Recovery of Residual Organic Matter from In Vitro Digestion of Forages

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Abstract

After two-stage in vitro digestion of about .44 g of initial forage organic matter, residues in fermentation tubes were filtered sequentially through glass wool mats, sintered glass crucibles, and glass fiber discs. Only .002 g of residual organic matter which passed glass wool was retained by sintered glass. The residue on sintered glass did not include recognizable forage particles. Glass fiber retained .029 g of residual organic matter which had passed both glass wool and sintered glass. Calculations of in vitro organic matter digestion were not affected by including the residue retained by sintered glass in addition to that retained by glass wool. Inclusion of the residue retained by glass fiber reduced digestion percentages by 1.9 units. Glass wool mats are easily prepared in 25-ml porcelain Gooch crucibles, and filtration through them is rapid.

Introduction

The two-stage in vitro digestion procedure (5) generally has been adopted, with certain modifications, for routine quality screening of forages. In this procedure, fermentation with rumen microorganisms is followed by digestion with acid-pepsin, and the undigested residue is recovered and weighed. Recovery of undigested residues by filtration has been accomplished with sintered glass crucibles (2), glass fiber filters (1), filter papers (4, 5), or filter sticks (3). Slow filtration may occur with sintered glass and glass fiber. Paper may result in lower apparent digestion (4) perhaps because of retention of microbial matter synthesized during fermentation. Van Soest et al. (6) filtered rumen fluid through glass wool prior to inoculation, and this suggested that glass wool might retain undigested forage particles.

Our study evaluated the use of glass wool mats in porcelain Gooch crucibles in contrast to the use of sintered glass or glass fiber for recovery of in vitro residual organic matter.

Procedure

In vitro organic matter digestion (IVOMD) was determined on 12 forage samples, in duplicate in each of two replications. Four blanks (no sample) were included in each replication. Initial organic matter (OM) was about .44 g. Rumen fluid was strained through glass wool and mixed with CO₂-saturated McDougall's saliva (2), and 50 ml were used per fermentation tube (100 ml polyethylene) including blanks. After 48 h anaerobic incubation at 39 C, 6 ml 20% (vol/vol) HCl and 2 ml 5% (wt/vol) aqueous pepsin were added, and incubation was continued for 46 h. After pepsin digestion, residues were filtered through mats of Pyrex glass wool (Corning 3950) in 25-ml porcelain Gooch crucibles (Coors 270, size 3). Glass wool was cut into squares of approximately 20 mm by paper cutter. Only one layer of glass wool was cut at a time. It was essential that the glass wool be cut cleanly. Otherwise, tearing the pieces apart created small glass particles which irritated skin and throat. About six squares were placed in each crucible. Each glass wool mat was used only once.

In Experiment 1, the filtrates passing glass wool were filtered immediately through Pyrex sintered glass crucibles (Corning 39240-50C) to determine the amount of residue passing glass wool which was retained by sintered glass. In Experiment 2, the filtrates from eight sample and four blank tubes which had passed both glass wool and sintered glass were filtered through glass fiber discs (Reeves Angel 934 AH) in 25-ml porcelain Gooch crucibles. All crucibles, filters, and residues were dried at 110 C, and residual OM was determined as the loss in weight after ashing at 500 C for 4 h. The IVOMD was calculated with glass wool residues only, and glass wool residues plus the additional residues retained on either sintered glass (Exp. 1 and 2) or glass fiber (Exp. 2).

Results and Discussion

The residual OM passing glass wool but re-
tained by sintered glass was only about .002 g for both blanks and samples (Table 1). Glass wool mats were apparently effective in retaining undigested forage particles since microscopic examination revealed no recognizable forage particles in the residue on sintered glass. From sample tubes, glass fiber retained about .029 g of OM which had passed both glass wool and sintered glass (Exp. 2). The retention on glass fiber of residues from sample tubes was about .008 g greater than that from blank tubes due to retention of microbial matter synthesized during fermentation.

From glass wool residues only, IVOMD for all 48 determinations (Exp. 1) ranged from 37.7 to 61.4% with a mean of 51.82% (Table 1). Including the residual OM retained by sintered glass crucibles brought no appreciable change in the calculated IVOMD values. In Exp. 1, the mean difference was an increase of .03 ± .16 units, with the largest difference being a decrease of only .44 units, well within acceptable error limits. The inclusion of glass fiber residues resulted in IVOMD percentages about 1.9 units lower than those with glass wool residues alone or plus sintered glass residues (Exp. 2). Similar decreases have been reported for filter paper (4).

The glass wool recovery procedure has been used since 1971 at the University of Florida in the determination of IVOMD on several thousand samples of subtropical forages. One technician can prepare glass wool mats in 100 crucibles in about 30 min. Filtration over light vacuum is as rapid as the fermentation tube can be emptied and rinsed, and there has been no evidence of forage particles passing the mat. The 25-ml porcelain Gooch crucibles are less expensive than sintered glass crucibles and they occupy less space in ovens, furnaces, and desiccators.

References


Table 1. Residual organic matter recovered and in vitro organic matter digestion (IVOMD) using glass wool filters alone and with sintered glass and glass fiber filters.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Filter</th>
<th>Blank</th>
<th>Sample</th>
<th>IVOMD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glass wool</td>
<td>.0026 ± .0012&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.2133 ± .0374&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.82 ± 3.73&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>+ sintered glass</td>
<td>.0025 ± .0007</td>
<td>.0024 ± .0006</td>
<td>51.85 ± 3.79</td>
</tr>
<tr>
<td>2</td>
<td>glass wool</td>
<td>.0021 ± .0006</td>
<td>.2053 ± .0301</td>
<td>53.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>+ sintered glass</td>
<td>.0024 ± .0004</td>
<td>.0021 ± .0005</td>
<td>53.79</td>
</tr>
<tr>
<td></td>
<td>+ glass fiber</td>
<td>.0210 ± .0109</td>
<td>.0292 ± .0041</td>
<td>51.84</td>
</tr>
</tbody>
</table>

<sup>a</sup> Experiment 1: 8 blank and 48 sample tubes; initial organic matter = .4367 ± .0068 g. Experiment 2: 4 blank and 8 sample tubes; initial organic matter = .4375 ± .0040 g.

<sup>b</sup> Mean and standard deviation.

<sup>c</sup> Mean and sampling error.

<sup>d</sup> No sampling error available.