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Passage kinetics of dry matter and neutral detergent fibre through the gastro-intestinal tract of growing beef heifers fed a high-concentrate diet measured with internal $\delta^{13}C$ and external markers

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Abstract. Fractional rumen passage rates (K_1) are fundamental in feed evaluation systems for ruminants to predict the extent of nutrient degradation. Data on passage kinetics of growing beef cattle fed high-concentrate diets are scarce and mainly rely on external passage markers which do not provide nutrient-specific K_1 estimates. The present study describes the use of carbon stable isotopes ($\delta^{13}C$) as an internal marker to estimate K_1 of dry matter (DM) and neutral detergent fibre (NDF) fractions of a compound feed in a high-concentrate diet, and compares them to the external markers ytterbium (Yb)-actetate and chromium mordanted fibre (Cr-NDF). Four rumen-fistulated Holstein heifers received four times per day a basal diet consisting of barley straw and pelleted compound feed offered separately (ratio 10 : 90, DM basis). Compound feed in the basal diet was mainly based on wheat of low natural ^{13}C enrichment (−28.4 $\delta^{13}C$), which was exchanged with a single dose of a maize-based compound feed of higher natural ^{13}C enrichment (−18.9 $\delta^{13}C$). This difference in natural ^{13}C abundance was used to determine K_1 values from faecal ^{13}C excretion patterns. At the same time Yb-Acetate and Cr-NDF were introduced into the rumen to determine K_1 values from faecal excretions. Faeces were collected over 90 h after pulse dosing. The K_1 of $\delta^{13}C$-marked DM (0.062/h) did not differ (P = 0.745) from $\delta^{13}C$-marked NDF (0.060/h). The $\delta^{13}C$-based K_1 values also did not differ from Cr-NDF (0.056/h; P = 0.315). These results indicate similar passage behaviour of these fractions in the rumen of beef heifers fed a high-concentrate diet.

Additional keywords: digestion, feed evaluation, feedlot, rumen.

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Introduction

Southern European beef feedlot systems rely heavily on cereal-based diets. Cattle are typically fed straw and relatively high amounts of concentrate (70% and more), both offered *ad libitum* and separately (Devant *et al*. 2001; Rotger *et al*. 2005). The effect of concentrate level on fractional passage rate in beef cattle fed a high-concentrate diet is unclear, highlighted by empirical studies (Lescoat and Sauvant 1995; National Research Council 2001; Seo *et al*. 2006) and *in vivo* studies (Owens and Goetsch 1986; Colucci *et al*. 1990), which mainly addressed dairy cows and forage-based diets (Seo *et al*. 2006). However, accurate fractional passage rates are fundamental in feed evaluation models to predict absorption and excretion of nutrients, waste products as well as nutrition-related disorders (see review of Warner *et al*. 2014).

Fractional passage rate were traditionally estimated with the use of external markers. However, these markers have been criticised due to the potential increase in specific gravity of the feed particles they are bound to (Ehle *et al*. 1984) or due to migration of markers to rumen fluid (Hartnell and Satter 1979). Therefore, efforts have been made to identify suitable markers that better reflect the *in vivo* passage rate. Stable isotopes have been recently proposed as an internal marker to estimate passage kinetics (Huhtanen and Hristov 2001; Pellikaan *et al*. 2013). Based on the different natural abundance of the carbon stable isotope ^{13}C between maize and wheat species, fractional passage rates can be determined by exchanging a part of the daily wheat-based compound feed intake (C_3 origin) by a single dose of maize-based compound feed (C_4 origin; Südekum *et al*. 1995). This approach was previously applied to measure nutrient-specific passage kinetics in dairy cows fed varying levels of compound feed (Warner *et al*. 2013*a*).

The main objective of the present study was to assess the passage behaviour of various markers in growing beef heifers fed a forage-to-concentrate ratio of 10 : 90. For the solid fraction, two approaches were used and compared; one based on $\delta^{13}C$ as an internal marker in the dry matter (DM) and neutral detergent fibre (NDF) fraction for, respectively, DM and NDF passage kinetics,

and the other based on ytterbium (Yb)-acetate and chromium-mordanted NDF (Cr-NDF) as external markers. Additionally, the rumen liquid passage rate was estimated using cobalt (Co)-EDTA.

Materials and methods

The experiment was carried out at the Nutreco Ruminant Research Centre (Kempenshof, Boxmeer, The Netherlands). Experimental procedures were conducted under the approval of the ethical committee of Utrecht University, Utrecht, The Netherlands.

Animal, experimental design and diet

Four rumen-fistulated Holstein heifers (11–12 months of age; BW mean ± s.e.: 364 ± 22.7 kg) were fed a basal diet consisting of barley straw and pelleted compound feed offered separately. The adaptation period consisted of 24 days. During the first 14 days, heifers were offered the experimental diet on *ad libitum* basis. Afterward, feed was offered four times (0530 hours, 1100 hours, 1700 hours, and 2200 hours) per day. Amounts of feed (9.4 ± 0.42 kg/day) and the forage-to-concentrate ratio (10 : 90, DM basis) were based on the individual *ad libitum* intake from Days 7 through 14. Compound feed in the basal diet was mainly based on wheat of low ^{13}C enrichment (−28.4 $\delta^{13}C$), and exchanged with a single dose of a maize-based compound feed of higher natural ^{13}C enrichment (−18.9 $\delta^{13}C$). The difference in natural abundance of ^{13}C between the basal and pulse-dosed compound feeds was used to determine the fractional rumen passage rate (K_1) values for DM and NDF of the compound feed based on the respective faecal ^{13}C excretion patterns. A total of 3 kg (product basis) maize-based compound feed representing a pulse dose of 1.83 kg of maize DM and 0.55 kg of maize NDF was administered orally allowing voluntary consumption. After 2.5 h, orts were introduced into the rumen together with 60.0 g Cr-NDF (54.9 g Cr/kg DM), 20.0 g Yb-acetate (457.5 g Yb/kg DM) and 20.0 g Co-EDTA (166.2 g Co/kg DM). The amount of pulse-dosed compound feed inserted through the rumen cannula was on average 1.7 ± 0.17 kg (product basis) corresponding to 57 ± 5.8% of the total pulse dose.

Animals were housed individually in tie-stalls and had free access to water during the experiment. Orts were collected daily at 1100 hours, weighed (straw: 32 ± 13.3 g/day; compound feed: 54 ± 32.2 g/day) and inserted into the cannula to assure a constant forage-to-concentrate ratio. The compound feed composition and quality is presented in Table 1.

Preparation of external markers

Three external markers were used, including the solid particle phase markers Cr-NDF and Yb-acetate and the liquid phase marker Co-EDTA. The markers Cr-NDF (from wheat straw) and Co-EDTA were prepared as described by Udén *et al.* (1980); Yb-acetate was prepared as described by Siddons *et al.* (1985). The Cr-NDF was ground to pass a 0.5-mm screen to resemble particle size of the compound feed.

Sampling and chemical analyses

Barley straw and the two experimental compound feeds were sampled on the first day of the measurement period and analysed for DM, ash, crude protein (CP), NDF, acid detergent fibre (ADF), acid detergent lignin (ADL) and starch as described in detail by Abrahamse *et al.* (2008*a*, 2008*b*).

Table 1. Ingredients and chemical composition of pelleted compound feeds (basal and pulse dose) and barley straw

Item	Compound feed			Barley straw
	Basal		Pulse dose	
Diet composition (g/kg)	–	900	–	100
Ingredients (g/kg product)				
Wheat	352		–	–
Wheat middlings	274		–	–
Maize grits	–		350	–
Maize, heat treated	–		350	–
Soy hulls	150		7	–
Soy bean meal	111		179	–
Beet molasses	50		60	–
Hidropalm[A]	30		15	–
Limestone	9		11	–
Sodium bicarbonate	7		8	–
Monocalcium phosphate	7		7	–
Salt	5		7	–
Magnesium oxide	2		3	–
Mineral-vitamin mixture[B]	3		3	–
Chemical composition (g/kg DM)				
Dry matter (g/kg product)	866		879	928
Organic matter	935		931	931
Crude protein	174		206	35
Neutral detergent fibre	270		230	803
Acid detergent fibre	151		93	541
Acid detergent lignin	21		13	74
Starch	295		315	–

[A]Hydrogenated palm fatty acids (Norel, Madrid, Spain).
[B]Mineral-vitamin mixture (60%, product basis: premix intensive Beef, Hendrix UTD, The Netherlands; 40%: premix cobalt 1%, Hendrix UTD, The Netherlands).

Prior to pulse dosing, faeces (free defecation) were taken to determine background marker concentrations in faeces. Faecal samples were collected in blocks of 3 h each until 90 h after pulse dosing. Sampling intervals between blocks were 1 h within the first 43 h followed 3-h sampling intervals, resulting in 19 sampling points per animal. During a 3-h sampling block, faeces were collected quantitatively per individual animal, thoroughly mixed by hand, subsampled (~400 g) and immediately frozen at −18°C pending analyses.

Concentrations of Cr and Co were determined using an atomic absorption spectrophotometer (AA240FS, Varian Inc., Palo Alto, CA, USA) after oxidation with wet-destruction as described by Warner *et al.* (2013*b*). Concentrations of Yb were determined as described by Sterk *et al.* (2012). Faecal excretion patterns of ^{13}C were determined in the DM fraction ($\delta^{13}C$-DM) and in NDF ($\delta^{13}C$-NDF) as described by Pellikaan *et al.* (2013).

All fractions were analysed for ^{13}C enrichment by elemental analyses using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Bremen, Germany). The relative ^{13}C enrichment is expressed as the $^{13}C : ^{12}C$ ratio in the samples relative to the $^{13}C : ^{12}C$ ratio of the international

Vienna Pee Dee Belemnite standard. After correction for natural ^{13}C enrichment, faecal excretion patterns of atom percentage ^{13}C in excess were established, and scaled to marker peak concentration to improve model fit.

Rumen pH and volatile fatty acids (VFA) measurement were taken at times 0, 1, 3, 8, 20 and 27 h after pulse dosing in order to evaluate whether fractional passage rates were measured in comparable ruminal conditions for animals fed a high-concentrate diet. Rumen liquid was collected proportionally from a cranial, middle and caudal direction and the pH was immediately measured using a portable electronic pH meter. Concentrations of VFA were determined using gas chromatography (GC type Fisons HRGC MEGA2, Fisons Instruments, Milan, Italy) as described by Pellikaan *et al*. (2011).

Calculations and statistical analyses

Faecal excretion patterns of internal and external markers were fitted with a non-linear multi-compartmental model as proposed by Dhanoa *et al*. (1985):

$$C_t = A \times e^{(-K_1 \times t)} \times e^{[-(N-2) \times e((-K_2-K_1) \times t)]} \quad (1)$$

where C_t expresses the faecal marker concentration at time = t (h; defined as the mean time of each 3-h faecal collection period after marker administration); N denotes the number of compartments; K_1 and K_2 are the fractional passage rate constants for the compartments in the digestive tract with the, respectively longest (reticulo-rumen) and second longest retention time (large intestine); and A is a scalable parameter dependent on N, K_1, K_2. Transit time (TT), total mean retention time (TMRT) and the moment of peak concentration (PCT) were estimated from the parameters estimated in the multi-compartmental model as described by Dhanoa *et al*. (1985).

Table 2. Passage kinetics of different markers in beef heifers fed compound feed and straw separately at fixed forage-to-concentrate ratio of 10 : 90

K_1 = fractional passage rate constant (h) for the reticulorumen; K_2 = fractional passage rate constant (h) for the large intestine; PCT = peak concentration time (h); TT = transit time (h); TMRT = total mean retention time (h); δ^{13}C-DM = δ^{13}C-labelled dry matter; δ^{13}C-NDF = δ^{13}C-labelled neutral detergent residue; Cr-NDF = chromium-mordanted neutral detergent fibre; Yb-acetate = ytterbium acetate; Co-EDTA = cobalt ethylene diamine tetra-acetic acid

Marker	K_1	K_2	PCT	TT	TMRT
δ^{13}C-DM (I)	0.062	0.39	16.7	10.0	28.7
δ^{13}C-NDF (II)	0.060	0.38	17.4	10.3	29.9
Cr-NDF (III)	0.056	0.57	15.8	10.4	30.8
Yb-acetate (IV)	0.075	0.50	14.7	9.3	24.9
Co-EDTA (V)	0.096	0.57	13.7	9.1	21.5
s.e.	0.0051	0.054	0.90	0.69	1.67
P-values					
Marker	<0.001	0.045	<0.001	0.013	<0.001
I vs II	0.745	0.866	0.218	0.546	0.415
III vs (I, II)	0.315	0.013	0.009	0.524	0.237
IV vs (I, II, III)	0.007	0.368	<0.001	0.011	0.001
V vs (I, II, III, IV)	<0.001	0.064	<0.001	0.008	<0.001

Curve fitting was performed using the non-linear least-squares regression procedure PROC NLIN (version 9.3; SAS Institute Inc., Cary, NC, USA) based on the least square Levenberg–Marquardt algorithm. Initial values for the iterative procedure were obtained through a grid search. The array of initial values used were assessed from the excretion patterns and solved in 3–16 steps for each parameter. All curves converged. Curve fit accuracy was assessed by comparing predicted and observed marker concentrations based on the root mean square prediction error, which was decomposed into errors due to overall bias of prediction, errors due to regression bias and errors due to random disturbance (Bibby and Toutenburg 1977), and scaled to the observed mean (mean prediction error, MPE). Effect of marker type on passage kinetic parameters was tested using the mixed model procedure PROC MIXED (version 9.3; SAS Institute Inc.) with animal as the random variable. Differences between markers were assessed using orthogonal contrasts.

Results

Fractional passage rates were estimated for internal markers (δ^{13}C-DM and δ^{13}C-NDF) and external markers (Cr-NDF, Yb-acetate and Co-EDTA) based on faecal marker excretion

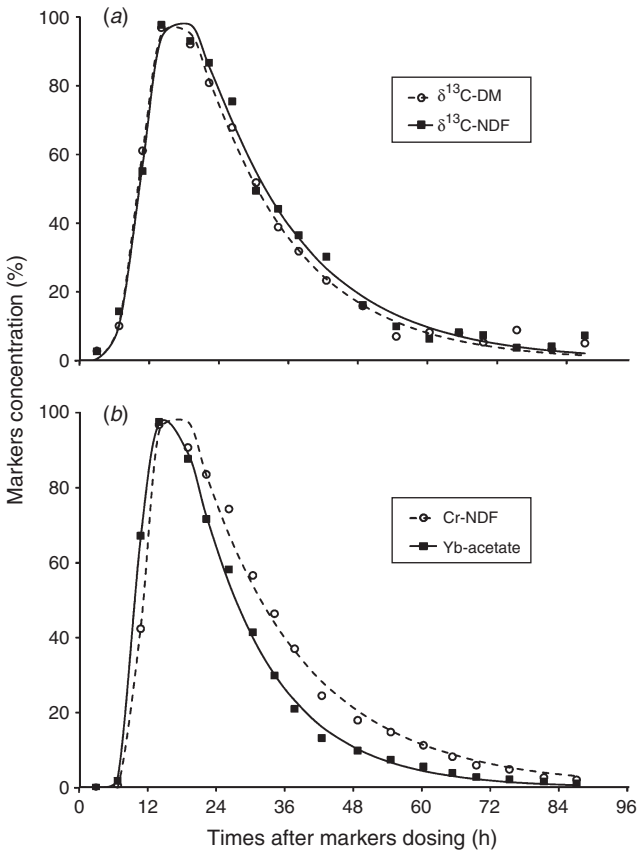

Fig. 1. Faecal mean solid marker concentrations relative to the marker peak concentration with model fits in beef heifers fed a high-concentrate diet: (*a*) δ^{13}C-DM = δ^{13}C-labelled dry matter from compound feed and δ^{13}C-NDF = δ^{13}C-labelled neutral detergent fibre from compound feed, (*b*) Cr-NDF = chromium-mordanted neutral detergent fibre and Yb-acetate = ytterbium acetate.

curves. The MPE across markers was 11.4 ± 2.6% (mean ± s.e.) of which 96.4% was related to errors due to random disturbance, 2.0% to errors in overall bias of prediction and 1.6% to errors due to regression bias (results not shown). The MPE was lower for the external markers than for the internal markers (respectively 8.8% and 15.2%; P = 0.004), suggesting a somewhat better model fit.

Liquid K_1 was 0.096/h based on Co-EDTA. A comparison between the markers δ^{13}C-NDF and δ^{13}C-DM did not show any difference (P = 0.745) for K_1 (Table 2). The K_1 estimated with Cr-NDF was not different from that of δ^{13}C-labelled fractions. However, K_1 of Yb-acetate was higher (P = 0.007) than that of δ^{13}C-labelled fractions. Concerning K_2, δ^{13}C-NDF and δ^{13}C-DM had considerably lower values (P = 0.013) than that estimated from Cr-NDF. The PCT was higher for δ^{13}C-NDF and δ^{13}C-DM than for Cr-NDF (P = 0.009). No significant differences were observed for parameters TT and TMRT between δ^{13}C markers and Cr-NDF. Yb-acetate yielded a lower PCT, TT and TMRT than δ^{13}C-labelled fractions.

Across animals, the average rumen pH value of 24 measurements over a 28-h period after pulse dosing was 5.75 ± 0.077 (mean ± s.e.) and the mean acetate-to-propionate ratio across was 2.51 ± 0.345. Whereas rumen pH fluctuated throughout the day and was characterised by a lower pH after feeding time, the acetate-to-propionate ratio was relatively constant over the day.

Discussion

To our knowledge, the present study is the first to establish fractional passage rates of ^{13}C-labelled compound feed fractions in beef cattle fed a high-concentrate diet. Therefore, we used external markers to obtain additional information on passage kinetics of small feed particles (Cr-NDF; Bruining and Bosch 1992), very small feed particles (Yb-acetate; Siddons *et al*. 1985) and the liquid phase (Co-EDTA). The ^{13}C-labelled compound feed was administered through the feed but a relatively high portion (57 ± 5.8%) was not eaten and inserted intra-ruminally. However, this procedure did likely not influence the ^{13}C faecal excretion curves as the compound feed was pelleted and thus most probably subjected to low chewing during eating (Beauchemin *et al*. 2008). As shown in Fig. 1, the ^{13}C marker excretion curves were comparable in shape to those of the external particulate markers, and followed the typical bell-shaped curve observed in previous digesta kinetics studies using a similar approach (Huhtanen and Hristov 2001; Pellikaan *et al*. 2013; Warner *et al*. 2013*a*; Lee and Hristov 2014).

Liquid phase K_1 based on Co-EDTA (0.096/h) was in the range of that reported by Rotger *et al*. (2005) and Devant *et al*. (2001). They reported K_1 values of 0.108 and 0.096/h with heifers receiving a similarly high-concentrate allowance (70% and 88%, respectively). With respect to particulate markers, we found similarly high K_1 values for ^{13}C as in the study of Warner *et al*. (2013*a*) using lactating dairy cows fed considerably lower concentrate levels (25% and 53%). The reported K_1 values for δ^{13}C-NDF are assumed to be representative of the true NDF passage because ^{13}C-labelled NDF will most likely not escape the reticulorumen with rumen bacteria. However, with regard to δ^{13}C-DM, we cannot excluded that some ^{13}C from the rumen DM pool might migrate to and escape the rumen with rumen bacteria at a different rate than the bulk DM (Pellikaan *et al*. 2013; Warner *et al*. 2014).

Fractional passage rates from the large intestine (K_2) were lower for ^{13}C than Cr-NDF. However, K_2 was estimated based on only 3 points in the ascending curve phase (see Fig. 1). A shorter sampling interval in the first few hours after pulse dosing would be desirable to increase the precision of K_2. We observed an average TMRT for δ^{13}C markers of 28.7–29.9 h, which was comparable to values observed by Warner *et al*. (2013*a*). However, we observed a lower TMRT for Cr-NDF (30.8 h) compared with their study (40.9 h) and, accordingly, a higher K_1 for Cr-NDF. Differences in rumen stratification due to the relative high-concentrate uptake in our study might explain the observed differences in K_1 for Cr-NDF between these two studies.

Further research should account for potential changes in passage rates in beef cattle fed varying proportions of concentrates and emphasise passage kinetics of δ^{13}C-labelled carbohydrates fractions in compound feed.

Conclusions

Naturally enriched compound feed, either in DM (δ^{13}C-DM) or NDF (δ^{13}C-NDF), gave rise to similar fractional rumen passage rates and did not differ from those of rumen solid fractions based on the more commonly used external marker Cr-NDF. These results indicate a comparable passage behaviour of these fractions in the rumen of cattle a fed high-concentrate diet.

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