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 ¹⁵N [acid detergent insoluble (ADI)-¹⁵N] as a solid digesta passage marker in dairy cows compared with chromium (Cr) and ytterbium (Yb) (as labeled fiber or forage, respectively). Intrinsically (ADI-¹⁵N) 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-¹⁵N, 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-¹⁵N (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-¹⁵N, which led to a shorter MRT of Yb in this compartment (15.6) versus ADI-¹⁵N (32.1) and Cr (24.8 h). The cumulative MRT was greater for ADI-¹⁵N versus Cr and Yb (46.0, 39.3, and 34.4 h, respectively). Total MRT of marker tended to be greater for ADI-¹⁵N 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-¹⁵N 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 ¹⁵N (**ADI-¹⁵N**), 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-¹⁵N (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

Received June 12, 2013.

Accepted December 5, 2013.

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dosed ADI-¹⁵N 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 $\pm$ 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-¹⁵N from ¹⁵N-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 (¹⁵NH₄)₂SO₄ (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 YbCl₃·6H₂O 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-¹⁵N labeled hay was from a different batch than that used in the Cr- and Yb-labeling process. Thus, results for ADI-¹⁵N 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 $\delta^{15}\text{N}$ 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 ¹⁵N. Cows in the main trial received ¹⁵N-Lys (Lee et al., 2011) and, although this could not have affected fecal ADI-¹⁵N data, it was judged that ¹⁵N from ¹⁵N-Lys could have been released in milk and urine, thus confounding the ¹⁵N secretion or excretion data.

Fecal ADI-¹⁵N, 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 R² values. The G₄G₁ 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 $\times$

marker in the model. We detected no diet $\times$ marker interaction for any of the variables ($P = 0.12$ to 0.87) and therefore the diet interaction term was removed from the final model. Data were checked for normality using the UNIVARIATE procedure of SAS. When the W statistic of the Shapiro-Wilk test was <0.90 (τ , time delay; **CMRT**, cumulative MRT; and **TMRT**, total MRT), log-transformed data were analyzed statistically. Statistical differences were declared at $P \leq 0.05$. Differences between treatments at $0.05 \leq P \leq 0.11$ were considered as a trend toward significance. Data are presented as LSM. When the main effect of marker was significant, means were separated by pairwise t -test (pdiff option of PROC GLM).

Marker excretions in fecal matter followed the typical bell-shaped curve (Figure 1) observed in previous studies, in which digesta kinetics were estimated using similar approaches (Huhtanen and Hristov, 2001; Ahvenjärvi et al., 2004; Pellikaan et al., 2013). Peak marker concentrations were reached at about 24 to 30 h for all markers, which was again similar to data reported by Huhtanen and Hristov (2001) and Ahvenjärvi et al. (2004). Marker rate of passage in the first, age-dependent compartment tended to be lower ($P = 0.11$)

for Yb versus Cr and ADI-¹⁵N (Table 1). This resulted in a trend for longer ($P = 0.07$) MRT of Yb in the age-dependent compartment, compared with Cr and ADI-¹⁵N. The rate constant for marker disappearance from the slow or age-independent compartment of digesta tended to be greater ($P = 0.06$) for Yb compared with Cr and ADI-¹⁵N. This led to a shorter ($P = 0.01$) MRT of Yb versus ADI-¹⁵N ($P = 0.05$, when compared with Cr) in this compartment. The cumulative MRT was longer ($P = 0.03$) for ADI-¹⁵N compared with Yb (a trend, $P = 0.08$, when compared with Cr). The total MRT in the digestive tract tended to be longer ($P = 0.10$) for ADI-¹⁵N compared with Yb.

Two of the markers tested in this study, Cr (as Cr-mordanted fiber) and ADI-¹⁵N, behaved similarly and resulted in similar compartmental or total MRT estimates. It is worth noticing that MRT in the age-independent compartment (described as pre-duodenal digestion sites by Pond et al., 1988) tended to be longer when ADI-¹⁵N was used as a marker versus Cr. This could be an artifact of the labeling procedure (i.e., NDF in Cr-mordanted fiber vs. acid detergent insoluble-N in ADI-¹⁵N), indicating longer MRT for ADF than NDF (see data and discussion in Huhtanen and Hristov, 2001).

Table 1. Marker passage kinetics in lactating dairy cows estimated from fecal samples

Parameter ¹	Marker ^{2,3}			SEM ⁴	P-value ^{5,6}
	Cr	Yb	ADI- ¹⁵ N		
λ , h ⁻¹	0.294	0.223	0.301	0.0262	0.11 ⁷
k , h ⁻¹	0.046	0.068	0.036	0.0079	0.06 ⁸
τ , h	2.2 ^a	2.6 ^a	0.6 ^b	0.71	0.01
CMRT ₁ , h	14.5	19.0	13.9	1.43	0.07 ⁹
CMRT ₂ , h	24.8 ^a	15.6 ^b	32.1 ^a	2.87	0.01 ¹⁰
CMRT, h	39.3 ^{ab}	34.4 ^b	46.0 ^a	1.97	0.03 ¹¹
TMRT, h	41.5	36.6	46.6	2.31	0.10 ¹²

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹ λ = rate constant for the first, age-dependent compartment; k = rate constant for the second, age-independent compartment; τ = time delay; CMRT₁ = mean retention time in the first, age-dependent compartment; CMRT₂ = mean retention time in the second, age-independent compartment; CMRT = cumulative mean retention time, CMRT₁ + CMRT₂; TMRT = total mean retention time, CMRT + τ .

²Markers: Cr = Cr-labeled fiber from alfalfa hay, Yb = Yb-labeled alfalfa hay, and ADI-¹⁵N = alfalfa hay intrinsically labeled with ¹⁵N.

³Data were processed for outlier identification based on an absolute studentized residual value >3 (PROC REG of SAS; SAS Institute Inc., Cary, NC). Two cow/observations for k were identified as outliers and were removed (together with the CMRT and TMRT estimates) from the statistical analysis.

⁴Largest SEM shown. Means are LSM; $n = 18$ to 21 , n represents the number of observations used in the statistical analysis.

⁵P-value for the main effect of marker.

⁶ τ , CMRT, and TMRT data were not normally distributed ($W < 0.90$; Shapiro-Wilk test, the UNIVARIATE procedure of SAS; SAS Institute Inc.) and the reported P-values are for log-transformed data.

⁷Yb vs. ADI-¹⁵N, $P = 0.06$; Yb vs. Cr, $P = 0.09$.

⁸Yb vs. ADI-¹⁵N, $P = 0.02$; Yb vs. Cr, $P = 0.08$.

⁹Yb vs. ADI-¹⁵N, $P = 0.03$; Yb vs. Cr, $P = 0.05$.

¹⁰Cr vs. ADI-¹⁵N, $P = 0.10$.

¹¹Cr vs. ADI-¹⁵N, $P = 0.08$.

¹²Yb vs. ADI-¹⁵N, $P = 0.04$.

Figure 1. Marker excretion in feces (mean $\pm$ SE): Cr = Cr-labeled fiber from alfalfa hay, Yb = Yb-labeled alfalfa hay (panel A), and ADI- ^{15}N = alfalfa hay intrinsically labeled with ^{15}N (panel B).

Recently, Pellikaan et al. (2013) reported contrastingly different compartmental and TMRT for Cr-NDF and ^{13}C -NDF (plants were grown in $^{13}\text{CO}_2$ -rich atmosphere). Those authors observed much greater fractional passage rates for Cr-NDF versus ^{13}C -NDF, which resulted in more than 2-fold greater (although not statistically significant) retention times for the latter (up to 100 h with grass silage). Warner et al. (2013) compared digesta kinetics data for labeled ryegrass silage obtained using Cr-NDF, ^{13}C -ADF, and ^{15}N -ADF (i.e., ADI- ^{15}N) as markers and reported highest fractional ruminal passage rate and lowest TMRT with Cr-NDF ($k = 0.047 \text{ h}^{-1}$ and 38.0 h, respectively, where k = rate constant for the second, age-independent compartment) and higher k and shorter TMRT for ^{15}N -ADF (0.034 h^{-1} and 46.4 h) than ^{13}C -ADF (0.023 h^{-1} and 61.1 h). In contrast to Cr, Yb (as Yb-labeled forage particles) produced longer MRT than both Cr and ADI- ^{15}N for digesta in the fast, age-dependent compartment and considerably shorter MRT in the slower, age-independent compartment in the current study. The ratio of cumulative mean re-

tention time in the first, age-dependent compartment (CMRT_1) to CMRT tended to be greater ($P = 0.06$; $\text{SEM} = 0.056$) for Yb (0.54) than for Cr and ADI- ^{15}N (0.38 and 0.33, respectively). Similar observations for Yb were reported by Ahvenjärvi et al. (2004). These authors estimated $\text{CMRT}_1:\text{CMRT}$ ratios of 0.58, 0.40, and 0.34 (Yb, Cr, and ADI- ^{15}N , respectively) for labeled timothy grass silage. The most likely explanation for this discrepancy between Yb and Cr or ADI- ^{15}N is dissociation of Yb from the particulate matter in the second, age-independent compartment of ruminal digesta. The fact that Yb does not associate exclusively with the particulate matter in digesta has been reported by Siddons et al. (1985) and Combs et al. (1992). In the current study, forage samples were processed as suggested by Owens and Hanson (1992) to address the dissociation problem (see Hristov and Broderick, 1996), but this was probably insufficient to prevent Yb migration and produce reliable estimates of MRT in the age-independent compartment of digesta with this marker. The slower disappearance rate and longer retention time of Yb compared with Cr and ADI- ^{15}N in the first, age-dependent compartment is difficult to explain. Ytterbium has been reported to primarily associate with smaller digesta particles (Siddons et al., 1985) and bind to microbial mass in the rumen (Combs et al., 1992). If Yb is binding to microbial mass and microbes are preferably retained in the age-dependent compartment of rumen digesta, this may explain the longer residence time of Yb in this compartment, which, according to Pond et al. (1988), is the first compartment encountered by feed residues in their 2-compartmental model of digesta kinetics. We are not aware of studies that specifically investigated the relationship between digesta and microbial compartments (Chen and McAllister, 1997) in the rumen. In a recent study with Yb-labeled fiber from corn and grass silages, Krämer et al. (2013) reported $\text{CMRT}_1:\text{CMRT}$ ratios of 0.25 to 0.38. Thus, it appears that Yb may be useful as a particulate marker if applied to isolated fiber, not to the entire forage mass, as in the current trial.

Average concentrations of Yb and Cr in urine and milk collected over a 48-h period following marker administration were $<0.002 \text{ mg/L}$ (both urine and milk) for Yb and $0.14 \pm 0.004 \text{ mg/L}$ in urine (0.12 and 0.18, minimum and maximum) and $1.08 \pm 0.251 \text{ mg/L}$ in milk (0.16 and 3.18, minimum and maximum) for Cr. Similar to our data, Siddons et al. (1985) infused Cr-EDTA and Yb-acetate intraruminally and reported about 3% urinary excretion of the infused Cr but no urinary losses of Yb in wethers. Based on urine volume and milk yield data from the main trial (Lee et al., 2011), Cr secretion or excretion in milk and urine in the 48-h period following intraruminal administration

of Cr-labeled feed was estimated at 0.79% (± 0.017 ; minimum = 0.73 and maximum = 0.86) of Cr dose. Collectively, these data indicate no measurable losses of Yb along the digestive tract of ruminants but some absorption and excretion or secretion of Cr.

In conclusion, this study confirmed previous observations that ADI- ^{15}N can be reliably used as a solid digesta passage marker for ruminants, producing pre-duodenal and total-tract retention times similar to Cr-labeled fiber. Retention time of marker in the pre-duodenal sites was underestimated when Yb was used as a marker, emphasizing the need to process forages to isolate fiber before labeling with Yb.

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