



## Passage kinetics of $^{13}\text{C}$ -labeled corn silage components through the gastrointestinal tract of dairy cows

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### ABSTRACT

Fractional passage rates form a fundamental element within modern feed evaluation systems for ruminants but knowledge on feed type and feed component specific passage rates are largely lacking. This study describes the use of carbon stable isotopes ( $^{13}\text{C}$ ) to assess component-specific passage kinetics of 6 intrinsically  $^{13}\text{C}$ -labeled corn silages varying in quality (2 cultivars  $\times$  3 maturity stages) in a  $6 \times 6$  Latin square design using 6 rumen-fistulated lactating dairy cows. An increase in maturity increased starch and decreased neutral detergent fiber and acid detergent fiber contents of corn silages. Passage kinetics were assessed for an external (chromium mordanted fiber; Cr-NDF) and an internal marker ( $^{13}\text{C}$  isotopes) collected in feces and omasal digesta. The best fit was obtained with a deterministic multicompartamental model compared with stochastic Gn and GnG1 models with increasing order of age dependency ( $n = 1$  to 5) for both sampling sites. The Cr-NDF marker yielded higher rumen fractional passage rates ( $K_1$ ) than did  $^{13}\text{C}$  in the dry matter ( $^{13}\text{CDM}$ ) in feces (0.042/h vs. 0.023/h). Omasal marker excretion patterns support the conclusions based on conventional fecal marker excretions. Component-specific passage was assessed for acid detergent fiber ( $^{13}\text{CADF}$ ) in feces and for starch ( $^{13}\text{CST}$ ) in omasal digesta. The fractional passage rate based on fecal  $^{13}\text{CDM}$  and  $^{13}\text{CADF}$  did not differ. Omasal  $^{13}\text{CST}$  provided higher  $K_1$  values (0.042/h) than omasal  $^{13}\text{CDM}$  (0.034/h) but lower values than omasal Cr-NDF (0.051/h). Fractional passage rates from the proximal colon-cecum ( $K_2$ ) based on fecal marker concentrations showed trends similar to  $K_1$ , with Cr-NDF providing a value (0.425/h) more than twice as high as that of  $^{13}\text{CDM}$  (0.179/h) and  $^{13}\text{CADF}$  (0.128/h). Total mean retention time in the gastrointestinal tract was approximately double for  $^{13}\text{CDM}$  (64.1 h) and  $^{13}\text{CADF}$  (77.6 h) compared with Cr-NDF (36.4 h). Corn silage quality did not affect

any of the estimated passage kinetic parameters. In situ fractional degradation rates did not differ among corn silages, except for a decreased fractional degradation rate of starch with advancing maturity. Results indicate that isotope labeling allows assessment of component-specific passage kinetics of carbohydrate fractions in corn silage.

**Key words:** rumen passage, marker, stable isotope, degradation

### INTRODUCTION

The competition between the processes of degradation and passage in the rumen has a major effect on digestibility of nutrients (Faichney, 1980; Allen and Mertens, 1988) and, therefore, on the formation of VFA and microbial protein, the cow's major sources of absorbed energy and protein, respectively. Hence, the protein supply to the animal largely depends on the balance between fractional degradation and fractional passage rates and is feed specific (Robinson et al., 1986). The fractional passage rate determines the time feed is retained in the gastrointestinal tract and is a major determining factor of the site and extent of degradation, as well as efficiency of microbial protein synthesis (Dijkstra et al., 2007).

Rumen degradation has been assessed for a wide range of corn silage qualities (De Boever et al., 2002) and rumen escape starch was not related to chemical composition or maturity. In contrast, Philippeau and Michalet-Doreau (1997) reported that starch degradation rates decrease with increasing corn maturity because of a concomitant increase in grain vitreousness, although this decrease with increased vitreousness may depend on the particle size of corn kernels (van Zwieten et al., 2008). Corn silage is commonly fed to dairy cows in intensive dairy systems, but quantitative information on the fractional passage rate of corn silage is limited. In the Dutch protein evaluation system for ruminants, a fixed fractional passage rate of protein and starch for forage is assumed independent of type of forage and forage quality, whereas the fractional passage rate constant for NDF is estimated from its fractional degrada-

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tion rate (van Duinkerken et al., 2011). Rumen passage of forages has been reported to depend on forage type (Lund et al., 2006) and to increase with increasing maturity of the grass silage (Rinne et al., 1997).

Fractional passage rates have been traditionally estimated using external markers collected in feces or in digesta from intestinally cannulated animals. External markers can describe the passage kinetics of a specific rumen particle pool, as shown by Bosch and Bruining (1995) for chromium-mordanted fiber (**Cr-NDF**), but do not provide component-specific fractional passage rates. Stable isotopes have been proposed as internal markers to study rumen passage rates in ruminants (Südekum et al., 1995; Huhtanen and Hristov, 2001; Sponheimer et al., 2003), and carbon isotopes ( $^{13}\text{C}$ : $^{12}\text{C}$  ratio) were recently shown to be suitable for determining component-specific fractional passage rates for grass silages (Pellikaan et al., 2013).

The aim of this study was to estimate feed component-specific fractional passage kinetics of 2 cultivars of corn silage, harvested at 3 maturity stages, through the gastrointestinal tract of dairy cows. Corn silages were intrinsically labeled with  $^{13}\text{C}$  by growing the corn plants under continuous elevated  $^{13}\text{CO}_2$  conditions. In addition to the external marker Cr-NDF,  $^{13}\text{C}$  isotopes were used as internal markers to specifically assess component-specific passage kinetics for starch and ADF based on the  $^{13}\text{C}$ : $^{12}\text{C}$  ratio in glucose in omasal digesta and ADF in feces, respectively.

## 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 tie stalls. At the start of the experiment, cows were  $53 \pm 11$  DIM (mean  $\pm$  SD), averaged  $571 \pm 66$  kg in BW, and produced  $39.1 \pm 4.9$  kg of milk/d. Animals were fed a TMR consisting of 625 g/kg of DM roughage (a 1:1 mixture of corn silage and grass silage on a DM basis) and 375 g/kg of DM compound feed (Table 1). The compound feed ingredients originated from cool-season  $\text{C}_3$  plants to keep the background level of  $^{13}\text{C}$  enrichment low and similar to that of the natural enrichment level of the grass silage mixed in the experimental diet. Corn silages were prepared from 2 corn cultivars, each harvested at 3 maturity stages (Table 1). Corn cul-

vars were Aastar (Limagrain Advanta BV, Rilland, the Netherlands) and Baleric (Syngenta Seed, Merelbeke, Belgium). Maturity stages were set to obtain target DM contents from 260 to 280 g/kg (early), 320 to 340 g/kg (mid), and 380 to 400 g/kg (late). Corn plants were sown on May 1, 2009, on sandy soil at Unifarm, Wageningen, the Netherlands, and harvested with a precision chop harvester from early September to early October 2009, and ensiled in sealed 30-kg bales.

Corn silage treatments were randomly distributed over 6 animals and 6 experimental periods according to a Latin square design with  $2 \times 3$  factorial arrangement of treatments. Each experimental period lasted 21 d starting with a 12-d adaptation period to the diet. From d 9 onward, animals were fed 95% of the individual DM intake measured during the preceding 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  $8^\circ\text{C}$  from April onward. Feed samples were collected each time the diet was prepared, and feed residues were collected daily before the morning 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 13 through 21. Feed intake and milk yield determined from d 13 through 21 of each experimental period were averaged per period for statistical analysis.

### *Marker Preparation*

Chromium mordanted fiber (Cr-NDF) and the stable isotope of carbon ( $^{13}\text{C}$ ) were used as external and internal passage markers, respectively. The Cr-NDF was prepared as described by Udén et al. (1980) from wheat straw, and was dried and ground to pass a 0.5-mm screen. The  $^{13}\text{C}$  markers were prepared as intrinsically labeled corn silage as described above for the field corn silage (2 corn cultivars  $\times$  3 maturity stages) under greenhouse conditions, as applied by Huhtanen and Hristov (2001). Corn seeds were sown in hermetically sealed and climate-controlled assimilation chambers, specifically designed to enable homogeneous atmospheric isotope labeling of herbage, resulting in uniformly labeled plants (Gorissen et al., 1996). Corn plants were continuously enriched under high levels of  $^{13}\text{CO}_2$  (released from  $^{13}\text{C}$ -bicarbonate) from plant emergence onward by IsoLife BV (Wageningen, the Netherlands). Growing corn plants were exposed to continuous moderate ventilation by a rotating fan to resemble wind-induced mechanical stress of field plants (Biddington, 1986). At harvest, the DM contents of the labeled corn plants were within the targeted range as

**Table 1.** Chemical composition of 2 corn silage cultivars (Aastar and Baleric, harvested at 3 maturity stages), grass silage, and compound feed

Item	Aastar			Baleric			Grass silage <sup>1</sup>	Compound feed <sup>2</sup>
	Early	Mid	Late	Early	Mid	Late		
Growing days <sup>3</sup>	124	139	152	125	140	154		
DM content (g/kg)	285	330	375	270	345	402	617	873
Chemical composition <sup>4</sup>								
OM	956	963	964	950	954	960	907	952
CP	87	81	78	83	78	76	207	253
Starch	286	375	426	295	368	400	—	216
NDF	410	357	341	407	376	361	485	248
ADF	229	197	188	234	208	198	254	158
DVE <sup>1</sup>	59	62	65	57	63	64	86	163
OEB <sup>1</sup>	−34	−45	−49	−36	−47	−50	56	8
OMD <sup>1</sup>	0.77	0.79	0.79	0.75	0.77	0.78	0.80	0.71
NE <sub>L</sub> <sup>1</sup> (MJ/kg of DM)	6.8	7.1	7.1	6.6	6.8	6.9	6.7	7.0

<sup>1</sup>Values determined by near-infrared spectroscopy (Blgg AgroXpertus, Wageningen, the Netherlands).

<sup>2</sup>Based on table values from Centraal Veevoeder Bureau (2007); ingredients (g/kg of DM): wheat (54.1), sunflower seeds (126.2), soybean hulls (26.5), palm kernel expeller (150.0), soybeans (100.0), sugar beet pulp (100.0), potato starch (200.0), MervoBest soybean meal (225.0; Pre-Mervo, Utrecht, the Netherlands), phosphoric acid limestone (7.6), salt (3.0), mineral premix (7.5).

<sup>3</sup>Interval in days from sowing to harvest date of corn silage.

<sup>4</sup>Chemical composition: NDF = ash-free amylase-treated NDF; ADF = ash-free ADF; DVE = intestinal digestible protein; OEB = rumen-degraded protein balance; OMD = OM digestibility coefficient. Values expressed as means (g/kg of DM unless specified otherwise) of 6 experimental periods (starch not determined for grass silage).

described above for the field corn plants. Kernels were gently broken using a mortar and pestle to simulate kernel bruising of harvested field corn plants, and stems and leaves were cut with a paper cutter into pieces of 1 cm. The collected corn plant portions were placed inside several bags of larger mashed grit gauze (pore size 212  $\mu$ m; PA-74, Sefar Nyltal, Heiden, Switzerland) and distributed over silage bales to be ensiled together with field corn plants. After an 8-wk ensiling period, the <sup>13</sup>C-labeled marker material was removed and stored at −20°C in sealed plastic bags.

### Marker Administration, Sampling, and Measurements

**Marker Administration.** On d 13, animals received a ruminal pulse dose of corresponding <sup>13</sup>C-labeled corn silage (30 g of DM) and Cr-NDF (100 g; 45.9 g of Cr/kg of Cr-NDF). Pulse dosing started at 0600 h with cow 1 and continued after an interval of 30 min for each consecutive animal. The mean degree of atom percentage <sup>13</sup>C (At%<sup>13</sup>C) as a proportion of total carbon in the DM of the labeled corn silage ranged between 8.68 and 12.36 At%<sup>13</sup>C for the different treatments (n = 6), whereas the natural level of enrichment of the unlabeled corn silage was 1.10 At%<sup>13</sup>C (SD = 0.002). Before being pulse dosed, the frozen and cut <sup>13</sup>C-labeled marker material was further cut to pieces of approximately 0.5 cm to resemble ingested bulk corn silage particles. From d 9 through 17, Co-EDTA (Udén et al., 1980) was continuously infused (0.5 L/h; 1.14 g of Co/d dissolved in 12 L of water) in the rumen after Faichney (1975)

to measure feed digestibility (from d 13 through 17), preceded by a primer dose of 1.5 times the daily dose.

**Fecal Sampling.** From d 13 onward, directly after administration of markers into the rumen, 22 spot samples of feces were collected after defecation. Fecal samples were collected in sampling blocks of 3 h each at average times  $t = 0, 3, 7, 11, 15, 19, 23, 29, 35, 41, 48, 54, 60, 66, 73, 79, 85, 91, 98, 104, 110, 116$  h after pulse dose administration. Feces were weighed and thoroughly homogenized by hand, and a representative sample of 300 to 400 g of fresh matter was stored at −20°C. An aliquot of 1% fresh feces was taken during each collection time and pooled over one experimental period to determine total-tract feed digestibility.

**Omasal Digesta Sampling.** At the same collection time as indicated above for feces, omasal digesta leaving the reticulorumen was collected by means of the omasal sampling technique as described by Huhtanen et al. (1997). The sampling device, introduced 24 h before pulse dosing, was designed based on modifications of Ahvenjärvi et al. (2000) and Sterk et al. (2012) to prevent blockage of the sampling device by coarse digesta. Blocking of the holes was prevented by injecting small volumes of pressurized carbon dioxide before sampling. The location of the sampling device in the omasal canal was confirmed each day before the morning feeding, as described by Brito et al. (2007). Omasal digesta samples (~750 mL) were collected and pH was measured. Collected omasal digesta was weighed, quantitatively poured into containers and stored at −20°C immediately after collection.

**Rumen Liquid Sampling.** During the first 24 h after administration of markers into the rumen, approximately 350 mL of rumen liquid was collected proportionally from a cranial, middle, and caudal directions after omasal sampling. Per treatment and cow, 6 rumen fluid samples were collected after average times  $t = 0, 3, 7, 11, 15, 19, 23$  h relative to moment of marker introduction. Subsamples of 0.75 mL each were taken for VFA and ammonia ( $\text{NH}_3$ ) analyses, and rumen pH was measured immediately using an electronic pH meter (pH electrode HI1230, Hanna Instruments BV, IJsselstein, the Netherlands). The VFA and  $\text{NH}_3$  samples were stored at  $-20^\circ\text{C}$  in 85% phosphoric acid (1:1, vol/vol) and 10% TCA (1:1, vol/vol), respectively. Excess fluid was immediately returned to the rumen.

### Rumen Degradability of Corn Silage

Rumen degradation of fresh corn silage was assessed per individual animal and per silage type treatment in situ, as described by Tas et al. (2006), over 2 consecutive series. The short-term incubations (0, 2, 4, 8, 12, 24, 48, 72 h; all-out procedure) of the first incubation series started at 60 h after administration of the passage rate markers to ensure similar conditions of degradation and passage rates. A previous isotope tracer study (D. Warner, unpublished data) showed that fecal baseline concentration of  $^{13}\text{C}$  after a pulse dose was reached at approximately 4 d after pulse dosing. Hence, the in situ incubations did not interfere with fractional passage rates. The prolonged incubation of 336 h was conducted on different rumen-cannulated animals fed a similar diet to the experimental diet after Tas et al. (2006). Effective rumen degradability (**ED**) was calculated as described by van Duinkerken et al. (2011).

### Chemical Analyses

Samples were freeze-dried and ground over a hammer-mill to pass a 1-mm screen (Peppink 100 AN, Olst, the Netherlands). Dry matter, ash, CP, starch, NDF, and ADF were analyzed as described by Abrahamse et al. (2008a,b). Concentrations of VFA were determined by gas chromatography with isocaproic acid as an internal standard using an EM-1000 column (30 m  $\times$  0.53 mm; Alltech, Deerfield, IL) and hydrogen as mobile phase. Concentrations of  $\text{NH}_3$  were determined calorimetrically using a spectrophotometer (Cary 50, Varian, Palo Alto, CA) based on the Berthelot reaction as described by Searle (1984) after deproteinizing the supernatant by addition of 10% TCA. Milk composition was analyzed by mid-infrared reflection spectroscopy (Milk Control Station VVB, Nunspeet, the Netherlands).

Fecal and omasal Co and Cr concentrations were determined using an atomic absorption spectrophotometer (AA240FS, Varian, Palo Alto, CA) after oxidation with wet destruction. Fecal and omasal excretion patterns of  $^{13}\text{C}$  were determined in the DM fraction ( $^{13}\text{CDM}$ ), ADF fraction ( $^{13}\text{CADF}$ ; feces only), and starch fraction ( $^{13}\text{CST}$ ; omasal digesta only). The ADF fraction was obtained by washing the dried and ground material with acid detergent in filter bags (type F57, porosity 25  $\mu\text{m}$ , Ankom Technology, Macedon, NY) but omitting the final combustion. The DM and ADF fractions were pulverized in a bullet mill (MM2000, Retsch, Haan, Germany) for 3 min at 85 Hz. The starch fraction was chemically isolated and  $^{13}\text{C}$  enrichment determined in the purified fraction by elemental analyses, as suggested by Wanek et al. (2001). Briefly, after extraction of soluble sugars with ethanol (40%, vol/vol), solubilization by autoclaving, and enzymatic hydrolyses with amyloglucosidase, the glucose extract was acidified by adding 3 N HCl to pH 2 and deionized by ion exchange (Amberlite MB-6113, Merck, Darmstadt, Germany) to separate amino acids, minerals, and acetic acid from glucose. Glucose was collected, washed 3 times with Millipore water to dispose of remaining acids, brought into tin cups, and evaporated. Amino acid analyses on random samples confirmed that collected residues did not contain amino acids. All fractions were analyzed for  $^{13}\text{C}$  enrichment by elemental analyses using an isotope ratio mass spectrometer (Finnigan MAT CN, Fisons Instruments, Milan, Italy). The relative  $^{13}\text{C}$  enrichment was expressed as the  $^{13}\text{C}:^{12}\text{C}$  ratio in the samples relative to the  $^{13}\text{C}:^{12}\text{C}$  ratio of the international Vienna Pee Dee Belemnite (VPDB) standard. After correction for natural  $^{13}\text{C}$  abundance, fecal and omasal excretion patterns of  $\text{At}\%^{13}\text{C}$  excess were established.

### Curve Fitting and Statistical Analyses

Fractional passage rates for the compartment with the longest retention time ( $\mathbf{K}_1$ ; assumed to be the reticulorumen) and for the compartment with the second longest retention time ( $\mathbf{K}_2$ ; assumed to be the proximal colon-cecum for fecal samples) were derived from excretion patterns of  $\text{At}\%^{13}\text{C}$  and Cr, fitted iteratively with nonlinear compartmental models (Table 2). Because of the inherently different profiles of fecal and omasal excretion curves, the following models were selected: 1-compartment models without (**G1** model) and with age dependency (**G2** model); 2-compartment models without (**G1G1** model) and with age dependency with increasing order of gamma distribution (**GnG1** models;  $n = 2$  to 5; Matis, 1972; Pond et al., 1988); and a multicompartmental model (**MC**; Dhanoa et al., 1985).



**Table 2.** Nonlinear compartmental models for fractional passage rate estimation

Model type <sup>1</sup>	Equation <sup>2</sup>
G1	$C_t = A \times e^{(-K_1 \times T)}$
G2	$C_t = A \times K_1 \times T \times e^{(-K_1 \times T)}$
G1G1	$C_t = A \times K_1 \times \left[ e^{(-K_1 \times T)} - e^{(-K_2 \times T)} \right] / (K_2 - K_1)$
GnG1	$C_t = A \times \left\{ \delta^n e^{(-K_1 \times T)} - \left\{ e^{(-K_2 \times T)} \times \left[ \sum (n; i = 1) \delta^i \times (K_2 \times T)^{n-1} / (n-i)! \right] \right\} \right\}$
MC	$C_t = A \times e^{(-K_1 \times T)} \times \exp \left\{ - (N - 2) \times e^{[-(K_2 - K_1) \times T]} \right\}$

<sup>1</sup>G1 = 1-compartment age-independent model; G2 = 1-compartment age-dependent model; G1G1 = 2-compartment age-independent model; GnG1 = 2-compartment models with increasing order of age dependency ( $n = 2$  to 5); MC = multicompartmental age-independent model.

<sup>2</sup> $C_t$  = marker concentration at time =  $t$  (h);  $A$  = scalable parameter;  $K_1$  = fractional rate constant for the compartment with the longest retention time (/h);  $K_2$  = fractional rate constant for the compartment with the second longest retention time (/h);  $N$  = model-derived number of mixing compartments;  $T = (t - TD)$ , where  $TD$  = time delay (h);  $\delta = K_2 / (K_2 - K_1)$ ;  $i$  = imaginary unit.

Predicted marker concentrations were compared with the observed values using the root mean square prediction error, which was decomposed into errors due to overall bias of prediction, errors due to deviation of the regression slope from unity, and errors due to random variation (Bibby and Toutenburg, 1977), and scaled to the observed mean (mean prediction error, **MPE**).

Before curve fitting, marker concentrations were scaled to the marker peak concentration following the procedure of Sponheimer et al. (2003). Excretion curves were superimposed and values were considered outliers if they deviated by more than twice the SD from the mean per sampling time point ( $n = 36$ ). Curve fitting was performed using nonlinear least squares regression procedures of SAS (version 9.2, SAS Institute Inc., Cary, NC) based on the least square Levenberg-Marquardt algorithm. Initial values for the iterative procedure were obtained through a grid search and curve fits were solved after, on average, 18 to 24 iterations for fecal marker concentrations and 13 to 30 iterations for omasal marker concentrations. Based on model fit parameters estimated with the MC model, transit time (**TT**; i.e., moment of first appearance of the marker in the feces) and moment of peak concentration (**PCT**) were calculated for fecal marker excretion patterns as described by Dhanoa et al. (1985). Total mean retention time (**TMRT**) in the reticulorumen (i.e., for omasal digesta) was calculated as the sum of the estimated retention times; TMRT in the entire gastrointestinal tract (i.e., for feces) was calculated as above including retention times associated to the remaining compartments (assumed to be TT). Total marker clearance time was calculated as 3 times the TMRT as suggested by France et al. (1993).

Passage kinetic parameters were log-transformed due to asymmetrical distribution patterns of residuals and

tested by analyses of variance in a Latin square split-plot design, with a  $2 \times 3$  factorial arrangement of treatments within main plots and type of marker assigned to subplots, with mixed model procedures of SAS (version 9.2, SAS Institute Inc.) according to the model:

$$Y_{ijklm} = \mu + A_i + P_j + C_k + M_l + (C \times M)_{kl} + (A \times P \times C \times M)_{ijkl} + T_m + (C \times M \times T)_{klm} + \varepsilon_{ijklm}$$

where  $Y_{ijklm}$  is the dependent variable;  $\mu$  is the overall mean;  $A_i$  (animal;  $i = 6$ ),  $P_j$  (period;  $j = 6$ ),  $C_k$  (cultivar;  $k = 2$ ),  $M_l$  (maturity;  $l = 3$ ) and its interaction term  $(C \times M)_{kl}$  represent effects assigned to the main plots in a Latin square;  $T_m$  (type of marker;  $m = 3$  per sampling site) and  $(C \times M \times T)_{klm}$  represent effects related to the subplots. Main plot variables were tested against the interaction term  $(A \times P \times C \times M)_{ijkl}$ , and subplot variables were tested against the pooled residual error ( $\varepsilon_{ijklm}$ ). Covariance parameters were estimated using the REML method and denominator degrees of freedom were estimated using the Satterthwaite approximation. Differences between marker types were assessed using orthogonal contrasts. Tables report back-transformed values (geometric means). Standard errors of the mean (SEM) were calculated by multiplying the log-transformed SEM by its geometric mean. Rumen pH and VFA and  $\text{NH}_3$  concentrations were considered repeated measurements and were analyzed after Yandell (1997) as a Latin square split-plot design as described above, with  $T_m$  (time after marker administration;  $m = 7$ ) as the subplots effects. All other data were tested by ANOVA as a Latin square with a  $2 \times 3$  factorial arrangement of treatments [ $Y_{ijkl} = \mu + A_i + P_j + C_k + M_l + (C \times M)_{kl} + \varepsilon_{ijkl}$ ].

## RESULTS

**Diet and Animal Characteristics**

**Corn Silage Quality and Rumen Fermentation End-Products.** The chemical composition of the 6 corn silages tested is presented as means over 6 experimental periods in Table 1. Within treatments, the starch and fiber content differed on average by 11 g/kg of DM (SD) throughout the 6 experimental periods. On average, the Aastar cultivar had higher CP and starch contents but lower NDF and ADF contents than Baleric. This pattern was reflected in the fermentation end-products present in the rumen liquid, with a significantly lower rumen pH ( $P = 0.023$ ) and tendency for lower acetic acid ( $P = 0.062$ ) and higher propionic acid molar proportions ( $P = 0.079$ ) for cultivar Aastar compared with Baleric (Table 3). Starch content increased markedly with prolonged maturation, which was more pronounced for Aastar. The contents of CP, NDF, and ADF in the corn silage decreased with maturity, the decrease in NDF and ADF being more pronounced for Aastar. Despite these changes in the nutrient composition toward increased starch with maturing corn cultivars, no effects on rumen fermentation end-products were observed, except for a decrease in butyric acid with increasing maturity ( $P = 0.036$ ).

**Animals.** Animal performance was not different between corn cultivars. However, DMI and milk fat proportion were affected by the maturity of the corn plants (Table 4). The DMI increased by 0.6 and 0.8 kg of DM/d from early to late harvest for Aastar and

Baleric, respectively ( $P = 0.006$ ). Although not significant, the highest DMI was observed for mid-maturity corn. Both DMI and milk yield decreased from the third experimental period onward with advancing lactation stage ( $P \leq 0.034$  for factor period). Milk fat proportion decreased with advancing maturity of the corn silage ( $-1.7$  g/kg of milk from early to late maturity;  $P = 0.007$ ), whereas milk protein was not affected. Milk yield tended to be higher for dairy cows fed the most mature corn silage by 1.3 kg of milk/d ( $P = 0.087$ ). As a result, milk fat and protein yields did not change with corn silage maturity. Total-tract feed digestibility after a continuous Co-EDTA infusion was highest for Baleric for all nutrients ( $P < 0.001$ ) except starch (Table 4). Digestibility coefficients were not affected by maturity stage.

**Passage Kinetics**

**Model Assessment and Accuracy of Curve Fits.** Fecal marker peak concentration (PCT) occurred on average at 17.1, 29.5, and 36.3 h after marker dosage for Cr-NDF, <sup>13</sup>CDM, and <sup>13</sup>CADF, respectively (Table 5). In general, fecal samples were almost fully depleted of Cr-NDF by the time sampling was terminated 116 h after pulse dose administration, as shown by the estimated total marker clearance time (109 h) from the gastrointestinal tract. Concentration of <sup>13</sup>C at 116 h was still above natural abundance level, as also indicated by the total marker clearance time of 192 h for <sup>13</sup>CDM and 233 h for <sup>13</sup>CADF. Omasal marker excre-

**Table 3.** Rumen liquid characteristics of dairy cows fed different corn silage qualities

Item	Subset	Rumen liquid characteristic <sup>1</sup>								
		pH	HAc	HPr	HBu	HVa	HBc	tVFA	NGR	NH <sub>3</sub>
Cultivar	Maturity									
Aastar	Early	6.13	65.4	18.7	11.8	1.48	2.54	124.0	4.29	3.87
	Mid	6.10	64.2	20.7	11.1	1.46	2.57	122.4	3.88	3.51
	Late	6.12	64.9	20.1	11.0	1.50	2.53	123.6	3.96	3.76
Baleric	Early	6.17	65.0	19.1	12.0	1.50	2.47	120.1	4.24	3.98
	Mid	6.19	66.0	18.9	11.0	1.42	2.62	116.7	4.28	4.02
	Late	6.23	66.2	18.7	11.1	1.44	2.62	116.9	4.31	4.23
SEM		0.040	0.56	0.64	0.36	0.037	0.109	0.15	0.147	0.292
$P$ -value <sup>2</sup>	Animal	<0.001	0.030	0.003	0.047	0.027	0.014	0.004	0.004	0.000
	Period	0.002	0.333	0.540	0.646	0.903	0.308	0.012	0.417	<0.001
	Cultivar (C)	0.023	0.062	0.079	0.833	0.398	0.761	0.033	0.068	0.123
	Maturity (M)	0.760	0.713	0.374	0.036	0.383	0.693	0.684	0.437	0.707
	C × M	0.719	0.124	0.201	0.981	0.551	0.751	0.896	0.275	0.753
	Time (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	C × M × T	0.981	0.599	0.523	0.981	0.967	0.643	0.927	0.671	0.990

<sup>1</sup>HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; HVa = valeric acid; HBc = branched-chain VFA (isobutyric + isovaleric acid); tVFA = total VFA (mmol/L; HAc + HPr + HBu + HVa + HBc); NGR = nonglucogenic to glucogenic VFA ratio [(HAc + 2 × HBu + 2 × isobutyric + HVa + isovaleric) / (HPr + HVa + isovaleric)]; NH<sub>3</sub> = ammonia (mmol/L). All values expressed in mol/100 mol unless specified otherwise.

<sup>2</sup>Analyses of variance based on log-transformed means with subplot T (= time; 0, 3, 7, 11, 15, 19, 23 h after marker administration) within factorial main plots in a Latin square.

Table 4. Animal performance data and total-tract feed digestibility coefficients for 6 dairy cows fed rations containing 2 corn silage cultivars harvested at 3 maturity stages

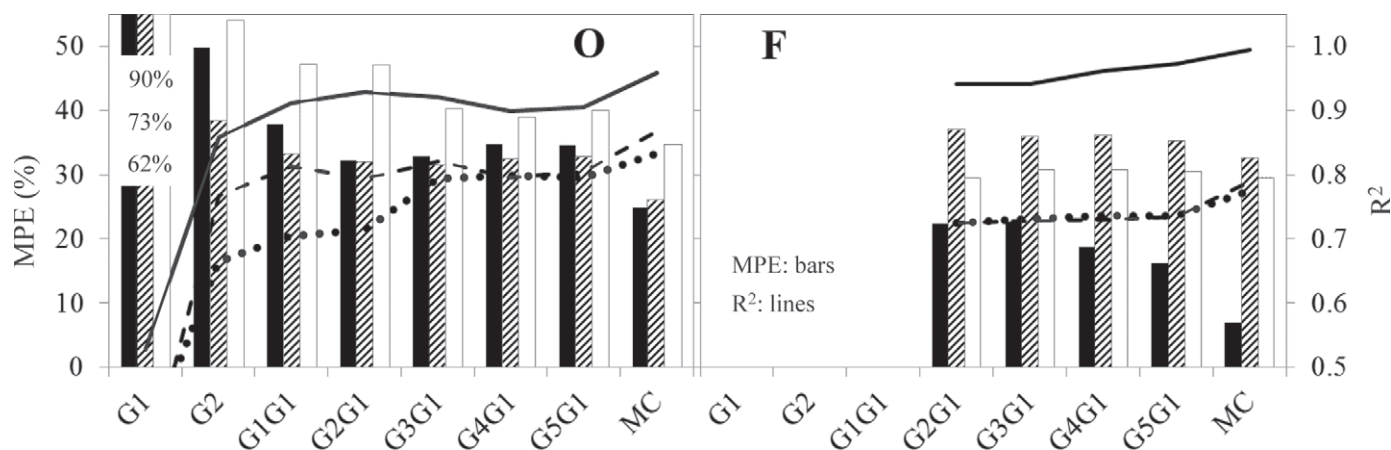
Total-tract feed digestibility coefficients										Milk				
Item	Subset	DMI (kg/d)	OM	CP	Starch	NDF	ADF	Yield (kg/d)	Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	Urea (mg/dL)	Fat (g/d)	Protein (g/d)
Cultivar Aastar	Maturity Early	21.4	0.666	0.554	0.949	0.568	0.463	34.4	43.5	34.3	45.6	25.4	1472	1171
	Mid	22.1	0.667	0.550	0.961	0.558	0.460	35.6	42.7	34.9	45.7	25.6	1499	1228
	Late	22.0	0.675	0.568	0.931	0.574	0.467	36.1	42.0	34.0	46.1	25.8	1522	1229
Baleric	Early	20.9	0.729	0.640	0.955	0.664	0.573	34.5	43.8	34.5	45.7	26.7	1506	1187
	Mid	22.0	0.747	0.658	0.959	0.673	0.593	35.3	42.1	33.8	45.8	26.0	1474	1180
	Late	21.8	0.769	0.696	0.950	0.707	0.622	35.4	41.9	34.0	46.4	25.2	1470	1194
SEM		0.26	0.0107	0.0152	0.0103	0.0150	0.0155	0.56	0.46	0.28	0.23	0.75	28	19
P-value	Animal	<0.001	0.199	0.022	0.675	0.022	0.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Period	0.006	0.413	0.251	0.246	0.251	0.290	0.034	0.009	<0.001	0.333	<0.001	0.005	0.185
	Cultivar (C)	0.193	<0.001	<0.001	0.364	<0.001	<0.001	0.482	0.724	0.243	0.446	0.534	0.532	0.163
	Maturity (M)	0.006	0.088	0.081	0.189	0.081	0.232	0.087	0.007	0.347	0.057	0.773	0.943	0.235
	C × M	0.865	0.371	0.179	0.602	0.179	0.374	0.817	0.591	0.083	0.906	0.489	0.312	0.242

tion patterns differed from fecal excretion curves by a markedly shorter PCT occurring at 4.4, 10.0, and 9.5 h after marker dosage for Cr-NDF, <sup>13</sup>CDM, and <sup>13</sup>CST, respectively (data not shown).

Because of the different marker excretion patterns between feces and omasal digesta, several compartmental models were evaluated and their accuracy estimated by calculating MPE and R<sup>2</sup> (Figure 1). Accuracy of fit was highest when the observed marker concentrations were fitted with the MC model. One-compartment models without (G1; MPE of 61.7–89.5%) and with age dependency (G2; MPE of 38.3–54.0%) did not adequately fit the observed omasal marker concentrations and did not converge when fecal marker concentrations were used. Two-compartment models with increasing order of age dependency (GnG1 models) performed somewhat better for omasal marker concentrations. The G2G1 model appeared to fit best among GnG1 models. A further increase in age dependency to *n* = 5 generally reduced model accuracy. The G1G1 model frequently did not converge when fecal marker concentrations were used. The MC model had generally the lowest MPE values for omasal and fecal marker concentrations. Furthermore, the MC model converged for all 36 curves for Cr-NDF and <sup>13</sup>CDM, respectively, showing high model robustness to fecal marker excretion patterns. Therefore, passage kinetics will be further discussed for fractional passage rates estimated by the MC model.

Among markers, MPE for fecal marker excretion profiles was lowest for Cr-NDF (7.1%; *P* < 0.001) compared with <sup>13</sup>CDM (32.5%) and <sup>13</sup>CADF (29.4%). For omasal marker excretion profiles, MPE was similar among markers, with values ranging from 24.9% for Cr-NDF, 26.0% for <sup>13</sup>CDM, and 34.6% for <sup>13</sup>CST (*P* = 0.266). The largest proportion of the MPE for fecal and omasal marker concentrations was due to random variation (96.9–97.2%) and only a minor part was explained by an error in the overall mean (1.2–1.3%) or regression bias (1.6–1.8%; data not shown).

**Fractional Passage Rates Through the Gastrointestinal Tract.** Marker choice affected fecally determined fractional passage kinetics (*P* < 0.001; Table 5). Fractional passage rates from the reticulorumen (*K*<sub>1</sub>) were, on average, 1.9 times higher for Cr-NDF than for the <sup>13</sup>C markers (*P* < 0.001) but were not different among the <sup>13</sup>C markers. Fractional passage rates from the proximal colon-cecum (*K*<sub>2</sub>) were highest for Cr-NDF (*P* < 0.001) and, among <sup>13</sup>C markers, lowest for <sup>13</sup>CADF (*P* = 0.008). Model parameters *A* and *N*, as estimated by the MC model, averaged 2.5 (SEM 0.70; *P* = 0.042) and 40 (SEM 36.5; *P* < 0.001) across markers, respectively (data not shown). Marker PCT and TT were lowest for Cr-NDF (*P* < 0.001). Total mean retention time in the gastrointestinal tract was higher for



**Figure 1.** Model accuracy (mean prediction error, MPE; coefficient of determination,  $R^2$ ) for different markers in omasal digesta (O) and feces (F) based on one-pool age-independent (G1) and age-dependent models (G2), 2-pool age-independent (G1G1) and age-dependent models with increasing order of gamma distribution (GnG1,  $n = 1$  to 5) and an age-independent multicompartamental model (MC). Markers: chromium mordanted fiber (Cr-NDF; solid bars and lines),  $^{13}\text{C}$  in DM ( $^{13}\text{CDM}$ ; dashed bars and lines),  $^{13}\text{C}$  in omasal starch ( $^{13}\text{CST}$ ) or fecal ADF ( $^{13}\text{CADF}$ ; white bars and dotted lines). G1, G2 and G1G1 models not shown for feces because of poor model convergence.

**Table 5.** Passage kinetics and residence times of different markers in the gastrointestinal tract of dairy cows fed rations containing 2 corn silage cultivars harvested at 3 maturity stages determined by fecal sampling

Item	Subset	Kinetic parameter <sup>1</sup>				
		$K_1$	$K_2$	PCT	TT	TMRT
Cultivar	Maturity					
Chromium mordanted fiber (Cr-NDF)						
Aastar	Early	0.042	0.383	16.8	9.1	35.8
	Mid	0.039	0.507	15.8	9.4	37.5
	Late	0.037	0.465	16.2	9.4	39.0
Baleric	Early	0.047	0.391	17.8	10.5	34.7
	Mid	0.043	0.422	17.1	10.1	35.8
	Late	0.044	0.382	17.5	9.9	35.5
$^{13}\text{C}$ isotopes in DM ( $^{13}\text{CDM}$ )						
Aastar	Early	0.022	0.196	29.1	14.6	66.2
	Mid	0.022	0.153	29.3	12.1	66.6
	Late	0.025	0.168	28.5	13.3	60.8
Baleric	Early	0.023	0.175	31.4	16.0	67.2
	Mid	0.025	0.162	30.4	14.6	61.6
	Late	0.022	0.219	24.8	11.1	62.0
$^{13}\text{C}$ isotopes in ADF ( $^{13}\text{CADF}$ )						
Aastar	Early	0.020	0.140	29.1	11.5	70.8
	Mid	0.016	0.202	26.0	10.6	79.5
	Late	0.022	0.088	41.9	17.1	75.8
Baleric	Early	0.024	0.143	37.1	19.8	68.9
	Mid	0.023	0.122	39.0	18.9	77.4
	Late	0.020	0.069	52.5	25.0	93.3
SEM		0.0061	0.0831	4.26	2.45	8.36
$P$ -value <sup>2</sup>	Animal	0.112	0.941	0.435	0.276	0.060
	Period	0.757	0.911	0.983	0.727	0.941
	Cultivar (C)	0.162	0.383	0.029	0.003	0.904
	Maturity (M)	0.730	0.325	0.280	0.396	0.620
	C × M	0.433	0.744	0.669	0.434	0.636
	Marker type (T)	<0.001	<0.001	<0.001	<0.001	<0.001
	C × M × T	0.887	0.397	0.058	0.025	0.673
	Cr-NDF vs. ( $^{13}\text{CDM}$ , $^{13}\text{CADF}$ )	<0.001	<0.001	<0.001	<0.001	<0.001
	$^{13}\text{CDM}$ vs. $^{13}\text{CADF}$	0.180	0.008	<0.001	0.010	0.001

<sup>1</sup>Fractional passage kinetic parameters:  $K_1$  = fractional passage rate constant (/h) for the reticulorumen;  $K_2$  = fractional passage rate constant (/h) for the proximal colon-cecum; PCT = marker peak concentration time (h); TT = transit time (h); TMRT = total mean retention time ( $1/(K_1 + 1/K_2 + TT)$ ; h).

<sup>2</sup>Analyses of variance with orthogonal contrasts based on log-transformed means in a split-plot arrangement with factorial main plots in a Latin square.



the  $^{13}\text{C}$  markers compared with Cr-NDF ( $P < 0.001$ ), and, among individual markers, TMRT was highest for  $^{13}\text{C}$ ADF, being 41.2 h longer than Cr-NDF and 13.5 h longer than  $^{13}\text{C}$ DM ( $P = 0.001$ ). Corn silage quality did not affect passage kinetic parameters.

**Rumen Fractional Passage Rates.** Rumen fractional passage rates for starch from corn silages were determined as  $^{13}\text{C}$ ST from marker excretion in the omasal digesta (Table 6). No effects of corn silage cultivar or maturity were found. However,  $K_1$  estimates were affected by choice of marker ( $P < 0.001$ ), with omasal Cr-NDF having the highest rates. Omasal  $^{13}\text{C}$ ST had higher  $K_1$  estimates than  $^{13}\text{C}$ DM ( $P = 0.017$ ) but lower rates than omasal Cr-NDF ( $P = 0.001$ ). Estimates of  $K_2$  (data not shown) were 2.91/h, 1.07/h, and 1.33/h for Cr-NDF,  $^{13}\text{C}$ DM, and  $^{13}\text{C}$ ST, respectively (SEM 1.480/h;  $P = 0.006$ ). Total mean retention time was highly affected by marker choice ( $P < 0.001$ ); the TMRT of  $^{13}\text{C}$ ST in the reticulorumen was 7.9 h shorter, on average, than that of  $^{13}\text{C}$ DM and 6.6 h longer, on average, than that of Cr-NDF. Model parameters  $A$  and  $N$  were 1.5 (SEM 0.29;  $P = 0.031$ ) and 6 (SEM 3.2;  $P = 0.283$ ) across markers, respectively (data not shown). Mean pH of omasal digesta was 6.52 (SEM 0.035).

### Rumen Degradability

In situ rumen degradation characteristics of corn silages are presented in Table 7. The potentially degradable fraction ( $D$ ) increased, whereas the undegrad-

able ( $U$ ) and washable ( $W$ ) fractions decreased with advancing maturity for all nutrients ( $P < 0.001$ ) except for  $U$  of NDF, which showed the opposite effect ( $P = 0.007$ ). Fractional degradation rate of the  $D$  fraction ( $K_D$ ) decreased with maturity for the starch fraction ( $P < 0.001$ ) but did not change for all other nutrients. Corn silage cultivar did not affect  $K_D$ . No direct relationship was observed between fractional degradation and marker passage rates (data not shown). The highest observed  $R^2$  values were 0.14 for  $K_D$  of starch and NDF with  $K_1$  of fecal Cr-NDF.

Based on the experimentally determined  $K_D$  values, effective rumen degradability (Figure 2) was calculated either from in vivo measurements (i.e., experimentally determined  $K_1$  estimates based on Cr-NDF and  $^{13}\text{C}$  isotopes) or from  $K_1$  estimates as assumed by the Dutch protein evaluation system for ruminants (DVE/OEB system; van Duinkerken et al., 2011). The DVE/OEB  $K_1$  values are fixed to 0.045/h for crude protein and starch, whereas  $K_1$  for NDF is estimated from its  $K_D$ . Effective nutrient degradability calculated from DVE/OEB  $K_1$  values was similar to the effective degradability measured in vivo with fecal Cr-NDF. Effective rumen CP degradability was lower with the DVE/OEB  $K_1$  values (49.4%) than with fecal  $^{13}\text{C}$ DM (55.8%). Similarly, effective rumen NDF degradability was lower using the DVE/OEB equation (20.6%) than when measured in vivo with fecal  $^{13}\text{C}$ ADF (28.4%). In contrast, effective starch degradability calculated in vivo with omasal  $^{13}\text{C}$ ST (58.9%) was in close agreement with ef-

**Table 6.** Rumen passage of chromium-mordanted fiber (Cr-NDF) and  $^{13}\text{C}$  isotopes in DM ( $^{13}\text{C}$ DM) and starch ( $^{13}\text{C}$ ST) for dairy cows fed rations containing 2 corn silage cultivars harvested at 3 maturity stages determined by omasal sampling<sup>1</sup>

Item	Subset	$K_1$			TMRT		
		Cr-NDF	$^{13}\text{C}$ DM	$^{13}\text{C}$ ST	Cr-NDF	$^{13}\text{C}$ DM	$^{13}\text{C}$ ST
Cultivar	Maturity						
Aastar	Early	0.055	0.028	0.039	18.6	37.0	27.2
	Mid	0.057	0.053	0.044	18.3	24.4	26.1
	Late	0.044	0.027	0.042	22.8	39.0	24.4
Baleric	Early	0.053	0.031	0.042	19.3	35.6	26.6
	Mid	0.045	0.024	0.048	22.9	43.5	21.7
	Late	0.053	0.039	0.039	20.0	28.7	30.0
SEM			0.0096			7.11	
$P$ -value <sup>2</sup>	Cow		0.509			0.819	
	Period		0.842			0.523	
	Cultivar (C)		0.709			0.506	
	Maturity (M)		0.626			0.990	
	C $\times$ M		0.069			0.283	
	C $\times$ M $\times$ T		0.569			0.770	
	Marker type (T)		<0.001			<0.001	
	Cr-NDF vs. ( $^{13}\text{C}$ DM, $^{13}\text{C}$ ST)		0.001			<0.001	
	$^{13}\text{C}$ DM vs. $^{13}\text{C}$ ST		0.017			0.021	

<sup>1</sup>Fractional passage kinetic parameters:  $K_1$  = fractional passage rate constant (/h) for the reticulorumen; TMRT = total mean retention time ( $1/K_1 + 1/K_2 + \text{transit time; h}$ ), where transit time = 0.

<sup>2</sup>Analyses of variance with orthogonal contrasts based on log-transformed means in a split-plot arrangement with factorial main plots in a Latin square.

**Table 7.** Rumen degradation kinetics of different corn silage qualities

Degradation parameter <sup>1</sup>	Aastar			Baleric			SEM
	Early	Mid	Late	Early	Mid	Late	
OM							
$K_D$	0.024	0.023	0.021	0.022	0.026	0.025	0.0015
$D$	62.0	66.1	66.8	56.1	58.9	66.7	0.61
$W$	17.1	17.7	15.4	19.0	19.0	12.9	0.57
$U$	21.0	16.2	17.8	24.9	22.1	20.4	0.26
$lag$	1.8	0.7	1.0	0.9	4.3	5.3	1.03
CP							
$K_D$	0.028	0.021	0.022	0.025	0.024	0.022	0.0025
$D$	41.3	42.9	46.1	31.7	37.9	42.2	0.79
$W$	36.2	40.6	33.4	42.2	38.5	36.4	0.72
$U$	22.6	16.5	20.5	26.1	23.6	21.5	0.19
$lag$	3.0	1.0	6.8	5.3	10.8	11.2	2.79
Starch							
$K_D$	0.042	0.045	0.033	0.052	0.042	0.034	0.0019
$D$	68.5	71.1	75.4	51.8	70.3	85.7	1.87
$W$	31.6	29.0	24.6	48.2	29.7	14.4	1.87
$lag$	0.3	0.6	0.8	1.1	3.2	3.1	0.82
NDF							
$K_D$	0.018	0.016	0.017	0.021	0.020	0.020	0.0035
$D$	66.6	68.7	60.9	59.5	58.0	55.5	1.69
$U$	33.4	31.3	39.1	40.5	42.0	44.5	1.69
$lag$	6.3	11.6	20.6	12.9	10.1	15.7	7.24

<sup>1</sup>Fractional degradation kinetic parameters:  $K_D$  = fractional degradation rate constant (/h) of  $D$  fraction;  $D$  = potentially degradable and insoluble fraction (%;  $D = 100 - W - U$ );  $U$  = undegradable fraction at  $t = \infty$  (%; assumed zero for starch);  $W$  = washable fraction (%; assumed zero for NDF);  $lag$  = lag time (h).

fective degradability based on the DVE/OEB  $K_1$  values (57.7%).

## DISCUSSION

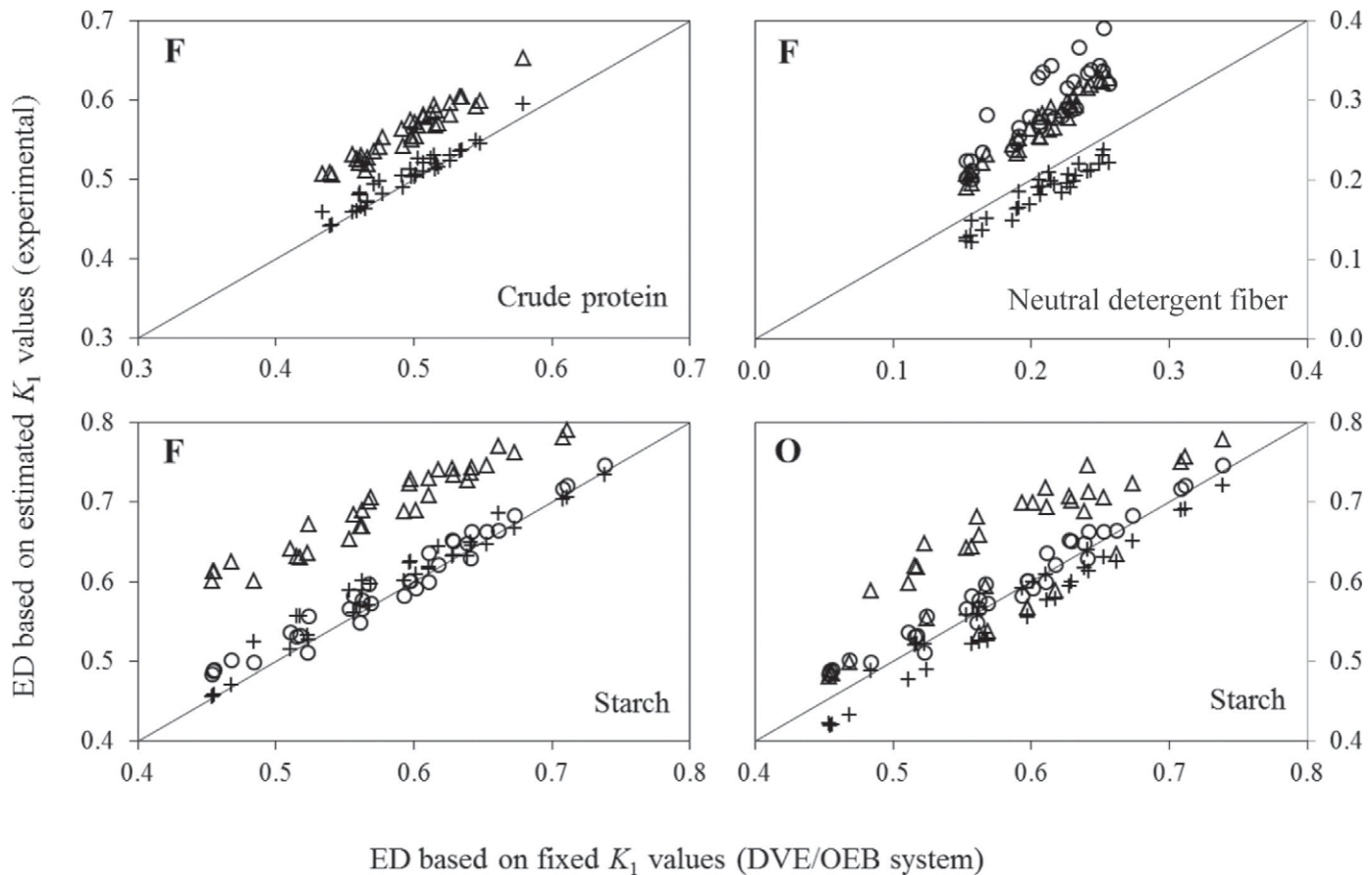
### Animal Performance

Fractional passage rates were reported to depend on the level of feed intake (Huhtanen and Kukkonen, 1995). In this study, daily mean DMI ( $21.7 \pm 0.26$  kg of DM) increased and milk fat ( $42.7 \pm 0.46$  g/kg of milk) decreased with advancing maturity (Table 4). The observed deviations in DMI and milk fat content can be explained by the higher inclusion of starch in the diet due to the maturing corn plants. Our findings are in line with observations of Phipps et al. (2000) and Khan et al. (2012). The increase in milk yield with advancing maturity agrees with observations reported by Sutton et al. (2000). An inconsistent response of molar proportions of VFA and ratio of nonglucogenic to glucogenic VFA was observed, despite the marked increase in starch with maturing corn silage, which was, however, coherent with the findings of Sutton et al. (2000). Corn maturity had no clear effect on feed digestibility, which was in line with data on corn silage at comparable maturity stages (Sutton et al., 2000). Total-tract apparent digestibility was slightly higher for starch and CP in their study compared with our observations, but no maturity effect was observed, except for a significant although small decrease for starch.

### Passage Dynamics of Corn Silages

Excretion patterns of an external (Cr-NDF) and an internal marker (<sup>13</sup>C isotopes) were established from fecal and omasal digesta grab samples upon a pulse dose into the rumen. A previous tracer study showed the suitability of <sup>13</sup>C as an internal marker to assess component specific passage kinetics of grass silage (Pellicaan et al., 2013). In our study, externally applied Cr-NDF from wheat straw (<0.5 mm) was used as a reference passage rate marker for corn silage as it has been widely used in passage studies. The particle size of Cr-NDF was chosen to be similar to the mean particle size in the rumen observed for corn silages of different maturity (approximately 0.5 mm; Fernandez and Michalet-Doreau, 2002). However, differences in marker type preparation and material, especially with regard to particle size, particle density, particle entrapment in the rumen, and possible migration of particulate marker to particles not originally labeled (Owens and Hanson, 1992), need to be considered when comparing markers within the present study and between studies.

**Rumen Passage Kinetics of Corn Silage.** Passage kinetics were assessed from marker concentrations in feces and omasal digesta based on the MC model. The model assigns a slow fractional passage rate to the compartment with the highest retention time ( $K_1$ ) and a fast fractional passage rate to the compartment with the lowest retention time ( $K_2$ ). With regard to fecal



**Figure 2.** Relations between different calculations of effective rumen degradability (ED) of different corn silage qualities. The x-axis shows a reference ED based on fixed fractional rumen passage rates ( $K_1$ ) as assumed by the Dutch protein evaluation system (DVE/OEB system; van Duinkerken et al., 2011); the y-axis shows ED based on  $K_1$  estimates determined experimentally from fecal (F) or omasal (O) excretion of chromium mordanted fiber (Cr-NDF; +),  $^{13}\text{C}$  in the DM ( $^{13}\text{CDM}$ ;  $\Delta$ ), and  $^{13}\text{C}$  in the respective feed component ( $\circ$ ) either as omasal starch ( $^{13}\text{CST}$ ) or fecal ADF ( $^{13}\text{CADF}$ ).

marker excretion,  $K_1$  and  $K_2$  are generally associated with the reticulorumen and proximal colon-cecum, respectively (Dhanoa et al., 1985). With regard to omasal marker excretion,  $K_2$  probably represents rumen-related processes, such as mixing time and gas entrapment of marker particles, rather than a major second particle-retaining compartment per se.

Passage kinetics did not change with corn silage maturity. However, marker type had a large effect on fecal and omasal  $K_1$  estimates, with the highest mean  $K_1$  estimates observed for the external marker Cr-NDF for both sampling sites. Similar observations were reported for grass silages using Cr-NDF,  $^{13}\text{CDM}$ , and  $^{13}\text{C}$ -labeled NDF in feces (Pellikaan et al., 2013). Our observed mean  $K_1$  values based on fecal Cr-NDF (0.042/h) were slightly lower than for grass silage (0.048–0.050/h; Pellikaan et al., 2013). With regard to fecal  $^{13}\text{CDM}$ , mean  $K_1$  values reported here for corn silage (0.023/h) were distinctly lower than for grass silage (0.035–0.039/h).

This in agreement with Mambrini and Peyraud (1994), who reported lower  $K_1$  values for corn silage (0.041/h) than for fresh grass (0.053/h) using rare earth elements as passage rate markers. However, their reported rate constants should be treated with caution because stage of lactation and concentrate levels differed among their dietary treatments. Mulligan et al. (2002) reported  $K_1$  values of 0.020/h for corn silage based on fecal Cr-NDF excretion but DMI was considerably lower (13.5 kg/d).

Interestingly, the mean  $K_1$  of fibers based on fecal  $^{13}\text{CADF}$  (0.021/h) was similar to that of  $^{13}\text{CDM}$  (0.023/h;  $P = 0.180$ ), and the  $K_1$  of starch based on omasal  $^{13}\text{CST}$  was comparable (0.042/h) to that of fecal Cr-NDF although lower than  $K_1$  of omasal Cr-NDF (0.051/h). Pellikaan et al. (2013) observed greater differences between the  $^{13}\text{C}$  markers for grass silages with  $K_1$  values for fibers (0.015–0.017/h as  $^{13}\text{C}$ -labeled NDF) being half the value of  $^{13}\text{CDM}$ . The different amplitude in variation between markers in the 2 studies might be

due to the specific physical structure of the 2 forages. Corn silage fiber was reported to cause a higher rumen fill than grass silage fiber (Mulligan et al., 2002). Furthermore, physical structure, derived by the ruminating index, was reported to be lower for corn silage than for grass silage (De Boever et al., 1993). This might induce a shorter retention time of corn silage fibers in the reticulorumen compared with grass silage fibers, which is shown by the higher  $K_1$  value of corn silage fiber (this study) compared with grass silage fiber (Pellikaan et al., 2013). Data on individual structural polysaccharides intrinsically labeled with  $^{13}\text{C}$  might provide a better understanding of the differential passage of fibers between those 2 forages.

**Total-Tract Passage Kinetics.** Passage behavior of markers in the 2 main mixing compartments was comparable, with  $K_2$  showing a similar trend to  $K_1$ , which was reflected in their TMRT through the gastrointestinal tract. Based on fecal Cr-NDF, no differences between TMRT of our corn silage (36.4 h) and that of grass silage (35.8–37.4 h; Pellikaan et al., 2013) could be detected, whereas TMRT based on fecal  $^{13}\text{C}$  was, on average, 26 h longer ( $^{13}\text{CDM}$ ) and 29 h shorter ( $^{13}\text{CADF}$ ) than that of grass silage. These results indicate that the external Cr-NDF marker is less sensitive to the nutritional quality of the forages and confirms early reservations on the use of Cr-NDF as not fully representing the rumen particle pool (Bosch and Bruining, 1995).

**Evaluation of Sampling Sites.** Sampling site had a considerable effect on fractional passage rates of corn silages. Estimates of  $K_1$  were 15% (Cr-NDF) and 21% ( $^{13}\text{CDM}$ ) higher in omasal digesta than in feces. The higher amplitude in variation for  $^{13}\text{CDM}$  between sampling sites is in line with the higher retention time of  $^{13}\text{CDM}$  in the proximal colon-cecum (equivalent to a lower  $K_2$ ) compared with Cr-NDF. Therefore, differences between sampling sites might be due to particle retention in the proximal colon-cecum. Similar discrepancies among sampling sites were observed between fecal and duodenal sampling (Wylie et al., 2000; Huhtanen and Hristov, 2001), and between fecal and ruminal sampling (Beauchemin and Buchanan-Smith, 1989). Estimated  $K_1$  values of Cr-NDF were moderately related between feces and omasal digesta ( $R^2 = 0.51$ ;  $P < 0.001$ ) but not the  $K_1$  values of  $^{13}\text{CDM}$  ( $R^2 = 0.01$ ). Possible factors contributing to the low  $R^2$  value between fecal and omasal  $^{13}\text{CDM}$  might be the small pulse dose size of  $^{13}\text{C}$ -labeled corn silage compared with Cr-NDF, resulting in a differential mixing behavior of the 2 markers in the rumen.

**Model Comparison.** Marker concentrations in feces and omasal digesta upon a ruminal pulse dose were fitted using several nonlinear compartmental models. As

profiles of excretion curves differed between sampling sites, several stochastic and one deterministic compartmental model were chosen to best describe fractional passage rates. The first type of model assumes a time (or age) dependency of ingested particles required for their escape from the reticulorumen (Matis, 1972) in line with observations made on required fermentative particle size reduction (Poppi et al., 1980) and buoyancy characteristics of particles (Sutherland, 1988). Age-dependent turnover of particles was described by an increasing order of discrete gamma distribution lifetimes ( $Gn$ ;  $n \geq 1$ ) for 1-compartment ( $Gn$ ) and 2-compartment systems ( $GnG1$ ). Because of the quickly occurring peak time and fast initial increase in marker concentrations in the omasum upon ruminal pulse dosage, 1-compartment models without ( $G1$ ) or with age dependency ( $G2$ ) would suggest an adequate curve fit allowing an estimation of the rumen fractional passage rates from the descending curve phase. However, the MPE as an indicator of the goodness of fit of the model suggests poor accuracy for both the  $G1$  (74.7% across markers) and  $G2$  models (47.3%) with omasal marker excretion. Similar observations were reported for fecal excretion of Cr-mordanted hay particles (Moore et al., 1992), although  $G1$  and  $G2$  models did not converge based on our fecal marker concentrations. Increasing the age dependency generally improved the model accuracy for fecal marker to a certain extent. Similar observations have been reported for ruminally and abomasally dosed rare earth elements in duodenal chyme and feces (Wylie et al., 2000). On the other hand, the MC model is based on a deterministic model approach and fitted the marker concentrations best among chosen models. The overall MPE for fecal and omasal marker excretion with the MC model was 23.0 and 28.5%, respectively. Similarly, Bernard et al. (1998) concluded that the  $K_1$  estimates of external markers in the duodenum and feces of sheep were, in general, more accurately determined with the MC model than the  $G2G1$  model when compared with the algebraic sum method that served as their reference method.

**Model Accuracy.** Model accuracy for parameter estimates from the MC model was generally better for fecal than for omasal marker excretion due to the larger variability of marker excretion profiles in the omasal digesta, a circumstance described also for duodenal chyme by Wylie et al. (2000). Model accuracy seemed to be affected by marker choice and was generally better for the external than internal markers. Model accuracy was somewhat less accurate for fecal  $^{13}\text{C}$  isotopes (29.4–32.5%) than for  $^{13}\text{C}$ -labeled grass silage (11.8–17.0%; Pellikaan et al., 2013) and concentrates (9.9–13.9%; D. Warner, unpublished data). The conditions for the different studies varied, particularly with



regard to the pulse dose size, the  $^{13}\text{C}$  enrichment, and the route of marker administration. The present study used the smallest (30 g of DM) but continuously and highly enriched pulse dose (8.68–12.36 At% $^{13}\text{C}$ ). In contrast, Pellikaan et al. (2013) used a larger (170–252 g of DM grass silage) but less enriched pulse dose (1.65–1.97 At% $^{13}\text{C}$ ), and D. Warner (unpublished data) used the largest (9 kg of DM corn bran) but less enriched pulse dose (i.e., the difference of natural  $^{13}\text{C}$  abundance of  $\text{C}_4$  corn bran relative to that of a  $\text{C}_3$  concentrate mixture), which was administered orally. Model accuracy for fecal Cr-NDF was comparable among the 3 studies, which all applied a similar large ruminal dose (90–100 g) of 0.5-mm-ground Cr-NDF. Based on these results, we suggest increasing the pulse dose size of  $^{13}\text{C}$ -labeled material to obtain a better mixing of the marker with the rumen digesta.

An uneven distribution of isotopes in growing plants not labeled continuously might restrict the use of isotopes as passage rate markers (Owens and Hanson, 1992). Previous studies applied  $^{13}\text{C}$ -labeled forage enriched under field conditions, either as alfalfa labeled on one single occasion (Svejcar et al., 1993) or as grass silage labeled on 6 to 8 occasions (Pellikaan et al., 2013). Although the latter study did not find any effects of the labeling procedure on rumen passage rates based on fecal grab samples, early time points before PCT in their fecal excretion curves were affected. This might restrict the use of isotopes if they were to be collected more proximate to the reticulorumen (e.g., in the omasal digesta) as omasal marker excretion curves were shown to follow an exponential decay curve or having a quickly ascending phase in our study. The procedure used in our study, based on continuous isotope dosing of growing plants resulting in a homogeneous distribution of isotopes (Gorissen et al., 1996), will, therefore, circumvent any potential effects of differential  $^{13}\text{C}$  enrichment in plant tissue on the passage kinetics (Owens and Hanson, 1992) from omasal digesta. Conversely, a closer resemblance of labeled plant material to unlabeled field plants might be more easily achieved under field conditions as the growing and climate conditions are more alike. Nonetheless, Huhtanen and Hristov (2001) proved the suitability of intrinsically labeled alfalfa under greenhouse conditions to assess passage kinetics of alfalfa. In our study, conditions between labeled and unlabeled corn plants were kept similar by inducing mechanical stress on plant cell walls to resemble the wind stress of field plants (Biddington, 1986), and by ensiling labeled plants with the unlabeled field plants.

### Effective Rumen Degradability

Rumen degradability of the corn silages by in situ nylon bag incubation revealed a considerable effect ( $P$

$\leq 0.007$ ) of corn silage maturity on the potentially degradable ( $D$ ), undegradable ( $U$ ), and washout fraction ( $W$ ) but less on the fractional degradation rate of  $D$  ( $K_D$ ). Estimates for  $K_D$  decreased for the starch fraction with advancing maturity ( $P < 0.001$ ) but did not change for all other nutrients. An in vitro incubation experiment in which the same corn silages were used [J. Oerlemans (Utrecht University, Utrecht, the Netherlands), D. Warner, and W. F. Pellikaan, unpublished data] confirmed our findings from the in situ experiment and revealed no effect of corn silage maturity on in vitro OM disappearance after 12 h (0.68–0.71) or 72 h (0.82–0.88) of incubation in rumen fluid, or on gas production as an indicator of in vitro fermentation measured at 12 h (207–230 mL/g of OM) and 72 h (291–328 mL/g of OM). It can be assumed that degradation is linked to passage kinetics because faster rumen degradation was associated with a higher rumen escape of feed particles (Welch, 1986; Hristov et al., 2003) by altering the buoyancy properties of feed particles (Sutherland, 1988). However, no direct link could be established for in vivo corn silage degradation and passage in our study, probably due to the low number of experimental units required for the comparison.

Effective rumen degradability measured in vivo with Cr-NDF was comparable to that calculated from the DVE/OEB equations. This is in line with our expectations, as feed evaluation systems typically rely on passage studies using external markers. The  $^{13}\text{C}$  isotopes gave higher effective rumen degradability values for CP and NDF but comparable values for starch relative to the DVE/OEB equations. Results on effective CP degradability should be considered with caution because  $^{13}\text{C}$  isotopes might not best describe component-specific fractional passage of feed proteins and the use of  $^{15}\text{N}$  isotopes should be rather considered. Nonetheless, these results suggest that although the use of stable isotopes should be considered to estimate component specific fractional passage rates, the rumen escape of nutrients might not be affected, depending on the component of interest and type of feed.

## CONCLUSIONS

Intrinsic isotope labeling of corn silages allows assessment of component-specific fractional passage rates. Corn silage cultivar and stage of maturity did not affect passage dynamics although chemical composition and in situ rumen degradability were affected to a large extent. Fractional passage rate from the rumen estimated with an external marker (Cr-NDF) was higher than that estimated with  $^{13}\text{C}$  feed components used as internal passage rate markers. Among  $^{13}\text{C}$  markers, fractional passage rate from the rumen estimated with

labeled corn silage DM ( $^{13}\text{CDM}$ ) did not differ from that with labeled fibers ( $^{13}\text{CADF}$ ) but was lower than that of labeled starch ( $^{13}\text{CST}$ ).

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