage samples were determined at 60°C.

Neutral Detergent Fiber Isolation

Isolated NDF for each forage was prepared by autoclaving 150-mL serum bottles containing 500 mg of forage and 100 mL of ND solution (Pell and Schofield, 1993). The isolated NDF from different bottles was combined and rinsed with hot water and 100 mL of ethanol using 37- μ m nylon mesh as a filter (Tetko®, Briarcliff Manor, NY). Residual detergent was removed by soaking the isolated NDF overnight at 39°C in a solution of 1 M (NH₄)₂SO₄ in the ratio of 1 g of fiber to 100 mL of 1 M (NH₄)₂SO₄, with the fiber weight calculated from 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. For these experiments, sodium sulfide was replaced by an equal weight of cysteine hydrochloride. The medium

was boiled to remove dissolved gases, cooled, cysteine added, and the pH was adjusted to 6.8 as necessary.

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 bottle contained 8 mL of medium, 2 mL of ruminal fluid, and either 100 mg of whole forage or 75 mg of isolated NDF. Previous research in our laboratory indicated that, for 100 mg of sample DM, a 20% inoculum produced the maximum digestion rate (our unpublished observations). To ensure that the pH of the medium did not fall below 6.0, a level that produces a nonlinear relationship between substrate disappearance and gas production (Beuvink and Spoelstra, 1992), we used samples of 75 to 100 mg of DM.

Gas production was measured during a 48-h fermentation using a computerized monitoring system (Pell and Schofield, 1993; Schofield and Pell, 1995a). In these experiments, 50-mL serum bottles were used for all fermentations. Pressure sensors, with a range of 0 to 103.4 kPa (185PC15DT, Micro Switch, Freeport, IL), which were inserted into the fermentation vessels using a needle, measured cumulative gas pressure every 30 min. Bottles and sensors were calibrated as described previously (Schofield and Pell, 1995a,b). At the end of each fermentation, the pH was measured, 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 and no correction was made for the presence of water-insoluble gas. Gas produced from the NDS fraction was determined by difference between the average gas yields of the unfractionated feed sample and its respective NDF preparation (Schofield and Pell, 1995a).

Volatile Fatty Acid Analysis

A 1.5-mL aliquot of the medium was centrifuged at $4,000 \times g$ for 5 min, and 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- μ L aliquot of each sample was transferred to a microcentrifuge tube containing 40 μ L of 50 mM H₂SO₄. After mixing and standing at room temperature for 10 min, samples were centrifuged as described above and the supernatant was analyzed for VFA by the HPLC method of Ehrlich et al. (1981) using a BioRad HPX-87H column (7.8 \times 300 mm) at 30°C, isocratic elution with 5 mM H₂SO₄, and UV detection at 210 nm. A mixture of succinic, lactic, acetic, propionic, isobutyric, and butyric acids was used as an external standard in all analyses. Values have been corrected to exclude the amount of VFA added to the medium initially from either ruminal fluid or the sample (as with the silages). Where total VFA

production is discussed, the sum of acetic, propionic, isobutyric, and butyric acids is implied.

Rate Calculation

The cumulative gas curves from the 48-h digestion provided 96 time points (i.e., one observation every .5 h). Kinetic analysis of the 48-h cumulative gas production was performed using the two-pool logistic model (Schofield et al., 1994). A model with more parameters always increases the fit (F statistic), but the t -values (parameter value divided by the corresponding standard error) of the individual parameters reflect changing standard errors. Therefore, the two-pool model was chosen if a better fit (F statistic) was combined with t -values similar to those obtained with the one-pool model. For cases when a two-pool model gave low t -values ($t < 12$), the one-pool logistic model was used. The NDS gas curves obtained by subtraction reached a plateau after 12 h, indicating that this fraction had been depleted. After this time, changes in gas volume from NDS are related to microbial turnover, changes in the rate of NDF disappearance, and some nonadditivity of the curve peeling approach (Cone et al., 1996; Stefanon et al., 1996). For these reasons, gas production data after this time were not used for calculating the digestion rates of the NDS fraction.

All curves were fitted using Table Curve (version 2.0, Jandel Scientific, San Rafael, CA).

Experimental Design

The determination of NDS by difference requires the fermentation of an unfractionated and a NDF preparation for each sample of interest. For this

experiment, fermentations were conducted in duplicate. The duplicate gas production curves then were averaged for curve subtraction. The available incubator space was sufficient for the fermentations needed to represent 11 treatments (four bottles per treatment). Each block (a set of 48-h fermentations) consisted of a partial replicate of the factorial arrangement (one third, nine treatments) and the duplication of two treatments (within the partial replicate). Three blocks were necessary to include the complete set of experimental treatments. The fourth block was a replication of one of the first three blocks. The replication of a block and the duplication of treatments within the blocks provided the degrees of freedom needed for the block by treatment interaction terms used in the statistical analysis. Table 1 presents the treatment allocation for the blocks.

Statistical Analyses

All statistical analyses were performed using the GLM procedure of SAS (1985). Based on the factorial arrangement of treatments and the fractional replicates within each block, the model was $Y_{ijklm} = \mu + B_i + F_j + M_k + P_l + I_{ijkl} + e_{ijklm}$, where μ is the population mean, B is block, F is forage, M is maturity, P is preservation method, I is the combined treatment by block interactions, and e is the residual error (for $i = 1$ to 4 and $j, k, l = 1$ to 3). Because each block contains only a fraction of the experimental treatments, the main effects are partially confounded with some portion of the two-way interaction terms. Also, two-way interactions between main effects were confounded with each other and not separated. Planned comparisons for different means were estimated using

Table 1. Experimental assignment of treatments to blocks for the in vitro fermentations^a

| Treatments | Block ^b | | | |
|------------|--------------------|------|------|------|
| | 1 | 2 | 3 | 4 |
| 1 | B1s | A3fd | A1fd | A1fd |
| 2 | A2fd | B3d | O1s | O1s |
| 3 | O3d | A1s | B1d | B1d |
| 4 | B2d | O2fd | O2d | B2fd |
| 5 | A1d | B1fd | B2fd | O2d |
| 6 | O1fd | O3s | A3d | B3s |
| 7 | B1s | A2d | O3fd | A3d |
| 8 | A3s | B1fd | O1s | A1fd |
| 9 | B3fd | B2s | O2d | A2s |
| 10 | O2s | O1d | B3s | O3fd |
| 11 | A2fd | O2fd | A2s | B2fd |

^aAbbreviations: A = alfalfa, B = bromegrass, O = orchardgrass, 1 to 3 indicates cutting dates; for grasses (May 20, May 30, June 7), or alfalfa (May 20, June 1, June 17), fd = freeze-dried, d = dried at 50°C, s = ensiled.

^bBlocks represent a one-third fractional replicate of a $3 \times 3 \times 3$ factorial arrangement of experimental treatments. Within each block two randomly chosen treatments are duplicated. The equation defining confounded treatment effects was $X_1 + X_2 + X_3 = \{0, 1, 2\}$ (modulo 3), where X_1 = species, X_2 = cutting date, and X_3 = preservation method (Lentner and Bishop, 1993).

Table 2. Silage characteristics

| Forage and maturity (date) | DM, ^a % | Bulk ^b density | pH | Lactate, ^c % DM | Acetate, % DM | NH ₃ %, DM |
|----------------------------|-----------------------|------------------------------|------|-------------------------------|------------------|-----------------------|
| Alfalfa | | | | | | |
| May 20 | 26.2 | .80 | 4.53 | 10.0 | 2.5 | 1.42 |
| June 1 | 30.6 | .78 | 4.32 | 9.3 | 2.2 | .92 |
| June 17 | 32.2 | .74 | 4.03 | 8.2 | 1.9 | .80 |
| Brome | | | | | | |
| May 20 | 23.1 | .74 | 3.78 | 12.7 | 1.3 | .28 |
| May 30 | 29.3 | .73 | 3.83 | 9.4 | 1.1 | .17 |
| June 7 | 30.6 | .71 | 3.78 | 7.3 | .3 | .12 |
| Orchardgrass | | | | | | |
| May 20 | 25.2 | .66 | 3.91 | 10.6 | .5 | .60 |
| May 30 | 25.8 | .69 | 3.85 | 7.9 | .4 | .17 |
| June 7 | 31.6 | .66 | 3.77 | 8.0 | .3 | .12 |

^aMeasured at 60°C.

^bkg/L.

^cPropionate and butyrate found in trace amounts (< .1% DM) only.

contrasts. Block effects were tested on the within-error term, and main effects were tested on the combined block by treatment interaction term.

Results

Forages

The silages were of good quality, as indicated by the analytical values presented in Table 2. Several DM values were below the target of 30%, but only trace amounts of butyrate were found in any of these silages. Ammonia and pH values were within reported ranges given the silage DM (McDonald, 1981). The fiber and protein composition of the experimental forages are shown in Table 3. The immature forages (first cutting date) had high soluble protein contents, in keeping with the low DM and high crude protein values. The pattern of changes in the protein fractions are in agreement with those previously reported (Kohn and Allen, 1992). The differences in NDF content between the final two cuttings of the grasses were less than 5% because of a warm spring and rapid maturation. Fiber content of the alfalfa samples increased between each cutting date. Analysis of the oven-dried samples revealed small increases ($P < .05$) in NDF, ADF, acid detergent insoluble protein, and lignin content and may indicate that some Maillard products might have been formed during drying. Even with these differences, NDF digestibility and gas production results were comparable to those of the freeze-dried samples.

Figure 1 shows the in vitro gas production from the fermentation of the unfractionated immature forages (first cutting date, freeze-dried). Bromegrass was more digestible with a greater final gas volume ($P < .05$) than alfalfa and orchardgrass (Table 4). Good statistical fits (t -values >12) were obtained using the

two-pool logistic model providing a description of two kinetic pools with significantly different numerical rates (fast pool > slow pool). Alfalfa had a slightly higher specific rate (by $.02 \text{ h}^{-1}$) associated with the fast pool than the two grasses, but less total gas production. In the grasses, approximately 2 mL more gas was associated with the slow pool than the alfalfa, but differences in digestion rates were very small. The larger amount of soluble fiber in alfalfa compared to

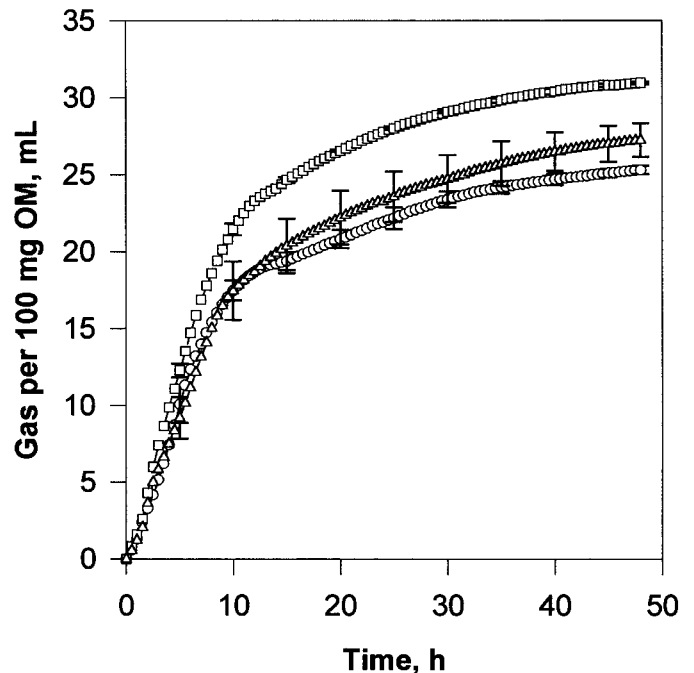


Figure 1. Gas production comparisons among the unfractionated freeze-dried samples of bromegrass (□), orchardgrass (△), and alfalfa (○) from the first cutting date. Gas production expressed on the basis of 100 mg of forage OM. Error bars represent ± 1 SD.

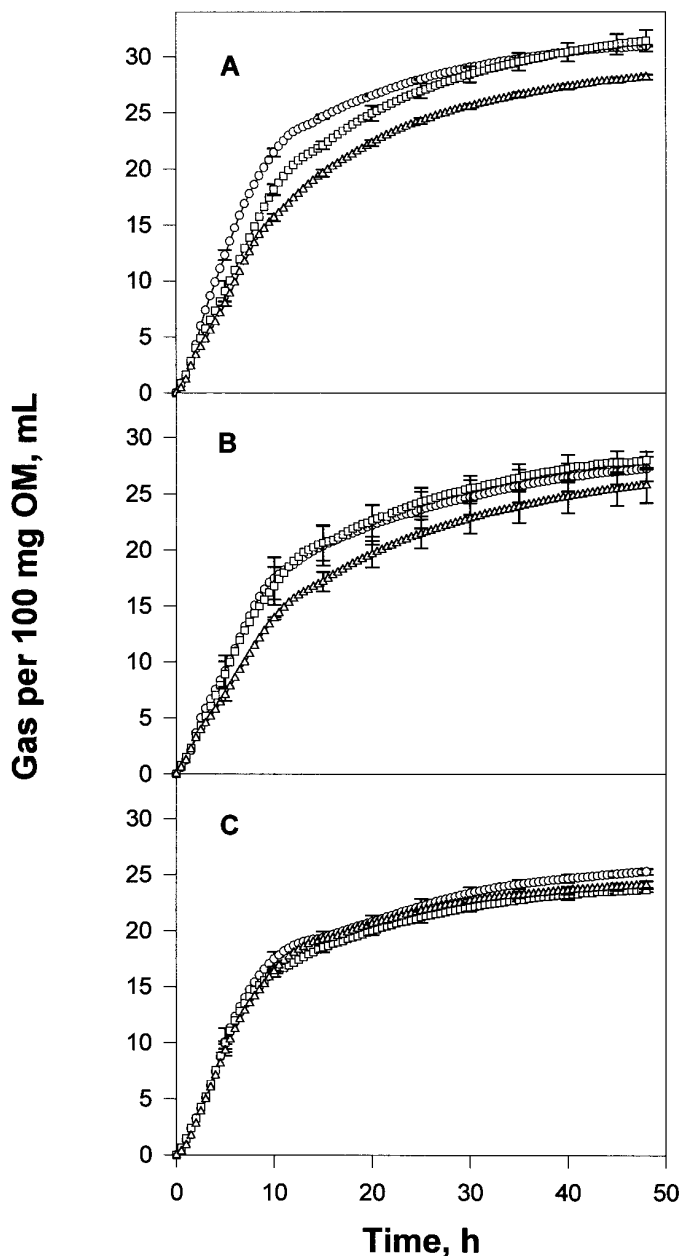


Figure 2. Differences in maturity (cutting date) among the unfractionated freeze-dried samples of forage. Cutting dates were May 20 (\circ), May 30 (\square), June 7 (\triangle) for grasses and May 20, June 1, June 17 for alfalfa (same symbol order). Panels A, B, and C present data from bromegrass, orchardgrass, and alfalfa, respectively. Error bars represent ± 1 SD.

grasses may account for the larger size of the fast pool (Table 4) but, because the fast pool was calculated mathematically and soluble fiber is a chemical entity, it is not possible to equate the two.

There were only small differences in gas production among the unfractionated forages due to maturity (cutting date). Gas production decreased with increasing maturity (Figure 2). Advancing maturity consistently reduced the proportion of gas yield in the

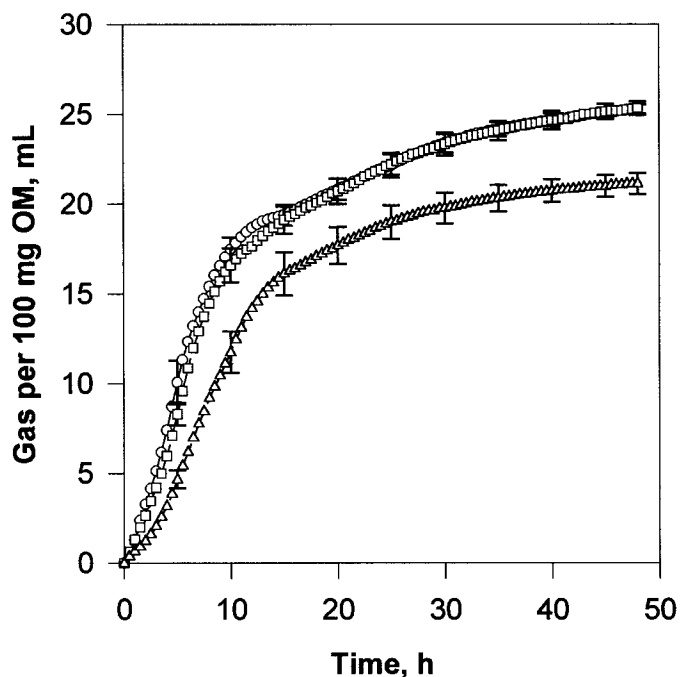


Figure 3. The effect of preservation method on the gas produced by the first cutting (May 20) of alfalfa. The preservation treatments are: dried at 50°C (\square), freeze-dried (\circ), and ensiled (\triangle). Error bars represent ± 1 SD.

fast pool and also decreased NDF digestibility for all species (Table 4). The isolated NDF fermentation also showed a decreased gas percentage associated with the fast pool (data not shown). In neither the unfractionated forage nor its isolated NDF was the specific rate of the fast pool altered by maturity.

Preservation Effects

The amounts of gas produced from the fermentation of the freeze-dried and oven-dried samples were similar (Figure 3). The small (1 mL) reduction in final gas volume was statistically significant (Table 4) but would be of small consequence biologically. The specific rate and proportion of the fast kinetic fraction were reduced in the oven-dried material compared to the freeze-dried treatment. When compared with the freeze-dried samples (a proxy for fresh material), ensiling reduced the final gas volume, increased the lag time, and also decreased the total VFA production on an OM basis.

Differences in gas production due to preservation method (ensiling) may be presented in two ways (Figure 4): 1) unfractionated freeze-dried forage – unfractionated ensiled forage, and 2) freeze-dried NDS – ensiled NDS. The maximum difference occurs within the first 12 h for these forage samples. The decreased gas production due to ensiling is nearly the same volume whether calculated from the NDS fraction or the unfractionated forages. This indicates

ensiling predominantly affected the NDS fraction. The differences in gas production between the freeze-dried and ensiled forages were greater in the immature forage that contained more NDS than in the older samples.

The NDS determination by subtraction is based on the assumption that preparation of the NDF residue does not alter the fermentation characteristics of the fiber. Changes in lag time or fermentation rate would bias the estimate of the NDS fraction. The curves shown in Figure 4 are nearly parallel. This indicates that the fermentation of the fiber within the unfractionated forage and the isolated NDF were similar. The average NDF digestibility values obtained from the unfractionated forages and the NDF preparations were similar (.74 and .73, respectively).

Table 5 presents the kinetic analysis of the NDS using the first 12 h of data. Similar to the results of the whole forage fermentations, ensiling reduced the final gas volume. Ensiling also clearly reduced the specific rate of fermentation for the soluble fraction by

approximately 30% ($.05 \text{ h}^{-1}$), which was not apparent in the whole forage data (Figure 5, Table 4). Similarly, the analysis of the whole forages indicated a reduction in fast specific rate due to drying that was not apparent in the NDS fraction.

Discussion

The energy values commonly reported for ensiled forages are often derived from the empirical relationship between energy and fiber (ADF) content (Linn and Martin, 1991). At the same time, programs for ration balancing have incorporated more carbohydrate fractions in the attempt to more accurately describe feed values across a spectrum of feeding situations (Sniffen et al., 1992; NRC, 1996). Few data are available on the digestion rates of soluble materials from ensiled forages. However, some limited data have been obtained from pure culture studies with single substrates (Russell and Van Soest, 1984).

Curve subtraction seems to be a promising technique to describe changes in the soluble fraction of forages. The reduction in gas yield from the

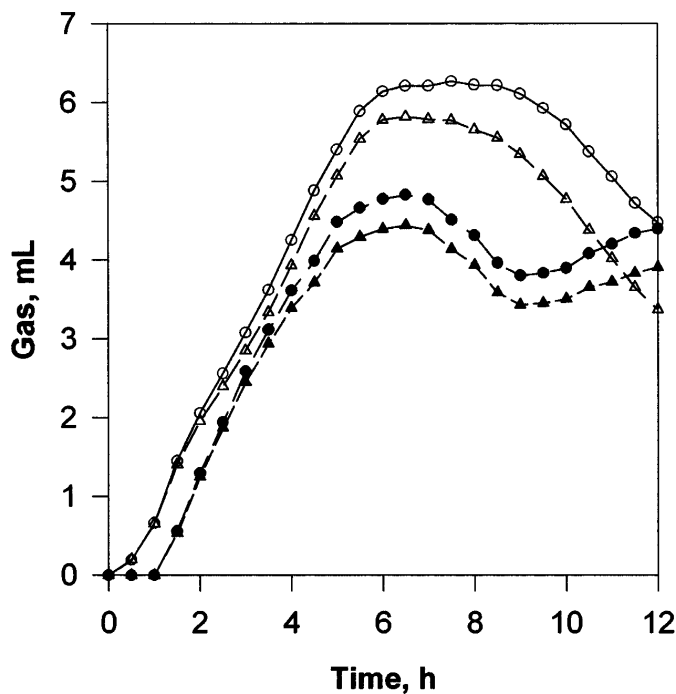


Figure 4. The discrepancy in gas produced by fermentation of the freeze-dried unfractionated forage and ensiled material (freeze-dried – ensiled). Calculations from the unfractionated alfalfa forages (\circ , \bullet) and the neutral detergent solubles (NDS) (\triangle , \blacktriangle) presented for two cutting dates; May 20 (open symbols), and June 17 (filled symbols). Gas production for the NDS was calculated by difference between the gas yield of unfractionated forage and the gas yield of its respective NDF preparation. Gas volumes are based on the milliliters of gas from 100 mg of unfractionated forage OM.

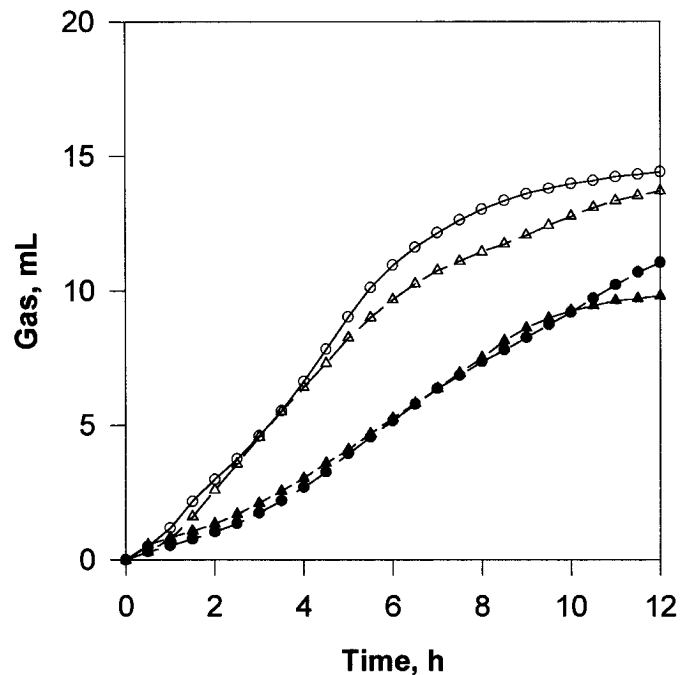


Figure 5. Gas production curves from the neutral detergent soluble fraction (NDS) of freeze-dried (open symbols) and ensiled (filled symbols) alfalfa from the first cutting (May 20, \circ , \bullet) and last cutting date (June 17, \triangle , \blacktriangle). Gas production for the NDS was calculated by subtracting the gas yield of the NDF preparation from the gas volume of its respective unfractionated forage. Gas volumes presented as the milliliters of gas from the NDS in 100 mg sample OM.

Table 3. Chemical analyses of forages

| Date ^a and pres. ^b | NDF | ADF | CP | NPN ^c | solCP | NDIP | ADIP | Lignin | Ash |
|--|------|------|------|------------------|-------|------|------|--------|-----|
| % of DM | | | | | | | | | |
| Alfalfa | | | | | | | | | |
| May 20 | | | | | | | | | |
| D | 26.2 | 20.0 | 32.1 | 12.2 | 12.4 | 2.0 | .5 | 4.6 | 7.8 |
| FD | 22.8 | 17.8 | 31.7 | 10.8 | 13.1 | 1.1 | .4 | 3.4 | 7.5 |
| S | 23.0 | 19.9 | 30.7 | 20.9 | 22.6 | 1.3 | .5 | 4.5 | 8.0 |
| June 1 | | | | | | | | | |
| D | 35.1 | 27.9 | 25.8 | 8.7 | 9.8 | 1.7 | .6 | 7.1 | 7.1 |
| FD | 31.8 | 25.0 | 24.6 | 8.4 | 11.9 | 1.2 | .4 | 5.4 | 7.0 |
| S | 32.1 | 27.4 | 25.1 | 14.7 | 15.6 | 1.6 | .6 | 6.9 | 7.2 |
| June 17 | | | | | | | | | |
| D | 43.9 | 36.5 | 18.3 | 6.3 | 7.0 | 1.9 | .7 | 9.4 | 8.1 |
| FD | 40.2 | 32.6 | 17.8 | 4.0 | 8.1 | 1.5 | .5 | 8.1 | 8.0 |
| S | 39.0 | 33.2 | 17.5 | 10.7 | 11.6 | 1.4 | .7 | 8.3 | 7.7 |
| Bromegrass | | | | | | | | | |
| May 20 | | | | | | | | | |
| D | 49.1 | 26.8 | 15.4 | 4.3 | 4.8 | 2.7 | .2 | 2.6 | 6.8 |
| FD | 47.8 | 25.5 | 15.1 | 4.1 | 6.2 | 2.4 | .2 | 2.5 | 6.6 |
| S | 43.6 | 26.0 | 14.8 | 11.4 | 12.1 | .6 | .2 | 3.0 | 7.0 |
| May 30 | | | | | | | | | |
| D | 58.9 | 31.7 | 12.4 | 1.6 | 2.8 | 4.0 | .2 | 3.8 | 6.5 |
| FD | 55.4 | 30.8 | 12.7 | 2.3 | 4.9 | 2.1 | .1 | 2.9 | 6.9 |
| S | 54.4 | 31.1 | 12.4 | 5.6 | 7.3 | 2.3 | .2 | 4.0 | 6.9 |
| June 7 | | | | | | | | | |
| D | 63.9 | 35.4 | 9.6 | 1.7 | 2.6 | 3.4 | .3 | 4.4 | 6.3 |
| FD | 61.1 | 34.3 | 8.6 | 1.7 | 3.1 | 1.6 | .1 | 2.8 | 6.0 |
| S | 60.4 | 34.5 | 8.8 | 4.8 | 4.8 | 1.7 | .3 | 4.0 | 6.1 |
| Orchardgrass | | | | | | | | | |
| May 20 | | | | | | | | | |
| D | 44.1 | 24.3 | 25.8 | 6.6 | 11.6 | 3.8 | .2 | 2.4 | 6.8 |
| FD | 41.1 | 22.0 | 25.0 | 6.7 | 10.9 | 3.3 | .2 | 1.9 | 6.6 |
| S | 39.3 | 22.9 | 24.0 | 17.4 | 18.4 | 1.1 | .1 | 2.1 | 6.5 |
| May 30 | | | | | | | | | |
| D | 66.1 | 37.6 | 10.0 | 2.5 | 3.0 | 3.1 | .4 | 5.2 | 6.4 |
| FD | 60.2 | 34.5 | 12.0 | 2.3 | 4.6 | 1.7 | .2 | 4.0 | 7.4 |
| S | 59.7 | 34.5 | 10.5 | 3.4 | 5.9 | 2.3 | .2 | 4.0 | 6.6 |
| June 7 | | | | | | | | | |
| D | 67.9 | 39.7 | 7.9 | 1.7 | 2.2 | 2.3 | .4 | 5.9 | 5.3 |
| FD | 64.6 | 38.6 | 8.3 | 2.1 | 3.5 | 1.4 | .2 | 5.5 | 5.0 |
| S | 65.1 | 38.3 | 7.8 | 3.5 | 4.0 | 1.6 | .3 | 5.5 | 5.2 |

^aCutting date.

^bPres. = method of preservation. D = dried at 50°C, FD = freeze-dried, S = silage.

^cNPN = nonprotein nitrogen by tungstic acid precipitation ($N \times 6.25$), solCP = buffer soluble CP, NDIP = ND insoluble protein, ADIP = AD insoluble protein.

fermentation of the unfractionated forages due to ensiling was similar to the reduced gas yield from the fermentation of the NDS fraction. This suggests the main effects of ensiling were on the NDS fraction and agrees with the general expectation of small changes in the fiber fraction (McDonald, 1981). Gas production of the ensiled forage was 3 mL (approximately 10%) lower than that of the freeze-dried material. However, when expressed as a percentage of the NDS in the freeze-dried sample, ensiling reduced the gas produced from the NDS fraction between 7 and 36% (bromegrass and orchardgrass, May 30 cutting date, respectively, data not shown). This is lower than the

theoretical values of Van Soest and Allen (1993). However, these silages contained more lactic acid, and, perhaps, more soluble sugars than was assumed for the theoretical calculations.

The solubles were rapidly fermented and the rates for the freeze-dried samples were comparable to previously published data (Schofield and Pell, 1995a; Stefanon et al., 1996). In agreement with Stefanon et al. (1996), maturity had no effect on the digestion rate of the soluble fraction, but the volume of gas produced decreased (Table 5).

Our data show that ensiling reduces the logistic NDS fermentation rate by approximately $.05 \text{ h}^{-1}$.

Table 4. Least squares means representing the main treatment effects for the unfractionated forage samples

| Class ^c | Gas ^b , mL | Fast, % | FSR, h ⁻¹ | SSR, h ⁻¹ | Lag, h | NDF, dig. ^c | C ₂ ^d , mM | C ₃ , mM | C ₄ , mM | VFA, mM | A:P |
|--|--------------------------|-------------------|-------------------------|-------------------------|------------------|---------------------------|-------------------------------------|------------------------|------------------------|--------------------|--------------------|
| Effect of forage species | | | | | | | | | | | |
| Alfalfa | 22.6 ^g | 59.7 ^e | .150 ^e | .034 ^e | 2.3 ^f | .60 ^g | 34.4 ^f | 19.4 ^f | 5.4 | 59.2 ^f | 1.76 |
| Bromegrass | 28.8 ^e | 53.4 ^f | .125 ^f | .032 ^{ef} | 2.3 ^f | .85 ^e | 40.6 ^e | 22.4 ^e | 5.5 | 68.4 ^e | 1.80 |
| Orchardgrass | 25.2 ^f | 52.8 ^f | .129 ^f | .030 ^f | 2.5 ^e | .76 ^f | 35.3 ^{ef} | 20.5 ^e | 5.2 | 61.0 ^{ef} | 1.72 |
| SE | .1 | .4 | .002 | .001 | .05 | .002 | 1.7 | .8 | .3 | 2.7 | .02 |
| Effect of maturity ^h (cutting date) | | | | | | | | | | | |
| Early | 26.3 ^e | 60.0 ^e | .138 | .031 | 2.2 ^f | .84 ^e | 37.5 | 22.4 ^e | 5.7 | 65.6 | 1.68 ^f |
| Middle | 26.1 ^e | 54.4 ^f | .134 | .032 | 2.5 ^e | .73 ^f | 37.0 | 20.4 ^{ef} | 5.2 | 62.6 | 1.79 ^{ef} |
| Late | 24.3 ^f | 51.2 ^g | .132 | .032 | 2.5 ^e | .64 ^g | 35.7 | 19.5 ^f | 5.2 | 60.4 | 1.82 ^e |
| SE | .1 | .4 | .003 | .001 | .1 | .002 | 1.8 | .8 | .3 | 2.8 | .02 |
| Effect of preservation method | | | | | | | | | | | |
| Dried 50°C | 26.4 ^f | 53.6 ^g | .132 ^f | .032 | 2.2 ^g | .73 ^f | 40.0 ^e | 21.1 ^{ef} | 5.6 ^{ef} | 66.7 ^e | 1.91 ^e |
| Freeze-dried | 27.3 ^e | 55.5 ^f | .141 ^e | .031 | 1.8 ^f | .75 ^e | 40.0 ^e | 22.3 ^e | 5.7 ^e | 68.0 ^e | 1.79 ^f |
| Silage | 23.0 ^g | 56.8 ^e | .131 ^f | .033 | 3.2 ^e | .73 ^f | 30.2 ^f | 18.9 ^f | 4.7 ^f | 53.9 ^f | 1.58 ^g |
| SE | .1 | .4 | .002 | .001 | .1 | .002 | 1.8 | .8 | .3 | 2.8 | .02 |

^aClass variables for the main treatment effects.

^bAbbreviations for the kinetic parameters from the two-pool logistic model (Schofield et al., 1994): Gas from the digestion of 100 mg forage OM, Fast (%) = the percentage of the total gas in rapid kinetic pool, FSR = the specific rate for the rapid fraction, SSR = the specific rate for the slower kinetic fraction.

^cFraction of NDF digested.

^dTotal VFA (millimolar) from 100 mg forage OM, fluid volume was 10 mL, C₂ = acetate, C₃ = propionate, C₄ = butyrate, A:P = acetate to propionate ratio.

^{e,f,g}Class means with different superscripts differ ($P < .05$).

^hCutting dates for alfalfa (May 20, June 1, June 17) and grasses (May 20, May 30, June 7).

Because the silage NDS fraction is a heterogeneous mixture of organic acids, sugars, volatile fatty acids, and lactate in addition to nitrogenous compounds and lipids, it is difficult to determine the reason for this rate reduction. Lactate, an important component of ensiled forages, may play a significant role. It is well

established that the ATP yield from lactate is lower than that for glucose for both aerobic and anaerobic systems (Stouthamer, 1979), but few data are available on the rate of lactate utilization in mixed cultures.

Other organic acids may also contribute to the observed rate reduction. Russell and Van Soest (1984) reported rapid fermentation of several organic acids found in plants, many of which were fermented within 12 h. When we calculated the logistic digestion rates of organic acids from their data, the rates were between .14 and .24 h⁻¹. The specific rates we obtained for the complex NDS fraction (.10 to .14 h⁻¹) were slightly lower than those that they obtained from their experiments using pure cultures and purified organic acids. These rates are lower than those obtained from fermentations of glucose by mixed cultures (unpublished data from our laboratory).

The gas yield from ensiled feeds was less than from freeze-dried material. Because fermentation of lactate contributes less ATP to bacterial growth, it follows logically that gas production also will be reduced in ensiled feeds compared with freeze-dried material. The data of Peltekova and Broderick (1996), who found that more microbial protein was produced from the fermentation of alfalfa hay compared with silage from the same source, agree with this hypothesis.

Table 5. Least squares means of the kinetic^a parameters for the NDS fraction

| Class ^b | Gas, mL | Rate, h ⁻¹ | Lag, h |
|--|-------------------|--------------------------|------------------|
| Effect of forage species | | | |
| Alfalfa | 12.3 ^c | .146 | 1.4 ^c |
| Bromegrass | 8.8 ^d | .155 | .5 ^d |
| Orchardgrass | 7.7 ^e | .151 | .6 ^d |
| SE | .2 | .004 | .06 |
| Effect of maturity ^f (cutting date) | | | |
| Early | 11.1 ^c | .156 | .9 ^c |
| Middle | 9.2 ^d | .148 | .8 ^{cd} |
| Late | 8.4 ^e | .148 | .7 ^d |
| SE | .2 | .004 | .06 |
| Effect of preservation method | | | |
| Dried 50°C | 9.5 ^d | .166 ^c | .7 ^d |
| Freeze-dried | 10.9 ^c | .170 ^c | .6 ^d |
| Silage | 8.4 ^e | .117 ^d | 1.2 ^c |
| SE | .2 | .004 | .06 |

^aKinetic parameters for the one-pool logistic model (Schofield et al., 1994).

^bClass variables of the main treatment effects.

^{c,d,e}Class means with different superscripts differ ($P < .05$).

^fCutting dates for alfalfa (May 20, June 1, June 17) and grasses (May 20, May 30, June 7).

Implications

Many of the methods used to evaluate silage quality do not permit measurement of fermentation kinetics of

the soluble carbohydrate fractions. This is a serious shortcoming because a poor silage fermentation process can significantly reduce the amount of energy available from the feed and the rate at which this energy is available. The curve subtraction approach described in this paper is a promising solution to these problems. The new approach may provide a better way to accurately predict the energy value of silages than the prevalent method of using acid detergent fiber to predict energy values.

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