



Changes in ruminal volatile fatty acid production and absorption rate during the dry period and early lactation as affected by rate of increase of concentrate allowance

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ABSTRACT

The aim of the present experiment was to study changes in volatile fatty acid (VFA) production using an isotope dilution technique, and changes in VFA fractional absorption rate (k_a VFA) using a buffer incubation technique (BIT) during the dry period and early lactation, as affected by the postpartum (pp) rate of increase of concentrate allowance. The current results are complementary to previously reported changes on rumen papillae morphology from the same experiment. From 50 d antepartum to 80 d pp, VFA production rate was measured 5 times and k_a VFA was measured 10 times in 12 rumen-cannulated Holstein Friesian cows. Cows had free access to a mixed ration, consisting of grass and corn silage, soybean meal, and (dry period only) chopped straw. Treatment consisted of either a rapid (RAP; 1.0 kg of DM/d; $n = 6$) or gradual (GRAD; 0.25 kg of DM/d; $n = 6$) increase of concentrate allowance (up to 10.9 kg of DM/d), starting at 4 d pp, aimed at creating a contrast in rumen-fermentable organic matter intake. For the BIT, rumen contents were evacuated, the rumen washed, and a standardized buffer fluid introduced [120 mM VFA, 60% acetic (Ac), 25% propionic (Pr), and 15% butyric (Bu) acid; pH 5.9 and Co-EDTA as fluid passage marker]. For the isotope dilution technique, a pulse-dose of ^{13}C -labeled Ac, Pr, and Bu and Co-EDTA as fluid passage marker was infused. The rate of total VFA production was similar between treatments and was 2 times higher during the lactation (114 mol/d) than the dry period (53 mol/d). Although papillae surface area at 16, 30, and 44 d pp was greater in RAP than GRAD, Bu and Ac production at these days did not differ between RAP and GRAD, whereas at 16 d pp RAP produced more Pr than GRAD. These results provide little support

for the particular proliferative effects of Bu on papillae surface area. Similar to developments in papillae surface area in the dry period and early lactation, the k_a VFA (per hour), measured using the BIT, decreased from 0.45 (Ac), 0.53 (Pr) and 0.56 (Bu) at 50 d antepartum to 0.28 (Ac), 0.34 (Pr) and 0.38 (Bu) at 3 d pp. Thereafter, k_a VFA (/h) rapidly increased up to 0.67 (Ac), 0.79 (Pr), and 0.79 (Bu) at 80 d pp. Although papillae surface area was greater at 16, 30, and 44 d pp in RAP than GRAD, no differences in k_a VFA between RAP and GRAD were observed during these days showing papillae surface area is not the limiting factor for k_a VFA during early pp adaptation.

Key words: transition dairy cow, volatile fatty acid absorption, volatile fatty acid production, rumen papillae, rumen adaptation

INTRODUCTION

After calving, feed intake as well as ration quality generally increases. Consequently, the production of VFA from the microbial fermentation of OM in the rumen increases (Bergman, 1990). This results in a rise in VFA concentration and a decrease in pH. To maintain favorable conditions for rumen fermentation, the removal of VFA from the rumen is an essential process (Penner et al., 2009; Aschenbach et al., 2011; Dijkstra et al., 2012). Removal of VFA occurs by passage with the rumen fluid, but mainly across the rumen epithelium (Gäbel et al., 2002; Aschenbach et al., 2011), either through passive diffusion or facilitated transport (Aschenbach et al., 2009, 2011). The VFA fractional absorption rate is affected by the carbon-chain length of the VFA, rumen fluid pH, VFA concentration, rumen fluid volume (Thorlacius and Lodge, 1973; Dijkstra et al., 1993), epithelial blood flow (Storm et al., 2011), and epithelial capacity for facilitated transport (Penner et al., 2011; Schurmann et al., 2014). The rate of absorption of VFA has been shown to increase as papillae surface area increases (Dirksen et al., 1984; Melo et al.,

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2013), and papillae surface area has been shown to increase when animals were transitioned to a higher plane of nutrition (Dirksen et al., 1984; Liebich et al., 1987; Bannink et al., 2012). However, evidence also exists that, in response to a higher plane of nutrition, VFA absorption rate can increase independent from changes in papillae surface area (Sehested et al., 2000; Etschmann et al., 2009; Schurmann et al., 2014). These adaptive responses, possibly aided by an increased blood flow (Reynolds et al., 2003; Storm et al., 2011), would result in an increase of the VFA absorption capacity after calving. However, our knowledge of the changes in the VFA fractional absorption rate measured in vivo concomitant with nutritionally induced changes in papillae surface area is limited (Dirksen et al., 1984; Martens et al., 2012). Moreover, no such studies cover both the dry period and early lactation.

The aim of the present experiment was to study changes in daily VFA production rate and VFA fractional absorption rate in the rumen of dairy cows from the start of the dry period up to 80 d postpartum (**pp**), and to evaluate the effect of the **pp** rate of increase of concentrate allowance on VFA production and absorption. Previously, in the same experiment, it was shown that the rumen papillae surface area increased with the **pp** increase in intake of rumen-fermentable organic matter (**FOM**), and this surface area increase was faster with a more rapid rate of increase of concentrate allowance (Dieho et al., 2016). Therefore, daily VFA production rate and VFA fractional absorption rate were expected to increase from the dry period to early lactation and were also expected to increase faster with a more rapid rate of increase of concentrate allowance.

MATERIALS AND METHODS

The experimental procedures were approved by the Animal Care and Ethics Committee of Wageningen UR and conducted under the Dutch Law on the Animal Experiment.

Animals, Experimental Design and Housing

Full details on the animals, experimental design, housing, feeding, feed and milk sampling, and feed and milk chemical analyses have been presented by Dieho et al. (2016). Briefly, 12 rumen-cannulated, first-parity Holstein Friesian dairy cows were dried-off and entered the experiment 8 wk before the expected calving date. The experiment had a randomized block design with repeated measurements. Prior to the start of the experiment, cows were blocked by expected calving date and, within each block, cows were randomly assigned to either a rapid or a gradual rate of increase of concen-

trate allowance **pp**. The ruminal fractional absorption rate of VFA was measured using a buffer incubation technique during the pretreatment period at 50, 30, and 10 d antepartum (**ap**) and 3 d **pp**, as well as during the treatment period on 9, 16, 30, 44, 60, and 80 d **pp**. The ruminal VFA production and absorption rates were measured using an isotope dilution technique on 50 and 10 d **ap**, and on 16, 44, and 80 d **pp**. Dry and lactating animals were housed in separate groups in a freestall barn, with lactating cows from both treatments sharing the same pen, and were moved to a tiestall for the duration of the experimental procedures.

Rations and Experimental Treatments

Cows had free access to water and to either a dry period ration or basal lactation ration consisting of grass and corn silage, soybean meal, and (dry period only) chopped wheat straw (Dieho et al., 2016). Rations were freshly mixed and fed once a day throughout the experiment. The dry period ration (603 g of DM/kg) provided 5.3 MJ of NE_L (calculated according to the Dutch NE-system; van Es, 1978) and 455 g of FOM (calculated according to the Dutch DVE/OEB-system; Tamminga et al., 1994) per kilogram of DM. The basal lactation ration (466 g of DM/kg) provided 6.7 MJ of NE_L and 561 g of FOM per kilogram of DM.

From calving up to 3 d **pp**, 0.9 kg of DM/d concentrate was fed; thereafter the concentrate treatment started, in which concentrate allowance increased at either a rapid rate of 1.0 kg of DM/d (**RAP**), or a gradual rate of 0.25 kg of DM/d (**GRAD**). Maximum concentrate allowance was 10.9 kg of DM/d, irrespective of rate of increase, achieving the maximum concentrate allowance at 13 and 43 d **pp** for **RAP** and **GRAD**, respectively. Daily intake of the dry period ration or the basal lactation ration was measured individually using feed-bins (Insentec, Marknesse, the Netherlands), and concentrate was fed using a dispenser (Manus VC5, DeLaval, Steenwijk, the Netherlands) with individual daily allowance available in equal portions over six 4-h periods, recording the quantity actually dispensed (kg/d). During visits cows were shielded from herd-mates and the concentrate was fed as a series of small portions, effectively preventing the possibility of other cows stealing concentrate. The concentrate (892 g of DM/kg) provided 7.4 MJ of NE_L and 682 g of FOM per kilogram of DM.

Measurement of Production and Absorption of VFA

Isotope Dilution Technique. The isotope dilution technique (**IDT**) was used to measure the ruminal VFA production rate based on the fractional clearance

rate of $1\text{-}^{13}\text{C}$ -VFA and the VFA pool size; in addition, the fractional fluid passage rate was measured using Co-EDTA as fluid passage marker (France and Dijkstra, 2005). The measurements were made under the assumption that the rumen fermentation and rumen fill were in steady state (constant fractional passage rate and fractional rate of VFA production and absorption), which was established by feeding small meals once every hour before introduction of the stable isotopes. For each cow the hourly meal size (kg) was calculated from the intake of the dry period ration or the basal lactation ration recorded for the 3 d preceding the measurement. The amount of concentrate to be fed each hour was calculated from the allowance for the day of the measurement (for GRAD: 0.2 kg/h at 16 d pp, and 0.5 kg/h at 44 and 80 d pp; for RAP: 0.5 kg/h at 16, 44, and 80 d pp). At 0600 h, the first meal was fed and a pH logger was introduced in the rumen (15-s reading interval; model T4, Dascor Inc., Escondido, CA; Penner et al., 2006). At 0700 h, and every hour up to 1400 h, subsequent meals were fed.

At 1100 h, a 200-mL rumen fluid sample was taken from the ventral rumen sac followed by the quantitative introduction of 2 L of water at 39°C containing 30 g of Co-EDTA (Udén et al., 1980) and $1\text{-}^{13}\text{C}$ -Na-VFA salts into the ventral rumen sac. The quantity of $1\text{-}^{13}\text{C}$ -Na-VFA introduced varied between the pretreatment and treatment period, and between treatments at 16 d pp, but was calculated to achieve an initial ^{13}C enrichment of 1.30% of total C for the acetic (**Ac**), propionic (**Pr**), and butyric (**Bu**) acid pools. For $1\text{-}^{13}\text{C}$ -Na-acetate 1,000 to 1,400 mg, for $1\text{-}^{13}\text{C}$ -Na-propionate 500 to 1,000 mg, and for $1\text{-}^{13}\text{C}$ -Na-butyrate 400 to 900 mg was introduced (99% enriched, Sigma-Aldrich, Zwijndrecht, the Netherlands). The Co-EDTA and $1\text{-}^{13}\text{C}$ -Na-VFA were dissolved in water just before introduction into the rumen (Sutton et al., 2003). At 1400 h, a 200-mL rumen fluid sample was taken from the ventral rumen sac and a 600- μL aliquot was taken (acidified with 600 μL 5% vol/vol H_3PO_4 , with 19.68 mM isocaproic acid as internal standard) for measurement of the VFA concentration, and a 10-mL aliquot was taken for measurement of the Co concentration. Samples were stored at -20°C pending analysis. Subsequently, the pH logger was retrieved and the buffer incubation technique (**BIT**) procedure was started.

Buffer Incubation Technique. The BIT was used to measure the fractional rate of absorption of Ac, Pr, and Bu as well as fractional fluid passage rate under standardized conditions in an empty washed rumen (Dijkstra et al., 1993). At the start of each measurement, 0 to 1 h after last access to feed, the rumen contents were completely evacuated, stored in an insulated tub to prevent cooling, and weighed. After

evacuating approximately half of the rumen contents, a sample of whole rumen content (stored at -20°C) was taken for determination of DM content. After evacuation, the rumen was washed twice with 10 kg of tap water at 39°C and subsequently with 5.1 kg (5.0 L) of buffer solution. The wash buffer fluid in the rumen was completely removed using the vacuum of a hand-held milking system. A visual and manual inspection of the rumen was performed to confirm complete removal of rumen contents and fluid. Then, 46.7 kg (46.0 L) of buffer solution (39°C, pH 5.9) was introduced into the empty rumen using an electric bailing pump (2,200 L/h, Hozelock, Birmingham, UK). The buffer solution (adapted from Dijkstra et al., 1993) was freshly prepared immediately before each assessment and contained 39 mM Na_2HPO_4 , 70 mM NaHCO_3 , 14 mM NaCl, 4.6 mM KCl, 0.9 mM CaCl_2 , 0.7 mM MgCl_2 , and 20 mM NaOH, with 72 mM Ac, 30 mM Pr, and 18 mM Bu, with 0.17 mM Co-EDTA (0.07 g/L) as a marker of fluid outflow rate. Buffer fluid (200 mL) was sampled immediately before introduction into the rumen and after 60 min of incubation in the rumen. Then the buffer fluid was recovered using the vacuum system, and a visual and manual inspection of the rumen was performed to confirm complete recovery. The recovered buffer fluid was weighed before returning the rumen contents. In the buffer fluid samples, pH was measured immediately after collection and aliquots taken as described earlier. Complete emptying of the rumen before buffer introduction and at the end of the procedure was assumed for the calculations.

Chemical Analyses

The concentration of Ac, Pr, and Bu in the rumen fluid taken during the IDT was measured by gas chromatography as described by van Gastelen et al. (2015). The concentration of Ac, Pr, and Bu in the buffer fluid samples taken during the BIT measurements was also measured by gas chromatography with slight modifications. Briefly, after thawing (at ambient temperature, 20°C) and 5 min of centrifugation ($14,000 \times g$), 0.1 μL of supernatant was injected into the gas chromatograph (Trace GC Ultra, Thermo Scientific, Milan, Italy). The inlet temperature was 260°C with a split ratio of 1:9. Hydrogen gas flowed (25 kPa, constant pressure) through a capillary column (Agilent HP-FFAP, Agilent Tech., Santa Clara, CA; 30 m length, 0.53 mm i.d., 1 μm film) to a flame-ionization detector (260°C). Initial column temperature was 80°C for 1 min, increasing with $20^\circ\text{C}/\text{min}$ to 120°C , and subsequently with $6.1^\circ\text{C}/\text{min}$ to 205°C and held for 2 min. Quantification was based on a reference solution after internal standard correction.

For ^{13}C enrichment in the rumen fluid samples, Ac, Pr, and Bu were separated using a gas chromatograph and subsequently ^{13}C was measured by continuous flow isotope ratio mass spectrometry (Finnigan Delta V Plus, Thermo Scientific, Bremen, Germany). After thawing (at ambient temperature, 20°C) and 5 min of centrifugation ($14,000 \times g$), 1.0 μL of supernatant was injected into the gas chromatograph (Trace GC Ultra, Thermo Scientific, Milan, Italy). The inlet temperature was 225°C with a split ratio of 1:5, with helium carrier gas flow set to 2.5 mL/min (vacuum compensated) through a capillary column (Agilent HP-FFAP, Agilent Tech.; 30 m length, 0.32 mm i.d., 0.25 μm film) with the column outlet fitted to a combustion interface (Thermo Finnigan GC Combustion III, Thermo Scientific, Bremen, Germany) that was connected to the isotope ratio mass spectrometer. Initial column temperature was 110°C for 2 min, increasing $18^\circ\text{C}/\text{min}$ to 200°C , and held for 2 min.

Cobalt was determined in rumen and buffer fluid after thawing (at ambient temperature, 20°C) and 10 min of centrifuging ($5,000 \times g$) using an atomic absorption spectrophotometer (AA240FS, Varian Inc., Palo Alto, CA) at 240.7 nm (Udén et al., 1980).

Calculations and Statistical Analysis

Total VFA concentration was defined as the sum of Ac, Pr, and Bu. Fractional absorption rate (k_a ; per hour) of Ac ($k_a\text{Ac}$), Pr ($k_a\text{Pr}$), and Bu ($k_a\text{Bu}$), fractional fluid passage rate (k_i ; per hour), and net influx of water (I ; L/h) for the BIT were calculated using the following equations (Dijkstra et al., 1993):

$$\text{Co}(t) = \text{Co}(0) \exp(-k_i \times t),$$

$$V(t) = [V(0) - I/k_i] \exp(-k_i \times t) + I/k_i,$$

$$\text{VFA}(t) = \text{VFA}(0) \exp[-(k_i + k_a) \times t],$$

where $\text{Co}(t)$ is the amount of cobalt in the rumen (g), $V(t)$ is rumen fluid volume (L), $\text{VFA}(t)$ is the amount of VFA (Ac, Pr, or Bu) in the rumen (mmol; all after 60 min incubation), and $\text{Co}(0)$, $V(0)$, and $\text{VFA}(0)$ denote the amount of Co, the volume, and the amount of VFA in the rumen immediately after introduction of the solution in the rumen, respectively. For the BIT, pool size of Co and VFA at the start and at the end of the incubation were known, and the equations were solved to calculate k_i , k_a , and I . Average VFA fractional absorption rate, $k_a\text{VFA}$, was calculated as the average of $k_a\text{Ac}$, $k_a\text{Pr}$, and $k_a\text{Bu}$. The $k_a\text{VFA}$ represents the average fractional absorption rate during the 1-h incubation period.

For the IDT, the k_a , k_i , and I were calculated by solving the equations used for the BIT. Fractional clearance rate, k_c (per hour), was calculated by addition of k_i and k_a . For k_a , $\text{VFA}(0)$ was the amount of ^{13}C added to the rumen at $t = 0$ h (for Ac, Pr, and Bu) and $\text{VFA}(t)$ was the amount of ^{13}C at $t = 3$ h (for Ac, Pr, and Bu). For k_i , $\text{Co}(0)$ was the amount of Co added at $t = 0$ h, and $\text{Co}(t)$ the amount of Co at $t = 3$ h. The amount of ^{13}C $\text{VFA}(t)$ at $t = 3$ h (for Ac, Pr, and Bu) was calculated from the rumen fluid volume, the VFA concentration in the rumen fluid, and the ^{13}C -enrichment (corrected for the natural ^{13}C enrichment). The rumen fluid volume, $V(t)$, was calculated from the rumen content mass and the DM content of the mid-rumen samples, and $V(0)$ was assumed to be equal to $V(t)$. Rate of VFA production (for Ac, Pr, and Bu; mol/d) was calculated from k_c and amount of VFA in the rumen. Amount of VFA in the rumen was assumed to be constant during the IDT and calculated from the rumen fluid volume, $V(t)$, and the average VFA concentration in the rumen fluid at $t = 0$ h and $t = 3$ h. Rumen fluid pH data, logged during the steady-state feeding and IDT, was averaged per minute before analysis.

All variables were assumed to be related to sampling day and treatment. Data were analyzed using the MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC) with the model (Littell et al., 2006):

$$Y_{ij} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ij},$$

where variable Y_{ij} was dependent on μ as the average experimental value and fixed main effects of concentrate treatment T_i ($i = \text{RAP, GRAD}$), of sampling day D_j ($j = -50, -30, -10, 3, 9, 16, 30, 44, 60$, and 80 for BIT, and $j = -50, -10, 16, 44$, and 80 for IDT), and fixed interaction $(T \times D)_{ij}$. Errors e_{ij} were assumed to be autocorrelated for repeated observations on the same cow, using a spatial power covariance structure over sampling days (Littell et al., 1998). As described in detail by Dieho et al. (2016), the experimental design could not be treated as a complete factorial design with respect to concentrate treatment and sampling day. Therefore custom CONTRAST statements were constructed for (1) the pretreatment period (sampling d -50 through 3 or -50 and -10 , if applicable), to compare the 2 future treatment groups, thereby evaluating the success of the random treatment allocation [no differences between treatment groups were found at the end of the pretreatment period ($P \geq 0.19$), except for the fractional fluid passage rate during the BIT ($P = 0.03$)]; (2) the treatment main effect, T_i , over the treatment period; (3) the sampling day main effect, D_j , over the total experimental period; and (4) the interac-

tion between treatment and sampling day, $(T \times D)_{ij}$, by testing sampling day by sampling day difference between concentrate treatment groups over the treatment period (Dieho et al., 2016). Specific hypotheses for separating means were tested by formulating CONTRAST and ESTIMATE statements. The strength of estimated squared coefficients of correlation (r^2) was interpreted as weak if $r^2 < 0.50$, moderate if $0.50 \leq r^2 < 0.70$, strong if $0.70 \leq r^2 < 0.90$, and very strong if $0.90 \leq r^2 \leq 1.00$. All results are reported as least squares means with their standard error unless indicated otherwise. Significance of effect was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

RESULTS

One cow (RAP) calved early and missed the sampling day at 10 d ap, otherwise all measurements were completed as planned. One cow was treated for mastitis (from RAP) and 7 for (chronic) endometritis (4 from RAP and 3 from GRAD). All cows fully recovered after veterinary treatment, and all cows completed the experiment. Actual sampling days (mean \pm SD) for the pretreatment period were 46.3 ± 5.1 , 26.5 ± 5.7 , and 8.6 ± 3.4 d ap, and 3.0 ± 0.0 d pp and for the treatment period 9.0 ± 0.0 , 16.1 ± 0.3 , 30.2 ± 0.3 , 44.2 ± 0.4 , 60.0 ± 0.9 , and 79.3 ± 2.2 d pp. Repeated rumen evacuations and buffer incubations during the experiment had no adverse effects on feed intake and milk production.

Feed Intake and Rumen Contents

Feed intake is presented in detail by Dieho et al. (2016). Intake of DM (11.9 ± 0.54 kg/d; Figure 1) and FOM (5.7 ± 0.27 kg/d) did not vary during the pretreatment period, and increased during the treatment period to 24.5 ± 0.54 and 15.0 ± 0.27 kg/d at 80 d pp, respectively. Concentrate treatment and sampling day interacted for FOM intake but not for DMI. Intake of FOM was 21.8% larger for RAP compared with GRAD at 16 d pp and was 12.3 ± 0.27 and 10.1 ± 0.27 kg/d, respectively.

During the pretreatment period, the average mass of the rumen contents was 64 ± 2.8 kg containing 133 ± 3.6 g of DM/kg. During the treatment period, mass of the rumen contents increased to 77 ± 2.8 kg containing 140 ± 3.6 g of DM/kg at 80 d pp ($P < 0.01$). Neither concentrate treatment ($P = 0.79$) nor the interaction between concentrate treatment and sampling day ($P = 0.20$) affected mass of the rumen contents. The DM fraction of the rumen contents tended ($P = 0.06$) to be affected by sampling day, but was not affected ($P = 0.26$) by the interaction between concentrate treat-

ment and sampling day. The DM fraction of the rumen content mass was slightly higher for GRAD compared with RAP (144 vs. 136 ± 3.6 g of DM/kg; $P = 0.02$; data not shown).

VFA Production Rate

Rumen Fluid Composition and pH. The total VFA concentration in rumen fluid (Table 1) was affected by sampling day ($P < 0.01$), increasing from on average 77 mM during the pretreatment period to on average 120 mM during the treatment period. Sampling day affected the fractions of Ac, Pr, and Bu of total VFA ($P < 0.01$), with the fraction of Ac being higher and that of Pr being lower in the pretreatment period (on average 71.9 and 17.8 mol/100 mol, respectively) compared with the treatment period (on average 64.0 and 24.0 mol/100 mol, respectively), which was also reflected by the ratio between the fraction of Ac and Pr. Although total VFA concentration was neither affected by concentrate treatment nor by the interaction between concentrate treatment and sampling day; the fractions of Ac and Pr, but not of Bu, were affected by the interaction ($P \leq 0.02$). At 16 d pp, the fraction of Ac was smaller ($P < 0.01$) and the Pr fraction greater ($P < 0.01$) in RAP compared with GRAD. This was also reflected in the Ac-to-Pr ratio, which was smaller for RAP at 16 d pp compared with GRAD. Rumen fluid pH was affected by sampling day ($P < 0.01$), decreasing

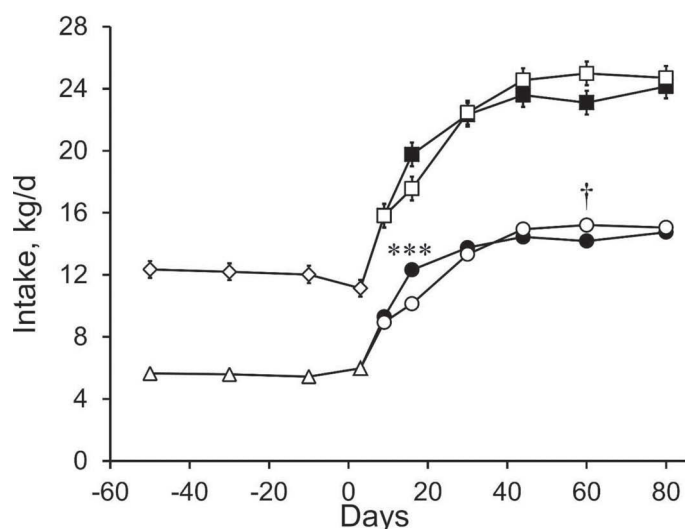


Figure 1. Dry matter intake (kg/d; \blacktriangle , \blacksquare) and fermentable organic matter intake (kg/d; \triangle , \circ) in the pretreatment period (\triangle , $n = 12$), and in the treatment period for a rapid (1.0 kg of DM/d; \blacksquare , $n = 6$) and gradual (0.25 kg of DM/d; \square , $n = 6$) rate of increase of concentrate allowance postpartum. Values represent LSM \pm SE; *** $P < 0.001$, $\dagger P < 0.10$, significance of difference in LSM of RAP or GRAD. Adapted from Dieho et al. (2016).

from pH 6.72 during the pretreatment period to 6.17 during the treatment period. The fraction of time of rumen fluid below pH 6.3 (below pH 6.3, proportion of Ac, Pr, and Bu produced from starch and sugar, and NDF digestion change; Erdman, 1988; Bannink et al., 2008) and pH 5.8 (associated with subacute rumen acidosis; Zebeli et al., 2012) was affected by sampling day ($P \leq 0.03$), with a larger fraction of time below the threshold values during the treatment period than the pretreatment period. Concentrate treatment and sampling day interacted for fraction of time below pH 5.80 ($P = 0.02$). At 16 d pp, fluid pH was 18 min/h below pH 5.80 for RAP compared with 2 min/h for GRAD ($P = 0.01$).

VFA Fractional Clearance Rate and VFA Production Rate. Only the fractional clearance rate of Ac (k_c Ac) was affected by sampling day ($P < 0.01$), decreasing from 0.60 to 0.49/h during the pretreatment period ($P = 0.02$), increasing to 0.60/h at 16 d pp ($P = 0.02$), and tending to further increase to 0.68/h at 80 d pp ($P = 0.09$; Table 2). The k_c Pr and k_c Bu were

not affected by sampling day ($P \geq 0.14$), averaging 0.66 and 0.47/h, respectively. The k_c of Ac, Pr, and Bu were neither affected by the concentrate treatment nor by the interaction between concentrate treatment and sampling day ($P \geq 0.40$).

Total VFA production rate was affected by sampling day ($P < 0.01$) but was neither affected by concentrate treatment nor by the interaction between concentrate treatment and sampling day ($P \geq 0.45$; Table 3). Total VFA production rate increased from on average 53 mol/d during the pretreatment to 103 mol/d at 16 d pp ($P < 0.01$), and increased further to 116 mol/d at 44 d pp (tendency only; $P = 0.06$) and 122 mol/d at 80 d pp ($P < 0.01$), when compared with 16 d pp. Production rates of Ac and Bu were only affected by sampling day, with an increased production rate during the treatment period. However, the production rate of Pr was affected by an interaction between concentrate treatment and sampling day ($P = 0.02$), with a 35% higher production rate on 16 d pp for RAP (34 mol/d) compared with GRAD (22 mol/d; $P < 0.01$). The fractions of

Table 1. Rumen fluid composition and pH from the VFA production rate measurement using the isotope dilution technique for acetic (Ac), propionic (Pr), and butyric acid (Bu) during the pretreatment and treatment period with a rapid (1.0 kg of DM/d; RAP, $n = 6$) and a gradual (0.25 kg of DM/d; GRAD, $n = 6$) rate of increase of concentrate allowance postpartum

Item	Pretreatment period ¹		Treatment period ¹			SE ²	Fixed effects ³		
	d -50	d -10 ⁴	d 16	d 44	d 80		T	D	T × D
Total VFA, ⁵ mM									
RAP	70	79	118	125	115	4.6	0.76	<0.01	0.93
GRAD	74	87	121	122	120				
Fraction Ac, mol/100 mol			***						
RAP	70.8	72.8	60.2	64.4	64.7	0.73	0.09	<0.01	<0.01
GRAD	71.4	72.3	66.3	64.1	64.2				
Fraction Pr, mol/100 mol			***						
RAP	18.0	17.2	28.8	24.2	22.8	0.73	0.03	<0.01	<0.01
GRAD	17.9	18.1	21.9	23.2	23.2				
Fraction Bu, mol/100 mol									
RAP	11.2	10.0	11.0	11.4	12.5	0.35	0.06	<0.01	0.15
GRAD	10.7	9.6	11.8	12.7	12.6				
Ratio Ac:Pr			***						
RAP	4.0	4.3	2.1	2.7	2.9	0.13	0.11	<0.01	<0.01
GRAD	4.1	4.0	3.1	2.8	2.8				
Average rumen fluid pH									
RAP	6.75	6.81	6.06	6.17	6.18	0.069	0.24	<0.01	0.28
GRAD	6.62	6.69	6.34	6.11	6.18				
Time pH <6.30, min/h									
RAP	5	0	47	37	30	5.1	0.53	<0.01	0.12
GRAD	8	7	23	41	40				
Time pH <5.80, min/h			**						
RAP	0	0	18	7	12	2.6	0.03	0.03	0.02
GRAD	0	0	2	11	5				

¹Sampling day relative to calving.

²Standard error for LSM, pooled by sampling day, $n = 12$.

³T = treatment, P -value for treatment period (d 9 to 80); D = sampling day, P -value for pretreatment and treatment period (d -50 to 80); T × D, P -value for treatment period (d 9 to 80).

⁴Group GRAD: $n = 5$; 1 cow no measurement at d -10 due to early calving.

⁵Sum of Ac, Pr, and Bu.

*** $P < 0.001$, ** $P < 0.01$, significance of difference in LSM of RAP or GRAD for the same sampling day and variable.

Table 2. Fractional rate (per hour) of VFA clearance (k_c), fluid passage (k_i), and absorption (k_a) from the VFA production rate measurement using the isotope dilution technique for acetic (Ac), propionic (Pr), and butyric acid (Bu), and fluid inflow, during the pretreatment and treatment period with a rapid (1.0 kg of DM/d; RAP, n = 6) and a gradual (0.25 kg of DM/d; GRAD, n = 6) rate of increase of concentrate allowance postpartum

Item	Pretreatment period ¹		Treatment period ¹			SE ²	Fixed effects ³		
	d -50	d -10 ⁴	d 16	d 44	d 80		T	D	T × D
k_c Ac, /h									
RAP	0.63	0.51	0.58	0.61	0.68	0.032	0.99	<0.01	0.73
GRAD	0.57	0.47	0.62	0.57	0.68				
k_c Pr, /h									
RAP	0.77	0.64	0.63	0.67	0.72	0.039	0.62	0.16	0.74
GRAD	0.68	0.57	0.64	0.59	0.71				
k_c Bu, /h									
RAP	0.56	0.48	0.48	0.46	0.49	0.033	0.40	0.14	0.56
GRAD	0.50	0.44	0.45	0.37	0.48				
k_i , /h									
RAP	0.18	0.18	0.20	0.19	0.18	0.016	0.45	0.03	0.09
GRAD	0.21	0.17	0.23	0.16	0.22				
Fluid inflow, L/h									
RAP	8.2	9.0	12.8	12.2	11.6	0.81	0.43	<0.01	0.24
GRAD	10.9	9.6	13.2	11.7	14.6				
k_a Ac, /h									
RAP	0.46	0.33	0.38	0.43	0.50	0.027	0.54	<0.01	0.89
GRAD	0.36	0.30	0.39	0.41	0.46				
k_a Pr, /h									
RAP	0.59	0.46	0.43	0.48	0.54	0.032	0.29	0.07	0.72
GRAD	0.46	0.40	0.41	0.43	0.49				
k_a Bu, /h									
RAP	0.38	0.30	0.28	0.28	0.31	0.027	0.12	0.20	0.47
GRAD	0.29	0.27	0.22	0.21	0.26				

¹Sampling day relative to calving.

²Standard error for LSM, pooled by sampling day, n = 12.

³T = treatment, *P*-value for treatment period (d 9 to 80); D = sampling day, *P*-value for pretreatment and treatment period (d -50 to 80); T × D, *P*-value for treatment period (d 9 to 80).

⁴Group GRAD: n = 5; 1 cow no measurement at d -10 due to early calving.

Ac (60.0 vs. 68.0 mol/100 mol) and Pr (31.1 vs. 23.2 mol/100 mol) differed ($P < 0.01$) on 16 d pp for RAP and GRAD, respectively, whereas on all other days the fractions of Ac, Pr and Bu were not affected by treatment (data not shown). Sampling day, concentrate treatment, and their interaction did not affect VFA production expressed per unit intake of DM or FOM.

Fractional Rate of Fluid Passage and of VFA Absorption

IDT. The fractional fluid passage rate (k_i ; Table 2) was affected by sampling day, with the highest fluid passage rate at 16 d pp. Concentrate treatment did not affect k_i ($P = 0.45$) and a tendency was observed for an interaction between concentrate treatment and sampling day ($P = 0.09$). The inflow of water into the rumen was affected by sampling day ($P < 0.01$) and was lower during the pretreatment (9.4 L/h) compared with the treatment period (12.7 L/h). Concentrate treatment and interaction between concentrate treatment and sampling day did not affect water inflow ($P \geq 0.24$).

The fractional absorption rate (k_a ; Table 2) was affected by sampling day for Ac ($P = 0.01$) but not for Bu ($P = 0.20$), whereas it tended to affect k_a of Pr ($P = 0.07$). Both k_a Ac and k_a Pr decreased from 50 to 10 d ap ($P \leq 0.04$). The k_a Ac averaged 0.36/h in the pretreatment period, increasing by 26% during the treatment period to 0.48/h at 80 d pp ($P = 0.02$). The k_a Ac was greater during the treatment period (averaging 0.43/h) compared with the pretreatment period ($P = 0.01$). The fractional absorption rates of Ac, Pr, and Bu were neither affected by concentrate treatment nor by the interaction between concentrate treatment and sampling day ($P \geq 0.12$).

BIT. The fractional fluid passage rate (k_i , Table 4) was affected by sampling day ($P < 0.01$), generally increasing during the pretreatment period and decreasing during the treatment period. Concentrate treatment affected k_i ($P < 0.01$), and was on average greater in GRAD (0.20/h) compared with RAP (0.13/h). Concentrate treatment interacted with sampling day for k_i , with a higher fractional passage rate for GRAD at 16, 30, and 80 d pp compared with RAP ($P \leq 0.05$). Net influx of water was affected by sampling day ($P < 0.01$).

and generally was higher during the treatment period. Net influx of water was neither affected by concentrate treatment ($P = 0.36$) nor the interaction between concentrate treatment and sampling day ($P = 0.30$).

Fractional absorption rates (k_a) of Ac, Pr, and Bu (Table 4) were affected by sampling day ($P < 0.01$), and decreased during the pretreatment period by approximately 35% to 0.28, 0.34, and 0.38/h for Ac, Pr, and Bu at 3 d pp, respectively ($P \leq 0.01$). During the treatment period, the k_a of Ac, Pr, and Bu increased to 0.67, 0.79, and 0.79/h at 80 d pp, respectively ($P < 0.01$). The k_a of Ac, Pr, and Bu were neither affected by concentrate treatment ($P \geq 0.85$) nor by the interaction between sampling day and concentrate treatment ($P \geq 0.40$). Similarly, the pH of the buffer fluid at the end of the incubation period was only affected by sampling day. It decreased during the pretreatment period from pH 7.11 at 50 d ap to pH 7.01 at 3 d pp ($P = 0.03$), increasing during the treatment period to pH 7.24 at 80 d pp ($P < 0.01$).

DISCUSSION

The present study provides a comprehensive overview of changes in ruminal VFA production rate and VFA fractional absorption rate in dairy cows during the

dry period and the first 80 d of lactation. In addition, the effect of the postpartum rate of increase of concentrate allowance on VFA production and absorption was examined. The current results are complementary to previously reported changes on rumen papillae morphology from the same experiment (Dieho et al., 2016).

VFA Production

In line with our expectations, daily VFA production and rumen fluid VFA concentration increased with increased DM and FOM intake after onset of lactation. Concentration of a VFA in rumen fluid is mainly a reflection of its rate of production and rate of clearance, and to lesser degree rumen fluid volume. However, fractions of the VFA produced (mol/100 mol) are not sensitive to the volume of rumen fluid. In line with results of Sutton et al. (2003), fractions of Ac and Pr of VFA produced were strongly related (Ac: $r^2 = 0.85$; Pr: $r^2 = 0.95$) to fractions of Ac or Pr in rumen fluid, whereas this relationship for Bu was weak ($r^2 = 0.17$; Figure 2). These relationships were established for dry period and lactation rations with daily feed intakes ranging from 11 to 25 kg of DM, confirming that fractions of Ac and Pr in rumen fluid are good indicators of the proportion in which they are produced. For Bu, the poor relationship

Table 3. Total VFA production rate, production rate of acetic (Ac), propionic (Pr), and butyric acid (Bu), and VFA production per unit of feed intake calculated from the VFA production rate measurements using the isotope dilution technique during the pretreatment and treatment period with a rapid (1.0 kg of DM/d; RAP, $n = 6$) and a gradual (0.25 kg of DM/d; GRAD, $n = 6$) rate of increase of concentrate allowance postpartum

Item	Pretreatment period ¹		Treatment period ¹			SE ²	Fixed effects ³		
	d -50	d -10	d 16	d 44	d 80		T	D	T × D
Total VFA production rate, mol/d									
RAP	52	49	108	117	119	3.8	0.64	<0.01	0.45
GRAD	49	58	98	115	126				
Ac production rate, mol/d									
RAP	36	35	65	76	79	2.6	0.33	<0.01	0.79
GRAD	34	41	67	76	83				
Pr production rate, mol/d			***						
RAP	11	10	34	31	29	1.3	0.03	<0.01	<0.01
GRAD	10	12	22	28	32				
Bu production rate, mol/d									
RAP	5	4	10	10	11	0.4	0.76	<0.01	0.63
GRAD	5	5	9	10	11				
VFA production, mol/kg of DM									
RAP	4.6	4.2	5.5	5.0	4.9	0.32	0.99	0.11	0.96
GRAD	4.2	4.8	5.6	4.7	5.1				
VFA production, mol/kg of FOM ⁴									
RAP	10.2	9.3	8.8	8.1	8.1	0.68	0.77	0.22	0.89
GRAD	9.2	10.7	9.7	7.7	8.3				

¹Sampling day relative to calving.

²Standard error for LSM, by sampling day, $n = 12$.

³T = treatment, P -value for treatment period (d 9 to 80); D = sampling day, P -value for pretreatment and treatment period (d -50 to 80); T × D, P -value for treatment period (d 9 to 80).

⁴FOM = fermentable organic matter (Tamminga et al., 1994).

*** $P < 0.001$, significance of difference in LSM of RAP or GRAD for the same sampling day and variable.

Table 4. Fractional rate (per h) of absorption (k_a) of acetic (Ac), propionic (Pr), and butyric acid (Bu), fractional fluid passage rate (k_f), buffer fluid end pH, and net influx of water, measured using the buffer incubation technique in the empty washed rumen during the pretreatment and treatment period with a rapid (1.0 kg of DM/d; RAP, n = 6) and a gradual (0.25 kg of DM/d; GRAD, n = 6) rate of increase of concentrate allowance postpartum

Item	Pretreatment period ¹					Treatment period ¹					Fixed effects ³			
	d -50	d -30	d -10 ⁴	d 3	d 9	d 16	d 30	d 44	d 60	d 80	SE ²	T	D	T × D
<i>k_a</i> Ac, /h														
RAP	0.44	0.31	0.36	0.28	0.38	0.55	0.52	0.61	0.47	0.64	0.032	0.92	<0.01	0.40
GRAD	0.46	0.32	0.33	0.28	0.34	0.51	0.44	0.61	0.60	0.70				
<i>k_a</i> Pr, /h														
RAP	0.52	0.38	0.43	0.34	0.45	0.62	0.63	0.74	0.60	0.77	0.035	0.94	<0.01	0.45
GRAD	0.53	0.39	0.40	0.34	0.41	0.58	0.53	0.73	0.72	0.82				
<i>k_a</i> Bu, /h														
RAP	0.56	0.44	0.49	0.38	0.46	0.62	0.63	0.74	0.61	0.78	0.033	0.85	<0.01	0.40
GRAD	0.57	0.45	0.46	0.38	0.44	0.58	0.52	0.72	0.72	0.81				
Buffer fluid end pH														
RAP	7.09	6.97	7.00	6.96	7.11	7.12	7.17	7.26	7.26	7.25	0.033	0.60	<0.01	0.22
GRAD	7.13	7.04	7.02	7.05	7.03	7.21	7.21	7.27	7.33	7.23				
<i>k_f</i> , /h						***	*			*				
RAP	0.13	0.17	0.12	0.22	0.23	0.09	0.19	0.10	0.14	0.05	0.018	<0.01	<0.01	0.02
GRAD	0.14	0.23	0.14	0.30	0.27	0.22	0.26	0.14	0.18	0.13				
Net influx water, L/h														
RAP	2.3	4.6	3.0	5.0	6.5	3.4	6.6	3.9	7.0	3.5	0.51	0.36	<0.01	0.30
GRAD	3.6	5.0	3.2	4.8	5.0	3.9	7.1	5.2	7.6	4.9				

¹Sampling day relative to calving.
²Standard error for LSM, pooled by sampling day, n = 12.
³T = treatment, P-value for treatment period (d 9 to 80); D = sampling day, P-value for pretreatment and treatment period (d -50 to 80); T × D, P-value for treatment period (d 9 to 80).
⁴Group GRAD: n = 5; 1 cow no measurement at d -10 due to early calving.
†P < 0.10, *P < 0.05, ***P < 0.001, significance of difference in LSM of RAP or GRAD for the same sampling day and variable.

and regression parameters indicate Bu production to be lower than that expected based on the fraction of Bu in rumen fluid. The fraction of Ac and Pr in the rumen fluid is slightly underestimated by the fraction in which Ac and Pr were produced, whereas Bu is overestimated.

In contrast to Sutton et al. (2003), who continuously infused ^{14}C -labeled VFA for 22 h into the rumen, we introduced ^{13}C -labeled VFA and fluid passage marker as a pulse dose at the start of the IDT measurements. We neither disturbed normal rumen function nor stratification by mixing the rumen contents by hand, and therefore a delay in the start of the clearance of ^{13}C -labeled VFA and fluid passage marker may have occurred (Teeter and Owens, 1983) leading to possible underestimation of VFA clearance and, thus, production rates in our experiment. The net total VFA production per kilogram of DM pp (on average 5.1 mol/kg of DM) was somewhat lower than that reported by Sutton et al. (2003; 6.2 mol/kg of DM for a 60% concentrate, 40% roughage diet), but within the range reported by Siciliano-Jones and Murphy (1989; range = 4.8 to 6.3 mol/kg of DM in two 80% concentrate, 20% roughage and two 20% concentrate, 80% roughage treatments). In contrast, the net VFA production per kilogram of DM calculated from Markantonatos et al. (2009; 2.6 and 2.9 mol/kg of DM for the dry period and lactation, respectively) is substantially lower than in our study and the studies of Sutton et al. (2003) and Siciliano-Jones and Murphy (1989).

Based on previous results from buffer incubation studies (Sutton et al., 1963; Thorlacius and Lodge, 1973; Dijkstra et al., 1993), the lower fractional absorption rate of Bu compared with Ac observed in the present experiment using the IDT was not expected. The use of a pulse-dose, which simultaneously enriches Ac, Pr, and Bu and does not allow for quantification of the C fluxes between Ac, Pr, and Bu, may explain the low fractional absorption rate of Bu. Interconversion of VFA would result in a net efflux of C from the Ac pool, and net influx of C into the Pr and in particular into the Bu pool (Sutton et al., 2003; France and Dijkstra, 2005; Markantonatos et al., 2008). The clearance rate of Ac during the IDT is therefore overestimated, as ^{13}C not only leaves the Ac pool through absorption across the rumen wall and passage with the fluid but also by conversion to other VFA. In contrast, the fractional clearance rates of Pr and in particular of Bu are likely underestimated, as there is a net flow of ^{13}C from Ac into the Pr and Bu pools. Due to the difference in ^{13}C pool sizes of Ac and Bu added at $t = 0$ h, a net flow of 10 to 30% ^{13}C from Ac to Bu (Sutton et al., 2003; Markantonatos et al., 2008) is sufficient to replenish 33 to 100% of the initial ^{13}C pool of Bu, explaining a significant part of the low fractional rate of absorption

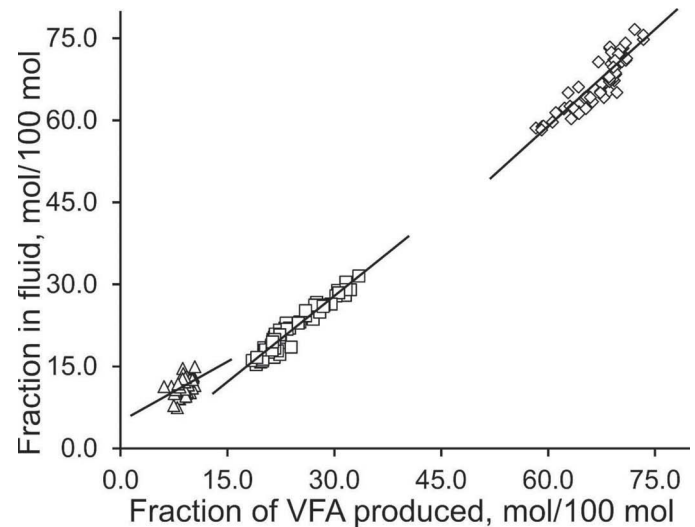


Figure 2. Relationship between the fraction of acetic (Ac), propionic (Pr), and butyric acid (Bu) of total VFA produced (mol/100 mol) and fraction of Ac, Pr, or Bu of total VFA concentration in rumen fluid (mol/100 mol) during the isotope dilution technique, for Ac (\diamond ; solid line: $b_0 = -14.3 \pm 4.62$, $b_1 = 1.21 \pm 0.069$, squared correlation (r^2) = 0.85, $P < 0.01$), Pr (\square ; solid line: $b_0 = -4.0 \pm 0.78$, $b_1 = 1.07 \pm 0.032$, $r^2 = 0.95$, $P < 0.01$), and Bu (\triangle ; solid line: $b_0 = 4.7 \pm 1.94$, $b_1 = 0.75 \pm 0.216$, $r^2 = 0.17$, $P < 0.01$) ($n = 12$). b_0 : intercept; b_1 : slope of the regression equation.

of Bu. A difference in ^{13}C flow from Ac to Bu for RAP and GRAD might mask the treatment effect on Bu production, although differences in ration composition were limited compared with the earlier studies suggesting a difference in ^{13}C flow was likewise limited.

All major VFA promote rumen papillae development in vivo (Sutton et al., 1963; Sakata and Tamate, 1979; Suárez et al., 2006), but Bu is generally believed to have a particular proliferative effect (Mentschel et al., 2001; Malhi et al., 2013; Kowalski et al., 2015). Given the greater papillae surface area in early lactation (16, 30, and 44 d pp) associated with a rapid compared with a gradual increase in concentrate allowance (Dieho et al., 2016), it was expected that Bu production would be larger at 16 and possibly 44 d pp in RAP compared with GRAD. However, neither the Bu production nor its concentration and proportion in the rumen fluid at 16 d pp appeared to be affected by rate of increase of concentrate allowance, which did result in a greater starch and sugar intake for RAP (Dieho et al., 2016). In contrast, the production, concentration and molar proportion of Pr in the rumen fluid was higher in RAP at 16 d pp. Whereas this experiment did not aim to examine the specific effects of Pr or Bu on papillae proliferation and no causal relationships can be made, a larger Pr production rate coinciding with papillae proliferation suggests that Bu is not the sole VFA implicated with this adaptive process.

VFA Fractional Absorption Rate

For the BIT, the k_a Ac, k_a Pr, and k_a Bu approximately doubled from the end of the dry period (10 d ap) to the end of the experimental period (80 d pp), supporting our expectations based on the observed increase in papillae surface area. For the IDT, however, changes in k_a of VFA were less pronounced, showing only modest increases of 55% for Ac, 20% for Pr, and no increase for Bu. Major methodological differences exist between the BIT and IDT. Basically, BIT reflects the capacity of the rumen to absorb VFA under standardized conditions, whereas IDT reflects absorption of VFA during the actual physiological conditions at time of measurement. The k_a of VFA is affected by factors including concentration of VFA in rumen fluid, VFA carbon-chain length, fluid pH, and rumen fluid volume (Sutton et al., 1963; Thorlacius and Lodge, 1973; Dijkstra et al., 1993). A standardized buffer fluid was used for the BIT to study changes in k_a of VFA related to differences in papillae surface area, rather than related to other factors. For the current experiment, buffer fluid VFA concentrations and pH were chosen to represent rumen fluid typically observed in lactation dairy cows (Rabelo et al., 2003; Abrahamse et al., 2008; Steele et al., 2012). The total VFA concentration, type of VFA, and initial pH measured in the BIT buffer resembled those measured in the rumen fluid pp (Table 1). Therefore, comparable k_a of VFA for the BIT and IDT during the lactation were expected. However, k_a Ac, k_a Pr, and k_a Bu with the BIT were approximately 20, 25, and 45% larger than those found with the IDT, respectively. In the dry period there seems to be more agreement between the methods, as k_a Ac (BIT = 0.40/h; IDT = 0.36/h) and k_a Pr (BIT = 0.47/h; IDT = 0.48/h) were rather similar between both methods, although not for k_a Bu (BIT = 0.52/h; IDT = 0.31/h). The good agreement in fractional absorption rate of Ac or Pr between both methods during the dry period was unexpected. The conditions for absorption of VFA from the rumen fluid during the IDT in the dry period (higher pH, lower VFA concentration) were less favorable compared with the buffer fluid, suggesting higher k_a of VFA would be found for the BIT. However, as discussed in section VFA Production, it is likely that the IDT overestimates fractional clearance rate of Ac and underestimates that of Bu, resulting in corresponding over- or underestimation of the fractional absorption rates. During the dry period this might explain the similar k_a Ac for the BIT and IDT, and explain part of the difference in k_a Bu.

Overall, the fractional absorption rates measured during the BIT had weak relationships with those measured during the IDT (k_a Ac, $r^2 = 0.19$; k_a Pr, $r^2 = 0.08$;

k_a Bu, $r^2 = 0.01$; Figure 3), and fractional absorption rates during BIT were usually higher than those during IDT. The correlation between the BIT and IDT, when determined separately for the dry period and lactation period, marginally improved for Pr only, increasing to $r^2 = 0.21$ and $r^2 = 0.16$, respectively. The generally higher k_a of VFA measured using the BIT may also result from a more efficient mixing of the buffer fluid during the incubation. Feed particles, which physically inhibit fluid flow, were absent and frequent rumen contractions caused turbulence within the buffer fluid which could easily be observed, reducing intraruminal differences or gradients in VFA concentration. During lactation, the greater rumen fill would have impaired free movement of rumen fluid to greater extent than during the dry period. However, during the dry period, the greater k_a VFA of the BIT is better explained by the higher VFA concentration and lower pH compared with the IDT. The capacity of the rumen epithelium to absorb VFA appears to have been used to a larger extent with BIT than IDT. These results, and differences in VFA concentration observed within the rumen (Tafaj et al., 2006), supports the concept that movement of rumen fluid and transport of VFA to the rumen wall may significantly influence VFA absorption (Storm and Kristensen, 2010). This may also imply that SARA is not just related to a lack of intrinsic capacity of the rumen epithelium for VFA absorption, but is also related to limitations of intraruminal movement of VFA toward the rumen epithelium.

Rumen Papillae Surface Area and VFA Fractional Absorption Rate

Although a larger rumen papillae surface area has been shown to coincide with a higher rate of VFA absorption (Dirksen et al., 1984; Gaebel et al., 1987; Melo et al., 2013), a limited amount of in vivo data are available examining this relation in dairy cows during the transition period (Bannink et al., 2012; Martens et al., 2012). The previously reported results from the present experiment show that papillae surface area decreased during the dry period and increased after calving (Dieho et al., 2016). Generally, the observed increase in papillae surface area coincides with an increase in VFA absorption capacity during the dry period up to 80 d pp in the present experiment and confirms earlier reports (Dirksen et al., 1984; Gaebel et al., 1987; Melo et al., 2013). However, in contrast to the greater rumen papillae surface area at 16, 30, and 44 d pp with a rapid increase of concentrate allowance pp (Dieho et al., 2016), the measured k_a of VFA was not affected by the rate of increase of concentrate allowance, neither

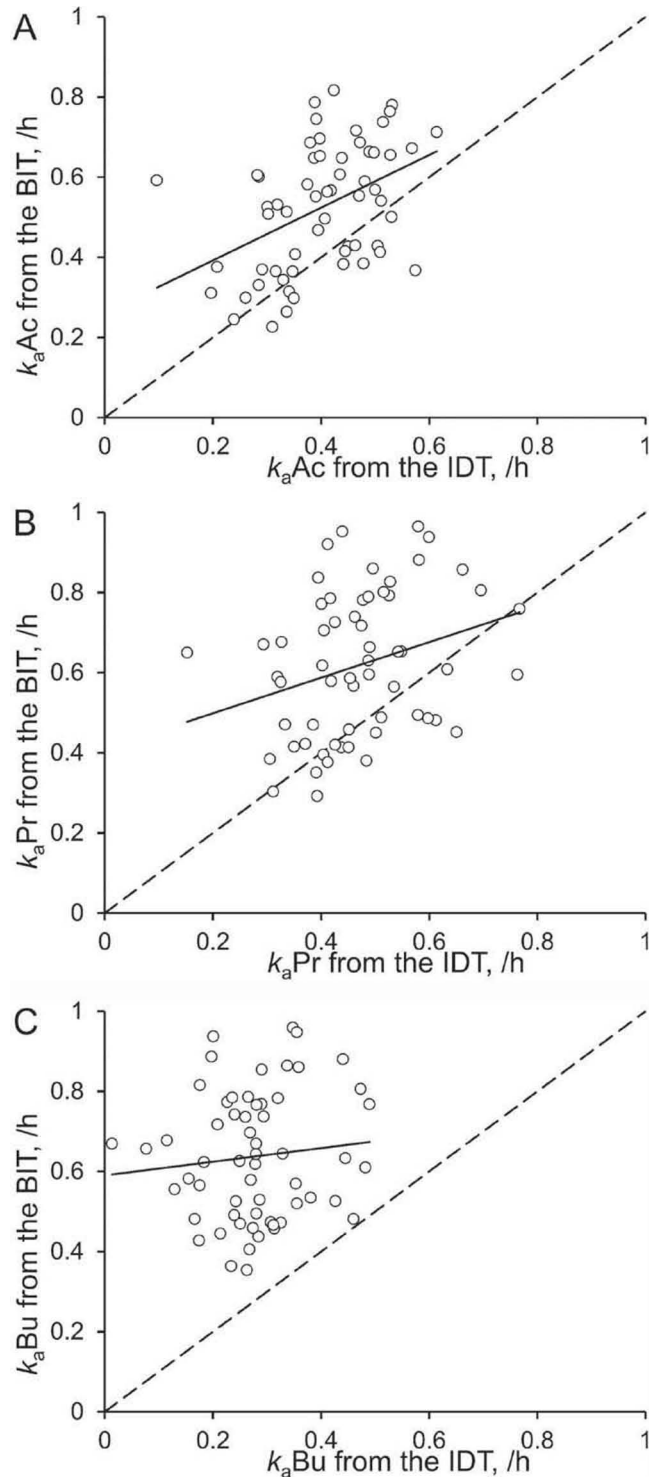


Figure 3. Relationships between the fractional absorption rate (k_a , per hour) obtained using the isotope dilution technique (IDT) in the naturally filled rumen and the buffer incubation technique (BIT) in the empty washed rumen, for (A) acetic acid [k_a Ac; solid line: $b_0 = 0.26 \pm 0.074$, $b_1 = 0.66 \pm 0.180$, squared correlation (r^2) = 0.19, $P < 0.01$], (B) propionic acid (k_a Pr; solid line: $b_0 = 0.41 \pm 0.095$, $b_1 = 0.44 \pm 0.196$, $r^2 = 0.08$, $P = 0.03$), (C) butyric acid (k_a Bu; solid line: $b_0 = 0.59 \pm 0.064$, $b_1 = 0.17 \pm 0.216$, $r^2 = 0.01$, $P = 0.43$). Dashed line indicates unity. b_0 : intercept; b_1 : slope of the regression equation.

for the BIT (16, 30 and 44 d pp) nor the IDT (16 and 44 d pp). An increased papillae surface area without a concomitant rise in fractional absorption of VFA was unexpected. It may suggest a diminished capacity of VFA absorption per unit of surface area; however, this is not supported by the literature (Sehested et al., 2000; Etschmann et al., 2009; Schurmann et al., 2014). Also, a decrease in true rumen surface area, despite growth of individual papillae, is unlikely, as papillae density does not differ between the dry period and early lactation (Reynolds et al., 2004). However, epithelial blood flow is reported to affect VFA absorption (Storm et al., 2011), and blood flow rather than papillae surface area might be a limiting factor for the increase in VFA absorption with rapid increase of concentrate allowance during early lactation. The large increase in splanchnic blood flow after calving (80% increase by 11 d pp, compared with 9 d ap; Reynolds et al., 2003) might be a factor explaining the rapid increase in k_a VFA between 10 d ap and 16 d pp in the present experiment. A greater capacity for VFA metabolism by the rumen epithelium might also aid k_a VFA by decreasing intracellular VFA concentrations, although metabolism of Ac and Pr is limited and inhibited by Bu (Kristensen, 2005).

Papillae surface area and morphology differ markedly between individual cows even on the same ration and with similar feed intake (Reynolds et al., 2004; Dieho et al., 2016). The repeated measurement approach used in the present study allows determination of the relationship between observed papillae surface area and VFA fractional absorption rate for individual cows. This relationship per cow ranged from weak (minimum $r^2 = 0.32$) to strong (maximum $r^2 = 0.87$), with generally a lower correlation for individual cows in RAP compared with cows in GRAD (data not shown). Using the mixed-model approach for multiple experiments described by St-Pierre (2001), repeated measurement data from multiple cows were adjusted for the random effect of cow. For the entire experimental period, 50 d ap to 80 d pp, papillae surface area was moderately related to k_a VFA obtained using the BIT ($r^2 = 0.50$; Figure 4A). However, for both the pretreatment period (Figure 4B) and treatment period (Figure 4C) the relationship was weak (pretreatment period, $r^2 = 0.23$; treatment period, $r^2 = 0.38$). The moderate relationships between papillae surface area and VFA fractional absorption rate for the entire experimental period contrasts with the weak relationships between papillae surface area and VFA fractional absorption rate during the period with rapid changes in FOM intake. This suggests that, besides papillae surface area, other factors should be considered as indicators for rumen wall adaptation and concomitant VFA absorption during the transition period in dairy cattle.

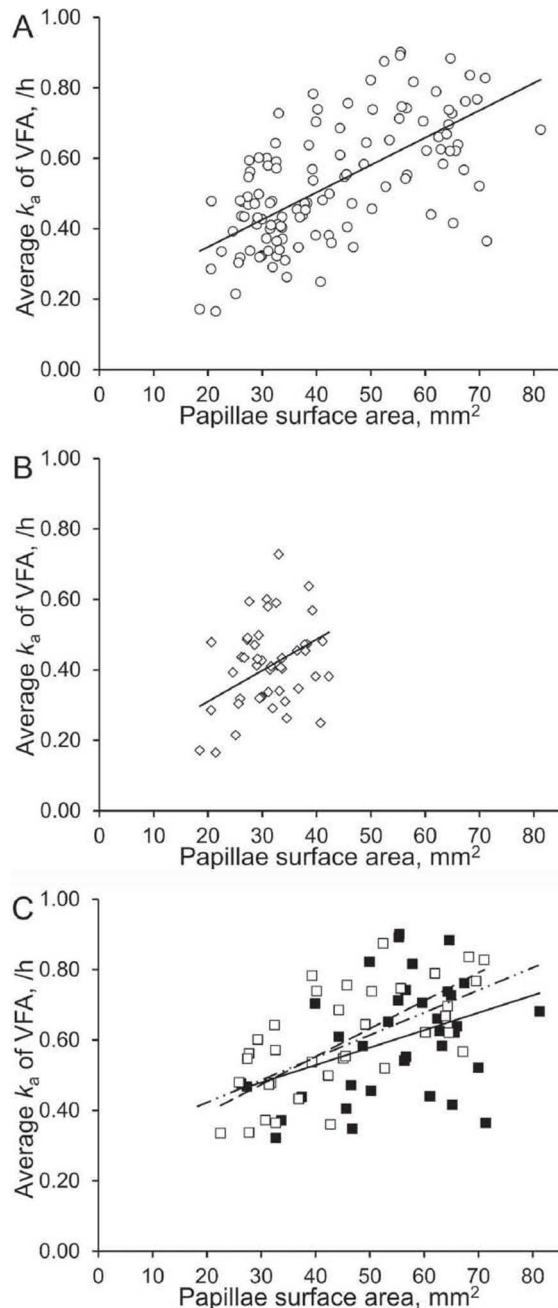


Figure 4. Relationship between rumen papillae surface area (mm^2) and rumen fractional absorption rate of VFA (k_a of VFA, per hour; average of k_a of acetic, propionic, and butyric acid) obtained using the buffer incubation technique for (A) the entire experimental period (\circ ; solid line: $b_0 = 0.19 \pm 0.033$, $b_1 = 0.0078 \pm 0.0007$, squared correlation (r^2) = 0.50, $P < 0.01$; $n = 12$), (B) the pretreatment period (\diamond ; solid line: $b_0 = 0.13 \pm 0.077$, $b_1 = 0.0089 \pm 0.0024$, $r^2 = 0.23$, $P < 0.01$; $n = 12$), and (C) the treatment period for cows receiving a rapid rate of increase of concentrate allowance postpartum (1.0 kg of DM/d; \blacksquare ; solid line: $b_0 = 0.33 \pm 0.095$, $b_1 = 0.0050 \pm 0.0017$, $r^2 = 0.20$, $P < 0.01$; $n = 6$), a gradual rate of increase of concentrate allowance postpartum (0.25 kg of DM/d; \square ; dashed line: $b_0 = 0.24 \pm 0.095$, $b_1 = 0.0081 \pm 0.0017$, $r^2 = 0.40$, $P < 0.01$; $n = 6$), and both concentrate treatments combined (dash-and-dotted line: $b_0 = 0.29 \pm 0.047$, $b_1 = 0.0063 \pm 0.0010$, $r^2 = 0.38$, $P < 0.01$; $n = 12$). b_0 : intercept and b_1 : slope of the regression equation.

CONCLUSIONS

The ruminal VFA fractional absorption rate measured under standardized (BIT) and physiological (IDT) conditions decreased during the dry period and increased after calving, and generally followed a pattern similar to changes in papillae surface area. However, unlike papillae surface area, the VFA fractional absorption rate was not affected by rate of increase of concentrate allowance pp. This suggests only a limited effect of papillae surface area on fractional VFA absorption rate during the transition period in dairy cattle. Fractional absorption rates pp obtained with the BIT were in general greater than those obtained with the IDT, suggesting the presence of rumen contents affects VFA fractional absorption rate. The increased papillae surface area with a faster rate of increase of concentrate allowance in early lactation coincided with a higher Pr production rate, whereas the production rate of Ac and Bu did not differ.

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