Chemical and Physical Characteristics of Corn Silages and Their Effects on In Vitro Disappearance

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ABSTRACT
Estimating the available energy in corn silage provides a unique challenge because the silage contains variable proportions of grain and stover, each of which can differ in availability due to chemical composition and physical form. The objectives of this study were to investigate relationships among chemical components and their relationships with in vitro disappearance of ground and unground dried silages, and to quantify minimally fragmented starch in corn silage and investigate its impact and that of mean particle size (MPS) on in vitro disappearance of unground silages. Thirty-two corn silages were selected to provide diversity in dry matter, protein, fiber, and MPS. Detergent fibers were highly correlated with each other and with nonfiber carbohydrates, and were used to develop prediction equations between these constituents. Sieves with apertures ≥4.75 mm were used to isolate intact kernels and large kernel fragments, which were collected and analyzed to measure minimally fragmented starch (Starch >4.75). Dividing Starch >4.75 by total starch defined the proportion of minimally fragmented starch (Starch >4.75/Total), which ranged from 9 to 100% with a mean of 52%. Starch >4.75/Total was positively correlated with MPS (r = 0.46). The inverse of Starch >4.75/Total is an index of kernel fragmentation. Silages were prepared as whole material or ground to pass through a 4- or 1-mm screen of a cutter mill. In vitro dry matter disappearance (IVDMD) was greater for ground than for whole samples (71.7 and 61.2%, respectively). Increased IVDMD for ground samples was attributed to greater in vitro neutral detergent fiber (NDF) and neutral detergent solubles (NDS) disappearances. The IVDMD of ground samples was related to NDF and acid detergent lignin (R² = 0.80). The IVDMD of whole corn silage was related to acid detergent lignin, Starch >4.75, MPS, and dry matter. When IVDMD was partitioned into in vitro digestible NDS (IVdNDS) and in vitro digestible NDF, the IVdNDS of whole was not uniform or completely fermented. The difference in IVdNDS between ground and whole was related to Starch >4.75/Total. In conclusion, the proportion of minimally fragmented starch provides a corn silage fragmentation index that is related to the in vitro digestion of whole silages that, if validated by in vivo trials, may be a useful quantitative substitute for the qualitative processing adjustment factor that is used currently in summative equations for estimating the total digestible nutrients of corn silages.

INTRODUCTION
Processing of corn silage through rollers during chopping often increases starch digestibility (Rojas-Burrilllon et al., 1987; Bal et al., 2000; Weiss and Wyatt, 2000; Andrae et al., 2001; Schwab et al., 2002), although this effect has been inconsistent (Johnson et al., 2003; Cooke and Bernard, 2005). Variable responses may be related to differences in the extent of kernel fragmentation. Cooke and Bernard (2005) observed 12% lower starch digestibility for corn silage processed with an 8-mm clearance between rollers compared with corn silage processed with a 2-mm clearance between rollers. Johnson et al. (2003) reported that corn silage processing did not affect total tract starch digestibility in one of their studies, and suggested that this lack of effect might have been related to the similar proportions of
intact corn kernels observed for processed and unprocessed corn silages. These observations show the importance of describing the effectiveness of kernel fragmentation, given that not all processed corn silage results in complete kernel fragmentation and not all unprocessed corn silage results in incomplete kernel fragmentation (Johnson et al., 2003; Cooke and Bernard, 2005). Methods are needed to quantitatively describe the extent of kernel fragmentation in corn silage so the effect of kernel fragmentation can be related to DM and starch digestibility in corn silage.

Corn silage contains variable proportions of grain and stover, each of which can differ in chemical composition and physical form. These variable constituents of corn silage create problems when attempting to predict energy value using chemical composition in empirical or mechanistic equations (Weiss, 1994, 1997; Undersander, 1997). Summative equations are simple mechanistic models that are based on the concentration and digestibility of different nutrients. The mechanistic equation adopted by the NRC (2001) estimates available energy as the sum of digestible NDF, CP, fat, and NFC. This complex summative equation requires chemical information that may not be routinely available. Developing relationships among components in corn silage would be useful when necessary information is missing.

In vitro digestibility assays can be used to investigate factors affecting forage digestion. For standard in vitro procedures (Tilley and Terry, 1963; Goering and Van Soest, 1970), forage materials are often ground before fermentation. Consequently, the effects of physical characteristics of corn silage may not be reflected in standard in vitro disappearance determinations. In this study we used a “macro” in vitro system to determine in vitro disappearance of corn silages in their native form, allowing us to test the hypotheses that grinding increases in vitro disappearance of corn silage and that the magnitude of this increase is related to the extent of kernel fragmentation.

The objectives of our study were: 1) to use a diverse set of corn silage samples to investigate relationships among chemical components and their relationships to in vitro disappearance of corn silage; 2) to evaluate how grinding affects in vitro disappearance of corn silage; and 3) to measure particle size and quantify the intact kernels and large kernel fragments in diverse corn silages and determine how they affect in vitro disappearance of unground corn silage.

MATERIALS AND METHODS

Corn Silages

Thirty-two corn silages were obtained from a commercial feed analysis laboratory (Dairyland Laboratories, Inc., Arcadia, WI) to be diverse in chemical and physical characteristics. All silages were selected from an initial set of 51 corn silages. Six corn silages were selected for fine, medium, and coarse particle size based on visual appraisal. The remaining corn silages were selected based on the amount of available material and on diversity of DM, CP, NDF, ADF, and starch concentrations. For each component, 2 samples representing the mean, 2 samples representing 2 standard deviations above the mean, and 2 samples representing 2 standard deviations below the mean were selected, while attempting to keep all other components as close as their means as possible. Four of these corn silages met 2 selection criteria resulting in the final selection of 26 corn silages based on diverse composition. No information regarding genotype or harvesting procedures was received, but visual appraisal (e.g., broken cobs and fractured kernels in silages that were coarsely chopped) indicated that a majority of the corn silages had been processed through rollers.

Chemical Analysis and Particle Size Determination

Dry matter concentration was determined by drying the samples in a forced-air oven for 24 h at 55°C. Concentrations of ADF, acid detergent lignin (ADL) using sulfuric acid, and ash were measured as described by AOAC (1990). Concentration of amylase-treated NDF (aNDF) was determined using both amylase and sodium sulfite (Mertens, 2002a). Concentration of CP was measured as N × 6.25 after analysis with N analyzer (Leco FP-2000; Leco Corp., St. Joseph, MI). Nonfiber carbohydrate was calculated as NFC = 100 - (aNDF + CP + ether extract + ash), where aNDF was not corrected for CP or ash and ether extract was considered constant for all silages and equal to 3.2% (NRC, 2001). Concentrations of starch were measured by a commercial feed analysis laboratory (Dairyland Laboratories, Inc.) using a YSI Biochemistry analyzer (Yellow Springs Instrument Inc., Yellow Springs, OH). Mean particle size (MPS) of corn silages was determined by sieving dry samples for 20 min using a vertical shaker (model RX-24, W. S. Tyler Inc., Mentor, OH). Sieves (ATM Corporation, Milwaukee, WI) with square apertures of 19.00, 13.20, 9.50, 6.70, 4.75, 2.36, and 1.18 mm, in addition to the bottom pan, were used. Mean particle size was calculated as described by ANSI (1993), except the square instead of the diagonal dimension of apertures were used (Mertens et al., 1984).

Kernel Collection and Analysis

In a preliminary study, we observed that sieves with square apertures of 6.25 mm and larger retained most...
intact corn kernels and those with apertures of 4.75 mm retained kernel fragments bigger than one-fourth of a kernel. The proportion of the corn silage DM that is intact kernels and kernel fragments retained on sieves with apertures of 4.75 mm and larger was defined as “minimally fragmented kernels” (CG$_{>4.75}$), and the concentration of starch in CG$_{>4.75}$ expressed as a percentage of total corn silage DM was defined as “minimally fragmented starch” (Starch$_{>4.75}$).

To measure CG$_{>4.75}$, a portion of undried corn silage (233 ± 89 g for each silage) was sieved for kernel collection. Materials were separated by shaking for 15 min using a vertical shaker and sieves with square apertures of 19.00, 13.20, 9.50, 6.70, and 4.75 mm (in addition to a pan). Kernels and kernel fragments retained on all sieves were manually collected and dried in a forced-air oven for 24 h at 55°C. The nonkernel material remaining on the sieves was dried in a forced-air oven for 24 h at 55°C. The proportion of minimally fragmented kernels was calculated as dry weight of collected kernels and divided by dry weight of the total sample. Starch$_{>4.75}$ was calculated as the product of CG$_{>4.75}$ and the starch concentration of collected kernels and kernel fragments. The percentage of starch minimally fragmented (Starch$_{>4.75}$/Total) was calculated by dividing Starch$_{>4.75}$ by total starch in silage DM and expressing as a percentage.

Sample Preparation for In Vitro Disappearance

Samples from each of the 32 corn silages were dried at 55°C and prepared as whole or ground to pass through a 4- or 1-mm screen of a Willey cutter mill (Arthur H. Thomas, Philadelphia, PA). Each sample was inserted into porous (50 ± 15 µm) Dacron bags using a sample amount to bag surface ratio of approximately 9 mg/cm². For whole samples, 2 R1020 bags (Ankom Technology Corp., Fairport, NY) were filled with 3.6 g of dried whole silage. For 4-mm ground samples, 2 R510 bags (Ankom Technology Corp.) were filled with 0.9 g of dried and ground corn silage. For 1-mm ground samples, 2 R510 bags that were previously cut in half were filled with 0.5 g of ground corn silage. To test the effects of incubator, jar position within incubator, and day of incubation on in vitro disappearance, 64 R510 bags that were previously cut in half were filled with 0.5 g of an “in-house” corn silage standard. To determine in vitro disappearance of kernels, a portion of collected kernels was dried and ground to pass through a 4-mm screen of a Wiley cutter mill. Four bags that were previously cut in half were filled with 0.5 g of ground corn kernels.

In Vitro Disappearance

Corn silage samples were fermented using the Daisy II rotating jar in vitro incubator (Ankom Technology Corp.). In vitro fermentations were conducted on 4 d using 2 incubators. For each corn silage, one replicate was fermented on either d 1 or d 2, and a second replicate was fermented on either d 3 or d 4 (i.e., across treatments replication). Corn silages fermented in incubator 1 during replicate 1 were fermented in incubator 2 during replicate 2 and vice versa. Sets of all treatments of 2 different silages were fermented in a jar during each fermentation. Each treatment set for a silage consisted of 1 bag containing whole samples, 1 bag containing 4-mm ground samples, 1 bag containing 1-mm ground samples, and 2 bags containing ground kernels. In addition, 2 bags containing the corn silage standard and blank bags of each type were included in each jar. Residues were corrected for the changes in blank bags during fermentation, washing, and extraction with neutral detergent solution.

In vitro media and reducing agent were prepared as described by Goering and Van Soest (1970). On the day of fermentation, Dacron bags containing samples were placed in their respective fermentation jar with 1200 mL of media and warmed in a waterbath at 39°C under continuous purging with carbon dioxide. A 4-L flask containing 3360 mL of media for blending was also placed in a waterbath at 39°C and purged with carbon dioxide. Reducing solution was prepared, 60 mL was added to each fermentation jar, and 168 mL was added to the media for blending. A composited inoculum was prepared with rumen fluid and rumen solids collected from 3 cannulated lactating Holstein cows that were fed a diet containing 30% corn silage, 30% alfalfa silage, and 40% concentrate mix (DM basis). The composited inoculum was prepared as follows. For each of the 3 cows, two 2-L thermos flasks (previously warmed with hot tap water) were filled with approximately 1 L of rumen fluid and the remaining space was filled with rumen solids. Once in the laboratory, each thermos was opened and the first portion of solids (surface solids exposed to the air) was discarded. The remaining mix of rumen fluid and rumen solids was strained through 1 layer of cheesecloth into a flask. Then, 550 mL of strained rumen fluid from each thermos was collected and strained through a 2-layer cheesecloth into the composite flask. From each thermos, approximately 280 g of strained solids was collected, mixed with 560 mL of preheated and reduced media, and then blended (Waring blender HGB-300, Waring Commercial, New Hartford, CT) for 15 and 45 s at low and high speed, respectively. The resulting blend was strained through a 2-layer cheesecloth into the composite flask. Every step was done under constant purging with carbon dioxide. After adding 800 mL of the resulting inoculum to each fermentation jar, the jars were sealed with lids and placed into the designated incubator.
After 24 h of fermentation, bags were removed from the jars, rinsed (twice by hand with ice and water and once in washing machine for 3 min), and dried in a forced-air oven for 24 h at 55°C. Single-stage in vitro DM disappearance (IVDMD) was calculated using equation [1] after determining the weight of the undigested residue corrected for the changes in the blank.

\[
\text{IVDMD}(_{\%}) = \frac{\text{Initial DM}(_g) - \text{Undigested Residue}(_g)}{\text{Initial DM}(_g)} \times 100
\]

Bags containing the undigested residue of whole and 4-mm ground treatments were emptied and the material was then ground to pass through a 2-mm screen of an abrasion mill (Udy Corporation, Fort Collins, CO). Approximately 0.35 g of residue were weighed and inserted into F57 bags (Ankom Technology Corp.) for NDF analysis with an ANKOM200 fiber analyzer (Ankom Technology Corp.). Undigested residues of 1-mm ground samples were extracted in their original Dacron bags using the fiber analyzer. Residues remaining after extraction with neutral detergent were weighed to determine in vitro DM true disappearance (IVDMTD) as described by equation [2]. In vitro NDF disappearance (IVNDFD) was calculated using equation 1 except that aNDF replaced DM. In vitro neutral detergent solubles (NDS) disappearance (IVNDSD) was calculated using equation [3]. The aNDF concentration of each corn silage was assumed constant for all treatments.

\[
\text{IVDMTD}(_{\%}) = \frac{\text{Initial DM}(_g) - \text{Undigested NDF Residue}(_g)}{\text{Initial DM}(_g)} \times 100
\]

\[
\text{IVNDFD}(_{\%}) = \frac{\text{IVDMD}(_{\%}) \times \text{IVNDSD}(_{\%})}{100} \times 100
\]

\[
\text{IVNDSD}(_{\%}) = \frac{\text{IVDMD}(_{\%}) - \text{NDF}(_{\%}) \times \text{IVNDFD}(_{\%})}{100} \times 100
\]

**Statistical Analyses**

The effect of sample grinding on in vitro disappearance was tested using the MIXED procedure of SAS (SAS Institute, 2001). The model included the effect of treatment (fixed, df = 2), the effect of silage (random, df = 31), and the interaction of treatment by silage or residual error (random, df = 62). Orthogonal contrasts were used to test the effects of grinding (whole vs. 4- and 1-mm ground) and particle size during grinding (4-mm ground vs. 1-mm ground) on in vitro disappearance. The variation of IVDMD among incubators, jar positions, and days was determined using data from the corn silage standard. The model used in the MIXED procedure of SAS included the effect of incubator (random, df = 1), the effect of jar position in the incubator (fixed, df = 3), the interaction of incubator by jar position (random, df = 3), the effect of day (random, df = 3), the interaction of incubator by day (random, df = 3), and the residual error (random, df = 18).

For multiple regression analysis, models were obtained using the stepwise method of Minitab (Minitab Inc., 2000). Variables were added or removed with a significance level of \( P < 0.05 \). All coefficients of determination \( R^2 \) were adjusted to the number of variables included in the model (Chatterjee et al., 2000) to avoid artifact improvement in \( R^2 \) associated with variable addition (Weiss, 1993). Models were selected based on the highest adjusted \( R^2 \) with the least number of variables. Model selection was also based on visual appraisal of residual plots. Unless stated, all regression coefficients were significant at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

**Chemical Composition**

The average chemical composition of the 32 selected corn silages (Table 1) was similar to that reported by NRC (2001) for typical corn silage, except for ash, which was higher than that reported by NRC (5.1 vs. 4.0%) due to one sample with a high value. The ranges and standard deviations indicated large diversity in the sample set for each component. High correlations (Table 2) were observed between aNDF and other fiber fractions (ADF and ADL), and between fiber fractions (either aNDF or ADF) and nonfiber fractions (NFC and starch). The slope of the relationship between aNDF and ADF was nearly identical (0.62 and 0.61) to that reported by NRC (2001); however, the intercept of our equation was not different from zero. High correlations were also observed between ADL and nonfiber fractions (NFC and starch), although these correlations were lower (−0.74 and −0.65, respectively). From these relationships, empirical equations were obtained for estimating NFC, starch, and ADL concentrations from standard fiber analyses (Table 3).

Nonfiber carbohydrates calculated by difference was proposed by Mertens in the early 1980s as a crude estimate of rapidly available carbohydrate that could be estimated from routine chemical analysis. It was used to validate starch analyses (which must be less than NFC as a portion of DM) and to ensure that feed inputs for rumen models would sum to 100% of DM. Mertens
(1988) reported that calculated NFC differs from non-structural carbohydrates, which are measured analytically as starch and soluble sugars, and recommended that the terms not be used interchangeably. Although NFC consists of a mixture of sugars, starch, pectins, soluble fibers, and organic acids, it is a crude estimate of available carbohydrate, which is used to describe maximum recommendations for rapidly fermentable matter in dairy rations. In corn silage, it appears that NFC is most highly correlated with fiber fractions (Table 2) and that it can be predicted from NDF or ADF with good precision (Table 3) in situations when other chemical components are missing or suspect (although it should be recalled that this precision may be overestimated by the use of a constant ether extract concentration for all corn silages). In corn silage, the major NFC is starch, which can be predicted reliably from NFC when it is not measured (Table 3). Starch in corn silage also can be predicted by aNDF because of the inverse relationship between fiber and grain in corn silage, although the estimate is less precise than for NFC (Table 3). Both NFC and starch are used in current summative equations to estimate total digestible nutrients (NRC, 2001; Shaver, 2002).

In most forages, maturity is highly positively correlated with fiber concentration. However, in corn silage, the accumulation of grain dilutes the fiber concentration in the whole plant. Thus, we expected maturity in corn silage to be positively correlated with grain and starch, and negatively correlated with fiber concentration. In the diverse set of corn silages (Table 2), DM concentration was positively correlated with NFC concentration (r = 0.55, P < 0.01) and starch concentration (r = 0.48, P < 0.01) and negatively correlated with aNDF concentration (r = −0.49, P < 0.01). In the corn plant, both the stover and grain portions dry with advanced maturity (Cummins, 1970; Hunt et al., 1989). Therefore, in contrast to other forages, DM concentration and not carbohydrate composition may be the best chemical indicator of maturity in corn silage. In this study, we observed that DM concentration of the collected intact kernels and kernel fragments (DMCG) was highly correlated (r = 0.88) with DM concentration of the corn silage (DMCS):

\[
\text{DMCG} = 36.7 + 0.56 \text{DMCS} \quad (r^2 = 0.77, S_{x,y} = 2.4).
\]

### Kernel Collection and Composition

The concentration of minimally fragmented kernels (CG₄₇₅) was highly variable (CV = 47%) and ranged from 4.2 to 48.4% of corn silage DM (Table 4). The concentration of minimally fragmented starch (Starch₄₇₅) was also highly variable (CV = 50%) and ranged

### Table 1. Chemical and physical characteristics of 32 corn silages.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>34.7</td>
<td>7.8</td>
<td>22.5</td>
<td>19.2</td>
<td>48.1</td>
</tr>
<tr>
<td>Amylase-treated NDF, % DM</td>
<td>44.2</td>
<td>6.4</td>
<td>14.5</td>
<td>30.0</td>
<td>56.3</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>26.9</td>
<td>4.4</td>
<td>16.4</td>
<td>17.7</td>
<td>34.7</td>
</tr>
<tr>
<td>Acid detergent lignin, % DM</td>
<td>2.3</td>
<td>0.6</td>
<td>26.0</td>
<td>1.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Neutral detergent solubles, % DM</td>
<td>55.8</td>
<td>6.4</td>
<td>11.5</td>
<td>43.7</td>
<td>70.0</td>
</tr>
<tr>
<td>NFC, % DM</td>
<td>41.6</td>
<td>7.1</td>
<td>17.1</td>
<td>27.4</td>
<td>57.7</td>
</tr>
<tr>
<td>Starch, % DM</td>
<td>25.2</td>
<td>5.7</td>
<td>22.5</td>
<td>12.2</td>
<td>36.2</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>7.8</td>
<td>1.4</td>
<td>17.4</td>
<td>5.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Ash, % DM</td>
<td>5.1</td>
<td>1.5</td>
<td>19.9</td>
<td>3.1</td>
<td>9.6</td>
</tr>
<tr>
<td>Mean particle size, mm</td>
<td>4.2</td>
<td>1.4</td>
<td>33.5</td>
<td>2.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

### Table 2. Correlations between chemical components¹ of 32 corn silages.

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>Starch</th>
<th>NFC</th>
<th>aNDF</th>
<th>ADF</th>
<th>ADL</th>
<th>CP</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>—</td>
<td>0.48**</td>
<td>0.55**</td>
<td>−0.49**</td>
<td>−0.55**</td>
<td>−0.37*</td>
<td>NS</td>
<td>−0.40*</td>
</tr>
<tr>
<td>Starch</td>
<td>−0.48**</td>
<td>—</td>
<td>0.93**</td>
<td>−0.89**</td>
<td>−0.84**</td>
<td>−0.65**</td>
<td>NS</td>
<td>−0.63**</td>
</tr>
<tr>
<td>NFC</td>
<td>−0.96**</td>
<td>−0.91**</td>
<td>—</td>
<td>−0.74**</td>
<td>−0.74**</td>
<td>NS</td>
<td>−0.63**</td>
<td>NS</td>
</tr>
<tr>
<td>aNDF</td>
<td>−0.96**</td>
<td>−0.74**</td>
<td>−0.74**</td>
<td>—</td>
<td>0.76**</td>
<td>NS</td>
<td>0.49**</td>
<td>NS</td>
</tr>
<tr>
<td>ADF</td>
<td>−0.96**</td>
<td>−0.74**</td>
<td>−0.74**</td>
<td>0.76**</td>
<td>—</td>
<td>NS</td>
<td>0.66**</td>
<td>NS</td>
</tr>
<tr>
<td>ADL</td>
<td>−0.96**</td>
<td>−0.74**</td>
<td>−0.74**</td>
<td>0.76**</td>
<td>0.66**</td>
<td>NS</td>
<td>0.43*</td>
<td>NS</td>
</tr>
<tr>
<td>CP</td>
<td>−0.40*</td>
<td>NS</td>
<td>−0.63**</td>
<td>−0.63**</td>
<td>0.49**</td>
<td>NS</td>
<td>0.66**</td>
<td>NS</td>
</tr>
<tr>
<td>Ash</td>
<td>−0.63**</td>
<td>NS</td>
<td>−0.63**</td>
<td>−0.63**</td>
<td>0.66**</td>
<td>NS</td>
<td>0.43*</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹aNDF = Amylase-treated NDF; ADL = acid detergent lignin.

*P < 0.05; **P < 0.01; NS = P > 0.05.
Table 3. Linear relationships between chemical components of 32 corn silages.

<table>
<thead>
<tr>
<th>y</th>
<th>x</th>
<th>Intercept (b0)</th>
<th>Slope (b1)</th>
<th>r²</th>
<th>P</th>
<th>Sx,y</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADF</td>
<td>aNDF¹</td>
<td>0.66</td>
<td>0.61</td>
<td>NA</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>ADL²</td>
<td>aNDF</td>
<td>0.11</td>
<td>0.05</td>
<td>NA</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>NFC</td>
<td>aNDF</td>
<td>0.03</td>
<td>0.01</td>
<td>NA</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>ADF</td>
<td>87.5</td>
<td>0.01</td>
<td>0.01</td>
<td>NA</td>
<td>0.93</td>
<td>1.86</td>
</tr>
<tr>
<td>Starch</td>
<td>aNDF</td>
<td>0.01</td>
<td>0.01</td>
<td>NA</td>
<td>0.73</td>
<td>3.11</td>
</tr>
<tr>
<td>Starch</td>
<td>54.4</td>
<td>0.01</td>
<td>0.01</td>
<td>NA</td>
<td>0.76</td>
<td>2.12</td>
</tr>
</tbody>
</table>

¹aNDF = Amylase-treated NDF.
²NI = No intercept; H0: b0 = 0 (P > 0.10).
³NA = Not applicable.
⁴ADL = Acid detergent lignin.

from approximately 2 to 33% of corn silage DM. No correlation was found between MPS and CG > 4.75 (P < 0.31) or Starch > 4.75 (P < 0.42), because these variables are influenced by both the proportion of grain in the silage and its particle size. The percentage of minimally fragmented starch (Starch > 4.75/Total) averaged approximately 52% and ranged from approximately 9 to 100% (CV = 40%). Contrary to CG > 4.75 and Starch > 4.75, Starch > 4.75/Total was positively correlated with MPS (r = 0.46, P < 0.01) because the variation in grain concentration among silages is reduced when the index is expressed as a percentage of total starch in the silage. The low correlation between Starch > 4.75/Total and MPS is probably related to different degrees of fragmentation within the same chop length (Roberge et al., 1998; Shinners et al., 2000), although other factors such as maturity (Shinners et al., 2000) and differences in grain fragility may alter the proportion of fragmented kernels at a given MPS. Because vigorous vertical shaking of dried forages bounces particles on end so they are separated by the minimum cross-sectional dimension (Mertens et al., 1984) and the square instead of the diagonal aperture dimension was used, the MPS in Table 1 represents the geometric average of the smallest (i.e., width) dimension of the particles in each corn silage.

Although processing can reduce the amount of starch excreted in feces (Johnson et al., 1996; Dhiman et al., 2000), not all silage processing is equally effective in enhancing starch digestibility (Johnson et al., 2003; Cooke and Bernard, 2005). Quantitative methods are needed to describe the differences in starch physical properties among silages. In vitro disappearance of whole corn silage indicates that Starch > 4.75 negatively affects DM fermentation (discussed later), which suggests that this starch may be poorly fermented in vivo. We propose that the inverse of Starch > 4.75/Total (which is a function of Starch > 4.75) be used as a corn silage fragmentation index (CSFI = 100 – Starch > 4.75/Total), which represents the proportion of total starch that is in particles that are less than one-fourth of a kernel and provides a quantitative measure of kernel fragmentation in corn silage. For our diverse set of corn silages, the CSFI ranged from 0 to 91% (Table 4). Data from Ferreira (2002) showed 5.6% greater total tract starch

Table 4. Concentration and chemical composition of intact kernels and large kernel fragments from 32 corn silages.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG &gt; 4.75¹</td>
<td>26.5</td>
<td>5.0</td>
<td>13.9</td>
<td>22.2</td>
<td>32.0</td>
</tr>
<tr>
<td>Kernels DM, %</td>
<td>56.2</td>
<td>5.0</td>
<td>8.9</td>
<td>44.8</td>
<td>62.6</td>
</tr>
<tr>
<td>Kernels starch, %</td>
<td>67.2</td>
<td>6.6</td>
<td>13.6</td>
<td>50.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Starch &gt; 4.75²</td>
<td>13.3</td>
<td>4.9</td>
<td>7.8</td>
<td>8.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Starch &gt; 4.75/Total³</td>
<td>52.1</td>
<td>20.8</td>
<td>39.9</td>
<td>7.6</td>
<td>91.3</td>
</tr>
<tr>
<td>Corn silage fragmentation index, %</td>
<td>47.9</td>
<td>—</td>
<td>—</td>
<td>0.0</td>
<td>91.3</td>
</tr>
</tbody>
</table>

¹CG > 4.75 = Minimally fragmented kernels (i.e., intact kernels and large kernel fragments retained by sieves with square apertures of 4.75 mm and larger).
²Starch > 4.75 = Minimally fragmented starch (calculated as the product of CG > 4.75 by kernels starch concentration).
³Calculated as Starch > 4.75 divided by total starch.
digestibility for cows consuming diets containing processed corn silage (CSFI = 90%) than for cows consuming diets containing unprocessed corn silage (CSFI = 50%).

In our set of corn silages, high variation in CSFI at a given MPS was observed (Figure 1). Corn silage harvesting conditions such as theoretical length of cut and roller processing likely alter kernel fragmentation and, consequently, may affect starch digestibility. Bal et al. (2000) observed lower MPS (6.7 and 9.4 mm, respectively) for processed than for unprocessed corn silages when harvested at a theoretical length of cut of 9.5 mm. This reduction in MPS was accompanied by an increase in total tract starch digestibility (99.4 and 95.1%, respectively). Bal et al. (2000) also observed that the MPS of processed corn silage harvested at a theoretical length of cut of 19 mm was similar to that of unprocessed corn silage harvested at a theoretical length of cut of 9.5 mm. Despite the similar MPS, a greater total tract starch digestibility was observed for processed corn silage than for unprocessed corn silage (99.3 and 95.1% respectively). This difference in starch digestibility despite the similar MPS would likely be attributed to differences in kernel fragmentation due to processing.

Johnson et al. (2003) reported that, when harvested at the same theoretical length of cut, the difference in MPS between processed and unprocessed corn silages is greater at longer than at shorter theoretical lengths of cut (21 and 7% difference for theoretical lengths of cut of 39.7 and 11.1 mm, respectively). These observations were accompanied by a processing × chop length interaction, which showed that corn silage processing affects total tract starch digestibility at longer, but not at shorter, theoretical lengths of cut. These observations and the variation in corn silage fragmentation index at a given MPS (Figure 1) reflect the importance of having a quantitative index of kernel fragmentation in corn silage that would help to explain the inconsistent effect of corn silage processing on starch digestibility.

In Vitro Disappearance

A 24-h fermentation time was chosen for 2 reasons. First, complete fermentation of the amount of corn silage placed in each fermentation jar may have exceeded the buffer capacity of the media. Second, after 48 h of fermentation, most of the DM may have been degraded, and the effects of potential extent of digestion are measured primarily. Selecting 24-h fermentation times measures the combined effects of rate and potential extent of digestion.

System variability. The in vitro trial was designed to maximize the ability to detect differences between preparation treatments by fermenting all treatments of a silage in the same jar within a single incubator and on a single day. Because of the high number of treatments and samples, corn silages were fermented in different incubators, in different jar positions within incubators, or on different days. To test the effects of these factors on IVDMD, replicates of the standard corn silage sample were placed in every jar from each incubator in each day. The standard error for IVDMD among replicates of the standard corn silage was greater (2.5 vs. 1.8% units, respectively) within jars than across jars (i.e., across days, jars, and incubators). Based on these standard errors of replication, high repeatability within jars and across days, incubators, and jars was achieved for IVDMD. Mixed model analysis showed that the variations among days and between incubators were not different from zero. Jar position within the incubator had no effect on IVDMD ($P < 0.51$). Based on these results, we concluded that IVDMD was not affected by day of incubation, incubator, or jar positions within incubators in our trial. We therefore assumed that differences in IVDMD among preparation treatments were not confounded by incubators, jar positions within incubators, or days of incubation.

Sample processing and in vitro disappearance. As expected, IVDMD was greater ($P < 0.001$) for ground samples than for whole samples (Table 5). This increase in IVDMD for ground samples was related to greater IVNDFD ($P < 0.001$) and IVNDSD ($P < 0.001$). In vitro DM disappearance tended to be greater ($P < 0.107$) for 1-mm than for 4-mm ground samples. This difference resulted from greater IVNDFD ($P < 0.001$) for 1-mm compared with 4-mm ground samples. Increased
Table 5. In vitro disappearance as affected by sample grinding.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contrast</th>
<th>SEM</th>
<th>P value</th>
<th>Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>4-mm</td>
<td>1-mm</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>IVDMD, %</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>61.2</td>
<td>70.8</td>
<td>72.6</td>
<td>1.14</td>
<td>0.107</td>
</tr>
<tr>
<td>IVDMDT, %</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>72.3</td>
<td>75.7</td>
<td>77.4</td>
<td>0.81</td>
<td>0.014</td>
</tr>
<tr>
<td>IVNDFD, %</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>37.4</td>
<td>44.9</td>
<td>48.7</td>
<td>1.23</td>
<td>0.010</td>
</tr>
<tr>
<td>IVNDSD, %</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>79.7</td>
<td>90.9</td>
<td>91.2</td>
<td>1.29</td>
<td>0.852</td>
</tr>
</tbody>
</table>

1IVDMD = In vitro DM disappearance; IVDMDT = in vitro DM true disappearance; IVNDFD = in vitro NDF disappearance; IVNDSD = in vitro neutral detergent solubles disappearance.
2Treatments: 4-mm = corn silage samples ground to pass a 4-mm screen, 1-mm = corn silage samples ground to pass a 1-mm screen.
3Grinding: Whole vs. 4- and 1-mm ground; Particle size: 4-mm ground vs. 1-mm ground.

IVNDFD of 1-mm ground samples may be related to increased fermentation of fiber particles in response to particle size reduction (Wilson and Mertens, 1995), although it is possible that increased loss of fine fiber particles through the bag pores could have occurred. No difference was observed on IVNDSD between 1-mm and 4-mm ground samples (P < 0.852).

Factors affecting in vitro disappearance. In vitro DM disappearance of the ground silages was correlated mainly to the fiber components aNDF, ADF, and ADL, with correlation coefficients ranging from −0.77 to −0.87 (Table 6). Stepwise procedure indicated that the models for estimating IVDMD of 4- and 1-mm ground samples were similar and that aNDF and ADL explained most of the variation of IVDMD of ground samples (equation [5]). These observations suggest that the components having the greatest impact on IVDMD of ground silages are related to fiber (aNDF) and its digestibility (ADL). The variables selected in equation [5] also agree with those proposed by Mertens (2002b), who used the concept of potential digestibility observed in digestion kinetics to show that theoretically digestible NDF, a component of DM digestibility, should be a linear function of aNDF and ADL concentration.

\[ \text{IVDMD}_{\text{Ground}} = 95.3 - 0.32 \text{aNDF} - 4.29 \text{ADL} \ (R^2 = 0.80, S_{x,y} = 2.2). \]

Compared with other forages, corn silage digestibility may be more susceptible to the effect of particle size.

Table 6. Correlation coefficients of the different chemical and physical characteristics of 32 corn silages when analyzed at different particle sizes with their in vitro disappearances.

<table>
<thead>
<tr>
<th></th>
<th>IVDMD Whole 4-mm 1-mm</th>
<th>IVNDFD Whole 4-mm 1-mm</th>
<th>IVNDSD Whole 4-mm 1-mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage DM, %</td>
<td>0.36* 0.36*</td>
<td>0.52** 0.43*</td>
<td></td>
</tr>
<tr>
<td>Kernels DM, %</td>
<td>0.46** 0.42**</td>
<td>0.54** 0.56**</td>
<td></td>
</tr>
<tr>
<td>Amylase-treated NDF, %</td>
<td>-0.83** -0.87**</td>
<td>-0.82** -0.69**</td>
<td></td>
</tr>
<tr>
<td>ADF, %</td>
<td>-0.86** -0.81**</td>
<td>-0.78** -0.73**</td>
<td></td>
</tr>
<tr>
<td>Acid detergent lignin, %</td>
<td>-0.78** -0.73**</td>
<td>-0.82** -0.73**</td>
<td></td>
</tr>
<tr>
<td>NFC, %</td>
<td>0.44* 0.78**</td>
<td>0.39* 0.53**</td>
<td></td>
</tr>
<tr>
<td>Corn silage starch, %</td>
<td>0.72** 0.58**</td>
<td>0.62** 0.50**</td>
<td></td>
</tr>
<tr>
<td>Kernels starch, %</td>
<td>0.50** 0.35*</td>
<td>0.71** 0.50**</td>
<td></td>
</tr>
<tr>
<td>Mean particle size, mm</td>
<td>-0.47** -0.47**</td>
<td>-0.49** -0.40**</td>
<td></td>
</tr>
<tr>
<td>Ash, %</td>
<td>-0.37* -0.37* -0.35*</td>
<td>-0.35*</td>
<td></td>
</tr>
<tr>
<td>CG&lt;sub&gt;4.75&lt;/sub&gt;, %</td>
<td>0.54** 0.41*</td>
<td>-0.51** 0.48** 0.38*</td>
<td></td>
</tr>
<tr>
<td>Starch&lt;sub&gt;4.75&lt;/sub&gt;, %</td>
<td>0.56** 0.43*</td>
<td>0.51** 0.40*</td>
<td></td>
</tr>
<tr>
<td>Mean particle size, mm</td>
<td>-0.47** -0.47**</td>
<td>-0.49** -0.40**</td>
<td></td>
</tr>
</tbody>
</table>

1Whole = Unground corn silage samples, 4-mm = corn silage samples ground to pass a 4-mm screen, 1-mm = corn silage samples ground to pass a 1-mm screen.

2IVDMD = In vitro DM disappearance; IVNDFD = in vitro NDF disappearance; IVNDSD = in vitro neutral detergent solubles (NDS) disappearance.

3CG<sub>4.75</sub> = Minimally fragmented kernels (i.e., intact kernels and large kernel fragments retained by sieves with square apertures of 4.75 mm and larger).

4Starch<sub>4.75</sub> = Minimally fragmented starch (calculated as the product of CG<sub>4.75</sub> by kernels starch concentration).

5Calculated as Starch<sub>4.75</sub> divided by total corn silage starch.

*P < 0.05, **P < 0.01.
because kernels may be inadequately chewed during ingestion and rumination and consequently pass out of the digestive tract before digestion is completed (McAllister et al., 1990). Buck et al. (1969) reported that the use of recutter screens with forage choppers reduced the passage of kernels into feces. Miller et al. (1969) observed that rechopping the corn at the silo blower or grinding the ear before ensiling also reduced the kernels appearing in the feces. More recently, Johnson et al. (1996) and Dhiman et al. (2000) reported that processing corn silage reduced kernels in feces. These observations suggest that starch digestibility in corn silage is affected by physical properties like the amount of minimally fragmented grain. Correlations between IVDMD of whole materials and silage characteristics were low (absolute r < 0.63, Table 6). Moreover, IVDMD of the whole materials was poorly explained (R² = 0.40) by the variables included in equation [5]. However, the adjusted R² was increased by the inclusion of Starch₄₄.₇₅ or CG₄₄.₇₅ (equations [6] and [7], respectively). The negative regression coefficients for Starch₄₄.₇₅ and CG₄₄.₇₅ indicated that most of the starch in intact kernels and large kernel fragments is not fermented in vitro after 24 h, which suggests that some of this starch might escape fermentation in vivo and be excreted in the feces, as indicated by in vivo observations (Dhiman et al., 2000). These observations suggest that starch digestibility of corn silage should be discounted for minimally fragmented starch. The NRC (2001) uses a processing adjustment factor to alter the digestibility of NFC in its summative equations depending on starch characteristics of the feed. More recently, Shaver (2002) proposed a summative equation for corn silage in which the NFC fraction is divided into a starch fraction of uniform and almost complete (98%) digestibility. The variable digestibility of starch was related to DM with separate relationships for processed and unprocessed silages (Shaver, 2002). The inclusion of Starch₄₄.₇₅ or CG₄₄.₇₅ in equations [6] and [7] indicates that these variables may provide a quantitative alternative to processing adjustment factors or classification as processed or unprocessed to describe the impact of physical form of the starch on corn silage starch digestibility.

\[
\text{IVDMD}_{\text{Whole}} = 121 - 12.1 \text{ ADL} - 0.50 \text{ Starch}_{44.75} \quad [6] \\
- 2.86 \text{ MPS} - 0.41 \text{ DMCS} (R^2 = 0.67, S_{x,y} = 5.0).
\]

\[
\text{IVDMD}_{\text{Whole}} = 121 - 12.0 \text{ ADL} - 0.34 \text{ CG}_{44.75} \quad [7] \\
- 2.78 \text{ MPS} - 0.41 \text{ DMCS} (R^2 = 0.67, S_{x,y} = 5.0).
\]

The inclusion of both MPS and corn silage DM concentration (DMCS), in addition to the inclusion of Starch₄₄.₇₅ or CG₄₄.₇₅, increased the adjusted R² for estimating IVDMD of whole materials (equations [6] and [7]), suggesting that both particle size and maturity affect the IVDMD of whole dried silages. The biological interpretation of stepwise multiple regression coefficients is difficult because variables are added to account for the residual variation remaining after preceding variables were included. The inclusion of Starch₄₄.₇₅ or CG₄₄.₇₅ in the model may be explained by their effects on IVNDSD of whole samples (r = -0.50; Table 6). The addition of MPS after Starch₄₄.₇₅ or CG₄₄.₇₅ were included indicates a more general effect of particle size on IVDMD beyond that related to digestion of starch in kernels. Physical form is important for the estimation of total digestible nutrients in corn silage because length of chop and kernel processing vary among silages and influence nutrient availability (Rojas-Bourrillon et al., 1987; Bal et al., 2000; Weiss and Wyatt, 2000; Andrae et al., 2001; Schwab et al., 2002). The difference in variables between equation [5] for ground samples and equations [6] and [7] for whole samples indicates that grinding of corn silages obliterates some of the important physical differences between silages. However, animals more or less effectively chew corn silage and the in vitro digestibility of whole silages may not reflect in vivo digestibility. Nonetheless, in vitro digestibility of whole forages can be used to identify those physical characteristics of silages that may influence in vivo digestion. The inclusion of corn silage DM concentration in equations [6] and [7] can be related to corn silage maturity, which may affect both starch and fiber digestibilities (Hunt et al., 1989; Bal et al., 1997; Philippeau and Michalet-Doreau, 1997).

In vitro DM disappearance of collected kernels (ground form) ranged from 81.2 to 96.7%, with a mean and a median of 93.9 and 95.2%, respectively. Two silages contained no intact kernels and consisted of kernel fragments that were mainly seed coats with little or no germ or endosperm. When these 2 samples of collected kernel fragments were excluded, the minimum IVDMD of the remaining samples was 90.2%, which reduced the observed range. In our study, there was a significant correlation between silage and kernel DM concentrations (equation [4]), which indicated that kernel DM was generally higher than silage DM at typical corn silage maturities. This observation suggests that at maturities typical of corn silages, IVDMD of ground kernels does not vary with DM concentration. Shaver (2002) suggested, based on in vivo data, that corn silage DM concentration explains 77 and 85% of the variation in starch digestibility for processed and unprocessed corn silages, respectively. However, these relationships may be a function of the effects of matu-
 Partition of DM digestibility. Because digestible DM is the sum of digestible NDF and digestible NDS (Van Soest, 1967), DM digestibility can be partitioned into these 2 components when 2 of the 3 measurements are determined. In our system, both IVDMD and in vitro digestible NDF (IVdNDF, % of DM) were measured, so in vitro digestible NDS (IVdNDS, % of DM) could be determined by difference and the independent effects of IVNDFD and IVNDSD can be evaluated.

In most forages, NDS have uniform and almost complete digestion or availability. To evaluate IVNDSD in corn silages, IVdNDS was plotted against ash-free NDS concentration to estimate the true digestibility (slope) and uniformity of digestion (standard error of regression), which is the Lucas test of uniform availability (Van Soest, 1967, 1994; Weiss, 1994). For ground samples, the intercept was not different from zero ($P < 0.62$), the slope was not different from 1.0 ($P < 0.66$), and the standard error of the regression was 1.7 (Figure 2A). These results suggest that NDS of ground forms had a complete and uniform availability. For whole samples (Figure 2B), the standard error of the regression was 6.4, suggesting that NDS availability was not uniform. In addition to this, the slope of 0.88 in Figure 2B suggests that a portion of the NDS in whole silages was not fermented completely. In agreement with the hypothesis that the magnitude of the effect of grinding is related to the extent of kernel fragmentation, the difference between IVdNDS of ground samples (IVdNDS\textsubscript{Ground}) and IVdNDS of whole samples (IVdNDS\textsubscript{Whole}) was related ($r = 0.74$) to Starch\textsubscript{>4.75/Total} (Figure 3), which may explain why Starch\textsubscript{4.75} or CG\textsubscript{4.75} were included in equations [6] and [7]. The low coefficient of determination ($r^2 = 0.58$) between Starch\textsubscript{4.75/Total} and difference between IVdNDS\textsubscript{Ground} and IVdNDS\textsubscript{Whole} suggests that other variables such as maturity or genotype (Harrison et al., 1996; Philippeau and Michalet-Doreau, 1997) may also affect NDS fermentation.

When stepwise regression was used to identify variables related to IVdNDS of whole samples, both NDF (i.e., the direct determinant of NDS) and Starch\textsubscript{>4.75} were selected in the first 2 terms (equation [8]).

$$\text{IVdNDS}_{\text{Whole}} = 125 - 1.56 \text{aNDF} - 0.91 \text{Starch}_{>4.75} \quad (R^2 = 0.82, S_{x,y} = 3.9).$$

When stepwise regression was used to identify variables related to IVdNDF, both aNDF and ADL were selected in the first 3 terms for all sample preparation methods, and the intercepts typically were not different from zero. The observations that aNDF and ADL were selected to predict both IVDMD and IVdNDF suggests that there is a fundamental relationship between digestibility and the concentrations of aNDF and ADL in corn silage as theorized by Mertens (2002b). When a no-intercept model with aNDF and ADL was fit to our in vitro data the following equations were obtained:

$$\text{IVdNDF} = 0.63 \text{aNDF} - 3.77 \text{ADL} \quad (S_{x,y} = 1.8).$$

CONCLUSIONS

Minimally fragmented starch can be quantified by separating corn silage by vertical shaking and measur-
Figure 3. Difference on in vitro neutral detergent solubles (NDS) disappearance (Delta-IVdNDS) between ground and whole corn silage samples (Delta-IVdNDS = IVdNDSGround – IVdNDSGrounded) as affected by the proportion of the total starch that was minimally fragmented (Starch_{4.75/Total}).

In vitro DM disappearance of whole samples was lower and more variable than the ground samples. Fiber and lignin concentration described most of the variation for in vitro disappearance of ground silages in which starch was completely digested. In vitro DM disappearance of whole corn silage was related to minimally fragmented starch and mean particle size in addition to lignin and DM concentration. Part of the decrease of in vitro disappearance of whole compared with ground silages was related to lower disappearance of NDS in whole silages. The proportion of minimally fragmented starch is a quantitative index of kernel fragmentation in corn silage that is related to the in vitro disappearance of whole silages. This fragmentation index offers potential for adjusting the digestibility of starch in silage when predicting total digestible nutrients using summative equations.

REFERENCES


