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# Passage of stable isotope-labeled grass silage fiber and fiber-bound protein through the gastrointestinal tract of dairy cows

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## ABSTRACT

Fractional passage rates are required to predict nutrient absorption in ruminants but data on nutrient-specific passage kinetics are largely lacking. With the use of the stable isotope ratio (δ) as an internal marker, we assessed passage kinetics of fiber and fiber-bound nitrogen (N) of intrinsically labeled grass silage from fecal and omasal excretion patterns of $\delta^{13}C$ and $\delta^{15}N$. In a 6 × 6 Latin square, lactating dairy cows received grass silages [455 g/kg of total diet dry matter (DM) ] in a 2 × 3 factorial arrangement from ryegrass swards fertilized at low (45 kg of N/ha) or high (90 kg of N/ha) levels of N and harvested at 3 maturity stages. Feed intake (16.7 ± 0.48 kg of DM/d; mean ± standard error of the mean) and milk yield (26.7 ± 0.92 kg/d) increased at the high level of N fertilization and at decreasing maturity. Nutrient digestibility decreased with increasing plant maturity, particularly at the high level of N fertilization, essentially reflecting dietary treatment effects on the nutritional composition of the grass silage. Fractional rumen passage rates ($K_1$) were highest and total mean retention time in the gastrointestinal tract (TMRT) was lowest when based on the external marker chromium mordanted fiber (Cr-NDF; 0.047/h and 38.0 h, respectively). Fecal $\delta^{13}C$ in the acid detergent fiber fraction ($^{13}$CADF) provided the lowest $K_1$ (0.023/h) and the highest TMRT (61.1 h) and highest peak concentration time (PCT; 24.3 h) among markers. In comparison, fecal fiber-bound N ($^{15}$NADF) had a considerably higher $K_1$ (0.032/h) and lower TMRT (46.4 h) than $^{13}$CADF. Total N (measured with $^{15}$NDM) had a comparable $K_1$ (0.034/h) to that of $^{15}$NADF but provided the highest fractional passage rates from the proximal colon-cecum ($K_2$; 0.37/h) and lowest PCT (17.4 h) among markers. A literature review indicated unclear effects of grass silage maturity on $K_1$ and unknown effects of N fertilization on $K_1$. Our study indicated no effect of advancing maturity on fecal $K_1$ and a trend for $K_1$ to increase with the high level of N fertilization. Parameter $K_2$ increased, whereas PCT and TMRT generally decreased with the high level of N fertilization. Omasal digesta sampling largely confirmed results based on fecal sampling. Results indicate that the use of $\delta^{13}C$ and $\delta^{15}N$ can describe fiber-specific passage kinetics of forage.

**Key words:** passage marker, $^{13}$C, $^{15}$N, perennial ryegrass

## INTRODUCTION

Quantitative knowledge on fractional rumen passage rates ($\boldsymbol{K_1}$) is required to determine ruminal VFA and microbial protein yields and to determine site and extent of degradation of ingested feed (Dijkstra et al., 2007). Therefore, $K_1$ is an essential parameter in several feed evaluation systems and mechanistic rumen models that predict absorption of nutrients in the small intestine as well as excretion of nutrients with respect to environmental and metabolic load (Kebreab et al., 2009). Fractional rumen passage rates are conventionally determined by marker techniques involving external or inert internal markers. External markers are not inherent to the feed and have, therefore, been criticized for not fully representing the passage behavior of the diet (Smith, 1989; Tamminga et al., 1989). In particular, external markers do not provide passage rates specific to feed chemical components. In principle, internal markers are preferred because they are inherent to the feed ingested by the animal; however, inert markers require time- and labor-intensive rumen evacuations to determine the rumen pool size. By labeling specific feed fractions with stable isotopes, fractional passage rates can be determined from stable isotopes as internal markers determined in feces (Svejcar et al., 1993; Südekum et al., 1995). Based on this principle, Huhtanen and Hristov (2001) determined fractional passage rates of fiber-bound nitrogen (N) from $^{15}$N-labeled alfalfa (Huhtanen and Hristov, 2001). A subsequent study has shown that the carbon isotope ratio ($^{13}$C:$^{12}$C); that is, $\delta^{13}C$, can be used to assess fiber passage kinetics of

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a $\delta^{13}$C-labeled grass silage and that the $\delta^{13}$C did not change with microbial rumen fermentation under in vitro conditions (Pellikaan et al., 2013). Disappearance of the labeled fiber fractions in the gastrointestinal tract did not, therefore, affect respective passage kinetics estimated from the $\delta^{13}$C in the apparent undigested fecal fraction. This has been validated in vivo by quantifying the carbon isotopes from feed and fecal output [L. M. M. Ferreira, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal, unpublished data] resulting in an equal $\delta^{13}$C between the isotope pool flowing into the rumen and that in feces.

In the study of Pellikaan et al. (2013), changing the diet from a low to high digestible grass silage had no effect on passage kinetics, based on 2 cows. Previous studies based on external or inert markers suggest that grass silage quality affects passage kinetics, although effects were not clear and results from literature range from slightly negative or no effects (Mambrini and Peyraud, 1994; Lamb et al., 2002; Lund et al., 2006; Kuoppala et al., 2009; Bayat et al., 2010, 2011) to clearly positive effects (Gasa et al., 1991; Bosch et al., 1992a; Rinne et al., 1997a, 2002) of advancing plant maturity on $K_1$. Effects of N fertilization were not specifically investigated with regard to passage kinetics, but results from in situ degradation studies indicate clear effects of N fertilization level on fractional degradation rates and the potentially rumen digestible fraction (van Vuuren et al., 1991; Valk et al., 1996; Peyraud et al., 1997). As rumen degradation of feed particles might reduce the probability of their rumen escape by increasing particle buoyancy (Sutherland, 1988), fractional rumen degradation might be indirectly related to fractional rumen passage.

Previously, we used intrinsic isotope labeling of corn silages varying in nutritional quality to assess component-specific fractional passage rates (Warner et al., 2013a). The aim of the present study was to assess feed component-specific passage kinetics of grass silage from early through late maturity from ryegrass swards fertilized at 2 different N levels. Ryegrass plants were uniformly labeled with $\delta^{13}$C and $\delta^{15}$N under greenhouse conditions, and passage kinetics of fiber and fiber-bound N were determined based on the respective δ values, determined in fecal and omasal digesta samples, and compared with the external marker Cr-NDF.

## MATERIALS AND METHODS

### Animals and Diet

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Wageningen University (Wageningen, the Netherlands) and carried out under the Dutch Law on Animal Experimentation. Six multiparous Holstein-Friesian dairy cows in their second to fourth lactation, fitted with permanent rumen cannulas (10 cm i.d., Type 1C, Bar Diamond Inc., Parma, ID), were individually housed in tiestalls. At the start of the experiment, cows were 63 ± 13 DIM (mean ± SD), averaged 549 ± 64 kg of BW, had an average daily feed intake of 17.2 ± 2.60 kg of DM, and produced 33.1 ± 5.76 kg of milk/d. Animals were fed a TMR consisting of 455 g/kg DM grass silage, 195 g/kg DM corn silage, and 350 g/kg DM compound feed (Table 1). The compound feed ingredients mainly originated from cool-season $C_3$ plants to keep the background level of $^{13}$C low and similar to that of the natural enrichment level of the grass silage mixed in the experimental diet. Grass silage was prepared from a perennial ryegrass (*Lolium perenne*) sward sown as a 70:30 mixture (Havera; Limagrain Nederland, Rilland, the Netherlands) of late-heading tetraploid cultivars ('Pomposo' and 'Alcander') and diploid cultivars ('Frisian 1 and 'Jalinas') on clay soil. Grass was fertilized at 2 N fertilization levels and harvested at 3 maturity stages (Table 1). Levels of N fertilization were either 45 kg of N/ha (**N45**) or 90 kg of N/ha (**N90**) per cut, applied as an N-P-K complex. Maturity stages were set to obtain a target DM yield in the range of 1,800 to 2,000 kg/ha (early), 3,200 to 3,400 kg/ha (mid), and 4,600 to 4,800 kg/ha (late). The phenological development stages for the respective maturity stages were stem elongation (early maturity), begin heading (mid maturity), and full heading (late maturity). The field received 50 kg of N/ha before the first regrowth. Fresh grass from the second (mid to late maturity) and third regrowth (early maturity) was harvested from August to September 2010, wilted for 12 h, cut to a theoretical chop length of 40 mm, and ensiled in bales without addition of inoculants.

Grass silage treatments were randomly distributed over 6 animals and 6 experimental periods according to a Latin square design with 2 × 3 factorial arrangement of treatments. Each experimental period lasted 21 d starting with a 14-d adaptation period to the diet. From d 12 on, animals were fed 95% of the individual DMI measured during the adaptation period to minimize feed refusals during the measuring days. Animals received their daily rations in 2 equal meals at 0600 and 1700 h. The diet was prepared twice weekly and stored in a cooling unit at 8°C from April onwards. Feed samples were collected each time the diet was prepared. Feed residues were collected daily before the afternoon feeding. Feed samples and residues were pooled per animal over each experimental period. Animals were milked twice daily during feeding times and milk samples were collected from d 15 through 21. Feed