Measuring DM and NDF Digestibility and Defining Their Importance

David R. Mertens
USDA-ARS
U.S. Dairy Forage Research Center

Presented at the 2002 NIRS Consortium Meeting
Why do we measure digestibility

• Digestibility is important because feces represent the greatest loss of ingested energy
  – Feces = 20-50% loss of energy (DE)
  – Gasses + Urine = 15-25% loss of energy (ME)
  – Heat = 5-15% loss of energy (NE)

• Uses (value) of measuring digestibility
  – As an indicator of feed nutritive value
  – As a predictor of animal performance
Digestibility as a Measure of Animal Performance

• Want to maximize the accuracy of measuring animal performance
  – Lab results must mimic field performance
  – Animal and diet must match field conditions

• Animal differences are an integral part of the measurement
  – Performance is determined by both feed and animal characteristics
  – Want to duplicate actual performance
Digestibility as a Measure of Animal Performance

- **In vivo production digestibility protocol**
  - Performance status of animals
  - Production level of intake (1-5X Mnt)
  - Ad libitum (free choice) intake with refusals = selection
  - Measures digestibility during production
  - Much greater variability = difficult to measure inputs and outputs
Digestibility as a Measure of Feed Nutritive Value

- Want to maximize the accuracy and precision of measuring feed’s nutritive value
  - Must be repeatable within labs
  - Must be reproducible among labs
- Must minimize animal differences (within and among labs)
  - Animals are the measuring device
  - Want to measure feed, not animal differences
Digestibility as a Measure of Feed Nutritive Value

- Standardized in vivo digestibility protocol
  - Mature animals
  - Maintenance level of intake (1X Mnt)
  - No selection or refusals
  - Measures maximum digestibility
  - Weigh feed, refusals and feces for 5-7 days
In Vivo Digestibility

- Is a biological evaluation of a feed
- Is not a constant, but varies with
  - Species
  - Size
  - Production level
  - Intake
  - Selection and sorting
  - Methodology
In Situ / In Sacco Digestibility

• Is a biological evaluation of a feed
• Feed is sealed in a porous bag and suspended in the rumen of fistulated cows
• Assume in situ = in vivo
  – But only measures fermentative digestion
    • Not adequate for low fiber feeds
    • Losses from the bag may compensate for the lack of intestinal digestion
In Situ / In Sacco Digestibility

- Apparent value is in mimicking ruminal digestion for production levels and diets
- More difficult to standardize, especially among labs when used for feed evaluation
  - Bag dimensions and pore sizes
  - Washing of bags and removal of fines
  - Cyclic and variable ruminal conditions
  - Variability among animals
In Vitro Digestibility

• **Single-stage IVDMD**
  – Incubate ruminal fluid with feed in buffer
  – Dry residues and weigh

• **Two-stage Tilley & Terry IVDMD**
  – Incubate ruminal fluid with feed in buffer
  – Incubate undigested residue in acid pepsin
  – Dry residues and weigh
In Vitro Digestibility

Two-stage Van Soest IVDMTD
- Incubate ruminal fluid with feed in buffer
- Extract undigested residue in neutral detergent
- Dry NDF residues and weigh

• In vitro methods measure different things
  - Single and two-stage T&T IV measure apparent DM digestibility
  - Two-stage Van Soest IV measures true DM digestibility
In Vitro Digestibility

Two-stage T&T IVDMD

- 48 hr fermentation highly correlated with in vivo DMD at 1xMaintenance
- DOES NOT mean that IVDMD = in vivo DMD
- Will be lower value than 2-stage VS IVDMTD because undigested residues contain microbial debris (part of in vivo endogenous loss)
In Vitro Gas Production and Digestibility

- Usually a closed system
  - Buffers do not work well and pH drops after 12-24 hr
- Used to measure fermentation curves
  - Assume that production of fermentation gas is proportional to DM disappearance
In Vitro Fermentation Time versus In Vivo Retention Time

- In vivo Retention Time DOES NOT equal in vitro fermentation time
  - i.e., digestion at 30 hr retention time DOES NOT digestion at 30 hr fermentation time
  - In vivo digestion = $kd / (kd + kp)$
  - In vitro digestion = $1 - DM \times \exp(-k \times t)$
Apparent versus True Digestibility

- Feed DM
  - ND Sol
  - Pot Dig NDF
  - Indig NDF

- Fermented DM
  - Digested DM

- Feces DM
  - Undig. Feed
  - Endogenous Loss
    - Int. Secr. + Micr. Debris

USDA-ARS
US Dairy Forage Research Center
Apparent versus True Digestibility

- Apparent DM digestibility (% DMD) =
  \[100 \times \frac{\text{Feed DM} - \text{Fecal DM}}{\text{Feed DM}}\]

- DM true digestibility (% DMTD) =
  \[100 \times \frac{\text{Feed DM} - \text{Undig Feed DM}}{\text{Feed DM}}\]

- DMTD > DMD, e.g., 78% vs 65%
Apparent versus True Digestibility

• Measuring true digestibility is difficult because there are limited ways of estimating or measuring endogenous losses
  – Regression for ideal nutritive entities
    • Have constant slope (estimates true dig.)
    • Have constant intercept (estimates End. Loss)
  – Analytically remove endogenous losses from feces using neutral detergent
Using Regression to Estimate True Digestibility and Endogenous Loss

\[ y = 0.9815x - 12.351 \]

\[ R^2 = 0.9998 \]
Ideal Nutritive Entities

• Have constant slope (true dig) near 0 or 1
• Have a negative intercept = endogenous loss
• Include
  – \( dCP = -3.5 + 0.95 \times CP \)
  – \( dEE = -1.5 + 0.98 \times EE \)
  – \( dSolCHO = -2.0 + 1.00 \times SolCHO \)
  – \( dNDS = -12.9 + 0.98 \times NDS \)
Summative Equations for Calculating Digestibility

• Based on the concept of Ideal Nutritive Entities
  – Identify them
  – Determine their true digestibilities and endogenous losses
  – Sum them

• Largest Ideal Nutritive Entity is Neutral Detergent Solubles

• Other Ideal Nutritive Entities are CP, EE, Sugars, Soluble Carbohydrates, & Lignin
Summative Equations for Calculating Digestibility

• Largest Ideal Nutritive Entity is Neutral Detergent Solubles (NDS = 100 - NDF)
  – True Digestibility = 0.98
  – Endogenous Loss = -12.9
  – \( d_{NDS} = -12.9 + 0.98 \times NDS \)

• Remaining fraction (NDF) is not ideal and its digestibility must be determined
  – \( d_{NDF} = NDFD \times NDF \)

• \( d_{DM} = DMD = d_{NDS} + d_{NDF} \)
Summative Equations for Calculating Digestibility

- VS DMD = 0.98*NDS + NDFD*NDF – 12.9
- Dairy NRC (2001) and Milk2000 are an expansion of the VS summative equation
- NRC2001 calculated Total Digestible Nutrients (TDN) by subdividing NDS
  - TDN1x = tdCP + tdFA*2.25 + tdNFC + tdNDF – 7
  - FA = (EE – 1), NFC = 100 – (NDF – NDFICP) – CP – EE – Ash, CPTD = exp(-1.2*ADICP/CP), FATD = 1.0, NFCTD = 0.98*PAF and tdNDF = 0.75*(NDF – NDFICP – Lignin)*[1 – (Lignin/NDF)\(2/3\)] or ??*IVNDFD*NDF
Summative Equations for Calculating Digestibility

• Milk2000 equation indicates that Starch is not an Ideal Nutritive Entity and removes it from NFC
  – TDN1x = tdCP + tdFA + tdNSNFC + tdST + tdNDF - 7
  – Non-starch NFC (NSNFC) = (NFC – Starch) has a variable Starch Digestibility depending on corn silage %DM and processing
Summative vs Empirical Equations

• Empirical equations assume that the true digestibility of fiber is constant (or correlated with fiber content)
  – Works best for ADF vs NDF because ADF contains a higher proportion of lignin and indigestible residues

• Summative allows NDFD to vary
  – DMD = \(0.98 \times \text{NDS} + \text{NDFD} \times \text{NDF} - 12.9\)
  – = \(0.98 \times (100 - \text{NDF}) + \text{NDFD} \times \text{NDF} - 12.9\)
  – = (98 – 12.9) - (98 – NDFD)\(\times\)NDF
Apparent versus True Digestibility

- **ND Sol**
- **Pot Dig NDF**
- **Indig NDF**
- **Feed DM**

**Digested DM**

**Fermented DM**

**Undig. Feed**

**Endogenous Loss**

Int. Secr. + Micr. Debris

**Feces DM**

USDA-ARS US Dairy Forage Research Center
Use Neutral Detergent to Remove Endogenous Losses from Feces

• ND will dissolve intestinal secretions
• ND will dissolve microbial debris
• But ND also dissolves undigested solubles
  – Only a problem in starchy feeds when starch is poorly digested, e.g., undamaged or coarsely cracked mature corn grain
Use Neutral Detergent to Remove Endogenous Losses from Feces

• In most feeds undigested feed in feeds is primarily fiber (aNDF)
• Procedure is to extract feces with ND to remove endogenous losses and recover undigested feed
• % DMTD = 100*[(Feed DM – (ND extracted fecal DM)] / Feed DM
Apparent versus True Digestibility

Feed DM

ND Sol Pot Dig NDF Indig NDF

Digested DM

Fermented DM

Endogenous Loss

Int. Secr. + Micr. Debris

Undig. Feed

Feces DM

+
Digestible Nutrient vs Nutrient Digestibility

• Nutrient Digestibility IS NOT the same as digestible Nutrient – UNITS ARE IMPORTANT

• Nutrient digestibility is always expressed as a percentage of the nutrient, i.e., it is the fraction of the nutrient that is digested, a digestion coefficient

• digestible Nutrient is always expressed as a percentage of the feed DM, i.e., it is the fraction of feed DM that is digested nutrient
Digestible Nutrient vs Nutrient Digestibility

- To distinguish between them I suggest the following terminology and abbreviations
  - dNut = digestible nutrient = % of digested nutrient in feed DM
  - NutD = nutrient Digestibility = % of nutrient that is digested
- DM is an exception, dDM = DMD because digestible DM is expressed per unit of itself
Digestible Nutrient vs Nutrient Digestibility

• Example:
  - Cow eats 50 lb of feed containing 20% CP and excretes 15 lb of feces containing 15% CP
  - \%CPD = 100\times(\text{Feed CP} - \text{Fecal CP}) / \text{Feed CP}
  - \text{Feed CP} = 50 \times 20/100 = 10 \text{ lb CP}
  - \text{Fecal CP} = 15 \times 15/100 = 2.25 \text{ lb CP}
  - \%CPD = 100\times(10 - 2.25) / 10 = 77.5\%
Digestible Nutrient vs Nutrient Digestibility

• Example:
  – digestible CP is always expressed in % of DM
  – \( dCP = 100 \times \frac{(\text{Feed CP} - \text{Fecal CP})}{\text{Feed DM}} \)
  – \( dCP = 100 \times \frac{(10 - 2.25)}{50 \text{ lb Feed DM}} \)
  – \( dCP = 15.5\% \text{ of DM} \)
  – \( dCP = \text{CP} \times \text{CPD/100} = 20 \times \frac{77.5}{100} = 15.5\% \text{ of DM} \)
**dNDF versus NDFD**

- dNDF is better than NDFD (Mertens’ opinion)
  - dNDF=(100-iNDF) are actually measured in vitro
  - dNDF is actually used to calculate DMD
- dNDF = NDF*(NDFD/100)
  - NDFD separates the affect from NDF
- iNDF (and dNDF) related to Lignin as % of DM
- NDFD related to Lignin as % of NDF
dNDF & NDFD Equations

**Procedure:**
- Determine NDF of the original samples ($\text{NDF, } \%\text{DM}$);
- Run ~0.5 g (DMwt, g) IV for 48h, followed by a NDF ($\text{NDFres, g}$);
- \[ \text{IVDMTD} = 100 \times \left[ \frac{\text{DMwt} - \text{NDFres}}{\text{DMwt}} \right] \] (%DM)
- \[ \text{iNDF} = 100 - \text{IVDMTD} \] (%DM)
- \[ \text{dNDF} = \text{NDF} - \text{iNDF} \] (%DM)
- \[ \text{NDFD} = 100 \times \frac{\text{dNDF}}{\text{NDF}} \] (%NDF)
- \[ = 100 \times \left[ \frac{\text{NDF} - (100 - \text{IVDMTD})}{\text{NDF}} \right] / \text{NDF} \]
dNDF & NDFD Equations

• Example:
  • NDF = 50 %DM
  • Sample DMwt = 0.5 g
  • NDFres = 0.1 g
  • IVDMTD = 100*[(0.5 - 0.1)/0.5]; = 80% DM
  • iNDF = 100 - 80 = 20% DM
  • dNDF = 50 - 20 = 30% DM
  • NDFD = 100*(30 / 50) = 60% NDF
# Calculating IVDMTD and IVNDFD

<table>
<thead>
<tr>
<th></th>
<th>Rep1</th>
<th>Rep2</th>
<th>Rep3</th>
<th>Rep4</th>
<th>Avg</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample wt</td>
<td>0.51</td>
<td>0.505</td>
<td>0.495</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample %DM</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Sample DM wt (A)</td>
<td>0.4692</td>
<td>0.4646</td>
<td>0.4554</td>
<td>0.4600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample %NDF</td>
<td>44.21</td>
<td>44.21</td>
<td>44.21</td>
<td>44.21</td>
<td>44.21</td>
<td></td>
</tr>
<tr>
<td>Sample NDF wt (B)</td>
<td>0.2074</td>
<td>0.2054</td>
<td>0.2013</td>
<td>0.2034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDF Res wt (C)</td>
<td>0.0802</td>
<td>0.0877</td>
<td>0.0836</td>
<td>0.0912</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dDM wt (A - C)</td>
<td>0.3890</td>
<td>0.3769</td>
<td>0.3718</td>
<td>0.3688</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample DM wt (A)</td>
<td>0.4692</td>
<td>0.4646</td>
<td>0.4554</td>
<td>0.4600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVDMTD</td>
<td>82.91</td>
<td>81.12</td>
<td>81.64</td>
<td>80.18</td>
<td>81.46</td>
<td>1.14</td>
</tr>
<tr>
<td>dNDF wt (B - C)</td>
<td>0.1272</td>
<td>0.1177</td>
<td>0.1177</td>
<td>0.1122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample NDF wt (B)</td>
<td>0.2074</td>
<td>0.2054</td>
<td>0.2013</td>
<td>0.2034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVNDFD</td>
<td>61.34</td>
<td>57.29</td>
<td>58.47</td>
<td>55.17</td>
<td>58.07</td>
<td>2.58</td>
</tr>
</tbody>
</table>
**dNDF equation for Corn silage**

\[
\text{NDFD} = 100 \times \left[\frac{\text{NDF} - (100 - \text{IVDMD})}{\text{NDF}}\right]
\]

<table>
<thead>
<tr>
<th>% NDF</th>
<th>Run1</th>
<th>Run2</th>
<th>Run3</th>
<th>Run4</th>
<th>Run1</th>
<th>Run2</th>
<th>Run3</th>
<th>Run4</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.76</td>
<td>81.02</td>
<td>82.15</td>
<td>80.60</td>
<td>81.32</td>
<td>58.52</td>
<td>61.00</td>
<td>57.61</td>
<td>59.18</td>
</tr>
<tr>
<td>46.29</td>
<td>80.67</td>
<td>80.41</td>
<td>80.14</td>
<td>80.24</td>
<td>58.24</td>
<td>57.69</td>
<td>57.10</td>
<td>57.32</td>
</tr>
<tr>
<td>43.94</td>
<td>82.10</td>
<td>82.65</td>
<td>78.99</td>
<td>80.38</td>
<td>59.26</td>
<td>60.51</td>
<td>52.19</td>
<td>55.35</td>
</tr>
<tr>
<td>51.51</td>
<td>76.53</td>
<td>78.78</td>
<td>80.57</td>
<td>80.88</td>
<td>54.43</td>
<td>58.80</td>
<td>62.27</td>
<td>62.87</td>
</tr>
<tr>
<td>40.42</td>
<td>81.98</td>
<td>82.39</td>
<td>82.94</td>
<td>82.37</td>
<td>55.41</td>
<td>56.43</td>
<td>57.80</td>
<td>56.38</td>
</tr>
<tr>
<td>35.30</td>
<td>85.52</td>
<td>86.01</td>
<td>87.51</td>
<td>86.38</td>
<td>58.99</td>
<td>60.35</td>
<td>64.62</td>
<td>61.41</td>
</tr>
<tr>
<td>43.46</td>
<td>81.00</td>
<td>79.73</td>
<td>82.07</td>
<td>80.70</td>
<td>56.28</td>
<td>53.37</td>
<td>58.75</td>
<td>55.60</td>
</tr>
<tr>
<td>34.15</td>
<td>85.59</td>
<td>86.74</td>
<td>83.59</td>
<td>85.92</td>
<td>57.81</td>
<td>61.16</td>
<td>51.94</td>
<td>58.76</td>
</tr>
<tr>
<td>38.55</td>
<td>84.40</td>
<td>83.54</td>
<td>82.49</td>
<td>82.20</td>
<td>59.54</td>
<td>57.30</td>
<td>54.58</td>
<td>53.83</td>
</tr>
<tr>
<td>34.43</td>
<td>84.36</td>
<td>86.13</td>
<td>84.24</td>
<td>84.01</td>
<td>54.57</td>
<td>59.71</td>
<td>54.22</td>
<td>53.55</td>
</tr>
<tr>
<td>47.89</td>
<td>80.42</td>
<td>80.45</td>
<td>81.08</td>
<td>80.78</td>
<td>59.12</td>
<td>59.19</td>
<td>60.50</td>
<td>59.87</td>
</tr>
<tr>
<td>48.92</td>
<td>79.86</td>
<td>77.52</td>
<td>76.12</td>
<td>76.44</td>
<td>58.83</td>
<td>54.06</td>
<td>51.18</td>
<td>51.84</td>
</tr>
<tr>
<td>39.27</td>
<td>83.60</td>
<td>84.21</td>
<td>83.00</td>
<td>83.89</td>
<td>58.23</td>
<td>59.80</td>
<td>56.71</td>
<td>58.98</td>
</tr>
</tbody>
</table>

**SD**

<table>
<thead>
<tr>
<th>SD IVDMD</th>
<th>SD NDFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.66</td>
<td>1.43</td>
</tr>
<tr>
<td>0.23</td>
<td>0.50</td>
</tr>
<tr>
<td>1.67</td>
<td>3.79</td>
</tr>
<tr>
<td>2.00</td>
<td>3.88</td>
</tr>
<tr>
<td>0.39</td>
<td>0.98</td>
</tr>
<tr>
<td>0.85</td>
<td>2.40</td>
</tr>
<tr>
<td>0.96</td>
<td>2.21</td>
</tr>
<tr>
<td>1.34</td>
<td>3.91</td>
</tr>
<tr>
<td>1.01</td>
<td>2.62</td>
</tr>
<tr>
<td>0.97</td>
<td>2.83</td>
</tr>
<tr>
<td>0.31</td>
<td>0.65</td>
</tr>
<tr>
<td>1.69</td>
<td>3.46</td>
</tr>
<tr>
<td>0.51</td>
<td>1.31</td>
</tr>
</tbody>
</table>
Conclusions

• Digestion is important
• Digestibility measurements are a function of method
• Know which IV method is used
  – Important for IVDMTD vs IVDMD
  – Only one way to measure NDFD
• dNDF is more important than NDFD (Mertens’ opinion)
• NDFD may have high SD and we may not be able to discriminate other than High, Medium and Low