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Short communication: Comparison of 3 solid digesta passage markers in dairy cows

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ABSTRACT

This study investigated the usefulness of acid-detergent fiber-bound ^{15}N [acid detergent insoluble (ADI)-^{15}N] as a solid digesta passage marker in dairy cows compared with chromium (Cr) and ytterbium (Yb) (as labeled fiber or forage, respectively). Intrinsically (ADI-^{15}N) or extrinsically (Cr, Yb) labeled alfalfa hay was pulse-dosed intraruminally to 7 lactating dairy cows. Following marker administration, spot fecal samples were collected for up to 72 h for marker analyses. Urine and milk samples were also collected and analyzed for Yb and Cr. Fecal marker excretion data were processed using 2-compartment mathematical age-dependent/age-independent ($G_n \rightarrow G_1$) models. The rate of passage of the marker in the first, age-dependent compartment tended to be slower for Yb compared with Cr and ADI-^{15}N, which resulted in a trend for longer mean retention time (MRT) in this compartment when Yb was used as a marker (19.0 h) compared with Cr and ADI-^{15}N (14.5 and 13.9 h, respectively). The rate constant of marker disappearance for the second or age-independent compartment tended to be greater for Yb compared with Cr and ADI-^{15}N, which led to a shorter MRT of Yb in this compartment (15.6) versus ADI-^{15}N (32.1) and Cr (24.8 h). The cumulative MRT was greater for ADI-^{15}N versus Cr and Yb (46.0, 39.3, and 34.4 h, respectively). Total MRT of marker tended to be greater for ADI-^{15}N than for Yb (46.6 vs. 36.6 h, respectively). Urine and milk analyses data suggested no measurable losses of Yb along the digestive tract, but about 0.79% of Cr dosed intraruminally was secreted or excreted in milk and urine in the 48-h period following marker administration. Collectively, this study confirmed previous observations that ADI-^{15}N can be used reliably as a solid digesta passage marker for ruminants, producing pre-duodenal and total-tract retention times similar to that of Cr-labeled fiber. Retention time in the age-independent compartment was underestimated when Yb was used as a marker, emphasizing the need to process forages to isolate fiber before labeling with Yb.
Key words: passage rate marker, ytterbium, chromium, nitrogen-15, dairy cow

Short Communication

Passage rate is a critical component of the digestion process in all farm species but is of particular importance in ruminants because of the extensive fermentation process occurring in the reticulo-rumen. Without reliable estimates of nutrient passage rates and retention time in the reticulo-rumen and the total digestive tract, accurate prediction of diet digestibility and its digestible energy and metabolizable protein value is not possible (NRC, 2001). Because of its importance, numerous experiments have been conducted and techniques and prediction models developed to study digesta passage rate (Shellenberger and Kesler, 1961; Ellis et al., 1994; Seo et al., 2006, 2009; Krizsan et al., 2010).

Several dietary (and animal) factors can influence passage rate in dairy cattle, but the most important one is DMI (Shellenberger and Kesler, 1961; Colucci et al., 1982; NRC, 2001). The size of the digestive tract can also be a factor [an example was provided by Aikman et al. (2008) for Holstein vs. Jersey cows]. Technique, mathematical model, and choice of digesta flow marker can also greatly affect passage rate estimates (Ellis et al., 1994; Hristov, 2005; Pellikaan et al., 2013). Extrinsic markers, for example, are likely to behave differently and yield shorter mean retention times (**MRT**) than intrinsic markers (Faichney et al., 1989). We proposed and tested an intrinsic digesta flow marker, acid-detergent insoluble ^{15}N (**ADI-^{15}N**), which gave reasonable estimates of MRT in dairy cows (Huhtanen and Hristov, 2001), comparable to passage rates derived using Cr-labeled grass silage (Ahvenjärvi et al., 2004). The objective of the current study was to further evaluate ADI-^{15}N (from alfalfa hay) as a solid digesta passage marker compared with common extrinsic markers such as Cr and Yb (as Cr-mordanted and Yb-labeled alfalfa hay). Our hypothesis was that MRT of digesta derived from fecal excretion of intraruminally

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dosed ADI-^{15}N would be comparable to those derived from extrinsic markers.

Animals involved in this experiment were cared for according to the guidelines of and the experimental procedures were approved by The Pennsylvania State University Animal Care and Use Committee. The study was part of a larger experiment (Lee et al., 2011) utilizing 8 ruminally cannulated multiparous Holstein cows (mean ± SD: parity, 2.8 ± 0.7 lactations; DIM, 102 ± 28.4 d; DMI, 26.4 ± 0.27 kg/d; milk yield, 43 ± 5.3 kg/d; and BW, 682 ± 47.7 kg). The design of the experiment was a replicated 4 × 4 Latin square, with 2 concurrent squares and four 21-d periods. Seven of the 8 cows on the main trial were used in the current experiment (1 cow was removed for health-related reasons that resulted in extremely low DMI). Treatments were as reported in Lee et al. (2011) and composition of the diets was similar to that reported in Lee et al. (2012). Briefly, the main trial had the following treatments: 15.6% CP diet (MP balance: −24 g/d), 14.0% CP diet (MP balance: −283 g/d), 14.0% CP diet supplemented with 100 g/cow per day of rumen-protected Lys, and 14.0% CP diet supplemented with 100 g/cow per day of rumen-protected Lys plus 24 g/cow per day of rumen-protected Met. The diets contained (% of DM): corn silage (31.7 to 32.7), alfalfa haylage (14.8 to 15.5), grass hay (5.0 to 5.2), ground corn grain (14.4 to 18.1), bakery by-product meal (8.4 to 8.8), whole, heated soybeans (7.5 to 8.4), solvent-extracted soybean meal (1.7 to 5.3), cottonseed hulls, molasses, a urea source, mineral-vitamin premix, and rumen-protected AA. The current experiment was conducted during the last 4 d of the last experimental period of the main experiment. For this part of the experiment, cows were moved and adapted to metabolic stalls. Urine was collected through urinary catheters (Hristov et al., 2010) and fecal samples were collected by stimulating defecation or from the rectum. Three solid digesta flow markers were studied: ADI-^{15}N from ^{15}N-labeled alfalfa hay and Yb- and Cr-labeled alfalfa hay. Nitrogen-15 labeled alfalfa was produced by fertilizing plants grown in a greenhouse with 10 atom % excess ($^{15}NH_4)_2SO_4$ (Isotec Inc., Miamisburg, OH) as described by Melgar (2012) following the procedure of Hristov et al. (2001). Ytterbium- and Cr-labeled forage was prepared from one batch of alfalfa hay ground in a Wiley mill (A. H. Thomas Co., Philadelphia, PA) through a 4-mm sieve. The hay was sieved through a 1.18-mm sieve, and the particles retained on the sieve were used in the labeling process. The Yb-labeled forage was prepared as described by Hristov and Broderick (1996). Briefly, alfalfa hay particles were incubated for 48 h in a neutral pH $YbCl_3 \cdot 6H_2O$ solution at 25°C, washed thoroughly with tap water, soaked for 1 h in an acetic acid solution, and then washed again with tap water. Chromium-labeled fiber was prepared according to Udén et al. (1980), except that, as indicated above, the hay was first sieved through a 1.18-mm sieve and the particles retained on the sieve were processed to isolate fiber. The ADI-^{15}N labeled hay was from a different batch than that used in the Cr- and Yb-labeling process. Thus, results for ADI-^{15}N versus Cr- and Yb-labeled forage have to be interpreted with caution.

Labeled forages were dried for 48 h at 65°C in a forced-air oven and aliquots were ground in a Wiley mill through a 1-mm sieve for marker and chemical analyses. Nitrogen concentration and δ^{15}N of alfalfa hay-ADF were (DM basis) 0.78% and 5,807‰, respectively; Cr concentration was 7.7%; and Yb concentration was 1.7%. A single pulse dose of 150 g of air-dry alfalfa hay or fiber (in the case of Cr) labeled with one of the above markers was mixed with about 10 kg of whole ruminal contents from each of the 7 cows on trial, returned to the rumen through the rumen cannula, and mixed with the ruminal contents. Following marker administration, fecal samples were collected at 0 h (background), 6, 8, 10, 12, 16, 24, 30, 42, 48, and 72 h postdose. Total urine collection was performed on d 1 (24 h) and 2 (48 h) following marker administration. Aliquots were analyzed for Cr and Yb by atomic absorption spectroscopy (Isaac and Johnson, 1985; University of Missouri-Columbia, Agricultural Experiment Station Chemical Laboratory, Columbia). Milk samples from d 1 and 2 following marker administration were also analyzed for Cr and Yb. The diets fed in the trial did not contain supplemental Cr and it was assumed that all Cr in urine and milk was from the Cr-labeled forage, which may have slightly overestimated Cr secretion or excretion. Urine and milk were not analyzed for ^{15}N. Cows in the main trial received ^{15}N-Lys (Lee et al., 2011) and, although this could not have affected fecal ADI-^{15}N data, it was judged that ^{15}N from ^{15}N-Lys could have been released in milk and urine, thus confounding the ^{15}N secretion or excretion data.

Fecal ADI-^{15}N, Cr, and Yb excretion data were fitted using 2-compartment mathematical age-dependent/age-independent ($G_n \rightarrow G_1$) models (Pond et al., 1988) as described in Huhtanen and Hristov (2001) and Hristov et al. (2003). Parameters were estimated using the nonlinear, least squares iterative process of SAS (SAS Institute Inc., Cary, NC). Criteria for best fit were (in order of priority) residual sum of squares, residuals distribution around the zero line, 95% confidence interval for the estimates, and R^2 values. The G_4G_1 model provided the best fit, and data based on this model were used in the statistical analysis and are presented in Table 1. Passage rate and MRT data were analyzed using the GLM procedure of SAS, with cow, diet, and marker as class variables and diet, marker, and diet ×