Comparisons of Karl Fischer Method with Oven Methods for Determination of Water in Forages and Animal Feeds

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In a comparative study of the Karl Fischer method with oven methods for determination of water in forages and animal feeds, oven methods yielded the following relative recoveries (expressed as a percentage of the recovery obtained by the Karl Fischer method) for hay, haylage, and corn silage, respectively: (1) drying at 135°C for 2 h (AOAC 930.15), 116 and 2746%; (2) drying at 104°C for 3 h (AOAC 935.29), 96, 122, and 113%; and (3) drying at 104°C for 6 h, 97, 129, and 117%. Relative recoveries for nonurea-containing and urea-containing feed, respectively, were as follows: (1) drying at 135°C for 2 h (AOAC 930.15), 116 and 2746%; (2) drying at 104°C for 3 h (AOAC 935.29), 88 and 239%; (3) drying at 95°C for 5 h under vacuum (AOAC 934.01), 83 and 727%; (4) drying at 104°C for 6 h, 90 and 427%; and (5) drying at 110°C for 3 h, 94 and 425%. Preliminary near-infrared reflectance calibrations for water (moisture) based on the Karl Fischer method were promising (r^2 = 0.98; standard error of calibration = 0.20).

Discrepancies in results obtained with various oven methods for determination of moisture and dry matter in animal feeds have been noted with American Association of Feed Control Officials check sample results and with National Forage Testing Association proficiency samples. Previous work (1, 2) indicated that a thorough evaluation of moisture methods was needed. To investigate the discrepancies, values obtained from moisture determinations using the Karl Fischer method were compared with values obtained by oven methods to determine moisture in dried, ground animal feed and forage samples. The types of forage tested include hay, haylage, and corn silage, and the feeds tested include various animal feeds with and without added urea.

Experimental

Forage samples (12 hay, 11 haylage, and 10 corn silage) were partially dried in a microwave oven and ground to pass a 1 mm sieve in a cyclone mill. Four samples each of grass hay, legume hay, and legume–grass mix made up the 12 hay materials. Haylage materials included 1 grass silage, 9 legume silages, and 1 legume–grass mixed silage. Materials of different compositions and were preserved by different techniques (drying versus ensiling). They were randomly selected from those received at the laboratory for routine nutritional analysis.

Feed materials (6 feeds containing urea and 10 feeds containing no urea) were ground to pass a 1 mm sieve in a Retsch centrifugal mill (Brinkmann Instruments, Westbury, NY) except for the soybeans and cat food, which were ground in a Tecator Knifetec mill (Foss North America, Eden Prairie, MN). Urea-containing feeds covered a range of urea levels, from very little to pure-feed grade urea. Nonurea-containing feeds represented a cross section of the types of feeds routinely analyzed for moisture content.

Moisture (as water) in all forage and feed materials was determined in triplicate with the Karl Fischer method. Moisture (as loss on drying) in forage materials was determined in duplicate by 3 oven methods as follows: drying at 135°C for 2 h (AOAC 930.15), drying at 104°C for 3 h (AOAC 935.29), and drying at 104°C for 6 h. Moisture (as loss on drying) in feed materials was determined in triplicate by 5 oven methods as follows: drying at 135°C for 2 h (AOAC 930.15), drying at 104°C for 3 h (AOAC 935.29), drying at 95°C for 5 h under vacuum (AOAC 934.01), drying at 104°C for 6 h, and drying at 110°C for 3 h.

Near-infrared reflectance (NIR) calibrations for moisture were made for the 33 hay, corn silage, and haylage materials by using moisture values generated by all methods in previous experiments. NIR calibrations for moisture were also made on the 16 feed materials by using the Karl Fischer moisture values generated in previous experiments. Additional NIR calibrations for moisture were made by combining the 16 feed materials with 33 forage materials (49 total samples).

Karl Fischer Method (1)

Reagents

(a) Karl Fischer reagent.—One component, based on imidazole, with titer of ca 5 mg H₂O/mL reagent;
Drying temperatures and times for oven methods

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Drying time after reaching required temperature, h</th>
<th>Oven</th>
</tr>
</thead>
<tbody>
<tr>
<td>135 ± 2</td>
<td>2</td>
<td>Forced draft</td>
</tr>
<tr>
<td>104 ± 1</td>
<td>3</td>
<td>Forced draft</td>
</tr>
<tr>
<td>104 ± 1</td>
<td>6</td>
<td>Forced draft</td>
</tr>
<tr>
<td>95–100</td>
<td>5</td>
<td>Vacuum oven (&lt;100 mm Hg)</td>
</tr>
<tr>
<td>110 ± 1</td>
<td>3</td>
<td>Forced draft</td>
</tr>
</tbody>
</table>

HYDRANAL, composite 5 (Riedel-de Haën, St. Louis, MO), or equivalent.

- **Methanol.**—Anhydrous, for moisture determinations, water content not to exceed 0.05% (EM Science, Gibbstown, NJ).
- **Formamide.**—ACS reagent grade (Fisher Scientific, Fair Lawn, NJ).
- **Sodium tartrate dihydrate.**—Primary standard; water content, 15.66 ± 0.05% H₂O; HYDRANAL, standard; sodium tartrate dihydrate (Riedel-de Haën), or equivalent.

**Apparatus**

- **Karl Fischer titration–homogenization system.**—Metrohm 720 KFS Titrino, or equivalent. Metrohm system includes titration vessel LP with water jacket (50–150 mL), snap-in buret unit (10 mL), 703 titration stand with pump, and cable with timer (Titrino-Polytron). All equipment is available from Brinkmann Instruments.
- **Circulating water bath.**—capable of maintaining 50° ± 1°C (Fisher Scientific, Pittsburgh, PA).
- **Homogenizer.**—Brinkmann Polytron homogenizer with PTA 20TSM foam-reducing generator with saw teeth and knives, assembled with generator extending into the titration vessel. Set to provide homogenization speed of 24 000 rpm.
- **Balance.**—Analytical, electronic, sensitive to 0.1 mg (Mettler Instruments, Highstown, NJ).
- **Glass weighing spoon.**—With opening for dispensing sample into the titration flask through the septum stopper (Brinkmann), or equivalent.

**Cell Conditioning**

Dry the titration cell by completing a pretitration as follows: Dispense 80 mL of the methanol–formamide solvent into the titration vessel. Close the cell to minimize addition of atmospheric moisture. Heat to 50° ± 1°C. Dry the cell (including solvent, cell walls, electrode walls, generator, and cell atmosphere) by performing a blank run, including homogenization and titration. Homogenize at 24 000 rpm for 60 s. Begin titration immediately. Before reaching the end point of the titration, homogenize momentarily to rinse untitrated methanol, which may have been sitting under the cap into solution. The end point is reached when no change in potential is observed for 10 s (titration system programmed for Stop criterion: time; Delay: 10 s). A dried titration cell has a maximum drift consumption of 5–10 µL Karl Fischer reagent/min.

**Standardization**

Heat cell to 50° ± 1°C. Dry the cell as described above. Immediately after drying the cell, quickly weigh 150–250 mg sodium tartrate dihydrate (Na₂C₄H₄O₆·2H₂O) standard into the glass weighing spoon and record weight of spoon and standard to the nearest 0.1 mg (S). Quickly transfer the weighted standard into the titration flask through the septum stopper. Reweigh empty sample spoon to obtain tare weight (T). Obtain the weight of standard material added by subtracting tare weight from weight of spoon plus standard (S – T). Homogenize at 24 000 rpm for 60 s. Begin titration immediately. Before reaching the end point of the titration, homogenize momentarily to rinse down moisture that may have been sitting under the cap to ensure that the moisture of the particles sitting under the cap or hanging on the glass walls above the liquid surface is titrated. Titrate to same end point as described in the conditioning step. Record volume of reagent required for the titration (mL reagent) to the nearest 0.001 mL. Repeat a total of 5 times. Calculate titer and then average the 5 values. The relative standard deviation (RSD) should be <2%.

\[
\text{Titer} = \frac{\text{mg H}_2\text{O}}{\text{mL reagent}} = \frac{\text{mg Na}_2\text{C}_4\text{H}_4\text{O}_6 \cdot 2\text{H}_2\text{O} \times 0.1566}{\text{mL reagent}}
\]

**Determination**

Heat cell to 50° ± 1°C. Dry the cell as described previously before running a sample or each time solvent is replenished in the titration cell. Check drift in the titration cell. A dried titration cell has a maximum drift consumption of 5–10 µL Karl Fischer reagent/min.

Analyze sample as follows: Immediately after drying the cell, quickly weigh ca 0.5 g sample (to contain ca 25–50 mg water) into the glass weighing spoon and record weight of the spoon plus the sample (W). Quickly add weighed sample into the titration flask through the septum stopper. Reweigh empty sample spoon and record tare weight (T). Obtain the sample weight by subtracting tare weight from weight of spoon plus sample (W – T). Homogenize at 24 000 rpm for 60 s. Begin titration immediately. Before reaching the end point of the titration, homogenize momentarily to rinse down moisture that may have been sitting under the cap to ensure that the moisture of the particles sitting under the cap or hanging on the glass walls above the liquid surface is titrated. The end point is reached when no change in potential is observed for 10 s (Stop criterion: time; Delay: 10 s). Record volume of titrant (V, mL). Repeat determination 3 times. The RSD of replicates should be <5%. The cell need not be emptied between each sample.
Table 2. Moisture in forage determined by the Karl Fischer method and various oven methods

<table>
<thead>
<tr>
<th>Forage</th>
<th>Moisture, %</th>
<th>Karl Fischer</th>
<th>135°C, 2 h</th>
<th>104°C, 3 h</th>
<th>104°C, 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legume hay 1</td>
<td>6.35 a</td>
<td>8.12</td>
<td>6.09 a</td>
<td>6.19 a</td>
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<tr>
<td>Legume hay 2</td>
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<td>7.88 a</td>
<td>7.42 a</td>
<td>7.42 a</td>
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<td>Legume hay 3</td>
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<td>5.05</td>
<td>4.12 a</td>
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<tr>
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<td>3.86 a</td>
<td>4.50 a</td>
<td>3.40 a</td>
<td>3.40 a</td>
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<tr>
<td>Legume–grass mixed hay 6</td>
<td>7.41 a</td>
<td>8.34</td>
<td>7.32 a</td>
<td>7.50 a</td>
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<td>3.52 a</td>
<td>3.16</td>
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<td>6.61 a</td>
<td>7.29</td>
<td>6.41 a</td>
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<td>Grass hay 10</td>
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<td>6.45</td>
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<tr>
<td>Grass hay 11</td>
<td>4.99 a</td>
<td>4.77 a</td>
<td>4.64 a</td>
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<td>Grass hay 12</td>
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<td>4.10 a</td>
<td>3.50 a</td>
<td>3.69 a</td>
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<tr>
<td><strong>Average hay</strong></td>
<td>5.36</td>
<td>6.09</td>
<td>5.17</td>
<td>5.23</td>
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<td><strong>Haylage</strong></td>
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<td>10.32</td>
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<td>5.49 a</td>
<td>4.17 a</td>
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<td>8.13</td>
<td>6.54</td>
<td>6.68</td>
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<tr>
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<td>5.92</td>
<td>9.34</td>
<td>7.00</td>
<td>7.40</td>
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<tr>
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<td>9.18</td>
<td>6.87</td>
<td>7.37</td>
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<td>Alfalfa haylage 11</td>
<td>5.42</td>
<td>8.84</td>
<td>6.47</td>
<td>6.97</td>
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<tr>
<td><strong>Average haylage</strong></td>
<td>6.01</td>
<td>9.69</td>
<td>7.29</td>
<td>7.80</td>
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<tr>
<td><strong>Corn silage</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn silage 1</td>
<td>8.48</td>
<td>11.58</td>
<td>10.22</td>
<td>10.65</td>
<td></td>
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<tr>
<td>Corn silage 2</td>
<td>8.67</td>
<td>11.79</td>
<td>10.31</td>
<td>10.62</td>
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<tr>
<td>Corn silage 3</td>
<td>4.75</td>
<td>8.95</td>
<td>6.80</td>
<td>7.30</td>
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<tr>
<td>Corn silage 4</td>
<td>4.49 a</td>
<td>5.42</td>
<td>4.70 a</td>
<td>4.83</td>
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</tr>
<tr>
<td>Corn silage 5</td>
<td>4.02</td>
<td>5.43</td>
<td>4.66</td>
<td>4.80</td>
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<td>Corn silage 6</td>
<td>6.87 a</td>
<td>7.11</td>
<td>6.77 a</td>
<td>6.77 a</td>
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<td>Corn silage 7</td>
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<tr>
<td>Corn silage 9</td>
<td>5.52</td>
<td>6.46</td>
<td>5.47 a</td>
<td>5.59 a</td>
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<tr>
<td>Corn silage 10</td>
<td>3.97</td>
<td>6.81</td>
<td>5.02</td>
<td>5.36</td>
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<tr>
<td><strong>Average corn silage</strong></td>
<td>6.08</td>
<td>7.87</td>
<td>6.82</td>
<td>7.04</td>
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</tr>
</tbody>
</table>

*a* Means within a feed with the same superscript letter are not statistically different ($P > 0.05$) from the Karl Fischer mean.
Usually about 3 titrations can be performed before the cell needs to be emptied and replenished.

\[ H_2O, \text{mg} = V \times \text{titer}; \quad H_2O, \% = \frac{V \times \text{titer}}{10 \times (W - T)} \]

**Oven Methods (3–5)**

**Apparatus**

(a) *Drying oven, forced draft convection.*—Capable of maintaining temperature at ±1°C. The oven should be equipped with a wire rod shelf to allow circulation of air. The exhaust vent on top of the oven should be wide open (Gallenkamp Model Plus II, Leicester, UK).

(b) *Drying oven, vacuum oven.*—Equipped with a pressure gauge and needle-type air valve. The oven should be equipped with system to dry input air (Thelco Model 19, Chicago, IL).

(c) *Vacuum pump.*—Connected to vacuum oven via vacuum tubing (Welch Model 1404, Skokie, IL).

(d) *Moisture pans.*—Aluminum, 50 mm diameter × 20 mm deep, lids are optional (Fisher Scientific, Itasca, NY).

(e) *Analytical balance.*—Electronic, sensitive to 0.1 mg (Mettler Instruments).

(f) *Desiccator.*—Corning (Corning, NY).

**Determination**

Dry moisture pan at >100°C for at least 2 h. Transfer moisture pan to a desiccator and cool. Weigh moisture pan and record the tare weight \((T)\) to the nearest 0.1 mg. Tare the pan to zero while on the balance. Add 1.5–2 g well-mixed sample to the pan and record sample weight \((F)\). Gently shake the pan to uniformly distribute the sample and place the samples in preheated oven. Dry at the temperatures and times indicated in Table 1.

Transfer dried samples to a desiccator, cool, and weigh. Record weight of pan and dried sample \((D)\).

\[ \text{Dry moisture,} \% = \frac{(D - T) \times 100}{F} \]

Moisture, % = 100 – % dry matter

**NIR Spectroscopy (6)**

**Apparatus**

(a) *Wavelength-scanning monochromator.*—NIRSystems 5000 (Perstorp Analytical, Inc., Silver Spring, MD).

(b) *Computer.*—Gateway 2000 Model 4DX2-50V (Gateway 2000, North Sioux City, SD).

(c) *Software.*—Infrasoft International (ISI) software, NIRS 2+, Version 3.00, Routine Operation and Calibration Software for Near Infrared Instruments (1993; Perstorp Analytical) to collect, store, and process NIR data.

(d) *NIRS sample holder.*—Nylon holder, 2.5 cm diameter and 1 cm thick, with IR transmittance quartz window. Sample capacity, 0.75–1.75 g. Sample is held in place with a backing made of foam core (Perstorp Analytical).
Determination

Mix dried, ground material and place 4 random portions in 4 quadrants of NIRS sample holder to ensure that portions of 3–4 different subsamples are scanned. Continue to add random portions until NIRS sample holder is 2/3 to level full. Press back into NIRS sample holder until it is tight. Immediately collect reflectance (R) measurements (log 1/R) of calibration samples from 1100 to 2500 nm and record at 2 nm intervals (saved into *.NIR file) with the ISI software. This process is performed at the same time as the samples are weighed for oven moisture or Karl Fischer determinations to minimize exposure to atmospheric moisture. All manipulations are performed with speed and care.

Average moisture values from reference methods are entered for each material into *.NIR file with the ISI software to generate a *.CAL file. Calibration procedure is performed by using the partial least squares regression method and the ISI software (6).

Statistical Analysis

Data were statistically analyzed by using the PROC GLM program in the SAS software (7).

Results and Discussion

Moisture in 12 hay, 11 haylage, and 10 corn silage materials was determined with the Karl Fischer method (as water; n = 3) and with 3 oven methods (as loss on drying; n = 2): 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h (Table 2).

Average moisture contents for hay materials obtained by the Karl Fischer method and by drying at 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer means. For one of the 12 hay materials, drying at 104°C for 3 h gave a mean that was significantly different (P ≤ 0.05) from the Karl Fischer mean. And for 2 of the 12 hay materials, drying at 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer means.

Average moisture contents for haylage materials obtained by the Karl Fischer method and by drying at 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer means. For 9 of the 11 haylage materials, drying at 104°C for 3 h gave a mean that was significantly different (P ≤ 0.05) from the Karl Fischer mean. And for 10 of the 11 haylage materials, drying at 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer mean.

Average moisture contents for corn silage obtained by the Karl Fischer method and by drying at 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer means. For 8 of the 10 corn silage materials, drying at 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer means.

Average moisture contents for corn silage obtained by the Karl Fischer method and by drying at 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h were 6.08, 7.87, 6.82, and 7.04, respectively. For 10 of the 10 corn silage materials, drying at 104°C for 6 h gave means that were significantly different (P ≤ 0.05) from the Karl Fischer means.

Table 4. Recovery of moisture from forage materials by various oven methods relative to moisture determined by the Karl Fischer method

<table>
<thead>
<tr>
<th>Forage</th>
<th>135°C, 2 h</th>
<th>104°C, 3 h</th>
<th>104°C, 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legume hay 1</td>
<td>128</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>Legume hay 2</td>
<td>99</td>
<td>94</td>
<td>94</td>
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<tr>
<td>Legume hay 3</td>
<td>120</td>
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<td>97</td>
</tr>
<tr>
<td>Legume hay 4</td>
<td>138</td>
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<td>110</td>
</tr>
<tr>
<td>Legume–grass mixed hay 5</td>
<td>117</td>
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<td>88</td>
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<td>Legume–grass mixed hay 6</td>
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<td>118</td>
<td>124</td>
</tr>
<tr>
<td>Grass haylage 3</td>
<td>159</td>
<td>123</td>
<td>130</td>
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<tr>
<td>Alfalfa haylage 4</td>
<td>167</td>
<td>119</td>
<td>129</td>
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<td>Alfalfa haylage 5</td>
<td>110</td>
<td>84</td>
<td>98</td>
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<tr>
<td>Alfalfa haylage 6</td>
<td>244</td>
<td>188</td>
<td>206</td>
</tr>
<tr>
<td>Alfalfa haylage 7</td>
<td>134</td>
<td>102</td>
<td>109</td>
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<td>Alfalfa haylage 8</td>
<td>136</td>
<td>109</td>
<td>112</td>
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<tr>
<td>Alfalfa haylage 9</td>
<td>158</td>
<td>118</td>
<td>125</td>
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<tr>
<td>Alfalfa haylage 10</td>
<td>161</td>
<td>121</td>
<td>112</td>
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<tr>
<td>Alfalfa haylage 11</td>
<td>163</td>
<td>119</td>
<td>129</td>
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<tr>
<td><strong>Average haylage</strong></td>
<td>162</td>
<td>122</td>
<td>129</td>
</tr>
<tr>
<td><strong>Corn silage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn silage 1</td>
<td>137</td>
<td>121</td>
<td>126</td>
</tr>
<tr>
<td>Corn silage 2</td>
<td>136</td>
<td>119</td>
<td>122</td>
</tr>
<tr>
<td>Corn silage 3</td>
<td>188</td>
<td>143</td>
<td>154</td>
</tr>
<tr>
<td>Corn silage 4</td>
<td>121</td>
<td>105</td>
<td>108</td>
</tr>
<tr>
<td>Corn silage 5</td>
<td>135</td>
<td>116</td>
<td>119</td>
</tr>
<tr>
<td>Corn silage 6</td>
<td>103</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Corn silage 7</td>
<td>105</td>
<td>101</td>
<td>102</td>
</tr>
<tr>
<td>Corn silage 8</td>
<td>111</td>
<td>103</td>
<td>105</td>
</tr>
<tr>
<td>Corn silage 9</td>
<td>117</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>Corn silage 10</td>
<td>172</td>
<td>126</td>
<td>135</td>
</tr>
<tr>
<td><strong>Average corn silage</strong></td>
<td>133</td>
<td>113</td>
<td>117</td>
</tr>
</tbody>
</table>
from the Karl Fischer means. For 5 of the 10 corn silage materials, drying at 104°C for 3 h gave means that were significantly different ($P \leq 0.05$) from the Karl Fischer means. And for 7 of the 10 corn silage materials, drying at 104°C for 6 h gave means that were significantly different ($P \leq 0.05$) from the Karl Fischer means.

The biases (or differences) between Karl Fischer means and oven method means are reported in Table 3. For hay materials, the differences between the Karl Fischer mean and the means obtained by drying at 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h were 0.73, –0.19, and –0.13, respectively. Corresponding biases were 3.68, 1.27, and 1.51, respectively, for haylage materials and 1.79, 0.74, and 0.96, respectively, for corn silage materials.

Recoveries of moisture by oven methods expressed as a percentage of the Karl Fischer mean are reported in Table 4. For hay materials, relative recoveries of water after drying for 135°C for 2 h, 104°C for 3 h, and 104°C for 6 h were 113, 96, and 90, respectively. Corresponding recoveries for haylage materials were 113, 96, and 90, respectively, and for corn silage materials were 113, 96, and 90, respectively.

### Table 5. Correlation between Karl Fischer moisture values and those obtained by oven methods for forage materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Karl Fischer vs 135°C, 2 h</th>
<th>Karl Fischer vs 104°C, 3 h</th>
<th>Karl Fischer vs 104°C, 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>Slope</td>
<td>$r^2$</td>
</tr>
<tr>
<td>Hay</td>
<td>0.85</td>
<td>0.76</td>
<td>0.97</td>
</tr>
<tr>
<td>Haylage</td>
<td>0.50</td>
<td>0.30</td>
<td>0.45</td>
</tr>
<tr>
<td>Corn silage</td>
<td>0.61</td>
<td>0.62</td>
<td>0.84</td>
</tr>
</tbody>
</table>

$a$ Correlation coefficient.

### Table 6. Moisture in feeds determined by the Karl Fischer method and various oven methods

<table>
<thead>
<tr>
<th>Feed</th>
<th>Karl Fischer</th>
<th>135°C, 2 h</th>
<th>104°C, 3 h</th>
<th>95°C, 5 h vacuum</th>
<th>104°C, 6 h</th>
<th>110°C, 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk replacer</td>
<td>4.19$^a$</td>
<td>10.99</td>
<td>3.68</td>
<td>2.64</td>
<td>4.14$^a$</td>
<td>4.22$^a$</td>
</tr>
<tr>
<td>Shelled corn</td>
<td>12.34</td>
<td>11.82</td>
<td>11.46</td>
<td>10.43</td>
<td>10.90</td>
<td>10.44</td>
</tr>
<tr>
<td>Cat food</td>
<td>8.39$^a$</td>
<td>8.23$^a$</td>
<td>7.23</td>
<td>7.00</td>
<td>7.63</td>
<td>7.96</td>
</tr>
<tr>
<td>Hog feed</td>
<td>11.81</td>
<td>11.42</td>
<td>10.84</td>
<td>10.62</td>
<td>10.78</td>
<td>10.94</td>
</tr>
<tr>
<td>Soybeans</td>
<td>12.58</td>
<td>11.61</td>
<td>10.9</td>
<td>10.45</td>
<td>11.47</td>
<td>11.71</td>
</tr>
<tr>
<td>Hulless barley</td>
<td>10.78</td>
<td>9.63</td>
<td>9.32</td>
<td>9.6</td>
<td>9.09</td>
<td>9.60</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>6.77$^a$</td>
<td>6.79$^a$</td>
<td>5.98</td>
<td>5.94</td>
<td>6.06</td>
<td>6.61$^a$</td>
</tr>
<tr>
<td>Golden Pig (OTC)</td>
<td>7.61$^a$</td>
<td>8.70</td>
<td>6.79</td>
<td>6.70</td>
<td>7.03</td>
<td>7.39$^a$</td>
</tr>
<tr>
<td>Hi NRG grass stretcher</td>
<td>9.41$^a$</td>
<td>9.59$^a$</td>
<td>8.35</td>
<td>8.46</td>
<td>8.46</td>
<td>8.84</td>
</tr>
<tr>
<td>EMF Phase III Med (Carbadox)</td>
<td>7.61</td>
<td>7.94</td>
<td>6.57</td>
<td>5.73</td>
<td>6.64</td>
<td>7.12</td>
</tr>
<tr>
<td>Average for feed with no urea</td>
<td>9.15</td>
<td>9.67</td>
<td>8.11</td>
<td>7.76</td>
<td>8.22</td>
<td>8.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feed</th>
<th>Karl Fischer</th>
<th>135°C, 2 h</th>
<th>104°C, 3 h</th>
<th>95°C, 5 h vacuum</th>
<th>104°C, 6 h</th>
<th>110°C, 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb grower (0.81% urea)</td>
<td>9.96</td>
<td>10.92</td>
<td>9.60</td>
<td>9.43</td>
<td>9.36</td>
<td>9.09</td>
</tr>
<tr>
<td>Forage 38 Block (3.3% urea)</td>
<td>11.18$^a$</td>
<td>12.63</td>
<td>11.28$^a$</td>
<td>10.39</td>
<td>11.43$^a$</td>
<td>11.25$^a$</td>
</tr>
<tr>
<td>Custom mix (6.6% urea)</td>
<td>6.83$^a$</td>
<td>9.28</td>
<td>6.79$^a$</td>
<td>7.18$^a$</td>
<td>7.14$^a$</td>
<td>6.86$^a$</td>
</tr>
<tr>
<td>Steak Maker 42% (10.0% urea)</td>
<td>6.45</td>
<td>11.26</td>
<td>7.14</td>
<td>8.47</td>
<td>7.83</td>
<td>7.26</td>
</tr>
<tr>
<td>Meal supplement (~37% urea)</td>
<td>8.89$^a$</td>
<td>23.16</td>
<td>10.40$^a$</td>
<td>14.39</td>
<td>13.05</td>
<td>12.41</td>
</tr>
<tr>
<td>Pure-feed-grade urea</td>
<td>0.193$^a$</td>
<td>30.26</td>
<td>1.76$^a$</td>
<td>7.29</td>
<td>3.85</td>
<td>3.87</td>
</tr>
<tr>
<td>Average for feed with urea</td>
<td>7.25</td>
<td>16.25</td>
<td>7.83</td>
<td>9.53</td>
<td>8.78</td>
<td>8.46</td>
</tr>
</tbody>
</table>

$a$ Means within a feed with the same superscript letter are not statistically different ($P > 0.05$) from the Karl Fischer mean.
and 97%, respectively. Corresponding relative recoveries were 162, 122, and 129%, respectively, for haylage materials and 133, 113, and 117%, respectively, for corn silage materials.

Correlations between Karl Fischer results and those of each oven method are reported for forage materials in Table 5. Correlation coefficients for hay, haylage, and corn silage were 0.85, 0.50, and 0.61 (and slopes were 0.76, 0.30, and 0.62), respectively, for Karl Fischer versus drying at 135°C for 2 h; 0.97, 0.45, and 0.84 (and slopes were 0.95, 0.41, and 0.79), respectively, for Karl Fischer versus drying at 104°C for 3 h; and 0.97, 0.42, and 0.78 (and slopes were 0.92, 0.38, and 0.74), respectively, for Karl Fischer versus drying at 104°C for 6 h.

Moisture in 10 feed materials containing no urea and in 6 feed materials containing different amounts of urea was determined by the Karl Fischer method (as water) and 5 oven methods (as loss on drying): 135°C for 2 h, 104°C for 3 h, 95°C for 5 h under vacuum, 104°C for 6 h, and 110°C for 3 h (Table 6).

Average moisture contents for feed materials without urea obtained by the Karl Fischer method and drying at 135°C for 2 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. For 10 of the 10 feed materials, drying at 104°C for 3 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. For 10 of the 10 feed materials, drying at 95°C for 5 h under vacuum gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. For 10 of the 10 feed materials, drying at 104°C for 6 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. And for 7 of the 10 feed materials, drying at 110°C for 3 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means.

Average moisture contents for feed materials with urea obtained by the Karl Fischer method and by drying at 135°C for 2 h, 104°C for 3 h, 95°C for 5 h under vacuum, 104°C for 6 h, and 110°C for 3 h were 7.25, 16.25, 7.83, 9.53, 8.78, and 8.46, respectively. For 6 of the 6 urea feed materials, drying at 135°C for 2 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. For 2 of the 6 urea feed materials, drying at 104°C for 3 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. And for 5 of the 6 urea feed materials, drying at 95°C for 5 h under vacuum gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means.

Average moisture contents for feed materials with urea obtained by the Karl Fischer method and by drying at 135°C for 2 h, 104°C for 3 h, 95°C for 5 h under vacuum, 104°C for 6 h, and 110°C for 3 h were 7.25, 16.25, 7.83, 9.53, 8.78, and 8.46, respectively. For 6 of the 6 urea feed materials, drying at 135°C for 2 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. For 2 of the 6 urea feed materials, drying at 104°C for 3 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. And for 5 of the 6 urea feed materials, drying at 95°C for 5 h under vacuum gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means. For 4 of the 6 urea feed materials, drying at 104°C for 6 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means.

### Table 7. Bias (difference) between various oven means and Karl Fischer mean for feeds

<table>
<thead>
<tr>
<th>Feed</th>
<th>135°C, 2 h</th>
<th>104°C, 3 h</th>
<th>95°C, 5 h</th>
<th>104°C, 6 h</th>
<th>110°C, 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Containing no urea</strong></td>
<td>Bias (difference) between oven method and Karl Fischer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk replacer</td>
<td>6.80</td>
<td>-0.51</td>
<td>-1.55</td>
<td>-0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Shelled corn</td>
<td>-0.52</td>
<td>-0.88</td>
<td>-1.91</td>
<td>-1.44</td>
<td>-1.90</td>
</tr>
<tr>
<td>Cat food</td>
<td>-0.16</td>
<td>-1.16</td>
<td>-1.39</td>
<td>-0.76</td>
<td>-0.43</td>
</tr>
<tr>
<td>Hog feed</td>
<td>-0.39</td>
<td>-0.97</td>
<td>-1.19</td>
<td>-1.03</td>
<td>-0.87</td>
</tr>
<tr>
<td>Soybeans</td>
<td>-0.97</td>
<td>-1.68</td>
<td>-2.13</td>
<td>-1.11</td>
<td>-0.87</td>
</tr>
<tr>
<td>Hulless barley</td>
<td>-1.15</td>
<td>-1.46</td>
<td>-1.18</td>
<td>-1.69</td>
<td>-1.18</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>0.02</td>
<td>-0.79</td>
<td>-0.83</td>
<td>-0.71</td>
<td>-0.16</td>
</tr>
<tr>
<td>Golden Pig (OTC)</td>
<td>1.09</td>
<td>-0.82</td>
<td>-0.91</td>
<td>-0.58</td>
<td>-0.22</td>
</tr>
<tr>
<td>Hi NRG grass stretcher</td>
<td>0.18</td>
<td>-1.06</td>
<td>-0.95</td>
<td>-0.95</td>
<td>-0.57</td>
</tr>
<tr>
<td>EMF Phase III Med (Carbadox)</td>
<td>0.33</td>
<td>-1.04</td>
<td>-1.88</td>
<td>-0.97</td>
<td>-0.49</td>
</tr>
<tr>
<td><strong>Average for feed with no urea</strong></td>
<td>0.52</td>
<td>-1.04</td>
<td>-1.39</td>
<td>-0.93</td>
<td>-0.67</td>
</tr>
<tr>
<td><strong>Containing urea</strong></td>
<td>Bias (difference) between oven method and Karl Fischer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb grower (0.81% urea)</td>
<td>0.96</td>
<td>-0.36</td>
<td>-0.53</td>
<td>-0.60</td>
<td>-0.87</td>
</tr>
<tr>
<td>Forage 38 Block (3.3% urea)</td>
<td>1.45</td>
<td>0.10</td>
<td>-0.79</td>
<td>0.25</td>
<td>0.07</td>
</tr>
<tr>
<td>Custom mix (6.6% urea)</td>
<td>2.45</td>
<td>-0.04</td>
<td>0.35</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>Steak Maker 42% (10.0% urea)</td>
<td>4.81</td>
<td>0.69</td>
<td>2.02</td>
<td>1.38</td>
<td>0.81</td>
</tr>
<tr>
<td>Meal supplement (~37% urea)</td>
<td>14.27</td>
<td>1.51</td>
<td>5.50</td>
<td>4.16</td>
<td>3.52</td>
</tr>
<tr>
<td>Pure-feed-grade urea</td>
<td>30.07</td>
<td>1.57</td>
<td>7.10</td>
<td>3.66</td>
<td>3.68</td>
</tr>
<tr>
<td><strong>Average for feed with urea</strong></td>
<td>9.00</td>
<td>0.58</td>
<td>2.27</td>
<td>1.53</td>
<td>1.21</td>
</tr>
</tbody>
</table>
different \((P \leq 0.05)\) from the Karl Fischer means. And for 4 of the 6 urea feed materials, drying at 110 °C for 3 h gave means that were significantly different \((P \leq 0.05)\) from the Karl Fischer means.

The biases (or differences) between Karl Fischer means and oven method means are reported in Table 7. For feed materials without urea the biases between the Karl Fischer mean and those obtained after drying at 135 °C for 2 h, 104 °C for 3 h, 95 °C for 5 h under vacuum, 104 °C for 6 h, and 110 °C for 3 h were 0.52, −1.04, −1.39, −0.93, and −0.67, respectively. Corresponding biases for feed materials with urea were 9.00, 0.58, 2.27, 1.53, and 1.21, respectively.

Recoveries of water by the oven methods expressed relative to the Karl Fischer mean are reported in Table 8. For feed materials without urea, relative recoveries after drying at 135 °C for 2 h, 104 °C for 3 h, 95 °C for 5 h under vacuum, 104 °C for 6 h, and 110 °C for 3 h were 116, 88, 83, 90, and 94%, respectively. Corresponding relative recoveries for feed materials containing urea were 2746, 239, 727, 427, and 425%, respectively.

### Table 8. Recovery of moisture from feeds by various oven methods relative to moisture determined by the Karl Fischer method

<table>
<thead>
<tr>
<th>Feed</th>
<th>135°C, 2 h</th>
<th>104°C, 3 h</th>
<th>95°C, 5 h vacuum</th>
<th>104°C, 6 h</th>
<th>110°C, 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Containing no urea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk replacer</td>
<td>262</td>
<td>88</td>
<td>63</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>Shelled corn</td>
<td>96</td>
<td>93</td>
<td>85</td>
<td>88</td>
<td>85</td>
</tr>
<tr>
<td>Cat food</td>
<td>98</td>
<td>86</td>
<td>83</td>
<td>91</td>
<td>95</td>
</tr>
<tr>
<td>Hog feed</td>
<td>97</td>
<td>92</td>
<td>90</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td>Soybeans</td>
<td>92</td>
<td>87</td>
<td>83</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td>Hulless barley</td>
<td>89</td>
<td>86</td>
<td>89</td>
<td>84</td>
<td>89</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>100</td>
<td>88</td>
<td>88</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>Golden Pig (OTC)</td>
<td>114</td>
<td>89</td>
<td>88</td>
<td>92</td>
<td>97</td>
</tr>
<tr>
<td>Hi NRG grass stretcher</td>
<td>102</td>
<td>89</td>
<td>90</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td>EMF Phase III Med (Carbadox)</td>
<td>104</td>
<td>86</td>
<td>75</td>
<td>87</td>
<td>94</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>116</td>
<td>88</td>
<td>83</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td><strong>Containing urea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb grower (0.81% urea)</td>
<td>110</td>
<td>96</td>
<td>95</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>Forage 38 Block (3.3% urea)</td>
<td>113</td>
<td>101</td>
<td>93</td>
<td>102</td>
<td>101</td>
</tr>
<tr>
<td>Custom mix (6.6% urea)</td>
<td>136</td>
<td>99</td>
<td>105</td>
<td>105</td>
<td>100</td>
</tr>
<tr>
<td>Steak Maker 42% (10.0% urea)</td>
<td>175</td>
<td>111</td>
<td>131</td>
<td>121</td>
<td>113</td>
</tr>
<tr>
<td>Meal supplement (~37% urea)</td>
<td>261</td>
<td>117</td>
<td>162</td>
<td>147</td>
<td>140</td>
</tr>
<tr>
<td>Pure-feed-grade urea</td>
<td>15769</td>
<td>912</td>
<td>3777</td>
<td>1995</td>
<td>2005</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>2746</td>
<td>239</td>
<td>727</td>
<td>427</td>
<td>425</td>
</tr>
</tbody>
</table>

### Table 9. Correlation between Karl Fischer moisture values and those obtained by oven methods for feeds

<table>
<thead>
<tr>
<th>Feed</th>
<th>Karl Fischer vs 135°C, 2 h</th>
<th>Karl Fischer vs 104°C, 3 h</th>
<th>Karl Fischer vs 104°C, 6 h</th>
<th>Karl Fischer vs 95°C, 5 h vacuum</th>
<th>Karl Fischer vs 110°C, 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r^2)</td>
<td>Slope</td>
<td>(r^2)</td>
<td>Slope</td>
<td>(r^2)</td>
</tr>
<tr>
<td><strong>Without urea</strong></td>
<td>0.30</td>
<td>0.86</td>
<td>0.99</td>
<td>1.08</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>With urea</strong></td>
<td>0.44</td>
<td>−0.30</td>
<td>0.96</td>
<td>1.11</td>
<td>0.75</td>
</tr>
</tbody>
</table>

\(^a\) Correlation coefficient.
Table 10. NIR calibrations for moisture in forages based on Karl Fischer and oven methods

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of samples&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of PLS terms&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Math treatment&lt;sup&gt;c&lt;/sup&gt;</th>
<th>SEC&lt;sup&gt;d&lt;/sup&gt;</th>
<th>r&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SECV&lt;sup&gt;e&lt;/sup&gt;</th>
<th>1-VR&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karl Fischer</td>
<td>32</td>
<td>5</td>
<td>1,4,4,1</td>
<td>0.203</td>
<td>0.980</td>
<td>0.306</td>
<td>0.959</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>4</td>
<td>2,10,10,1</td>
<td>0.214</td>
<td>0.979</td>
<td>0.273</td>
<td>0.968</td>
</tr>
<tr>
<td>135°C, 2 h</td>
<td>32</td>
<td>5</td>
<td>1,4,4,1</td>
<td>0.504</td>
<td>0.958</td>
<td>0.870</td>
<td>0.878</td>
</tr>
<tr>
<td>104°C, 3 h</td>
<td>31</td>
<td>5</td>
<td>2,10,10,1</td>
<td>0.432</td>
<td>0.969</td>
<td>0.760</td>
<td>0.907</td>
</tr>
<tr>
<td>104°C, 6 h</td>
<td>31</td>
<td>5</td>
<td>2,10,10,1</td>
<td>0.590</td>
<td>0.910</td>
<td>0.737</td>
<td>0.868</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>3</td>
<td>2,10,10,1</td>
<td>0.412</td>
<td>0.962</td>
<td>0.602</td>
<td>0.925</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of samples after removal of outliers.
<sup>b</sup> Number of terms in the partial least squares calibration equation.
<sup>c</sup> The first value indicates the derivative (2 = second derivative), the second value indicates the gap over which the derivative is calculated, the third value is the number representing the smoothing of points, and the fourth value indicates a second smooth (1 = no second smooth).
<sup>d</sup> Standard error of calibration.
<sup>e</sup> Correlation coefficient.
<sup>f</sup> Standard error of cross validation.

Correlations between Karl Fischer results and those of each oven method are reported for feed materials in Table 9. Correlation coefficients (r<sup>2</sup>) for feed with urea and feed without urea were 0.44 and 0.30 (and slopes were –0.30 and 0.86), respectively, for Karl Fischer versus drying at 135°C for 2 h; 0.96 and 0.99 (and slopes were 1.11 and 1.08), respectively, for Karl Fischer versus drying at 104°C for 3 h; 0.75 and 0.99 (and slopes were 1.03 and 1.15), respectively, for Karl Fischer versus drying at 104°C for 6 h; 0.30 and 0.95 (and slopes were 0.80 and 1.00), respectively, for Karl Fischer versus drying at 95°C for 5 h under vacuum; and 0.76 and 0.98 (and slopes were 1.09 and 1.19), respectively, for Karl Fischer versus drying at 110°C for 3 h.

Results of NIR calibrations for forage materials by all methods are reported in Table 10. Calibration equations based on partial least squares were generated by using 2 mathematical treatments: 1,4,4,1 and 2,10,10,1 where the first value indicates the derivative (2 = second derivative), the second value indicates the gap over which the derivative is calculated, the third value is the number representing the smoothing of points, and the fourth value indicates a second smooth (1 = no second smooth). The standard errors of calibration (SEC) and r<sup>2</sup> values for the Karl Fischer method were 0.203 and 0.980 for a 1,4,4,1 treatment and 0.214 and 0.979 for a 2,10,10,1 treatment, respectively. The SEC and r<sup>2</sup> for drying at 135°C for 2 h were 0.504 and 0.958 for a 1,4,4,1 treatment and 0.432 and 0.969 for the 2,10,10,1 treatment, respectively. The SEC and r<sup>2</sup> for drying at 104°C for 3 h were 0.356 and 0.968 for a 1,4,4,1 treatment and 0.365 and 0.969 for the 2,10,10,1 treatment, respectively. The SEC and r<sup>2</sup> for drying at 104°C for 6 h were 0.590 and 0.910 for the 2,10,10,1 treatment, respectively. The SEC and r<sup>2</sup> for drying at 95°C for 5 h under vacuum were 0.412 and 0.962 for the 1,4,4,1 treatment and 0.653 and 0.904 for the 2,10,10,1 treatment, respectively.

Results of NIR calibrations for feed materials and for all materials combined analyzed by the Karl Fischer method are reported in Table 11. Again, calibration equations were generated as described for forage materials. The SEC and r<sup>2</sup> for feed materials analyzed by the Karl Fischer method were 0.358 and 0.980 for a 1,4,4,1 treatment and 0.414 and 0.974 for the 2,10,10,1 treatment, respectively. The SEC and r<sup>2</sup> for all feed and forage materials combined analyzed by the Karl Fischer method were 0.298 and 0.982 for the 1,4,4,1 treatment and 0.313 and 0.980 for the 2,10,10,1 treatment, respectively.

Conclusions

On average for hay, drying at 104°C for 3 h or at 104°C for 6 h gave approximately the same results as the Karl Fischer method, whereas drying at 135°C for 2 h overestimated moisture in hay. All oven methods seriously overestimated moisture in haylage and corn silage, with drying at 135°C for 2 h giving the most dramatic overestimation.

For ensiled forages, loss of other volatile substances, such as volatile fatty acids, is drastic with oven heating. Overall, drying at 104°C for 3 h most closely approximated the Karl Fischer method. It appears that only 96% of the water is removed with this method. However, the loss of volatile substances other than water more than compensates for the incomplete removal of water from the ensiled products.

Estimation of moisture in feeds with oven methods is also very frustrating. Decomposition of urea in urea-containing feeds is observed with all oven methods and becomes very noticeable when urea concentration is 10%. Drying at 104°C for 3 h most closely approximated the Karl Fischer method for urea-containing feeds. All other oven methods overestimated moisture in urea-containing feeds. The feeds without urea pose a number of problems, from decomposition of milk replacer at 135°C to incomplete removal of water in other products, even at 135°C. Overall, drying at 135°C for 2 h and dry-
ing at 110°C for 3 h most closely approximated the Karl Fischer method. Bound water in corn, soybeans, and barley was difficult to remove, even at 135°C. Given the problems associated with drying at 135°C, we recommend drying at 110°C for 3 h for nonurea-containing feeds when an oven method is desired.

We believe that the proposed Karl Fischer method should be adopted as the official method for forages and feeds and that oven methods should be evaluated by comparison with the Karl Fischer method.

Preliminary NIR calibrations were made for 2 reasons: to develop an idea of the accuracy of the reference method to measure water (good calibrations cannot be obtained with a poor reference method) and to determine the feasibility of using the Karl Fischer method as a reference method for NIR calibrations for determination of moisture in animal feed or forage materials.

NIR calibrations for moisture in forages were made with no effort to optimize results or to select samples for calibration on the basis spectra. Samples used for experiments were used to determine the feasibility and relative accuracy of NIR calibrations for moisture (dry matter) based on the Karl Fischer and the 3 oven methods. Best calibrations were obtained with the Karl Fischer method because calibrations could be made directly for water (best calibration data), while calibrations for oven methods would be based on water and various other volatile components of the forage materials. We conclude that the Karl Fischer method would be the method of choice for calibrating NIR instruments for prediction of water content for all types of forages.

NIR calibrations based on the Karl Fischer method were also attempted for feeds even though the population was small (14 samples). Considering the diversity of the samples, the use of different grinding procedures, and the small population, good calibrations were obtained, indicating excellent feasibility for robust calibrations for moisture in animal feed. The 33 forage samples and 14 feed samples were combined to determine the feasibility of a mixed calibration. Calibrations were acceptable for combined feed and forage samples, indicating that one calibration for forages and feeds is feasible. For laboratories with NIR capabilities, NIR calibrations for moisture based on the Karl Fischer method appear to be a vast improvement over oven methods.

Acknowledgments

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References


Table 11. NIR calibrations for moisture in feeds, forages, and all samples based on the Karl Fischer method

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>No. of PLS terms</th>
<th>Math treatment</th>
<th>SEC</th>
<th>2e</th>
<th>SECV</th>
<th>1-VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeds</td>
<td>14</td>
<td>5</td>
<td>1,4,4,1</td>
<td>0.358</td>
<td>0.980</td>
<td>0.988</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>5</td>
<td>2,10,10,1</td>
<td>0.414</td>
<td>0.974</td>
<td>1.114</td>
<td>0.808</td>
</tr>
<tr>
<td>Forages</td>
<td>32</td>
<td>5</td>
<td>1,4,4,1</td>
<td>0.203</td>
<td>0.980</td>
<td>0.306</td>
<td>0.959</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>4</td>
<td>2,10,10,1</td>
<td>0.214</td>
<td>0.979</td>
<td>0.273</td>
<td>0.968</td>
</tr>
<tr>
<td>All samples</td>
<td>45</td>
<td>6</td>
<td>1,4,4,1</td>
<td>0.298</td>
<td>0.982</td>
<td>0.403</td>
<td>0.968</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>4</td>
<td>2,10,10,1</td>
<td>0.313</td>
<td>0.980</td>
<td>0.424</td>
<td>0.965</td>
</tr>
</tbody>
</table>

a Number of samples after removal of outliers.
b Number of terms in the partial least squares calibration equation.
c The first value indicates the derivative (2 = second derivative), the second value indicates the gap over which the derivative is calculated, the third value is the number representing the smoothing of points, and the fourth value indicates a second smooth (1 = no second smooth).
d Standard error of calibration.
e Correlation coefficient.
f Standard error of cross validation.
g One minus the variance ratio (explained variance divided by the total variance).