Comparison of Methods of In Vitro Dry Matter Digestibility for Ten Feeds

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ABSTRACT

The objectives were to determine if the in vitro dry matter (DM) digestibility was similar by using a traditional method compared to the new DAISY II system and to determine if in vitro DM digestibility was similar for sources of inoculum from two different donor cow diets, all forage or total mixed ration. Ten feeds were digested by the traditional method, the DAISY II method with same feeds in a digestion vessel, and the DAISY II method with different feeds in a digestion vessel. The study used a 2 × 3 factorial arrangement of treatments with two sources of inoculum and three methods. The study was replicated. Source of inoculum affected in vitro DM digestibility; the grass hay donor cow diet resulted in lower digestibility values in six of the 10 feeds tested. Method did not significantly affect the digestibility values, and there were no significant source by method interactions. The DAISY II method can be used to increase labor efficiency in the in vitro DM digestibility analysis, and forages and grains can be analyzed together in a single digestion vessel. (Key words: in vitro, digestion, forages, grains)

Abbreviation key: DD = DAISY II with different feeds, DS = DAISY II with same feeds, IVDMD = in vitro dry matter digestibility, and TM = traditional method.

INTRODUCTION

Measurement of in vitro DM digestibility (IVDMD) has been used extensively to analyze feeds because of a high degree of correlation to in vivo digestibility (1, 9). Over the years, various procedures to determine IVDMD have been developed and modified. The reagents used in the procedure of Tilly and Terry (15) have been modified to improve the precision of the IVDMD estimates, but methodologies have not permitted modifications that improve labor efficiency of assays or that incubate multiple samples in a single vessel. Additionally, research results (2, 4, 5, 6, 8, 11, 12) on the effect of source of inoculum with various combinations of donor cows diets vary widely.

The recent development of the DAISY II apparatus (ANKOM Technology Corp., Fairport, NY) allows multiple feed samples to be analyzed for IVDMD simultaneously, reducing labor demands and potentially improving precision of the assay. Our objectives were to determine if IVDMD values were similar for the traditional method and the new DAISY II methods and to determine if IVDMD values were similar with source of inoculum from two different donor cow diets, all forage and TMR.

MATERIALS AND METHODS

Ten feeds were dried in a forced air oven for 48 h at 55°C and ground through a 1-mm screen of a Wiley mill (Arthur H. Thomas, Philadelphia, PA) prior to analysis for IVDMD. Feeds were also analyzed for CP (1), ADF, and NDF (ANKOM 200 Fiber Analyzer, ANKOM Technology Corporation, Fairport, NY).

The two donor cow diets were all grass hay or an alfalfa haylage and corn silage-based TMR. The TMR was formulated according to production needs of the cow (10). The nutrient composition of the diets is shown in Table 1. Cows had been fitted with a rumen cannula; rumen fluid was collected 2 h after the morning feeding. Rumen fluid was collected from six areas of the rumen with a vacuum pump. The ruminal fluid and approximately 300 g of ruminal particulate matter were transported to the laboratory in a prewarmed container. Preparation of the inoculum included blending the ruminal fluid and particulate matter for 2 min in a Waring blender under constant purging with CO₂, followed by filtering with vacuum through 5 to 8 mm of glass wool, wrapped in one layer of cheesecloth with constant purging with CO₂.

The DAISY II apparatus contains four 4-L digestion vessels, which slowly rotate in a digestion chamber that is maintained at 39.5°C. Samples to be analyzed are heat sealed into ash-free and N-free filter bags and inserted into the digestion vessels. Each digestion vessel holds 25 bags, so that 100 bags can be analyzed simultaneously.
TABLE 1. Nutrient composition of the donor cow diets [all grass hay (GH)] or TMR.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>GH</th>
<th>TMR</th>
</tr>
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<tbody>
<tr>
<td>CP, % DM</td>
<td>11.8</td>
<td>16.0</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>41.7</td>
<td>20.1</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>68.8</td>
<td>30.2</td>
</tr>
<tr>
<td>NEL, Mcal/kg</td>
<td>1.25</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Methods tested in this study were traditional method (TM) with modification of the Tilly and Terry procedure (15) to use the same reagents as the DAISYII method with the same feeds in a digestion vessel (DS) and the DAISYII using different feeds in each digestion vessel (DD). The reagents used were: solution A (10 g of KH2PO4, 0.5 g of MgSO4·7H2O, 0.5 g of NaCl, 0.1 g of CaCl2·H2O, and 0.5 g of urea in 1 L of deionized water) and solution B (15 g of Na2CO3 and 1 g of Na2S·9H2O in 100 ml of deionized water). A buffer solution was made just prior to each digestion run by warming solutions A and B to 39°C and adding 20 ml of solution B to 1 L of solution A. The pH of the buffer solution was adjusted to 6.8 (if needed) by adding small additional amounts (1 to 2 ml) of solution B.

Each of the 10 feeds was digested in quadruplicate for each source of inoculum and each method, and each analytical run was replicated. For the TM method, 0.25 g of each sample per tube was used, and 0.5 g of sample per bag was used for the DS and DD methods. For the DS and DD methods, samples were heat sealed in Ankom F57 bags (ANKOM Technology Corp., Fairport, NY). The pore size of the bags was 57 microns. For the TM method, 15 ml of the buffer solution and 5 ml of the rumen inoculum were added to each 50-ml Nalgene tube, the tube was flushed with CO2, stoppered with a bunsen valve, and incubated for 48 h at 39°C. Tubes were swirled by hand at 0, 2, 4, 20 and 28 h of incubation. For the DS and DD methods, 25 bags were placed in each of the four digestion vessels, 1600 ml of the buffer solution and 400 ml of the rumen inoculum were added to each vessel, the vessels were flushed with CO2, and the vessels (with lids) were placed in the DAISYII for incubation at 39°C for 48 h. The DAISYII rotates the vessels continuously in the incubation chamber.

At the end of the 48 h, 1 ml of 6N HCl and 100 mg of pepsin powder was added to each tube in the TM method. Tubes were then incubated for an additional 24 h without stoppers and filtered through #54 Whatman filter paper. For the DS and DD methods, 40-ml of 6N HCl and 8 g of pepsin powder was added to each digestion vessel and incubated for another 24 h. For the three methods, filter paper and filter bags were dried at 100°C for 24 h, and blank tubes containing filter paper only and blank bags were used to correct for bacterial contamination (13). No time points were measured before 48 h, so no data is available for possible method by time interactions.

The statistical design was a completely randomized design with a 2 x 3 factorial arrangement of treatments with two sources of inoculum and three methods of analyses. Treatments included two sources of rumen inoculum, hay diet and TMR, and three methods of analysis of IVDMD, TM, DS, and DD. All analyses were replicated. The model was

IVDMD = source + method + (source*method) + replicate + (source*replicate) + (method*replicate) + error.

Differences between source and method were tested with (source*method) as the error term. Data were analyzed with the GLM procedures of SAS (14). Means separation was by the least squared means procedures of SAS. There were no significant interactions.

RESULTS AND DISCUSSION

The nutrient contents of the two donor cow diets—grass hay and TMR—are shown in Table 1. As expected, the nutrient content differed between the two diets, especially CP and NDF. The nutrient contents of the 10 feeds (alfalfa hay, grass pasture, grass hay, legume haylage, corn silage, TMR, grain mixture, high moisture shelled corn, steam flaked corn) and ground corn are shown in Table 2.

Source of inoculum significantly affected IVDMD for grass pasture and TMR (P < 0.05) and for alfalfa

<table>
<thead>
<tr>
<th>Feed</th>
<th>CP (%)</th>
<th>ADF (%)</th>
<th>NDF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alalfa hay</td>
<td>18.17</td>
<td>34.06</td>
<td>49.96</td>
</tr>
<tr>
<td>Grass pasture</td>
<td>23.65</td>
<td>24.55</td>
<td>46.37</td>
</tr>
<tr>
<td>Grass hay</td>
<td>14.17</td>
<td>40.70</td>
<td>58.47</td>
</tr>
<tr>
<td>Mixed haylage</td>
<td>15.24</td>
<td>44.15</td>
<td>56.29</td>
</tr>
<tr>
<td>Corn silage</td>
<td>7.67</td>
<td>22.13</td>
<td>37.70</td>
</tr>
<tr>
<td>TMR</td>
<td>15.97</td>
<td>20.07</td>
<td>30.21</td>
</tr>
<tr>
<td>Grain mixture</td>
<td>33.14</td>
<td>7.32</td>
<td>16.61</td>
</tr>
<tr>
<td>高三</td>
<td>9.84</td>
<td>2.62</td>
<td>7.88</td>
</tr>
<tr>
<td>Steam flaked corn</td>
<td>9.10</td>
<td>2.88</td>
<td>8.79</td>
</tr>
<tr>
<td>Dry ground corn</td>
<td>7.44</td>
<td>2.80</td>
<td>6.47</td>
</tr>
</tbody>
</table>

1. High moisture shelled corn.
hay, grass hay, steam flaked corn, and dry ground corn (P < 0.10; Table 3). For the remaining four feeds (mixed haylage, corn silage, grain mixture, and high moisture shelled corn), there were no statistically significant differences. For the six feeds that were affected by source of inoculum, IVDMD values were higher when the donor diet was TMR compared to grass hay. In some studies (8, 12), IVDMD values were similar for different donor diets. Other studies (4, 5, 6, 7) have shown that source of inoculum had significant effects on IVDMD of the feeds.

Despite the difference in IVDMD values, the ranking of feeds from highest to lowest was the same between the two sources of inoculum, except when pasture was included in the rankings. Ayres (2) found that 10 out of 15 donor cow diets, ranging between 45 and 82% NDF, ranked forages consistently for IVDMD.

The method of analysis (TM, DS, or DD) did not affect IVDMD of the 10 feeds tested (Table 4). Although it might be expected that incubating high starch grains with lower quality forages in the same digestion vessel might influence IVDMD, no differences were found between the DS and DD methods. Additionally, the relative ranking of IVDMD values from highest to lowest was the same for DS and DD when pasture was removed from the data. Likewise, the ranking with the TM was similar, although not exactly the same for the processed corn products analyzed. Research (8, 11, 16) has indicated the potential for decreased IVDMD values associated with gas accumulation in either in vitro or in situ bags. Although some gas accumulation was observed in the bags in the current study, the IVDMD values were not decreased.

### CONCLUSIONS

The DAISY II is an effective system for measuring IVDMD and produces data similar to the more traditional methodology using individual tubes. No significant differences were found when both grains and forages were incubated in the same digestion vessel. The DAISY II is more labor efficient than traditional methods and represents a significant advantage for analysis of forage, grain, and mixed samples.

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### REFERENCES

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