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# Effect of solid feed level and types of roughage on passage kinetics of milk replacer, concentrate, and roughage in veal calves

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## ABSTRACT

This study aimed (1) to provide estimates of total mean retention times of milk replacer (MR), concentrates, and roughage in veal calves fed a mixed diet; (2) to determine the effect of level and type of solid feed (SF) on passage kinetics of MR, concentrates, and roughages in veal calves; and (3) to compare passage kinetics in veal calves using the fecal excretion curves of indigestible markers and a noninvasive $^{13}C$ tracer breath test approach to determine whether the latter technique can serve as an alternative. At the start of the trial, 48 Holstein-Friesian calves (6 wk of age; 68 ± 7.7 kg of body weight; BW) were assigned to 1 of 4 dietary treatments (for statistical analysis, only 39 calf observations were used). Three treatments contained chopped wheat straw as roughage in the SF mixture in a concentrate:roughage ratio of 90:10 (dry matter basis). The SF level was 20 g/kg of metabolic BW per day (low straw), 30 g/kg of metabolic BW per day (middle straw), or 40 g/kg of metabolic BW per day (high straw). The fourth treatment (high hay) contained long perennial ryegrass hay as roughage in the SF mixture in a concentrate:roughage ratio of 70:30 (dry matter basis, at 40 g/kg of metabolic BW per day). The quantity of MR was fixed for the high straw treatment, whereas the amount of MR for the other treatments during the adaptation period was adjusted based on a pair gain strategy (i.e., exchanging ration components but keeping similar net energy). At the end of the adaptation period, calves ranged from 12 to 15 wk of age with an average BW of 123 ± 8.6 kg. Passage kinetics of concentrates were estimated by measuring $^{13}C$ enrichment excess of $CO_2$ in breath from a pulsed-dose of [1-$^{13}C$]octanoate. Passage kinetics of roughage, concentrates, and MR were also estimated using fecal excretion curves obtained after ingestion of chromium-mordanted roughage, $Yb_2O_3$, and Co-EDTA, respectively. We conclude that [1-$^{13}C$]octanoate cannot serve as a measure for oro-duodenal transit of concentrates because of unrealistic estimates. Based on the fecal excretion curves, we concluded that the total mean retention time of MR (i.e., time to peak; the moment that the excretion curve reaches peak concentration) was, on average, 12.4 h, and that the passage kinetics of MR was not affected by the level or type of SF. The mean retention time of concentrates was shorter (21.4 h) than that of both straw (59.1 h) and hay (36.8 h), and was not affected by the level or type of SF. Also, the mean retention time of the slowest compartment (i.e., the rumen) was shorter for concentrates (39.6 h) than that of straw (110.0 h) and hay (59.2 h). Contrary, the passage of roughage was affected by level and type of SF. Long hay increased time to peak by 22.3 h and decreased ruminal mean retention time by 50.8 h relative to chopped straw, indicating that the passage rate of long hay is faster than that of chopped straw. We conclude that the level and type of SF only affects the passage kinetics of roughage and not that of MR and concentrates.

**Key words:** calf, passage kinetics, solid feed level, roughage type

## INTRODUCTION

In veal calf nutrition, from a welfare and economic perspective, there is a strong incentive to replace a considerable portion of the milk replacer (**MR**) with solid feeds (**SF**; mixture of roughage and concentrates) in the diet (e.g., Webb et al., 2015). Feeding schedules in which 50% of the energy intake originates from SF are not an exception anymore (Gerrits, 2019). Feeding MR and SF simultaneously, however, poses new challenges. First, an increase in liquid DMI generally reduces the DMI of starter grains (Yunta et al., 2015; Gelsinger et al., 2016). Second, interactions between MR and SF

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occurring in the gastrointestinal tract (**GIT**), including influences of SF on the passage rate kinetics of the liquid and vice versa, complicate the prediction of the nutritional value of these ration components.

Quantitative information about passage kinetics of SF through the rumen and other gastrointestinal compartments of calves is limited. Broesder et al. (1990) demonstrated that decreasing MR intake by 30 and 60% relative to a control treatment in dairy calves (from 72 to 151 d of age, from 78 to 136 kg of BW) resulted in a linear increase of SF OM intake from 19.8 g/kg of BW for the control treatment to 23.1 g/kg of BW for the 60% MR reduction treatment, as well as increased ruminal fluid outflow rate (i.e., from 0.61 L/h for the control treatment to 0.74 L/h for the 60% MR reduction treatment), without affecting NDF and OM digestibility. Similarly, Berends et al. (2015) demonstrated that an increased SF level (from 1.2 to 3.0 kg DM/d) increased the rumen fractional passage rate of both concentrates and straw (3.3 and 1.3%/h for the low SF level, respectively, and 4.9 and 1.7%/h for the high SF level, respectively) in veal calves of about 250 kg of BW at 27 wk of age. This effect on ruminal passage rate appeared to be stronger for concentrates than that for straw. It was concluded that increasing SF level introduces large variation in passage kinetics of dietary components, predominantly in the rumen compartment (Berends et al., 2015).

Studies for measuring passage kinetics in ruminants traditionally involve (1) the recovery of a pulsed-dose indigestible tracer inside various compartments of the GIT (e.g., Berends et al., 2015), or (2) fecal excretion curves of pulsed-dosed indigestible markers (e.g., Richter and Schlecht, 2006). The first technique involves sacrificing experimental animals, thus preventing repeated measurements on a subject. The second technique requires frequent collection of feces, often performed during individual housing in balance cages. Novel technologies to measure passage kinetics include the measurement of abomasum emptying from appearance of an oral acetaminophen dose in blood plasma (e.g., Labussière et al., 2014; Stahel et al., 2017) as well as measurements of recovery of $^{13}C$ tracers in breath. Various $^{13}C$ breath test applications, an approach where $^{13}C$ in the exhaled $CO_2$ is measured, are used in human and animal research (McCue and Welch, 2016). Because $CO_2$ represents a waste product of substrate oxidation, $^{13}C$ breath tests can be used to study a variety of metabolic and physiological processes. When a diet, (naturally or artificially) enriched with $^{13}C$, is combined with $^{13}C$ breath testing, one can determine how soon after ingestion exogenous nutrients are used as metabolic fuels and how long after feeding they continue to provide energy (McCue and Welch, 2016). This technique has, however, not been tested and validated in calves, or other types of ruminants, for determination of passage rate kinetics of ration components.

We hypothesize that the level of SF feeding will affect ruminal passage rates, more so for concentrates compared with roughages, with estimates of the mean retention time (**MRT**) being considerable higher in veal calves than in calves exclusively fed on SF. Additionally, we hypothesize that the measurement of recovery of $^{13}C$ tracers in breath can serve as an alternative method to measure passage rate kinetics in calves. To test these hypotheses, the aims of this study were (1) to provide estimates of total-tract MRT as well as ruminal MRT of MR, concentrates, and roughage in veal calves fed a mixed diet, (2) to determine the effect of SF level and type of roughage on passage kinetics of MR, concentrates, and roughage in veal calves, and (3) to compare passage kinetics in veal calves using the fecal excretion curves of indigestible markers as well as a noninvasive $^{13}C$ tracer breath test approach to determine whether the latter technique can serve as an alternative.

## MATERIALS AND METHODS

The experiment was conducted from September 2017 until March 2018 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands), under the Dutch Law on Animal Experiments in accordance with European Union Directive 2010/63, and approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2017.W-0006.003 and 2017.W-0006.005).

### Animals

In total, 48 Holstein-Friesian calves (6 wk of age; 68 ± 7.7 kg of BW) were purchased from 2 commercial veal calf farms and selected based on uniformity and clinical health. The 48 calves could not be housed simultaneously; hence, calves arrived in 2 batches of 24 calves each (i.e., the first batch of calves originated from 1 veal calf farm and the second batch of calves originated from another veal calf farm), following the exact same procedure and timeline.

### Diets

Before arrival at the experimental facilities, calves received a commercial milk schedule and had free access to a muesli mix and chopped straw. Upon arrival, calves were assigned to 1 of 4 dietary treatments. These 4 dietary treatments consisted of 3 SF levels and 2 roughage types (Table 1). Three treatments contained chopped wheat straw (4–5 cm particle length) as rough-

age in the SF mixture, in a concentrate to roughage ratio of 90:10 (on a DM basis). The SF level was 20 g/kg of metabolic BW ($BW^{0.75}$) per day (low straw; **L-S**), 30 g/kg of $BW^{0.75}$ per day (middle straw; **M-S**), or 40 g/kg of $BW^{0.75}$ per day (high straw; **H-S**). The fourth treatment (high hay; **H-H**) contained long perennial ryegrass hay (20–30 cm particle length) as roughage in the SF mixture, in a concentrate to roughage ratio of 70:30 (on a DM basis). The SF level of the H-H treatment was set to the same level as the H-S treatment (40 g/kg of $BW^{0.75}$ per day). The H-H treatment was included as a high fibrous SF reference treatment with the level of SF comparable to the H-S treatment, but with long hay because (1) rumination behavior is more strongly stimulated with long hay than with chopped straw, and because (2) calves have shown to be highly motivated to consume hay, particularly long hay compared with chopped hay and chopped or long straw (Webb et al., 2014). The concentrate in all treatments was in meal form. Based on our expected maximum voluntary intake, the quantity of MR was fixed for the H-S treatment at 39 g/kg of $BW^{0.75}$ per day and during the adaptation period. The quantity of MR for the other treatments was calculated based on a pair gain strategy (i.e., exchanging ration components but keeping similar net energy; Berends et al., 2014) to achieve similar BW gain across treatments, and was adjusted weekly based on the realized BW gain in the preceding week (i.e., BW was measured on a weekly basis). During the adaptation period, the quantity of MR was 50.3 ± 1.15, 44.7 ± 0.58, and 41.6 ± 1.19 g/kg of $BW^{0.75}$ per day for treatments L-S, M-S, and H-H, respectively. After the adaptation period, the quantity of MR for the other treatments was fixed to the levels reported in Table 1.

The same MR (produced by Denkavit) was used for all 4 treatments, whereas the concentrate composition differed between the type of roughage fed (straw vs. hay) and was designed to provide an equal protein content of the SF mixture. Both concentrates were produced as a mash by Denkavit with mineral premix produced by Twilmij B.V. Table 2 shows the nutrient and chemical composition of the different feed components (i.e., MR, concentrates, and roughages).

### Adaptation

Within each batch, calves were allocated to pens (6 calves per pen; 4 pens; 3 × 3 m) to ensure similar mean BW across pens. The pens were randomly assigned to 1 of the 4 dietary treatments. Upon arrival, calves were exposed to the designated dietary treatment to allow the rumen to adapt to the levels and composition of SF. Pens used for the adaptation period were equipped with wooded-slatted floors and fences.

The experiment followed a staggered timeline due to the limited capacity of the climate respiration chambers (**CRC**). Hence, the adaptation period lasted 6 to 9 wk (Supplemental Figure S1, http://dx.doi.org/10.17632/32jrtd3dbr.1). After 4 wk of adaptation, the calves within a pen were paired according to BW, resulting in 3 pairs per pen (#1 to #3) with increasing mean BW. Striving for equal BW upon measurements, the pair of calves with the highest BW had the shortest adaptation period (i.e., 6 wk) and the pair of calves with the lowest BW had the longest adaptation period (i.e., 9 wk). The mean BW of the calves at the end of the adaptation period were 116 ± 3.7 kg, 115 ± 4.2 kg, 126 ± 7.4 kg for pairs 1, 2, and 3 of batch 1 and 127 ± 9.5 kg, 125 ± 3.7 kg, and 130 ± 9.6 kg for pairs 1, 2, and 3 of batch 2, respectively.

During the adaptation period, calves were allowed ad libitum access to water provided via drinking nipples. The concentration of MR was 130 g/L and was supplied individually in buckets at 40 to 42°C, provided twice daily in equally sized meals at 0730 and 1530 h throughout the experiment. The calves were allowed to drink for 15 min. Residual MR was subsequently

**Table 1.** Paired gain feeding schedule of the dietary treatments with a 90:10 concentrate to chopped straw ratio as solid feed mixture [at 20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] and with a 70:30 concentrate to long hay ratio as solid feed mixture [at 40 g/kg of metabolic BW per day; treatment high hay (H-H)]

| Treatment | MR[1] (g/kg of metabolic BW per day) | SF[2] (g/kg of metabolic BW per day) | Concentrate (g/kg of metabolic BW per day) | Roughage (g/kg of metabolic BW per day) | Concentrate to roughage ratio | Roughage type |
|---|---|---|---|---|---|---|
| L-S | 50.6 | 20 | 18 | 2 | 90:10 | Chopped straw |
| M-S | 44.8 | 30 | 27 | 3 | 90:10 | Chopped straw |
| H-S | 39.0 | 40 | 36 | 4 | 90:10 | Chopped straw |
| H-H | 41.4 | 40 | 28 | 12 | 70:30 | Long hay |

[1]Milk replacer (MR) mixed with water in a concentration of 130 g/L. The amount of MR for the H-S treatment was fixed. The amount of MR for the other treatments was based on pair gain strategy during the adaptation period, after which it was fixed as well.

[2]SF = solid feed.

**Table 2.** Nutrient and chemical composition of milk replacer (MR), concentrates, and roughages

| | | Concentrate | | Roughage | |
|---|---|---|---|---|---|
| Nutrient[1] | MR[2] | Conc1[3] | Conc2[4] | Chopped straw | Long hay |
| DM (g/kg of product) | 968 | 885 | 900 | 936 | 903 |
| OM | 940 | 953 | 953 | ND[5] | ND |
| CP | 184 | 168 | 181 | 41 | 101 |
| Crude fat | 212 | 52 | 48 | ND | ND |
| Starch | ND | 418 | 392 | 8 | 15 |
| NDF | ND | 161 | 154 | 850 | 641 |
| ADF | ND | 89 | 99 | 515 | 338 |
| Gross energy (MJ/kg of DM) | 21.1 | 18.7 | 18.6 | 18.9 | 18.7 |
| Calcium | 8.00 | 6.40 | 6.40 | ND | ND |
| Phosphorus | 6.56 | 4.62 | 4.93 | 0.39 | 2.55 |

[1]Presented as g/kg of DM unless stated otherwise.

[2]Ingredient composition of MR: 38% lactose, 21% fat-filled whey powder (i.e., 80% palm and 20% coconut), 20% whey protein concentrate, 10% vegetable oil (i.e., 70% palm, 20% coconut, and 10% rapeseed), 7% whey powder, and 4% mineral premix (i.e., vitamin A, 25,000 IU; vitamin $D_3$, 4,000 IU; vitamin E, 100 mg; vitamin $K_3$, 2.0 mg; vitamin $B_1$, 5.0 mg; vitamin $B_2$, 7.5 mg; vitamin $B_3$, 40 mg; vitamin $B_5$, 20 mg; vitamin $B_6$, 5.0 mg; vitamin $B_{12}$, 40 μg; biotin, 125 μg; choline, 350 mg).

[3]Ingredient composition of concentrates fed with straw-based low straw (L-S), middle straw (M-S), and high straw (H-S) diets: 55% corn, 37% lupins, 5% barley, and 3% mineral premix (i.e., magnesium, 1 g; vitamin A, 4,000 IU; vitamin $D_3$, 5,000 IU; vitamin E, 50 mg; vitamin $B_1$, 2.0 mg; vitamin $B_2$, 2.5 mg; vitamin $B_6$, 2.0 mg; manganese, 20 mg; copper, 10 mg; zinc, 25 mg; potassium iodide, 0.80 mg; cobalt, 0.10 mg; selenium, 0.15 mg).

[4]Ingredient composition of concentrates fed with the hay-based high hay (H-H) diet: 44% lupins, 22% maize feed flour, 26% corn, 5% barley, and 3% mineral premix (i.e., magnesium, 1 g; vitamin A, 4,000 IU; vitamin $D_3$, 5,000 IU; vitamin E, 50 mg; vitamin $B_1$, 2.0 mg; vitamin $B_2$, 2.5 mg; vitamin $B_6$, 2.0 mg; manganese, 20 mg; copper, 10 mg; zinc, 25 mg; potassium iodide, 0.80 mg; cobalt, 0.10 mg; selenium, 0.15 mg).

[5]ND = not determined.

collected and weighed. Except during the last week of adaptation, the SF was provided as a mixture (roughage and concentrates) directly after the MR meal, in a long feed trough in front of the pen. Refusals of the SF were removed twice daily and weighed (i.e., just before the next MR meal). During the last 5 d of the adaptation period, the pair of calves that was supposed to go the CRC for the breath test in the following week was separated from the other calves of their pen by placing a fence within the original pen (resulting in 1 pair of calves per pen; separating pair #1 from pair #2 and #3, and later separating pair #2 from pair #3). The pair of calves was subsequently accustomed to steady state feeding of the SF. During these 5 d, the SF was provided as a mixture in 4 equal portions per day (i.e., 0530, 0930, 1330, and 1730 h). Refusals of the SF were removed 4 times daily and weighed (i.e., just before the next SF meal).

## Breath Tests

After the adaptation period, 4 pairs of calves (1 pair from each pen; 1 pair from each dietary treatment) were transported to 1 of 4 identical CRC, located approximately 200 m from the barn, for a 7-d period to determine passage kinetics using the $^{13}C$ tracer breath test (Supplemental Figure S1). Within each CRC (area = 11.8 $m^2$, volume = 34.5 $m^3$), 1 pair of calves was housed in a pen (225 × 240 cm) equipped with a slatted, plastic flooring without bedding material. The calves were exposed to 10 h of light per day (from 0600 to 1800 h) and the relative humidity was maintained at 65% and temperature at 18°C.

***Feeding.*** During the 7-d measurement period in the CRC, calves were allowed ad libitum access to water provided via drinking nipples. The provision of MR in the CRC was identical to the adaptation period (see above). The SF was provided as a mixture of concentrates and roughage in 6 equal portions per day (i.e., 0000, 0400, 0800, 1200, 1600, and 2000 h) to ensure steady state feeding. Steady state feeding was implemented to differentiate between the $^{13}C$ enrichment of exhaled $CO_2$ originating from a pulse-dosed $^{13}C$ tracer and the $^{13}C$ enrichment originating of exhaled $CO_2$ originating from the SF. The $^{13}C$ tracer was administered in between the 2 MR meals to prevent the response in $^{13}C$ enrichment to the $^{13}C$ tracer to coincide with fluctuations in $^{13}C$ enrichment following a MR meal (Clouard et al., 2020). Refusals of the SF were removed and weighed just before the morning MR meal. The natural $^{13}C$ enrichment of chopped straw and long hay was analyzed (as described in the Analytical Procedures section) and was 1.074 and 1.076 atom%, respectively. The natural $^{13}C$ enrichment of both types

of concentrates was not measured, but estimated to be 1.080 atom%, based on previously reported $^{13}C$ enrichment of 2 corn starch sources by Gerrits et al. (2012) with corn being the major ingredient (>48%) within both concentrates in the current study.

***Measurements.*** The $^{13}C$ enrichment excess in expired $CO_2$ in breath measured in CRC was used to estimate passage kinetics of concentrates through the digestive tract of calves. A detailed description of the CRC design and gas measurements is reported by Heetkamp et al. (2015) and van Gastelen et al. (2015). In addition, $^{13}C$ enrichment in the expired $CO_2$ was measured every 12 min using nondispersive infrared spectrometry (Advance Optima Uras 14, ABB) as described by Alferink et al. (2003) to determine the passage kinetics of concentrates. The $^{13}C$ enrichment in expired $CO_2$ of the background breath (i.e., representing the $^{13}C$ enrichment originating from exhaled $CO_2$ originating from the steady state SF feeding) measured in the CRC was 1.082 ± 0.0009 atom% (mean ± SD). Feeding MR (with a different natural $^{13}C$ enrichment than SF) twice daily did not result in responses in the measured $^{13}C$ enrichment of exhaled $CO_2$ (data not shown). Hence, there was no need to account for a pattern in the $^{13}C$ enrichment of exhaled $CO_2$ due to the MR feeding schedule, when the effect of a $^{13}C$ tracer pulse dose was analyzed.

The order and time at which the $^{13}C$ tracers were administered is shown in Supplemental Figure S1. On d 2 in the CRC, at 1230 h, the delay in exhalation of $^{13}CO_2$ related to postabsorptive events was determined from $^{13}C$ enrichment excess of expired $CO_2$ after injection of a bolus dose of 4.7 mmol [$^{13}C$]sodium bicarbonate (99.1 atom%; Mass Trace; Buchem B.V.) into an ear vein. The infusate was prepared in 10 mL of sterile 0.15 mol/L NaCl and was injected within 2 min at 1300 h (i.e., 1 h after a SF meal was provided). On d 3 at 0800 h, 1 g of [1-$^{13}C$]octanoate (99.0 atom%; Mass Trace, Buchem B.V.; octanoate is not volatile and the $^{13}C$ can only appear in breath after removal of the carboxyl group from the octanoate) was added to the concentrates of 1 SF portion (i.e., 1/6 of the daily SF supply; representing 1 portion of the steady state feeding schedule). Calves were allowed 240 min (i.e., up to 1200 h in the afternoon) to consume the meal, which is when the next portion of the steady state feeding schedule would be provided. The completion of consumption of the [1-$^{13}C$]octanoate pulse dose was monitored hourly. If present after 240 min, the residual of the [1-$^{13}C$]octanoate dosed meal was removed and weighed to determine intake of the [1-$^{13}C$]octanoate. In total, 63% of the calves consumed the pulse dose [1-$^{13}C$]octanoate within the first 120 min and 84% of the calves consumed the pulse dose [1-$^{13}C$]octanoate within the total timeframe of 240 min. Only 16% of the calves did not consume the complete [1-$^{13}C$]octanoate dose. The $^{13}C$ enrichment excess of expired $CO_2$ was measured for 52 h after the pulse dose of [1-$^{13}C$]octanoate. On d 5 at 1600 h, bacterial protein (Feedkind, Calysta, partnered with Cargill) was pulse-dosed as a $^{13}C$ tracer. The bacterial protein was selected because it has a low natural $^{13}C$ enrichment (i.e., 1.057 atom%; analyzed) potentially resulting in a sudden drop in $^{13}C$ enrichment excess in the expired $CO_2$ after absorption and subsequent oxidation of nutrients originating from the bacterial protein. This bacterial protein was provided to the calves by replacing the complete concentrate part of the SF meal (i.e., 1/6 of the daily SF supply; representing 1 portion of the steady state feeding schedule) by a mixture containing 5.0% molasses, 46.7% barley, and 48.3% bacterial protein on a DM basis. Calves were allowed 240 min (i.e., up to 2000 h in the evening) to consume the meal with bacterial protein, which is when the next portion of the steady state feeding schedule would be provided. The completion of consumption of the bacterial protein pulse dose was monitored hourly. If present after 240 min, the residual of the bacterial protein containing meal was removed and weighed to determine intake of the bacterial protein. In total, only 50% of the calves consumed the pulse dose of the bacterial protein within 240 min. The $^{13}C$ enrichment excess of expired $CO_2$ was measured for 64 h after the pulse dose of the bacterial protein.

***Calculations.*** The $^{13}C$ enrichment excess of expired $CO_2$ from [1-$^{13}C$]octanoate and the bacterial protein was corrected for background enrichment, as described by Gilbert et al. (2016). The $^{13}C$ enrichment excess of expired $CO_2$ as response to [1-$^{13}C$]octanoate and the bacterial protein was expressed as atom percentage excess (**APE**, %) and was corrected for the delay in exhalation of $^{13}CO_2$ related to postabsorptive events from $^{13}C$ enrichment excess of expired $CO_2$ after injection of a bolus [$^{13}C$]sodium bicarbonate, similar to van den Borne et al. (2007). For the pattern of $^{13}C$ enrichment excess in time, $^{13}C$ enrichment excess was averaged per hour.

The derivative of a generalized Michaelis-Menten equation, as proposed by López et al. (2000) for growth curves, was used to express $^{13}C$ enrichment excess (APE, %) in time (similar to van den Borne et al., 2007). The generalized Michaelis-Menten model was fitted to the hourly means of $^{13}C$ enrichment excess in expired $CO_2$ (APE in %; corrected for background enrichment) of each pair of calves after ingestion of [1-$^{13}C$]octanoate and the bacterial protein and after infusion of [$^{13}C$]sodium bicarbonate:

$$y = \frac{\left[b_0 \times c \times (t - lt)^{(-c-1)} \times b_1^c\right]}{\left[1 + \left(\frac{b_1}{(t - lt)}\right)^c\right]^2}, \quad [1]$$

where $y = {}^{13}$C enrichment excess (APE, %) at time $t$ (min); $lt$ = lag time (min); $b_0$, $b_1$, and $c$ (all >0) are parameters that define the curve. The nonlinear least squares regression procedure (PROC NLIN, version 9.4, SAS Institute Inc., Cary, NC) was used for curve fitting. The time to peak ($t_{max}$; when $^{13}$C enrichment excess reaches peak) and the maximum $^{13}$C enrichment excess ($y_{max}$), were calculated as

$$t_{max} = lt + \left[b_1^c \times (1 - c) / (-c - 1)\right]^{\left(\frac{1}{c}\right)}, \quad [2]$$

$$y_{max} = b_0 \times c \times t_{max}{}^{(-c-1)} \times b_1^c \Big/ \left[1 + \left(b_1 / t_{max}\right)^c\right]^2. \quad [3]$$

Goodness of fit for predicted against experimental data were assessed via the root mean squared prediction error (**RMSPE**) as described by Ellis et al. (2010). The RMSPE and means were calculated over time until 90% of the total recovery was achieved. Values beyond this point were close to baseline and contributed disproportionately to the mean. The RMSPE, expressed as percentage of the observed mean, gives an estimate of the overall prediction error. The RMSPE was decomposed into errors due to overall bias, errors due to deviation of the regression slope from unity, and errors due to random variation (Bibby and Toutenburg, 1977).

### Fecal Excretion Curves of Co, Yb, and Cr from Mordanted Roughages

After the 7-d period in the CRC, the calves were transported back to the barn, where they were subsequently housed individually in pens of 1.5 by 3.0 m (equipped with wooded-slatted floors) and fitted with harnesses to which plastic bags were attached for the quantitative collection of feces. Feces was collected at defined intervals for 4 d after oral administration of indigestible markers (Supplemental Figure S1).

***Feeding.*** During this measurement period for fecal excretion curves, calves were allowed ad libitum access to water provided via drinking nipples. The provision of MR was identical to the adaptation period as well as the measurement period in the CRC. The SF was provided as a mixture of concentrates and roughages directly after the MR meal, in a long feed trough in front of the pen. Refusals of the SF were removed twice daily and weighed (i.e., just before the next MR meal).

***Measurements.*** Indigestible markers were used to estimate passage kinetics of roughage, concentrates, and MR through the digestive tract. The dose of the indigestible markers was determined using simulations before the study. We made assumptions for the level of DMI and fractional passage rate, and subsequently simulated the fecal excretion curves of the indigestible markers. An important criteria was that the concentration of the marker in the feces had to be detectable. Passage kinetics were assessed by measuring the concentration of external markers in fecal samples collected at defined intervals for 4 d (i.e., d 11 to 15; every 3 h during the first 24 h and every 4 h during the remaining 72 h). Each marker was provided as a pulse dose in a proportionate mixture with the other SF components corresponding to the treatment. The duration of the fecal collection period as well as the moment at which the pulse dose markers were provided relative to the fecal collection period, were based on the studies of Berends et al. (2015) and Gilbert et al. (2017). To assess passage kinetics of roughage, chromium-mordanted (**Cr-NDF**) straw (i.e., for L-S, M-S, and H-S treatments) and Cr-NDF hay (i.e., for H-H treatment) were prepared as described by Udén et al. (1980) with the same chopped straw and long hay as included in the SF (i.e., particular length roughages maintained). For the L-S treatment, the complete quantity of straw of the meal was replaced with Cr-NDF (i.e., on average 39 g/calf), whereas for the other treatments 60 g of Cr-NDF/calf was provided. The Cr-NDF was provided with the meal 24 h before the first fecal sample was collected (i.e., d 10 at 0730 h) and the calves were allowed 240 min to consume the Cr-NDF dosed meal. The completion of consumption of the Cr-NDF pulse dose was monitored hourly. If present after 240 min, the residual of the Cr-NDF containing meal was removed and weighed to determine intake. In total, 38% of the calves consumed the pulse dose containing Cr-NDF within the first 120 min and 75% of the calves consumed the pulse dose containing Cr-NDF within the total timeframe of 240 min. To assess passage kinetics of concentrates, 2 g of ytterbium(II)oxide ($Yb_2O_3$) was added to the concentrate of 1 SF portion (i.e., half of daily SF supply) 14 h before the first fecal sample was collected (i.e., d 10 at 1530 h). Calves were allowed 120 min to consume the meal, and in total, 90% of the calves consumed the pulse dose with $Yb_2O_3$ within 120 min. To assess passage kinetics of MR, 9 g of cobalt(II)EDTA (**Co-EDTA**; Udén et al., 1980) was dissolved into the last MR meal just before the collection of the fecal samples

started (i.e., d 11 at 0730 h). Calves were allowed 15 min to consume the meal and all calves consumed the pulse dose with Co-EDTA within 15 min.

***Calculations.*** The recovery of the indigestible markers Co, Yb, and Cr was calculated by measuring the recovery of the markers of each fecal sample separately as a percent (%) of the dose administered. Subsequently, the cumulative recovery per calf was calculated. The calculations described above for $^{13}C$ enrichment excess in expired $CO_2$, were also applied to the fecal concentrations of the ingestible markers. We worked with the concentrations of Co, Yb, and Cr on a g/kg air dry matter (**ADM**) feces basis. The generalized Michaelis-Menten equation, as proposed by López et al. (2000), was used to describe the pattern of fecal concentrations of the indigestible markers in time (see equation [1]), with the $y$ variable being the fecal concentrations of indigestible markers (in g/kg of ADM feces) at time $t$ (min). Also $t_{max}$ and $y_{max}$ were calculated, representing the moment that the excretion curve reaches peak concentration (minutes) and the maximum indigestible marker concentration (g/kg of ADM feces), respectively. Typical curves of measured concentrations of Co, Yb, and Cr in feces as well as the curve fitted using the generalized Michaelis-Menten equation are shown in Supplemental Figure S2 (http://dx.doi.org/10.17632/3yg3bpkz4f.1).

In addition, a multicompartmental model to describe the indigestible marker concentration patterns in the feces was applied. The fecal marker excretion technique is based on the principle that the pattern of marker excretion in the feces after an oral pulse dose of the marker reflects the cumulative effect of marker residence time in the various compartments of the digestive tract. Provided a satisfactory mathematical description of the excretion curve can be achieved and the compartment parts identified, the MRT in the rumen can be estimated (Dhanoa et al., 1985). Therefore, the fecal concentration patterns of Cr and Yb following an oral pulse dose were fitted to a multiplicative equation as proposed by Dhanoa et al. (1985):

$$y = A\mathrm{e}^{-C_1\times(t-lt)}\exp\left[-B\mathrm{e}^{-C_2\times(t-lt)}\right], \quad [4]$$

where $y$ represents the fecal marker concentration (g/kg ADM feces) at time = $t$ (h); $lt$ = lag time (h); $B$ refers to the model-derived number of mixing compartments; $C_1$ and $C_2$ resemble the fractional rate constants for the 2 compartments in the digestive tract with the longest retention times (most likely the reticulorumen and large intestines, respectively); $A$ forms a scalable parameter dependent on the $B$, $C_1$, and $C_2$. The curve fitting was conducted using the nonlinear least squares regression procedure PROC NLIN (version 9.4, SAS Institute Inc.). To aid with fitting, datapoints where the fecal marker concentration of Cr (for roughage) and Yb (for concentrates) were assumed to be 0 g/kg ADM were added for time 0, 6, and 12 h. This was based on the reported total-tract retention of 12.2 h for MR by Gilbert et al. (2017) with 117 veal calves with an age of 7 to 8 wk at the time of sampling, and the assumption that the total-tract retention for both roughage and concentrate will be longer than that of MR.

Parameter estimates generated from the fitting of the multicompartmental model were used to calculate transit time (**TT**; the moment of first appearance of the marker in the feces, in h), mean retention time (in h) of slowest compartment (**CMRT1**), mean retention time (in h) of the second slowest compartment (**CMRT2**), and total mean retention time (**TMRT**; in h), as described by Dhanoa et al. (1985):

$$\mathrm{TT} = \sum_{i=3}^{N-1}\frac{1}{C_2+(i-2)\times(C_2-C_1)}, \quad [5]$$

$$\mathrm{CMRT1} = \frac{1}{C_1},\ \mathrm{CMRT2} = \frac{1}{C_2}, \quad [6]$$

$$\mathrm{TMRT} = \frac{1}{C_1}+\frac{1}{C_2}+\sum_{i=3}^{N-1}\frac{1}{C_2+(i-2)\times(C_2-C_1)}. \quad [7]$$

## Analytical Procedures

Samples of MR, concentrates, straw, and hay were collected weekly, pooled over the experimental period, and stored at −20°C pending analysis. Also, the fecal samples collected after oral administration of indigestible markers were stored immediately at −20°C pending analysis. Samples of the feed components and feces were thawed at room temperature, oven-dried at 60°C until a constant weight was reached, ground to pass through a 1-mm screen using a cross beater mill (Peppink 100AN), and subsequently analyzed. The feed components were analyzed for DM, N, gross energy (**GE**), starch, NDF, ADF, and phosphorus, whereas the fecal samples and Cr mordants were analyzed for DM, and concentrations of Cr (from Cr-NDF; analyzed concentrations of 39.7 g of Cr/kg of DM for Cr-NDF straw and 38.1 g of Cr/kg of DM for Cr-NDF hay), Yb (from $Yb_2O_3$), and Co (from Co-EDTA).

Dry matter content was determined by drying to a constant weight according to ISO 6496 (ISO, 1998b). Bomb calorimetry (ISO 9831; ISO, 1998c) was used to

determine GE. Crude protein was calculated as N × 6.25, where N was determined using the Dumas principle (ISO 16634–1; ISO, 2008). Starch was determined enzymatically according to ISO 15914 (ISO, 2004). Both NDF and ADF were analyzed according to the methods described by van Soest et al. (1991). The phosphorus content was determined by incinerating a test portion at 550°C and digesting with concentrated hydrochloric acid. Molybdovanadate reagent was added, resulting in a characteristic yellow color after reacting with phosphorus, which was subsequently measured spectrophotometrically at 430 nm (ISO 6491; ISO, 1998a).

The DM, ash, and calcium content of the MR and concentrates were determined by Denkavit Nederland B.V. Dry matter content was determined by drying to a constant weight according to ISO 6496 (ISO, 1998b). Ash content was determined according to ISO 5984 (ISO, 2003) and calcium content was determined according to ISO 15510 (ISO, 2017). The Cr, Co, and Yb concentrations in the feces samples were determined by NutriControl using inductive coupled plasma–optical emission spectrometry after wet destruction (Berends et al., 2015). The $^{13}$C enrichment of the chopped wheat straw and long grass hay were analyzed by combustion-isotope ratio MS by using a Finnigan Delta C continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Thermo Electron Corporation).

### Statistical Analyses

The data set for the indigestible marker part of this study contained 39 observations (i.e., 39 individual calf observations with calf as experimental unit), whereas the data set for the $^{13}$C tracer part of this study contained 20 observations (i.e., 20 observations of pair-housed calves in the CRC with pair of calves as experimental unit). Due to health problems not related to the dietary treatments or experimental conditions (e.g., lung infections), 2 calves were excluded from the trial. Additionally, 1 calf appeared to be a ruminal drinker and was excluded from the trial (i.e., MR is generally assumed to bypass the rumen by means of the esophageal groove reflex; ruminal drinking refers to MR leaking into the rumen due to failure of the esophageal groove reflex or backflow of MR from the abomasum; Berends et al., 2015). Furthermore, 2 calves, who formed a pair in the CRC, were excluded from the $^{13}$C tracer part of this study because their MR refusals exceeded 10% of the MR provided while housed in the CRC. Also, due to a fire in the oven upon drying the fecal samples, results of 6 calves of the first batch could not be used for data analyses.

Dependent variables were analyzed by mixed model analysis in PROC MIXED (SAS 9.4, SAS Institute Inc.) with calf as experimental unit for the indigestible marker part of the study and the pair of calves housed in the CRC as experimental unit for the $^{13}$C tracer part of the study. Dietary treatment (i.e., L-S, M-S, H-S, and H-H), batch (i.e., 1 and 2), and group within batch (i.e., 1, 2, and 3) were treated as fixed effects. The covariance structure compound symmetry provided best fit with the lowest overall Akaike's information criterion values and the Kenward-Roger option was used to estimate the denominator degrees of freedom. Differences between treatment means were compared using the least squares means procedure and the Tukey-Kramer method for multiple comparisons when a treatment effect was detected at $P \leq 0.05$. All results are presented as least squares means and their standard error of the mean with significance of effects declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

## RESULTS

### Animal Performance

The DMI of MR, concentrates, and roughages as well as total DMI are consistent with the experimental design (targeted intake levels), and have thus not been statistically evaluated. Consistent with the paired gain feeding strategy, no differences where observed in BW or ADG (Table 3). The feed conversion ratio tended ($P = 0.064$) to be affected by the dietary treatments, tending to be higher for the H-H treatment compared with the L-S treatment.

### Response in $^{13}$C Enrichment Excess in Exhaled $CO_2$ After Ingestion of $^{13}$C Tracers

Despite the fact that the bacterial protein had a considerably lower $^{13}$C enrichment relative to the background breath (1.057 vs. 1.082 atom%, respectively), we did not detect any response in $^{13}$C enrichment excess in expired $CO_2$ (APE, %) after the oral dose of bacterial protein, and thus these data are not further presented.

Treatment means of the time to peak (h) of [1-$^{13}$C] octanoate, corrected for the postabsorptive delay in $^{13}$C exhalation calculated from intravenous infusion of [$^{13}$C] sodium bicarbonate, ranged from 1.77 to 2.54 h, and was not affected by the dietary treatments ($P = 0.854$; Table 4, Figure 1). The maximum $^{13}$C enrichment excess of expired $CO_2$ of [1-$^{13}$C]octanoate ranged from 0.0227 to 0.0304%, and was also not affected by the dietary treatments ($P = 0.344$). The RMSPE of the curve fits for $^{13}$C enrichment excess of expired $CO_2$ from [1-$^{13}$C]octanoate averaged 19.4% ($R^2 = 0.84$), with the majority of the prediction error (92.6%) attributable to random variation. The RMSPE of the curve fits for

**Table 3.** Animal performance of calves (13 to 17 wk of age) fed a solid feed mixture with a 90:10 concentrate to chopped straw ratio [20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] or a solid feed mixture with a 70:30 concentrate to long hay ratio [40 g/kg of metabolic BW per day; treatment high hay (H-H)] with an additional quantity of milk replacer to achieve equal rates of BW gain[1]

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Item | L-S | M-S | H-S | H-H | SEM | *P*-value |
| No. of animals | 9 | 10 | 10 | 10 | | |
| BW (kg) | 134.7 | 138.8 | 135.7 | 137.7 | 2.18 | 0.686 |
| DMI milk replacer (kg/d) | 1.94 | 1.75 | 1.51 | 1.60 | 0.021 | ND[2] |
| DMI roughages (kg/d) | 0.07 | 0.11 | 0.13 | 0.39 | 0.009 | ND |
| DMI concentrates (kg/d) | 0.60 | 0.89 | 1.07 | 0.91 | 0.039 | ND |
| DMI total (kg/d) | 2.60 | 2.74 | 2.70 | 2.91 | 0.052 | ND |
| ADG (kg/d) | 1.44 | 1.52 | 1.31 | 1.29 | 0.119 | 0.261 |
| Feed conversion ratio[3] | 1.85 | 1.91 | 2.14 | 2.62 | 0.219 | 0.064 |

[1]Animal performance was measured over a period of 7 d, identical to the period in which the fecal excretion curves were determined.

[2]ND = not determined; differences in DMI milk replacer, DMI roughages, and DMI concentrates between treatments are due to the design of the treatments. Values represent LSM.

[3]Ratio between DMI total (kg/d) and ADG (kg/d).

$^{13}$C enrichment excess of expired $CO_2$ from [$^{13}$C]sodium bicarbonate averaged 11.8% ($R^2$ = 0.98), with the majority of the prediction error (87.6%) attributable to random variation.

## Fecal Excretion Curves of Indigestible Markers

The results of the fecal excretion curves of the markers Co (for MR), Cr (for roughage), and Yb (for concentrate) are shown in Table 5 as well as Figure 2. The time to peak (h) represents the retention time of the total GIT. Differences in the maximum concentration (g/kg ADM) likely reflect differences in overall digestibility.

***Milk Replacer.*** The time to peak of Co ranged from 10.9 to 13.3 h, and tended to be affected by dietary treatment ($P$ = 0.071; Table 5). The RMSPE of the curve fits for fecal Co from orally dosed Co-EDTA averaged 36.4% ($R^2$ = 0.95), with the majority of the prediction error (84.6%) attributable to random variation. The treatment averages of the maximum concentration of Co (g/kg ADM feces) ranged from 9.52 to 13.63 g/kg of ADM feces and of the recovery (% of Co intake) ranged from 101.1 to 113.4% of intake. The maximum concentration of Co as well as the recovery of Co tended to be affected by dietary treatment ($P$ = 0.054 and $P$ = 0.065, respectively), with both being numerically lower for the H-H treatment (Table 5; Figure 2, upper panel).

***Concentrates.*** Treatment means of the time to peak of Yb ranged from 19.5 to 22.5 h and were not affected by dietary treatment ($P$ = 0.420, Table 5). The RMSPE of the curve fits for fecal Yb from orally dosed

**Table 4.** The time to peak (h), maximum $^{13}$C enrichment excess (atom percentage excess, APE, %), and recovery (%) of [1-$^{13}$C]octanoate after feeding a single dose of [1-$^{13}$C]octanoate of calves (12 to 16 wk of age) fed a solid feed mixture with a 90:10 concentrate to chopped straw ratio [20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] or a solid feed mixture with a 70:30 concentrate to long hay ratio [40 g/kg of metabolic BW per day; treatment high hay (H-H)] with an additional quantity of milk replacer to achieve equal rates of BW gain[1,2]

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Item | L-S | M-S | H-S | H-H | SEM | *P*-value |
| No. of groups | 4 | 5 | 6 | 5 | | |
| Total no. of animals | 8 | 10 | 12 | 10 | | |
| Time to peak (h) | 1.77 | 1.84 | 2.54 | 2.02 | 0.719 | 0.854 |
| Maximum $^{13}$C enrichment excess (APE, %) | 0.0304 | 0.0244 | 0.0227 | 0.0245 | 0.00287 | 0.344 |
| Recovery (%) | 62.3 | 61.1 | 55.9 | 62.7 | 4.11 | 0.585 |

[1]Results were obtained in climate respiration chambers measured over a period of 52 h for [1-$^{13}$C]octanoate and over a 24-h period for [$^{13}$C]sodium bicarbonate. Results are corrected for the delay in exhalation of $^{13}CO_2$ related to postabsorptive events from $^{13}$C enrichment excess of expired $CO_2$ after injection of a bolus [$^{13}$C]sodium bicarbonate.

[2]The time to peak (h) of [$^{13}$C]sodium bicarbonate was 1.34, 1.22, 1.37, and 1.44 h for treatments L-S, M-S, H-S, and H-H, respectively. The maximum $^{13}$C enrichment excess (APE, %) of [$^{13}$C]sodium bicarbonate was 0.049, 0.046, 0.042, and 0.043% for treatments L-S, M-S, H-S, and H-H, respectively. The recovery (%) of [$^{13}$C]sodium bicarbonate was 59.8, 58.6, 51.9, and 60.4% for treatments L-S, M-S, H-S, and H-H, respectively.

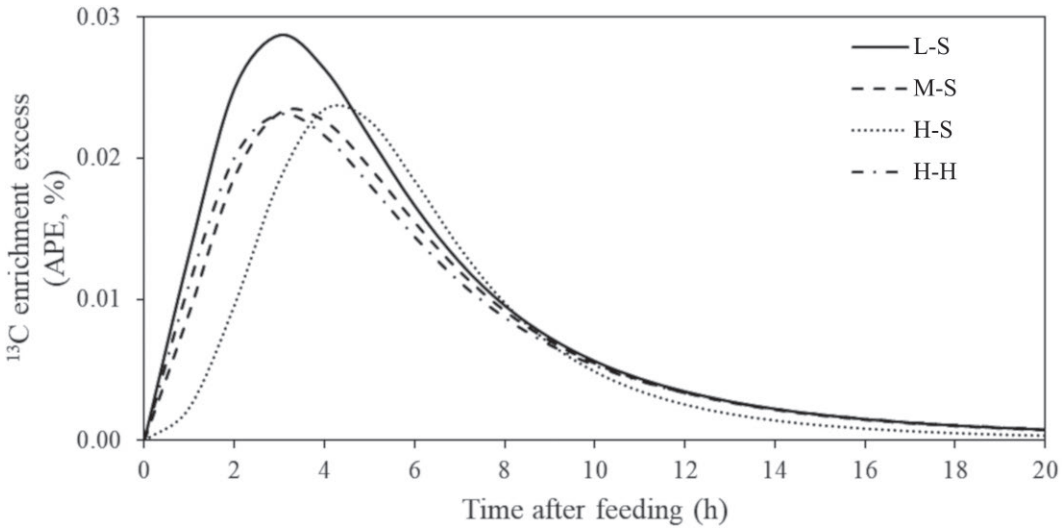


**Figure 1.** $^{13}$C enrichment excess (atom percentage excess, APE, %) after feeding a single dose of [1-$^{13}$C]octanoate of calves fed a solid feed mixture with a 90:10 concentrate to chopped straw ratio [20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] or a solid feed mixture with a 70:30 concentrate to long hay ratio (40 g/kg of metabolic BW per day; treatment high hay; H-H) with an additional quantity of milk replacer to achieve equal rates of BW gain. The curves are based on the model of López et al. (2000), using the parameter estimates averaged per treatment, uncorrected for [$^{13}$C]sodium bicarbonate.

$Yb_2O_3$ averaged 18.6% ($R^2$ = 0.81), with the majority of the prediction error (89.7%) attributable to random variation. The lag time [*lt*; time (h) of first appearance of $Yb_2O_3$ in the feces], being either 16.0 h for the L-S and M-S treatment or 15.9 h for the H-S and H-H treatment, was not affected by dietary treatment ($P$ =

**Table 5.** The concentration and recovery of pulse-dosed markers Co, Cr, and Yb in the feces of calves (13 to 17 wk of age) fed a solid feed mixture with a 90:10 concentrate to chopped straw ratio [20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] or a solid feed mixture with a 70:30 concentrate to long hay ratio [40 g/kg of metabolic BW per day; treatment high hay (H-H)] with an additional quantity of milk replacer to achieve equal rates of BW gain[1]

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Item | L-S | M-S | H-S | H-H | SEM | *P*-value |
| No. of animals | 9 | 10 | 10 | 10 | | |
| Milk replacer[2] | | | | | | |
| Time to peak (h) | 13.3 | 13.3 | 12.0 | 10.9 | 0.63 | 0.071 |
| Maximum concentration (g/kg of ADM feces) | 12.50 | 13.63 | 11.26 | 9.52 | 0.925 | 0.054 |
| Recovery (% of intake) | 113.4 | 108.9 | 108.1 | 101.1 | 2.80 | 0.065 |
| Concentrates[3] | | | | | | |
| Lag time (h) | 16.0 | 16.0 | 15.9 | 15.9 | 0.05 | 0.481 |
| Time to peak (h; including lag time) | 21.0 | 22.4 | 22.5 | 19.5 | 1.33 | 0.420 |
| Maximum concentration (g/kg of ADM feces) | 1.93[a] | 1.87[a] | 1.17[b] | 1.35[b] | 0.122 | <0.001 |
| Recovery (% of intake) | 82.0 | 81.1 | 68.9 | 80.2 | 4.57 | 0.243 |
| Roughage[4] | | | | | | |
| Lag time (h) | 23.6 | 23.6 | 23.8 | 23.9 | 0.23 | 0.811 |
| Time to peak (h; including lag time) | 64.3[a] | 59.5[a] | 53.4[ab] | 36.8[b] | 4.55 | 0.004 |
| Maximum concentration[5] (g/kg of ADM feces) | 1.42[a] | 1.10[ab] | 0.82[b] | 0.93[b] | 0.088 | <0.001 |
| Recovery (% of intake) | 69.6[a] | 69.3[a] | 71.4[a] | 100.1[b] | 6.58 | 0.009 |

[a,b]Values with different superscripts indicate a significant difference between the dietary treatments.

[1]The results were obtained by measuring the concentration of external markers in fecal samples collected at defined intervals for 4 d (every 3 h during the first 24 h and every 4 h during the remaining 72 h). The markers to assess passage kinetics of roughage, concentrate, and milk replacer were provided 24, 14, and 0 h, respectively, before the first fecal sample was collected.

[2]Co-EDTA was added to the milk replacer as marker.

[3]Ytterbium(III)oxide ($Yb_2O_3$) was added to the concentrates as marker.

[4]Chromium-mordanted chopped straw (for the L-S, M-S, and H-S treatments) or long hay (for the H-H treatment).

[5]The maximum concentration of Cr [g/kg of air dry matter (ADM) feces] for the L-S treatment was based on the fecal concentration of Cr corrected, by multiplying with (39/60), for the lower pulse dose Cr-NDF provided (i.e., Cr-NDF was on average 39 g/calf for treatment L-S and on average 60 g of Cr-NDF/calf for the other treatments).

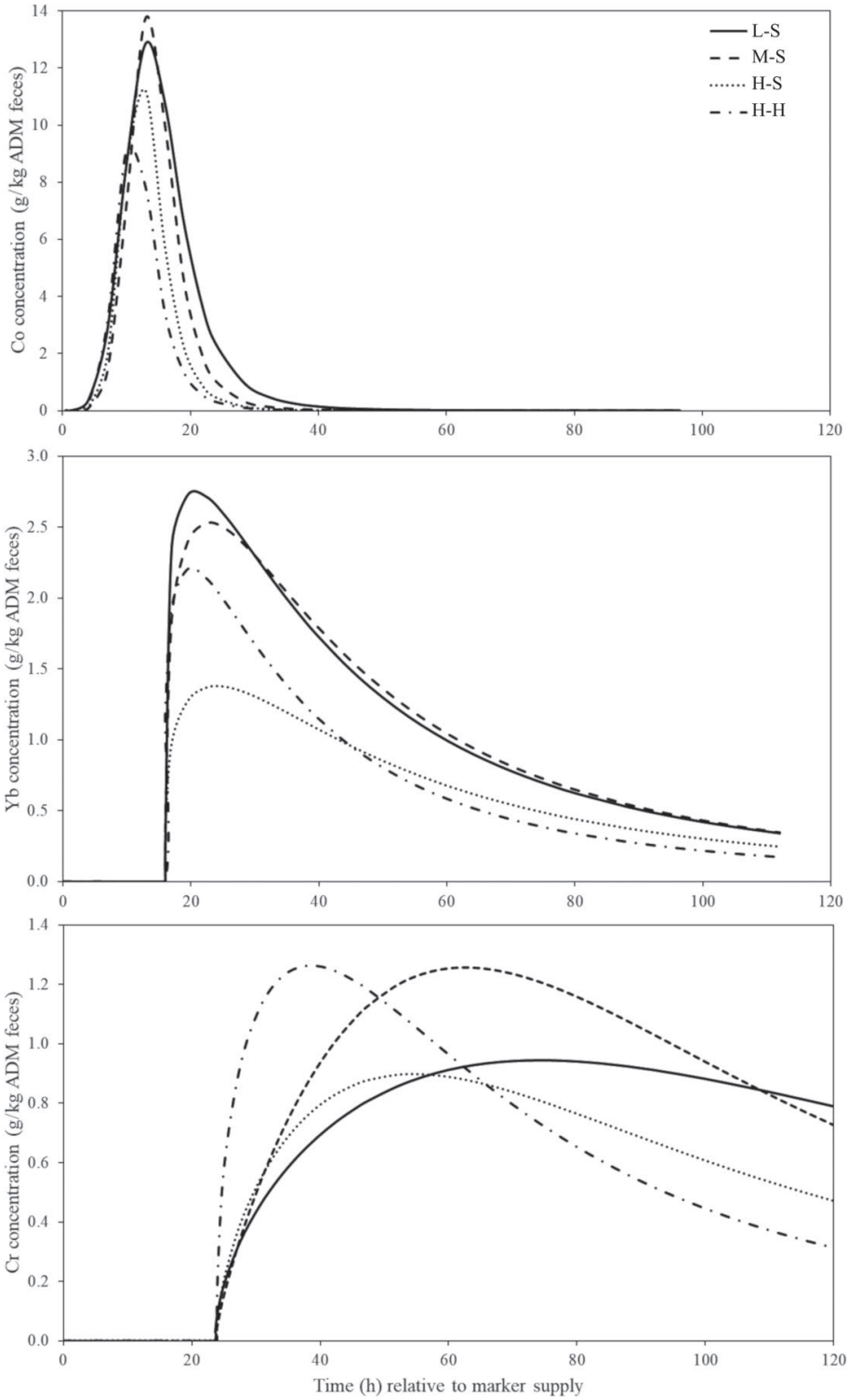


**Figure 2.** Excretion curves of Co (upper panel), Yb (middle panel), and Cr (lower panel) of calves fed a solid feed mixture with a 90:10 concentrate to chopped straw ratio [20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] or a solid feed mixture with a 70:30 concentrate to long hay ratio (40 g/kg of metabolic BW per day; treatment high hay; H-H) with an additional quantity of milk replacer to achieve equal rates of BW gain. Cobalt was pulse-dosed as Co-EDTA with milk replacer directly before sampling feces; Yb was pulse-dosed as $Yb_2O_3$ with concentrates at 14 h before sampling; Cr was pulse-dosed as Cr-NDF with chopped straw (for treatments L-S, M-S, and H-S) or long hay (for treatment H-H) 24 h before sampling. The excretion curves are based on the model of López et al. (2000), using the parameter estimates averaged per treatment. Results of the statistical analyses of these parameters are presented in Table 5.

**Table 6.** Passage kinetics and residence time of pulse-dosed markers Cr and Yb in calves (13 to 17 wk of age) fed a solid feed mixture with a 90:10 concentrate to chopped straw ratio [20, 30, and 40 g/kg of metabolic BW per day for treatments low straw (L-S), middle straw (M-S), and high straw (H-S), respectively] or a solid feed mixture with a 70:30 concentrate to long hay ratio [40 g/kg of metabolic BW per day; treatment high hay (H-H)] with an additional quantity of milk replacer to achieve equal rates of BW gain[1]

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Item | L-S | M-S | H-S | H-H | SEM | *P*-value |
| Yb, concentrates | | | | | | |
| No. of animals | 9 | 10 | 10 | 10 | | |
| Mean retention time slowest compartment GIT[2] (h) | 42 | 40 | 47 | 27 | 10.0 | 0.965 |
| Fractional passage rate slowest compartment GIT ($h^{-1}$) | 0.027 | 0.029 | 0.034 | 0.037 | 0.0042 | 0.343 |
| Mean retention time second slowest compartment GIT (h) | 0.59 | 0.50 | 1.10 | 0.74 | 0.337 | 0.330 |
| Fractional passage rate second slowest compartment GIT ($h^{-1}$) | 3.29 | 4.44 | 4.90 | 2.43 | 1.509 | 0.645 |
| Transit time[3] (h) | 15.7 | 16.0 | 14.5 | 14.8 | 0.63 | 0.069 |
| Total mean retention time (h) | 58 | 57 | 63 | 43 | 8.9 | 0.422 |
| Fractional passage rate total GIT ($h^{-1}$) | 0.018 | 0.019 | 0.021 | 0.023 | 0.0019 | 0.281 |
| Cr (roughages) | | | | | | |
| No. of animals | 7 | 10 | 9 | 10 | | |
| Mean retention time slowest compartment GIT (h) | 111$^{ab}$ | 128$^{a}$ | 91$^{ab}$ | 59$^{b}$ | 20.7 | 0.044 |
| Fractional passage rate slowest compartment GIT ($h^{-1}$) | 0.013$^{ab}$ | 0.009$^{a}$ | 0.014$^{ab}$ | 0.020$^{b}$ | 0.0033 | 0.021 |
| Mean retention time second slowest compartment GIT (h) | 17.5$^{a}$ | 10.1$^{ab}$ | 9.8$^{ab}$ | 4.4$^{b}$ | 2.99 | 0.038 |
| Fractional passage rate second slowest compartment GIT ($h^{-1}$) | 0.364 | 0.396 | 0.239 | 0.863 | 0.2490 | 0.125 |
| Transit time (h) | 12.4 | 16.4 | 13.0 | 19.3 | 2.23 | 0.121 |
| Total mean retention time (h) | 140$^{ab}$ | 154$^{a}$ | 114$^{ab}$ | 84$^{b}$ | 19.6 | 0.026 |
| Fractional passage rate total GIT ($h^{-1}$) | 0.009$^{ab}$ | 0.007$^{a}$ | 0.010$^{ab}$ | 0.013$^{a}$ | 0.0013 | 0.002 |

[a,b]Values with different superscripts indicate a significant difference between the dietary treatments.

[1]The results were obtained by measuring the concentration of external markers in fecal samples collected at defined intervals for 4 d (every 3 h during the first 24 h and every 4 h during the remaining 72 h). The markers to assess passage kinetics of roughage and concentrate were provided 24 and 14 h, respectively, before the first fecal sample was collected.

[2]GIT = gastrointestinal tract.

[3]Transit time: the moment of first appearance of the marker in the feces.

0.481). Also, the recovery (% of $Yb_2O_3$ intake), which ranged between 68.9 and 82%, was not affected by dietary treatment ($P = 0.243$). The maximum concentration of Yb (g/kg of ADM feces) was higher ($P < 0.001$) for both L-S and M-S (1.93 and 1.87 g/kg of ADM feces, respectively) compared with both H-S and H-H (1.17 and 1.35 g/kg of ADM feces, respectively; Table 5; Figure 2, middle panel).

***Roughage.*** The time to peak (h) of Cr was lower ($P = 0.004$; 36.8 h) for the H-H treatment compared with both L-S and M-S (64.3 and 59.5 h, respectively; Table 5). The RMSPE of the curve fits for fecal Cr from orally dosed Cr-NDF averaged 22.0% ($R^2 = 0.66$), with the majority of the prediction error (82.5%) attributable to random variation. Also, the recovery of Cr (% of Cr intake) was higher ($P = 0.009$; 100.1%) for the H-H treatment compared with the other 3 straw containing treatments L-S, M-S, and H-S (69.6, 69.3, and 71.4%, respectively; Table 5; Figure 2, lower panel). The maximum concentration of Cr (g/kg of ADM feces) was higher ($P < 0.001$) for L-S (1.41 g/kg of ADM feces; after correction for the lower dose Cr-NDF provided; Table 5) compared with treatments H-S (0.82 g/kg ADM feces) and H-H (0.93 g/kg ADM feces). The lag time [*lt*; time (h) of first appearance of Cr in the feces], which ranged from 23.6 to 23.9 h, was not affected by dietary treatment ($P = 0.881$).

## Multicompartmental Model

The rumen is the compartment of the digestive tract with the longest MRT. Based on the multicompartmental model of Dhanoa et al. (1985), the MRT of the slowest compartment can be estimated. This is of interest for the SF components only (i.e., concentrates and roughage) because these enter the rumen, whereas MR bypasses the rumen via the esophageal groove. The multicompartmental model could be successfully fitted, with the exception of 3 calves (2 calves receiving the L-S treatment and 1 calf receiving the H-S treatment) for the marker Cr. The RMSPE of the curve fits for fecal Yb and Cr averaged 18.8% ($R^2 = 0.91$) and 18.8% ($R^2 = 0.71$), respectively, with the majority of the prediction error (96.3% and 96.2%, respectively)

attributable to random variation. For the concentrates, none of the parameters were affected by dietary treatment (Table 6). For the roughage, both CMRT1 and TMRT for M-S were higher ($P < 0.044$; Table 6) and, subsequently, both fractional passage rate of the rumen and the total GIT for M-S were lower ($P < 0.026$) than for H-H. Additionally, CMRT2 was higher ($P = 0.038$) for L-S than for H-H.

## DISCUSSION

The primary objectives of this study were to determine the effect of SF level and type of roughage on passage kinetics of MR, concentrates, and roughage in veal calves, and to compare passage kinetics determined using the fecal excretion curves of indigestible markers as well as a noninvasive $^{13}C$ tracer breath test approach. Studying the effect of SF level and type of roughage on animal performance was thus not a primary objective. The results showed that the animal performance was in line with the paired gain strategy applied and hence was unaffected by the dietary treatments. The tendency for a difference in feed conversion ratio reflect expected differences in the intake of fermentable DM.

### Response in $^{13}C$ Enrichment Excess in Exhaled $CO_2$ After Ingestion of $^{13}C$ Tracers

The bacterial protein had a considerably lower $^{13}C$ enrichment relative to the background breath (1.057 vs. 1.082 atom%, respectively), but no response in $^{13}C$ enrichment excess in expired $CO_2$ was detected after the oral pulse dose of bacterial protein. The lack of response is most likely related with the low compliance observed, as only 50% of all calves consumed the pulse dose of the bacterial protein within 240 min (i.e., 1 pair received the L-S diet, 2 pairs received the M-S diet, 2 pairs received the H-S diet, and 5 pairs received the H-H diet). The calves that did not consume the bacterial protein within the timeframe offered had a residual of, on average, 80.9 ± 10.66% of the total amount of pulse dose provided. The low compliance may have been caused by the low palatability of the bacterial protein.

It was expected that [1-$^{13}C$]octanoate would be suitable for passage rate kinetic measurements via the $^{13}C$ breath test because of its potential to pass the rumen unchanged. First, according to Hird et al. (1966), there is little transport of the unmetabolized fatty acid octanoate across the rumen epithelium. Second, fatty acids are not fermented in the rumen (Beauchemin et al., 2008). Third, octanoate is a SFA, which does not enter the biohydrogenation pathway (Bauman and Griinari, 2003). Our results, however, suggest otherwise. There appears to be no relation between the time to peak of concentrates determined using the $^{13}C$ tracers in breath and the time to peak of concentrates determined using the fecal concentration of the marker $Yb_2O_3$ ($R = 0.135$ and $P = 0.433$). In addition, the $^{13}C$ breath test yielded unrealistic estimates of oro-duodenal TT, where the time to peak ranged between 1.77 and 2.54 h when using the $^{13}C$ tracers in breath and the time to peak ranged between 19.5 and 22.5 h when using the fecal excretion of $Yb_2O_3$ (in agreement with Ahvenjärvi et al., 2010), a difference of almost 20 h. Possible explanations for this discrepancy are the absorption of octanoate through the rumen wall or the exchange of the carboxyl-C by microbial metabolism (i.e., the $^{13}C$ carboxyl group of [1-$^{13}C$]octanoate was either detached from [1-$^{13}C$]octanoate or replaced by another carboxyl group in the rumen). It appears that the $^{13}C$ breath test can be used in nonruminant species (e.g., mice, pigs, and humans) to determine, among others, gastric emptying of liquids and solids (McCue and Welch, 2016), whereas we hypothesize, based upon our data, that microbial metabolism in the rumen prevents $^{13}C$ octanoate to be used as a suitable marker to quantify oro-duodenal TT.

### Fecal Excretion Curves of Indigestible Markers

For MR, the time to peak represents the abomasum, small intestine, and the large intestine, as MR is assumed to bypass the rumen by means of the esophageal groove reflex (Berends et al., 2015). For concentrates and roughage, the time to peak includes the rumen as well. The time to peak of MR in the current study averaged (taken all dietary treatments into account) 12.4 h. This is close to the total-tract retention time of 12.2 h for MR reported by Gilbert et al. (2017), a study involving 117 veal calves with an age of 7 to 8 wk at the time of sampling. As estimates by Gilbert et al. (2017) were based on visual scoring of colored feces after administration of an indigestible colored marker via the MR, the difference between the first appearance of the green color in the feces and the time to peak should be added.

In the present study, the MR was whey based, which is assumed not to clot in the abomasum. If MR would have been casein based, a clot would have formed in the abomasum with constant protein supply to the intestines. Hence, in practice there may be a difference in passage rate kinetics between whey-based versus casein-based MR. However, using an indigestible marker such as Co-EDTA, as in the present study, would not be able to discriminate this difference because the marker would not clot in the abomasum and just flow, identical to whey, to the intestines. Only a fat-soluble marker would be able to differentiate this difference in

passage rate kinetics between whey-based and casein-based MR.

The volume (in mL) of the abomasum is relatively constant in adult ruminants, but not in calves where the volume of the abomasum enlarges after ingestion of a liquid meal and that volume increases with meal size (Burgstaller et al., 2017). Also, abomasal emptying in calves is influenced by several factors, including the volume of the meal ingested (Burgstaller et al., 2017). Bell and Razig (1973) demonstrated that in solely milk-fed calves, the abomasal emptying rate is inversely related to meal volumes. This is in agreement with the result of the current study, where the time to peak tended to take place earlier (indicating a shorter TMRT) with decreasing level of MR. The decreasing level of MR in the current study, however, coincided with an increasing level of SF. To the best of our knowledge no study has investigated the effect of different SF levels nor the potential interaction between MR and SF on abomasal emptying in calves. Assuming that solids and fluid travel together from the small intestines onward, the tendency for a decreased time to peak for MR with the H-H treatment may also have been caused by a reduction in the colon MRT, which is supported by the reduced MRT of the second slowest compartment of the GIT (likely the intestines) for the H-H treatment.

The time to peak for concentrates, measured using $Yb_2O_3$, was not affected by dietary treatment and was (on average) 21.4 h. This is within the range reported by Ahvenjärvi et al. (2010; a TMRT of 24.7 h for rapeseed meal in dairy cows fed 40% concentrates, on a DM basis), but considerably lower than Warner et al. (2013; a TMRT of 40.9 to 42.0 h for concentrate in dairy cows fed 25 and 50% concentrates on a DM basis, respectively) and Colucci et al. (1990; a TMRT of 35.0 to 35.9 h for soybean meal in dairy cows fed 3 roughage to concentrate ratios). The comparison between the results of the current study for veal calves with values reported in literature for dairy cows should be interpreted with caution because of differences in feed intake level and rumen development. The level of feed intake is about 2 to 2.5 times maintenance for calves and up to 4 times maintenance in dairy cows. Additionally, the rumen of adult cattle is fully developed, resulting in improved utilization efficiency. Rumen development is, however, a long-term process and is related to the age of the calves as well as their cumulative feed intake. Veal calves, which are typically fed with MR (similar to the present study), gain a relatively large proportion of their ME from MR. Hence, their relatively low intake of SF potentially results in a slower rumen development process as VFA, end products of microbial fermentation of SF, are required for papillae growth and rapid rumen development (Flatt et al., 1958). Despite working with different types concentrates for the straw-based diets and the hay-based diet, the time to peak of the concentrates was not affected, most likely because the time to peak for concentrates depends on many factors, including physical feed characteristics (e.g., particle size; Poppi et al., 1980), animal-related factors (e.g., feed intake level relative to BW, body size, or gut volume; Colucci et al., 1990), and diet-related characteristics (e.g., concentrate proportion in the diet; Colucci et al., 1990).

The time to peak for chopped straw was (on average) 59.1 h and for long hay 36.8 h. These values are within the ranges reported in literature for lactating dairy cattle (e.g., Lund et al., 2006; Warner et al., 2013). Assuming that time to peak results from passage through all compartments in the GIT, hay appears to have a higher passage rate than straw. This is in agreement with the lower CMRT1 and TMRT for H-H compared with the straw-based treatment M-S. The earlier occurrence of the time to peak for hay also explains the higher recovery (% of intake) because with a shorter TMRT more Cr will be recovered in the feces within a fixed timeframe. It should be noted that the H-H treatment differed from the straw-based treatments in 3 ways: (1) the type of roughage (hay vs. straw), particle length (long vs. chopped), and concentrate to roughage ratio (70:30 vs. 90:10). Hence, the difference in time to peak between hay and straw is likely caused by a combination of these 3 factors. Hay is thought to have a beneficial influence on rumen function; due to increased fermentation, hay should lead to better papillae development (Suárez et al., 2007). Additionally, longer roughage particles require chewing and rumination before the particle length is sufficiently reduced to move from the reticulorumen to the abomasum, which improves rumen motility (Morisse et al., 1999).

When considering the time to peak of the roughage, excluding the H-H treatment, there is a numeric decline in the time to peak with increasing SF level. This is to be expected, as increased feed intake in adult ruminants results in an increased passage rate in the rumen. The negative relationship between SF level of the straw-based diets and the time to peak of the roughage in the current study appeared nonsignificant ($R = -0.252$, $P = 0.188$), suggesting that the level of SF level is not related, only numerically, with the passage kinetics of chopped straw. This is contrary to what was observed by Berends et al. (2015), where an increase in SF level (1,170 vs. 3,000 g of DM/d with a 20:80 roughage to concentrate ratio) increased the fractional ruminal passage rate of Cr-mordanted straw from 1.3%/h to 1.5%/h. This increase in fractional ruminal passage rate of Cr-mordanted straw was even greater (1.3 vs. 1.8%/h) with a 50:50 roughage to con-

centrate ratio. Discrepancies between the results of the current study and those from Berends et al. (2015) can be explained with several factors. First, we used the fecal excretion curve of Cr-NDF mordanted roughage, whereas Berends et al. (2015) slaughtered the calves 48 h after a pulse dose of Cr-NDF mordanted straw was provided and measured the recovery of that external marker from 4 compartments of the GIT; reticulorumen, abomasum, small intestines, and large intestines. Second, the level of SF of the current study was slightly lower than that of Berends et al., (2015), where the SF level was approximately 24 g/kg of $BW^{0.75}$ for the low SF treatments and 50 g/kg of $BW^{0.75}$ for the high SF treatments (based on calculations of Berends et al., 2014, 2015). Third, the roughage fraction in the SF was lower in the current study (90:10 concentrate to roughage ratio) compared with Berends et al. (2015; 80:20 as well as 50:50 concentrate to roughage ratio). In particular, the lower roughage fraction in the SF might be related to the lack of relationship found in the current study. When the concentrate to roughage ratio is different than 90:10, for example 80:20 or 70:30, resulting in a higher fraction of roughage in the SF, effects of SF level on passage kinetics of roughage might be found. This is especially the case when the roughage stimulates rumination and rumen motility, which appears to be true for the H-H treatment.

### *Multicompartmental Model*

For both concentrates and roughage, the reticulorumen represents the compartment with the longest retention time in the digestive tract (Warner et al., 2013), where the retention time of concentrate in the rumen is expected to be considerably less than that of roughage. This is also observed in the current study, where the average MRT of the slowest compartment (i.e., the rumen; CMRT1) was shorter for concentrates (39.6 h) than that of straw (110.0 h) and hay (59.2 h). This pattern is in agreement with the time to peak results of the generalized Michaelis-Menten equation, and also in terms of the difference observed between straw and hay, suggesting that hay has a faster passage rate than straw. Although the results of the multicompartmental model are generally in agreement with the results of the generalized Michaelis-Menten equation, the absolute values are considerably different, which can be explained by the shape of the fecal excretion curves. The multicompartmental model estimates the TMRT based on the complete curve (including both the ascending and descending part of the curve), whereas the time to peak of the generalized Michaelis-Menten equation represents the moment that the excretion curve reaches peak concentration. The time to peak can represent the TMRT accurately, but only when the fecal excretion curve is symmetric. When the fecal excretion curve is asymmetric with a steep ascending part and a slowly descending part (as it is for both concentrates and roughage; Supplemental Figure S2, middle and lower panel), the time to peak occurred relatively soon after the marker was provided (i.e., left side of the fecal excretion curve) and subsequently does not represent TMRT satisfactorily. In such cases, it is better to compare the TT of the multicompartmental model (Table 6) with the time to peak of the generalized Michaelis-Menten equation (Table 5).

The ruminal fractional passage rates of concentrates and straw are considerably lower than the ones reported in literature when considering differences in BW, rumen volume, and SF intake. For dairy calves around weaning, Hart and Polan (1984) reported values between 0.112 and 660/h for particulate digesta, and Lallès and Poncet (1990) reported on average 4.3 and 5.5%/h for hay and concentrates, respectively). Also, Berends et al. (2015) reported higher ruminal fractional passage rates for veal calves receiving 24 to 50 g of SF/kg of $BW^{0.75}$ and values greater than 3.2 and 1.3%/h for concentrates and straw, respectively. For adult ruminants fed only SF, Colucci et al. (1990) reported values between 2.69 and 8.32%/h for soybean meal, and Warner et al. (2013) reported values larger than 0.037/h for corn silage. This could be due to the relatively low SF intake in the current study where the calves still obtained the majority of their ME intake from MR. This illustrates that it is essential to determine the passage kinetics of ration components when ruminants are in different life stages as well when ruminants are fed different types of rations, and that one cannot simply extrapolate results obtained from a particular situation to another.

## CONCLUSIONS

We conclude that [1-$^{13}$C]octanoate cannot serve as a measure for oro-duodenal transit of concentrates in calves fed SF. Based on the fecal excretion curves, it can be concluded that the total-tract retention of MR was 12.4 h, and the passage kinetics of MR was not affected by the level or type of SF. The total-tract retention of concentrates was shorter (21.4 h) than that of both straw (59.1 h) and hay (36.8 h), and was not affected by the level or type of SF. Also the MRT of the rumen was shorter for concentrates (39.6 h) than for straw (110.0 h) and hay (59.2 h). The passage of roughage was affected by level and type of SF, where long hay increased time to peak by 22.3 h and decreased ruminal MRT by 50.8 h relative to chopped straw, indicating that the passage rate of long hay is faster than that of chopped straw.

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