A REVIEW OF THE USE OF INTRINSICALLY $^{14}$C AND RARE EARTH-LABELED NEUTRAL DETERGENT FIBER TO ESTIMATE PARTICLE DIGESTION AND PASSAGE

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ABSTRACT

Methodology that allows simultaneous measurement of dynamic events affecting NDF digestion in passage from the rumen should improve our understanding of factors influencing intake and digestion. Ideally, particle flow is measured with a marker indelibly attached to or intrinsically part of the feed. If flow measurements are to reflect physiological conditions, marked and unmarked feed must be digested and passed with identical fractional rates. Application of $^{14}$C-labeled plant fiber to the study of ruminal dynamics has been slow because of expense and difficulty in producing $^{14}$C-labeled plant material. Recently, alfalfa was intrinsically labeled with $^{14}$C under field conditions to produce plant material similar in composition to unlabeled material. Carbon-14 specific activity was similar in all particle sizes, and in indigestible and digestible NDF. Greater concentrations of ytterbium (Yb) were associated with smaller vs larger particles. Larger differences in turnover rates among animals than differences attributable to treatments force comparisons of markers to be made within animal or with in vitro systems. The uncertainty about how extrinsic markers respond under various environments resulting from interaction of feed properties and gut function, and the high error inherent in measuring dynamic systems, raise serious questions on the interpretability of results. Advantages of $^{14}$C-labeled NDF over other markers include simultaneous measurements of particle breakdown, digestion and passage rates as well as the potential to study microbial attachment, VFA, CO$_2$ and CH$_4$ production and rate of incorporation of labeled metabolites into tissues.

(Key Words: Fiber, Digestibility, Kinetics, Rumen Metabolism, Particle Size, Markers.)


Introduction

Markers are used commonly to estimate digestibility of feeds in animal nutrition. A more specific use in ruminant nutrition is to measure rate of passage of feed through the gastrointestinal (GI) tract and to provide data on the number and volume of organs (pools) or parts of organs through which feeds pass. Results of such research should be useful in developing concepts and elucidating plant and animal factors involved in limiting voluntary feed intake. Relevant data on pools and turnover may be obtained from input-output relationships, sampling GI contents from surgically altered animals or emptying GI contents at slaughter. However, there is merit in studying dynamic systems using kinetic approaches conducted under physiological conditions, rather than relying on static end-point measurements. Accuracy and precision of measurements of rate of passage in ruminants depends mostly on characteristics of the marker. An extrinsic marker should be tightly bound to the indigestible component of the feedstuff. Rare earth elements (Hartnell and Satter, 1979; Teeter et al., 1984) and chromi-
um-mordanted fiber (Uden et al., 1980) have been used to measure rate of passage. Under conditions in the rumen (Hartnell and Satter, 1979; Teeter et al., 1984), there is little exchange of cerium, samarium, lanthanum and ytterbium from one feed particle to another. However, Combs et al. (1984) indicated that substantial movement of marker and attachment to ruminal bacteria of marker occurs under conditions similar to those in the rumen and abomasum. McBurney et al. (1983) reported a widely varying cation exchange capacity of plant fiber depending on the source of feed and its preparation. The varying cation exchange capacity of plant fiber is likely a factor in observed extrinsic marker migration.

The technology for producing intrinsically 14C-labeled plant material has been available for 30 yr but has been applied only infrequently to the study of ruminant nutrition. Plant material intrinsically labeled with either 13C or 14C should provide equally applicable information to help unravel various mechanisms involved with consumption of feed by ruminants.

Differences in natural abundance of 13C to 12C in conventional feed was used by Tyrrell et al. (1984) to determine the source of metabolizable carbon and the contribution of dietary carbon to milk carbon. The ratio of 12C and 13C isotopes in the feces has been used to estimate the proportion of C3 and C4 plants consumed (Jones et al., 1979).

Application of 13C technology to ruminant nutrition is usually beyond the reach of animal scientists because of high costs of equipment and the maintenance and technical expertise needed to operate it. Boutton et al. (1986) produced 13C-enhanced plant material using 13CO2 to overcome the high natural background of 13C, a problem encountered with using differences in natural abundance in feedstuffs. High background is not a problem encountered with use of 14C.

Use of 14C methodology is less costly for both analysis and for use in production of labeled plant material. Disadvantages of using 14C are the need to comply with Nuclear Regulatory Commission rules for conducting research and complying with rules for safe disposal of radioactive wastes.

Production of 14C Plant Material

A simple, inexpensive, safe and scientifically sound method is needed for intrinsically 14C-labeled plant material to be used for investigating ruminal function and ruminant metabolism. Smith et al. (1963) reported on an improved chamber for full growth-cycle labeling of plants with uniform specific activity (SA). Uniform SA was achieved by placing seedlings in a closed-system chamber that was supplied a constant ratio of 14CO2 to CO2. Keith et al. (1963) designed a chamber used at Kansas State University to produce 14C alfalfa subsequently used in research reported by Yadava et al. (1964) and Alexander et al. (1969). Plant material produced in the chamber of Keith et al. (1963) was not uniform in SA, even though a uniform SA could have been achieved by maintaining a constant ratio of 14CO2/12CO2 in the chamber.

Chambers are more suitable for short-term labeling than for long-term, full growth-cycle labeling of plant material. Problems with controlling plant diseases, insects, low yield, and concern about chemical composition and nutritive properties of the plant material grown in chambers being different from field-grown forages have slowed application of 14C-labeled plant material.

Robles (1977) showed that 14CO2 from a single release was incorporated into various chemical constituents of the corn plant when done in a plastic bag under field conditions. Allocation patterns of carbon were studied under field conditions with 14CO2 released under airtight plastic canopies (R. E. Wyse, personal communication). Rapid 14CO2 uptake by photosynthesizing plants demonstrated the potential of this approach to intrinsically label forage crops.

Alfalfa (Smith and Erdman, 1986) and corn (Smith et al., 1988) were grown under field conditions and intrinsically labeled with 14CO2 so that the chemical composition would be similar to that of adjacent unlabeled plants. Labeling was accomplished using 14CO2 under an airtight plastic canopy four times during the growth period. The amount of 14C was increased with each additional release in an attempt to parallel plant weight accumulation.

Alfalfa leaf DM contained 51% of total plant weight and 52% of 14C activity, indicating gross uniformity in distribution of weight and activity. Neutral detergent fiber also was similar to total plant DM in SA, indicating a similar distribution of 14C in structural and nonstructural components. The difference in SA between leaves and stems was 22%, and
about the same difference existed between the upper and lower half of plant NDF in alfalfa. Differences in SA of plant parts also were observed in corn.

Smith and Erdman (1986) had concern that the nonuniform distribution of $^{14}$C in physical and chemical parts of alfalfa could invalidate its usefulness in research on particle breakdown. Because of the nonuniform distribution of $^{14}$C, two criteria were established for plant material to be acceptable. First, particle size distribution of the natural logarithm of $^{14}$C activity and weight (ln mean and ln sigma) should approximate each other in a coarsely ground sample. In other words, a plot of accumulative weight and activity in DM by particle size should appear as two parallel lines superimposed on each other (Figure 1). Second, the SA of the undigested NDF and indigestible NDF after 72 h of incubation in ruminal fluid should be constant. The 72-h in vitro NDF digestibility (%) of alfalfa shown in Figure 2 was 70 for leaves and 45 for stems. Constancy of specific activities in alfalfa NDF residue of leaves and of stems are shown in Figure 2. Plant material intrinsically labeled with $^{14}$C that possessed a similar distribution of activity and weight by particle size, and with a similar distribution in digestible and indigestible NDF, was assumed to be a suitable research material. Dugger and Palmer (1988) demonstrated incorporation of $[^{14}]$glucose into cell wall components of in vitro grown cotton fibers. Cellulose labeling continued to increase up to 4 h chase time, indicating that their approach may be useful for production of small amounts of experimental cellulose materials. The use of $^{15}$N incorporated into plant material or $^{15}$N-depleted plant material also may be useful in studying nitrogen use and metabolism in ruminants (Ta et al., 1988).

### Comparisons of Extrinsically and Intrinsically Labeled Feeds

Attempts to understand the complexities of digestive processes occurring in the GI tract have prompted the search for methodology to measure digesta flow (Kotb and Luckey, 1972; Warner, 1981). Mathematical formulations (Blaxter et al., 1956; Grovum and Phillips, 1973; Ellis et al., 1979) have been used to describe the rate of passage of marker through the digestive tracts of animals. Soluble markers move through the tract faster than particulate markers. Biological relevance of rate constants...
TABLE 1. MEAN RATES OF DISAPPEARANCE OF INTRINSIC (14C) AND EXTRINSIC (Yb, Cr) MARKERS FROM CONTINUOUS CULTURE FLASKS OF CONTROLLED FEED AND LIQUID INPUT

<table>
<thead>
<tr>
<th>Marker</th>
<th>Radiation emission</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Beta (h⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 14C NDF</td>
<td>-.042b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon-14 NDF-treated 169Yb</td>
<td>-.035c</td>
<td>-.039b;</td>
<td></td>
</tr>
<tr>
<td>Carbon-14 NDF-treated 51Cr</td>
<td>-.029c</td>
<td>-.025d</td>
<td></td>
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</tbody>
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^From Smith et al. (1987). Between-flask SE for comparison within a column = .0020. Within flask SE for comparison within a row = .0010.

Means with different superscripts differ (P < .05).

and compartments are still in dispute (Grovum and Williams, 1973; Dhanoa et al., 1985; France et al., 1985).

Use of rare-earth markers has been popular because of ease of analysis and the belief that these elements fulfill the requirements of an ideal marker (Crooker et al., 1982). Method of marker application, substrate applied and physical and chemical environment in the gut are factors that influence suitability of a marker for estimating passage of individual feedstuffs. Larger differences attributable to nutritional or physiological treatments (Hartnell and Satter, 1979) make comparisons of markers in animals impractical. Smith et al. (1987) compared the fractional turnover rates of intrinsically 14C-labeled NDF, Yb-treated NDF and Cr-mordanted NDF in a continuous culture system. Fractional rates (h⁻¹) of 14C NDF, Yb- and Cr-mordanted NDF are shown in Table 1. Treating 14C NDF (internal control) with Cr or Yb reduced disappearance rates. Smith et al. (1987) expressed caution regarding interpretation of kinetic data obtained with different markers because of the differences in rates of marker disappearance and interactions of markers, pH, fluid infusion rate and feed rates.

Teeter et al. (1984) showed large differences in the Yb binding capacity of various feedstuffs in vitro. Formation of complexes between Yb and soluble organic compounds affected marker transfer from labeled feeds. Feedstuffs saturated with Yb reduced in vitro and in situ digestibility of DM (Teeter et al., 1984). Erdman and Smith (1985) measured the relative binding of Yb among different sieve fractions of NDF from corn silage, orchard grass silages or alfalfa hay and alfalfa haylage. Recovery of Yb was greater from alfalfa than from corn silage or orchard grass. Concentration of Yb was greater in small particles than in large particles. They concluded that Yb should not be used as a marker to measure particle size reduction because differential passage of large and small particles and different concentration of marker by size could bias passage rate estimates.

**In Vivo Research with 14C Plant Material**

Several qualitative trials were conducted with 14C-labeled alfalfa (Yadava et al., 1964)
that demonstrated that a part of alfalfa is metabolized within minutes to VFA, another part (of hot-water insoluble alfalfa) is metabolized at a relatively rapid rate and soluble alfalfa is metabolized about four times faster than the insoluble residue. Wallnfer et al. (1966) observed that less than 10% of $^{14}$C cotton cellulose was fermented during 4 h in vitro incubation.

Using $^{14}$C NDF, Smith et al. (1967) reported that no significant breakdown of particles occurred past the duodenum. They observed that the SA of large particles that remained in the rumen decreased faster than small particles. However, they observed also that a dose with a smaller mean size decreased in SA faster than a dose of larger mean size. Ellis et al. (1984) demonstrated with a marked meal that rate of entry into the small particle pool was more rapid than turnover of the small particle pool resulting from passage, which was interpreted as evidence that the rate of particle reduction does not limit the rate of passage. In view of the scientifically substantiated concept that meals of small particles pass faster than larger particles (Blaxter et al., 1956; Meyer et al., 1959; Foot, 1964; Smith, 1968) and the greater affinity of marker to small particles than to large, the impact of rate of particle reduction on rate of passage is unresolved.

Smith et al. (1983) reported on the use of $^{14}$C NDF to study the rate of particle breakdown as influenced by initial particle size and degree of lignification. The materials were wheat plant, soybean plant and cotton stalk. Wheat plant contained 2.6% lignin and cotton stalk contained 6.3% lignin. Wheat plant ranged in ln mean particle size from 6.63 to 5.31 μm. The mean particle size of soybean plant and cotton stalk was similar to the finer particle size dose of wheat plant. An asymptotic model described NDF breakdown of particles with a ln mean size of 5.3 μm escaping the sheep rumen with essentially no reduction in size. The breakdown in mean particle size with time for a meal of $^{14}$C wheat plant NDF with an original ln mean size of 6.63 to 5.75 and the basal orchard grass pellet diet of 5.78 fed to a sheep support the asymptotic concept of fiber particle breakdown. Lignification was not a significant factor in particle breakdown because of the small size of particles in this study.

Disappearance of several chemically defined components of $^{14}$C-labeled alfalfa from the rumen of one Jersey cow was measured by Alexander et al. (1969). Structural components of prebud alfalfa disappeared from the rumen more rapidly than those components in bloom or seed stage-maturity alfalfa. The reported 30% to 50% of alfalfa cellulose disappearance from the rumen within 3 h of introduction into the rumen is more likely due to an error than it is a true representation of biology based on the slower later rates of disappearance (0.039 to 0.044 h⁻¹). The methodology used for measuring $^{14}$C activity may have caused the apparent rapid initial disappearance. Suspension techniques for counting $^{14}$C in insoluble materials usually are not quantitative because of self-absorption. Rapid combustion methods for $^{14}$C analysis are available (Smith, 1969).

**Future Research**

Use of intrinsically $^{14}$C-labeled plant material has application in heretofore unexplored areas of ruminant nutrition research. Incorporation of isotopes into plant material under field conditions expands the types and quantities of material available. Data are virtually nonexistent for simultaneously measured particle breakdown, digestion and passage rates in large animals fed practical diets. $^{14}$C-labeled plant material should be useful in separating the effects of chewing and rumination from other physiological phenomena in the study of rumen function. Critical experiments using $^{14}$C-labeled fiber should provide definitive information on lag time and microbial attachment as it relates to forage utilization and quality. Experimentation should be possible on associative effects that are not possible with other techniques. $^{14}$C-labeled feedstuffs offer a method to delve into the mechanisms and rates of production of methane and other metabolites.

In conclusion, use of $^{14}$C-labeled feedstuffs is quantitative, applicable to dynamic functions and should be useful in advancing knowledge about digestive physiology of ruminants.

**Literature Cited**


