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ABSTRACT
An experiment was conducted with labeled wheat plant cell walls intrinsically labeled with $^{14}$C and treated with extrinsic markers to measure their rate of disappearance from fermentors fed every hour. The systems were maintained at pH 7.0 in the first trial and at pH 2.5 in the second trial to mimic acidities for the rumen and abomasum. In each trial 12 fermentors were fed hourly .4 or .8 g orchardgrass hay pellets, infused continuously with 500 or 1000 ml of buffered solution daily, and dosed with three marked cell wall treatments. Treatments were 1) $^{14}$C cell walls, 2) $^{14}$C cell walls mordanted with Cr containing tracer $^{51}$Cr, and 3) $^{14}$C cell walls treated with Yb containing tracer $^{169}$Yb. Marker disappearance was faster at pH 7.0 than at pH 2.5. Marker disappearance was faster for the high than the slow liquid infusion rate. Disappearance was also faster for the high feed rate than the low feed rate. Fractional rates of $^{14}$C cell wall disappearance (h$^{-1}$) were -.042, -.035, and -.029 in the untreated, Yb-treated, and Cr-treated cell walls, and rates of $^{169}$Yb and $^{51}$Cr disappearance were -.039 and -.025, respectively. Treating $^{14}$C cell walls (internal control) with Cr or Yb reduced disappearance compared to nontreated $^{14}$C cell walls (external control). Differences in rates of marker disappearance and interactions of markers, pH, infusion, and feed rates support the need for caution in interpretation of kinetic data in general and comparison of results obtained with different markers.

INTRODUCTION
Various substances have been used as dietary markers to qualify and quantify digestive physiological phenomena. Use of markers in nutrition was reviewed by Kotb and Luckey (12) and Warner (25). Stained particles were used to measure rate of disappearance of particulate material from the rumen (1, 3), but small particles are difficult to count and the size collected and counted influences the mean retention time of feed particles (7). Rare earth elements have been used as particle markers but were shown to migrate (4, 9, 10) at pH occurring in the abomasum. These techniques suffer from difficulties in interpretation. Larger differences in turnover rates among animals than differences attributable to nutritional or physiological treatments (10) make comparison of markers in animals impractical. The objective of this study was to compare the fractional turnover rates of intrinsically $^{14}$C-labeled plant cell walls, Yb-treated cell walls, and Cr-mordanted cell walls in a continuous culture system.

MATERIALS AND METHODS

Marker Preparation
Cell walls prepared (19) from wheat plant grown in an atmosphere containing a constant ratio of $^{14}$CO$_2$ to CO$_2$ (17) were used as internal and external control marker. The
material had 11,000 dpm $^{14}$C/mg cell wall on a dry basis.

A portion of the $^{14}$C wheat plant cell walls was mordanted with 13% Cr (24) containing $^{51}$Cr tracer ($2 \times 10^6$ dpm/g dry cell walls). Another portion of wheat plant cell walls was treated with Yb (21, 22) containing $^{169}$Yb tracer (8).

In Vitro Fermentation

Two trials were conducted, each with 12 500-ml fermentors. Acidity was controlled (16) to mimic the rumen in trial 1 (pH 7.0) and the abomasum in trial 2 (pH 2.5). Wheat plant cell walls intrinsically labeled with $^{14}$C served as internal and external controls for C-labeled wheat plant cell walls mordanted with Cr or Yb markers. Each fermentor was automated for hourly addition of feed. Buffer was continuously infused. Effluent overflow was continuous and collected quantitatively at various intervals.

Cheesecloth-strained ruminal fluid from a rumen-fistulated steer fed orchardgrass hay (IFN 1-03-434) provided the inoculum to start trial 1. Inoculum was not used in trial 2. The basal feed for the fermentors was pelleted orchardgrass hay (IFN 1-03-434). After 5 d equilibration, .5 g of marked cell wall was administered in a single dose at 0800 h into each fermentor. Two infusion rates (500 and 1000 ml/d) and two feed rates (10 or 20 g orchardgrass hay pellets/d) were imposed.

The two feed rates were .42 or .83 g of pelleted hay hourly into each of six fermentors. The two flow rates were continuous infusion with 500 or 1000 ml of buffered solution daily giving liquid fractional turnover rates ($h^{-1}$) of −.0415 and −.083, respectively. A mixture of McDougall’s saliva (14):tap water in a 3:2 ratio maintained pH of incubations for the high infusion rate at both feed rates. Buffering capacity of McDougall’s saliva was modified by substitution of NaHCO$_3$ with Na$_2$CO$_3$ and substitution of Na$_2$HPO$_4$ with Na$_3$PO$_4$ equimolarly for the incubation on low flow rate and high feed rate. The artificial saliva part of the buffer used for incubation on low flow rate and low feed rate was composed of 58.5 mM each of NaHCO$_3$, Na$_2$CO$_3$ and 13 mM each of Na$_2$HPO$_4$:Na$_3$PO$_4$ instead of 117 mM NaHCO$_3$ and 26 mM Na$_2$HPO$_4$. The three buffer solutions contained 25 mg urea/100 ml. In trial 2, the McDougall’s was modified to contain 143 mM NaH$_2$PO$_4$ and sufficient HCl to maintain a pH of 2.5 in the fermentors regardless of turnover or feed input imposed.

Effluent was collected at 0800 and 1600 h each day for 5 d. Saturated HgCl$_2$ was added to effluent vessels to stop fermentation. Effluents were frozen, lyophilized, and equilibrated to ambient conditions. Dry matter was determined as stabilized weight after 24 h at 100 C. Carbon-14 was determined by combustion, by trapped CO$_2$, and by count in a liquid scintillation spectrometer (18). Concentrations of $^{51}$Cr and $^{169}$Yb were determined by count of about 1 g of sample packed to a height of 5 cm in a 12 x 74-mm plastic tube placed in a gamma scintillation spectrometer. Samples were counted until 100,000 counts accumulated or 10 min and corrected for background. Gamma counts attributable to beta activity in initial samples were corrected using appropriate controls.

Statistical Methods

Treatment design was a $2 \times 2 \times 2 \times 5$ factorial arrangement of pH, infusion rate, feeding rate, and the five marker combinations, respectively. The experimental design was a split plot with 24 whole units (fermentors), and 16 of the 24 fermentors were assigned two markers to make 32 subunits. Data for each fermentor and marker were collected at 10 times from 4 to 112 h postdosing in 8 and 16-h intervals.

Difficulties in maintaining satisfactory fermentor operation in trial 2 resulted in missing values for 10 of the 400 observations. A linear regression coefficient was determined for natural logarithm (dpm) versus time for each treatment and marker combination (15).

Analysis of variance was on the regression coefficients with certain three-factor and four-factor interactions pooled ($P>.25$) to provide an estimate of error variance between fermentors while other between markers within fermentor, three-factor, and four-factor interactions ($P>.25$) made up the residual error. For significant marker interactions, error variances from the ANOVA were used according to the rules for split plots to compute standard errors for the mean regression coefficients, and Duncan’s multiple range test was conducted to compare these coefficients (20).
RESULTS AND DISCUSSION

Fermentors functioned well at pH 7.0 in terms of automated feeding, buffer infusion, and effluent output. However, at pH 2.5 occasional clogging of the sample receiver prevented continuous effluent collection. Engorged fermentors were dislodged manually before sample collection. There was no pattern to this problem.

Observed fractional rate constants (h⁻¹) of marker disappearance are in Table 1. Mean retention time can be calculated as the reciprocal of the fractional disappearance rate (1/h⁻¹) for steady-state conditions. Steady state was maintained by hourly feeding, continuous buffer infusion, and maintenance of constant fermentor volumes by gravity overflow. Quantitative collection of fermentor effluent for marker analysis was analogous to sampling digesta passing from the rumen rather than sampling the rumen itself.

We assumed marker passage would be first order because marker concentration was determined in total dry matter. Digestion and disassociation of marker from cell walls could increase the rate of marker passage assuming the liquid phase has a higher passage rate. Inhibition of digestion or increased density caused by the presence of extrinsic markers could decrease rate of marker disappearance.

High coefficients of determination (CD) for natural logarithms (dpm/g) dry matter versus time regressions indicated that marker disappearance from fermentors fit the single exponential model. Fermentors controlled at pH 7.0 had CD ranging from .81 to .99. Fermentors controlled at pH 2.5 had CD ranging from .23 to .98 and reflected discontinuous outflow. Errors were much lower than Hartnell and Satter (10) observed in in vivo studies of mean ruminal turnover rates of cows in different stages of lactation. The large volume of material in the rumen relative to a fermentor would have increased error because of sampling, dilution, and mixing of digesta. Collecting the effluent is synonymous with sampling material leaving the rumen rather than that remaining which may influence error.

Analysis of variance for main effects (pH, infusion rate, feed rate, and flasks) and interactions (13) are in Table 2. Main effect means for pH (P<.01), infusion rate (P<.01), and feed rate (P<.025) are in Table 3. Maintaining fermentors at pH 7.0 and continuous infusion of 1000 ml of buffer solution daily resulted in about 50% higher rate of marker disappearance

<table>
<thead>
<tr>
<th>pH</th>
<th>Feed rate</th>
<th>14C</th>
<th>159Yb</th>
<th>51Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>High</td>
<td>-.062⁴</td>
<td>-.056</td>
<td>-.045</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-.053</td>
<td>-.052</td>
<td>-.036</td>
</tr>
<tr>
<td>2.5</td>
<td>High</td>
<td>-.074</td>
<td>-.029</td>
<td>-.041</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-.037</td>
<td>-.039</td>
<td>-.014</td>
</tr>
</tbody>
</table>

1 Standard error of estimates ranged from .0009 to .004 for rate constants at pH 7.0 and from .001 to .01 for rate constants at pH 2.5.

2Rate was 500 or 1000 ml of buffer solution daily with liquid fraction turnover of -.0415 and -.083, respectively.

Ten or 20 g of orchardgrass pellets daily.

Rate constant based upon measured beta emission in 10 serial samples.

Rate constant based upon measured gamma emission in 10 serial samples.

relative to the rate of disappearance at pH 2.5 and continuous infusion of 500 ml of buffer solution daily. Doubling the hourly feed rate from .4 to .8 g resulted in 19% higher marker disappearance rates. It is very unlikely that digestion influenced the rate of marker disappearance at pH 2.5 as the flasks were not inoculated and as growth of fiber digesting microbes is inhibited below pH 5.3 (11, 23). We suggest that particulate matter moves out of fermentors at this pH primarily due to displacement by liquid and solid inputs. The mean disappearance rates of the markers are in Table 4. Mean disappearances of control 14C cell walls and 169Yb were not different. Disappearance of 14C cell walls (based on beta emission) was reduced by treatment with either Yb or Cr. Reduced rates of disappearance presumably resulted from decreased dry matter loss by digestion or metabolism (24) and possibly by increased density (5) of cell walls caused by treatment with either Yb or Cr. Intrinsically labeled 14C cell walls in the treated 169Yb/14C cell walls disappeared 11% slower than 169Yb marker. However, intrinsic 14C cell walls disappeared 14% faster in the treated 51Cr/14C cell walls than 51Cr marker. The 169Yb and 51Cr disappearance rates were from gamma emission. Tracers aided analysis in this study, the stable elements were at amounts suggested in the literature (22, 24).

### TABLE 2. Statistical analysis of rates of marker disappearance.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares (%) of total model</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td>20.46</td>
<td>.01</td>
</tr>
<tr>
<td>Infusion rate</td>
<td>1</td>
<td>34.14</td>
<td>.01</td>
</tr>
<tr>
<td>Feed rate</td>
<td>1</td>
<td>5.23</td>
<td>.025</td>
</tr>
<tr>
<td>Flask¹</td>
<td>2</td>
<td>18.09</td>
<td>.01</td>
</tr>
<tr>
<td>Infusion rate × feed rate</td>
<td>1</td>
<td>4.97</td>
<td>.025</td>
</tr>
<tr>
<td>Infusion rate × flask</td>
<td>2</td>
<td>5.62</td>
<td>.10</td>
</tr>
<tr>
<td>pH × infusion rate × feed rate</td>
<td>1</td>
<td>3.31</td>
<td>.10</td>
</tr>
<tr>
<td>Within flask</td>
<td>2</td>
<td>1.68</td>
<td>.01</td>
</tr>
<tr>
<td>pH × within flask</td>
<td>2</td>
<td>.68</td>
<td>.10</td>
</tr>
</tbody>
</table>

¹ Three marker combinations: 14C, 14C/169Yb, and 14C/51Cr.

² Beta versus gamma emitter: 14C versus 169Yb versus 51Cr.

### TABLE 3. Main effect mean rates of disappearance (h⁻¹) of marker means.

<table>
<thead>
<tr>
<th>Source</th>
<th>n¹</th>
<th>High (h⁻¹)</th>
<th>Low (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH²</td>
<td>20</td>
<td>-.041a</td>
<td>-.027b</td>
</tr>
<tr>
<td>Infusion rate</td>
<td>20</td>
<td>-.042a</td>
<td>-.026b</td>
</tr>
<tr>
<td>Feed rate</td>
<td>20</td>
<td>-.037a</td>
<td>-.031b</td>
</tr>
</tbody>
</table>

¹ Observations per treatment mean, SEM = .0017.

² pH X infusion rate X feed rate interaction (P<.10).

a,b Means with different superscripts within source differ (P<.05).

### TABLE 4. Mean rates of disappearance of markers.¹

<table>
<thead>
<tr>
<th>Marker</th>
<th>Radiation emission (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 14C cell wall</td>
<td>- .042a</td>
</tr>
<tr>
<td>Carbon-14 cell wall-treated 169Yb</td>
<td>- .035b</td>
</tr>
<tr>
<td>Carbon-14 cell wall-treated 51Cr</td>
<td>- .029b</td>
</tr>
</tbody>
</table>

¹ Between flasks SE = .0020. Within flasks SE = .0010.
### TABLE 5. Mean marker disappearance for infusion rate × feed rate.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Infusion</th>
<th>High (h⁻¹)</th>
<th>Low (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>-0.048ᵃ</td>
<td>-0.026ᶜ</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>-0.036ᵇ</td>
<td>-0.026ᶜ</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ,ᵇ,ᶜMeans with different superscripts differ (P<.05).

A difference in the rate of disappearance between ¹⁴C cell walls (beta) and ⁵¹Cr/¹⁴C cell walls (gamma) was not anticipated. We propose that this difference, even though statistically significant, may not be biologically significant. The important comparison is that both ¹⁶⁹Yb and ⁵¹Cr and internal control cell walls (treated ¹⁴C cell walls) disappeared more slowly than external control cell walls (nontreated ¹⁴C cell walls). Migration of ¹⁶⁹Yb from the ¹⁴C cell walls at pH 2.5 could contribute in higher disappearance rates of ¹⁶⁹Yb than internal control ¹⁴C cell walls that could account for lack of significance.

Treatment of cell walls with Yb rather than treatment of total feed dry matter should reduce Yb migration (2, 6). Carbon-14 cell walls and ¹⁶⁹Yb cell walls would disappear at the same rate if neither were digested and metabolized to CO₂ and CH₄ and if the marker were indelibly associated with the cell walls. The methodology and design of this experiment were inadequate to partition the digestion and marker disassociation effects.

The interactions of actual infusion and feeding rates on fractional rates of marker disappearance are in Table 5. The interaction was significant because fractional rates of marker disappearance for feed rates were different under high infusion, but fractional rates of marker disappearance for feed rates were not different under low infusion. At least in this system, liquid volume input was more important in affecting marker disappearance than feed rates.

Interaction of marker and infusion rate was significant (P<.10). Means are in Table 6. The three markers, ¹⁴C cell walls, ¹⁶⁹Yb cell walls and ⁵¹Cr cell walls, disappeared from fermentors at a faster rate at the high infusion rate than at the low infusion rate, as was anticipated. At the low infusion rate, the ⁵¹Cr cell walls disappeared more slowly than the other two markers.

These results establish that treatment of cell walls with either Yb or Cr affects the rate of disappearance from continuously fed fermentors. A relatively large reduction in the fractional rate was observed except with Yb for which migration may have compensated for this reduction. Fluid dilution rate appears to influence these markers differently as suggested by their significant interaction (P<.1). These results support the need to exercise caution in choice of markers in nutritional studies; interpretation of results from different experiments involving different markers; and use of fractional turnover rates for rumen function models. Fractional turnover rate obtained with Yb and Cr marked feed may be useful for establishing relative differences between treatments but appear to be deficient for measuring passage of undigested feed residues. Complete description of the markers used, how they are applied to feeds, and how they are analyzed should improve understanding and aid evaluation of rumen kinetic data.

### ACKNOWLEDGMENTS

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REFERENCES


