Morphological adaptation of rumen papillae during the dry period and early lactation as affected by rate of increase of concentrate allowance

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ABSTRACT

Knowledge of the morphological adaptation of rumen papilla, which plays an important role in volatile fatty acid absorption, in dry and early lactation dairy cattle is limited. Therefore, macro- and microscopic changes in papilla morphology during the dry period and lactation and the effect of rate of increase of concentrate allowance were studied. Samples were collected from 12 rumen-cannulated Holstein Friesian dairy cows during a pretreatment period, 50, 30, and 10 d antepartum (the dry period) and 3 d postpartum (pp), and a treatment period, 9, 16, 30, 44, 60, and 80 d pp. Cows had free access to either a dry period ration [27% grass silage, 27% corn silage, 35% wheat straw, and 11% soybean meal on a dry matter (DM) basis] or a basal lactation ration (42% grass silage, 41% corn silage, and 17% soybean meal on a DM basis, and 0.9 kg of DM/d concentrate). Treatment consisted of either a rapid (1.0 kg of DM/d; RAP; n = 6) or gradual (0.25 kg of DM/d; GRAD; n = 6) increase of concentrate allowance (up to 10.9 kg of DM/d), starting at d 4 pp, aimed at creating a contrast in rumen-fermentable organic matter (FOM) intake. Papillae were collected from the ventral, ventral blind, and dorsal blind rumen sacs and measured digitally.

Intake of DM (11.9 kg/d) and FOM (5.7 kg/d) did not change during the pretreatment period, but increased during the treatment period to 24.5 and 15.0 kg/d at 80 d pp, respectively. Concentrate treatment and sampling day interacted for FOM intake, which was 22% greater in RAP at 16 d pp compared with GRAD. Papilla surface area decreased during the pretreatment period by 19% to 28.0 mm² at 3 d pp, thereafter increasing to 63.0 mm² at 80 d pp. Concentrate treatment and sampling day interacted for surface area, which was greater in RAP compared with GRAD at 16 (46.0 vs. 33.2 mm²), 30 (55.4 vs. 41.2 mm²), and 44 (60.5 vs. 49.7 mm²) days pp, showing that papillae can respond to a rapid rate of increase of FOM intake by increasing growth rate. Microscopic morphology was affected by sampling day, but neither by concentrate treatment nor by their interaction, with a decrease in papilla and epithelium thickness during the lactation. In conclusion, the rumen papillae respond to changes in FOM intake and the magnitude of this response depends on the rate of increase of FOM intake. This response in surface area of the rumen papillae potentially facilitates the absorption of the volatile fatty acids.

Key words: transition dairy cow, rumen papillae, rumen epithelium, rumen adaptation

INTRODUCTION

In ruminants, the reticulorumen is the major site for microbial fermentation of feed and, hence, production of VFA, which provide approximately 75% of total ME (Bergman, 1990). The luminal surface of the rumen wall is covered with papillae which increase the surface area for absorption of VFA. However, factors such as rumen VFA concentration and pH (Dijkstra et al., 1993), epithelial blood flow (Storm et al., 2012), and changes at the cellular level (Penner et al., 2011) may affect VFA absorption capacity as well. The absorption of VFA is seen as the primary process for maintaining rumen VFA concentration and pH within physiological limits, thereby supporting microbial fermentation of feed (Penner et al., 2009; Aschenbach et al., 2011; Dijkstra et al., 2012).

The proliferation of rumen papillae in young ruminants is triggered by the consumption of solid feed (Tamate et al., 1962; Sutton et al., 1963; Suárez et al., 2006), and thus associated with the intake of rumen fermentable organic matter (FOM) and its associated production of VFA. Furthermore, Dirksen et al. (1984) and Liebich et al. (1987) reported a positive relationship between the plane of nutrition and papilla surface area in the rumen. Therefore, it can be suggested that
differences in surface area of rumen papilla are associated with differences in intake of FOM.

Currently, knowledge of macroscopic morphological changes of the rumen papillae (papilla surface area, length, and width) in dairy cows in the dry period until early lactation is limited (Shen et al., 2005; Malhi et al., 2013; Steele et al., 2015), or experiments have yielded inconclusive results (Reynolds et al., 2004). Likewise, knowledge about changes on the microscopic scale in rumen papilla (thickness of the papilla and epithelial layer) in the aforementioned period is limited as well. Diet- and time-related changes in thickness and organization of rumen epithelium have been reported by Dirksen et al. (1984), Liebich et al. (1987), Steele et al. (2011), and Bannink et al. (2012), but a comprehensive description of those changes during the dry period and early lactation is currently lacking (Martens et al., 2012). Therefore, the aim of the present experiment was to study macro- and microscopic morphological changes of rumen papillae from 50 d before expected calving date until 80 d postpartum in dairy cattle. After calving, 2 rates of increase of concentrate allowance were applied to create a contrast in FOM intake and evaluate its effect on morphological changes of the papillae. It was hypothesized that a rapid versus a gradual rate of increase of surface area, length, and width of rumen papilla and a decrease in thickness of the papilla and its epithelial layer.

**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 Animal Experiments.

**Animals, Experimental Design and Management**

Three months before the start of the experiment 12 first-parity Holstein Friesian dairy cows were fitted with a rumen cannula (10 cm i.d., Bar Diamond Inc., Parma, ID). Eight weeks before the expected date of calving, cows were dried off and entered the experiment. The experiment had a randomized block design with repeated measurements. Cows were blocked by expected date of calving, and within each block cows were randomly assigned to either a rapid rate of increase of concentrate allowance or a gradual rate of increase, and was achieved at 13 and 43 d pp for RAP and GRAD, respectively.

Daily intake of the dry period ration or the basal lactation ration (kg/d) was measured individually using feed bins (Insentec, Marknesse, the Netherlands), with a maximum stocking density of 2 cows/bin. Cows had access to all feed bins. Concentrate was fed using a concentrate dispenser (Manus VC5, DeLaval, Steenwijk, the Netherlands) and made the individual daily allowance available in equal portions over six 4-h periods and recorded the quantity actually dispensed (kg/d). Concentrate and ration ingredient samples were taken once a week. One sample of concentrate and one of each ration ingredient was immediately used for DM determination by forced-air oven drying (105°C, 24 h); the remaining samples were stored at −20°C pending analyses. If necessary, basal ration formulation (on product basis) was adjusted for changes in ration ingredient DM content; DM content of concentrate was constant throughout the experiment.

After forced-air oven drying (60°C, 24 h) and determination of DM (ISO, 1999b), feed samples were ground (1-mm screen) and pooled (each pool containing samples of 4 consecutive weeks) before determination crude ash (ISO, 2002), crude fat (ISO, 1999a), starch (ISO, 2004),...
### Chemical Composition

<table>
<thead>
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<th>Ingredient</th>
<th>Dry period</th>
<th>Lactation</th>
<th>Concentrate</th>
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<tr>
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<tr>
<td>Soybean meal</td>
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<tr>
<td>Wheat straw</td>
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</table>

#### Ingredient Composition

- **DM, g/kg of product**: 603, 466, 892
- **Crude ash**: 80, 75, 68
- **Crude fat**: 109, 157, 178
- **Starch**: 24, 30, 17
- **Sugar**: 90, 139, 248
- **Crude fat**: 44, 67, 95
- **NDF**: 553, 392, 252
- **ADF**: 325, 235, 115
- **ADL**: 32, 16, 8

#### Calculated Values

- **NEL**: 5.3, 6.7, 7.4
- **IDP**: 57, 90, 114
- **RDPB**: 3, 14, 10
- **FOM**: 455, 561, 682

1. Concentrate composition (ingredients, g/kg): sugar beet pulp, 212; corn gluten, 200; barley, 150; wheat, 150; soybean meal, 120; citrus pulp, 100; molasses, 35; vinasse, 20; CaCO₃, 8; salt, 3; and mineral premix, 2.

2. Chemical composition of grass silage (g/kg of DM): crude ash, 105; CP, 110; crude fat, 31; sugars, 113; NDF, 497; ADF, 302; and ADL, 18.

3. Chemical composition of corn silage (g/kg of DM): crude ash, 44; CP, 64; crude fat, 31; starch, 330; NDF, 403; ADF, 231; and ADL, 17.

4. Calculated values for grass and corn silage based on near-infrared spectrometry. Blgg AgroXpertus (Wageningen, the Netherlands).

5. Calculated values for soybean meal and concentrate provided by Agrifirma Feed (Apeldoorn, the Netherlands).

6. Calculated values for wheat straw obtained from CVB Feed Tables 2011 (CVB, 2011). NEL values calculated according to the Dutch NE-system (Van Es, 1978). IDP = intestinal digestible protein; RDPB = rumen-degradable protein balance; FOM = fermentable organic matter; all calculated according to the Dutch DVE/OEB-system (Tamminga et al., 1994).

and sugars (van Vuuren et al., 1993). Crude protein was calculated from nitrogen (N × 6.25) obtained by the Kjeldahl method (ISO, 2005). The NDF (with heat stable α-amylase), ADF, and ADL were determined according to Van Soest et al. (1991) using an Ankom 2000 Fiber Analyzer (Ankom Technology Corp., Macedon, NY). The NEL was calculated according to Van Es et al. (1994).  

### Rumen Fluid Sampling and Papillae Collection

Approximately 1 h after last access to the feed bins and, during lactation, the concentrate dispenser, a 200-mL rumen fluid sample was taken from the ventral rumen sac using a stainless steel sampling probe (2-mm pore size). Immediately after collection, rumen fluid pH was measured and a 600-μL aliquot was taken, acidified with 600 μL of 5% H₃PO₄ (vol/vol), and subsequently stored at −20°C pending analysis for acetic, propionic, and butyric acid concentration by GC as described by van Gastelen et al. (2015). Thereafter, rumen contents were visually and manually inspected for remaining contents to ensure complete evacuation. The rumen contents were stored in an insulated tub and covered to prevent cooling until return into the rumen. Prior to the collection of rumen papillae, the rumen was washed twice with 10 L of tap water at 39°C. After introduction, the warm water was manually scooped against the rumen wall of all compartments. After each wash, water was removed using a 1-L wide-mouth plastic jar.

Biopsies were taken from 3 sites in the rumen (Dirksen et al., 1984; Lesmeister et al., 2004; Bannink et al., 2012): the ventral rumen sac, directly opposite of the rumen cannula, 15 cm cranial of the caudal coronary pillar, and 20 cm below the dorsal limit of the papillae mat; the caudoventral blind rumen sac, opposite the rumen cannula, halfway between the dorsal and ventral limit of the caudoventral blind rumen sac, and 15 cm caudal of the ventral coronary pillar; and the caudodorsal blind rumen sac, opposite the rumen cannula halfway between the dorsal and ventral limit of the caudodorsal blind rumen sac, and 15 cm caudal of the dorsal coronary pillar (Dyce et al., 1996). Papillae were extracted from the rumen wall using forceps (No. 631319, Stuemmer, Würzburg, Germany) and gently rinsed in 0.9% NaCl solution before storage in buffered 4% formalin solution. In line with Odongo et al. (2006) and Steele et al. (2011, 2015), at least 6 intact papillae per site per cow per sampling day were collected. Papillae damaged by the biopsy forceps were easily recognized as damaged papillae, as they showed distinctive circular cut marks.

### Macroscopic Measurements

For macroscopic measurements (per site per cow per sampling day), all papillae were photographed using a digital camera (Casio Exilim EX-Z70, Casio Computer Co., Tokyo, Japan), including a ruler in each photograph. The one-sided surface area of all papillae was measured using the Analyze Particles feature of ImageJ (Version 1.44n, National Institutes of Health, Bethesda, MD), comparable to the Geographical Information System (GIS) method described by Scocco et al. (2012). Length and width of all papillae was measured using ImageScope (Version 11.2.0.780, Aperio Technologies, Leica Biosystems, Nußloch, Germany). Papilla length
was measured from the tip to the base of the papilla along its axis, papilla width was measured halfway of and perpendicular to the papilla length.

**Microscopic Measurements**

Five papillae per site per cow per sampling day (Odongo et al., 2006; Steele et al., 2011, 2015) were processed (minimum of 6 mo after collection and storage in 4% buffered formalin) for histological examination (Veterinary Pathology, Faculty of Veterinary Medicine, Utrecht University). After stepwise dehydration and paraffin infiltration, papillae were mounted parallel to each other before being fully embedded in paraffin. Longitudinal sections, 4-μm thick, perpendicular to the flat surface (median sagittal plane), were made halfway across the papilla width (coronal plane). Two consecutive sections were mounted on glass slides and stained with hematoxylin and eosin (Dobson et al., 1956; Andersen et al., 1999). Slides were digitized at 40× magnification (Department of Pathology, Utrecht Medical Center) using an Aperio ScanScope slide scanner (Aperio Technologies, Leica Biosystems). Complementary software was used for measurements (ImageScope Version 11.2.0.780, Aperio Technologies, Leica Biosystems). Starting at the top-left of the slide, each pair of papilla sections was evaluated for impairing artifacts and the first 3 suitable papillae were used for subsequent assessments.

1. Total thickness of the papilla (measured perpendicular to papilla length) at 250, 500, 750, and 1,000 μm from the tip (Figure 1A).
2. Length of the luminal boundary of the stratum corneum and the length of the basal boundary of the stratum basale in 1,000 μm of papilla length, starting from the papilla tip (Figure 1B).
3. Total thickness of the papilla and thickness of the interstitium (both measured perpendicular to papilla length) was measured approximately halfway the papillae length, each 5 times with 250-μm intervals in both directions over a total length of 1,000 μm (Figure 1 C and D).

**Calculations and Statistical Analysis**

Daily DMI was calculated after correction for the weekly variation in DM content of ration components. For each sampling day, the reported DM and nutrient intakes are the averages of intakes recorded during the 3 preceding days.

For all papilla variables, averages were calculated per site per cow per sampling day [an average of 11.2 (SD = 2.9) papillae per site per cow per sampling day were collected]. Papilla surface area was calculated as 2 × one-sided surface area as measured. The thickness of the epithelial layer was calculated from the difference in thickness of the total papilla and thickness of the interstitium. Average of papilla surface area growth (mm²/d) and daily change in FOM intake (kg/d) were calculated by dividing the difference in surface area and FOM intake by the difference in days for 2 consecutive sampling days. Total VFA concentration in rumen fluid was calculated as the sum of acetic, propionic, and butyric acid.

Papilla variables were assumed to be related to sampling day, biopsy site, and treatment. Milk production and feed intake were assumed to be related to sampling day and treatment, and milk fat and protein content were assumed to be related to sample week and treatment. Data on papilla variables were analyzed using the MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC) with the model (Littell et al., 2006):

\[ Y_{ijkl} = \mu + T_i + S_k + D_l + (T \times D)_{il} + C_j + D(C)_{jl} + e_{ijkl} \]

where variable \( Y_{ijkl} \) was dependent on \( \mu \) as the average experimental value and fixed main effects of concentrate treatment \( T_i \) (i = RAP, GRAD), sampling day \( D_l \) (l = −50, . . . , 80), site \( S_k \) (k = ventral rumen sac, caudodorsal blind rumen sac, caudodorsal blind rumen sac), and fixed interaction \( (T \times D)_{jl} \). All other interactions were not significant and removed from the model. Cows, as experimental units, were represented with random effect \( C_j \) (j = 1, . . . , 12). Furthermore, random effects of sampling day within cows \( D(C)_{jl} \) were allowed, avoiding pseudo-replication due to multiple biopsy sites \( S_k \). Errors \( e_{ijkl} \) were assumed to be autocorrelated for repeated observations on the same cow and site using a spatial power covariance structure over sampling days (Littell et al., 1998). As concentrate treatment was only applicable to the treatment period (sampling d 9 to 80), the design could not be treated as a complete factorial design with respect to concentrate treatment and sampling day. The mixed model, however, was formulated as if a complete factorial design was in place, allowing treatment groups to differ before the treatment period. This structure was exploited by specifying custom hypotheses tests (ignoring default tests for main effects for sampling day, treatment, and their interaction, as made by PROC MIXED). Custom CONTRAST statements were constructed for: (1) the pretreatment period (sampling d −50 to 3), to compare the 2 future treatment groups, thereby evaluating the success of the random treatment allocation [neither dif-
ferences between groups were expected, nor found ($P \geq 0.268$); (2) testing the treatment main effect, $T_i$ (averaged over sampling days), over the treatment period; (3) testing the sampling day main effect, $D_l$ (averaged over treatments), over the total experimental period; and (4) testing the interaction between treatment and sampling day, $(T \times D)_il$, by testing sampling day by sampling day difference between concentrate treatment groups over the treatment period. To test specific hypotheses, CONTRAST and ESTIMATE statements were formulated to separate means. For analyses of feed intake, rumen pH and VFA, weekly milk production, milk fat and protein contents, papilla surface area by site, change in FOM intake, and change in papilla surface area, a simpler mixed model was used; fixed effect $S_i$ and random effect $D(C)_ij$ were removed, cow replaced site(cow) as subject of repeated measurement, and, where appropriate, $D_l$ was replaced by Week_l. All results are reported as least squares means with their standard error unless indicated otherwise. Significance of effect was declared at $P < 0.050$ and trends at $0.050 \leq P < 0.100$.

**Figure 1.** (A) Total thickness of the papilla at the tip (measured perpendicular to papilla length, indicated by vertical arrows) at 250, 500, 750, and 1,000 µm from the tip. (B) Length of the luminal boundary of the stratum corneum (thick outer line) and the length of the basal boundary of the stratum basale (thin inner line) in 1,000 µm of papilla length, starting from the papilla tip. (C and D) Total thickness of the papilla in the middle and thickness of the interstitium (both measured perpendicular to papilla length, approximately halfway the papilla length), measured 5 times with 250-µm intervals over a total length of 1,000 µm. A and B represent papillae at 16 d postpartum; C1 and C2 represent papillae at 10 d antepartum for future gradual (0.25 kg of DM/d; GRAD) and rapid (1.0 kg of DM/d; RAP) rate of increase of concentrate allowance groups, respectively; D1 and D2 represent papillae at 16 d postpartum for GRAD and RAP groups, respectively. All papillae shown were from the ventral rumen sac, 4-µm thick section, with hematoxylin eosin stain. Color version available online.
RESULTS

All cows completed the experiment. One cow in group GRAD calved earlier than expected and missed sampling at 10 d ap; otherwise all samples could be collected. Average (SD) actual sampling days were 46.3 (5.1), 26.5 (5.7), and 8.6 (3.4) d ap for the pretreatment and 3.0 (0.0) d pp and 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 for the treatment period. One animal was treated for mastitis (from RAP) and 7 were treated for (chronic) endometritis (4 from RAP, 3 from GRAD). All animals reacted favorably to veterinary treatment and followed their experimental treatments and sampling schedule as planned. The rapid or gradual rate of increase of concentrate allowance was readily accepted by all cows.

Intake of DM and Macronutrients

Intake of DM and macronutrients is presented in Table 2; in addition, daily DMI is shown in Figure 2A. Total DMI was affected by sampling day ($P < 0.001$). During the pretreatment period total DMI remained similar over the sampling days ($P = 0.277$), and during the pretreatment period future RAP and GRAD groups showed no differences in total DMI ($P = 0.416$) or, consequently, in intake of FOM, starch, sugars, and NE$_{L}$ ($P > 0.325$; data not shown). In the treatment period, total DMI increased from 3 to 30 d pp for group RAP and tended to increase further to 80 d pp ($P = 0.062$). For group GRAD, DMI increased to 44 d pp ($P < 0.004$), remaining similar thereafter. Total DMI was neither affected by the concentrate treatment nor by an interaction between concentrate treatment and sampling day. Concentrate treatment, sampling day, and their interaction affected DMI of the basal ration, which was higher for group GRAD at 9, 16, and 30 d pp, respectively ($P \leq 0.015$). In group RAP, DMI from the basal ration was 21.2% lower ($P \leq 0.006$) at 16 d pp compared with 9 or 30 d pp, whereas this decrease was not observed in group GRAD ($P \geq 0.156$).

In the treatment period, FOM intake was affected by sampling day ($P < 0.001$); in contrast to total DMI, an interaction between concentrate treatment and sampling day was observed ($P < 0.001$). Intake of FOM was 21.8% greater in group RAP than group GRAD at 16 d pp ($P < 0.001$). From 30 d pp onwards, FOM intake was similar for RAP and GRAD, but at 60 d

Table 2. Least square means of feed intake$^1$ (kg/d unless otherwise noted) for rapid rate of increase (1.0 kg of DM/d) of concentrate allowance (RAP; n = 6) and gradual rate of increase (0.25 kg DM/d) of concentrate allowance (GRAD; n = 6) treatments

<table>
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<th>Item</th>
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<th>Treatment period</th>
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<tr>
<td></td>
<td>$-50$</td>
<td>$-30$</td>
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<tr>
<td>Total DMI</td>
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<tr>
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<tr>
<td>GRAD</td>
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<td>GRAD</td>
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<td>GRAD</td>
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$^1$Reported mean is calculated over the 3 d preceding the sampling day.

$^2$D = sampling day relative to calving.

$^3$Standard error for LSM by sampling day; n = 12.

$^4$T = 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).

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

$^6$+$P < 0.100, **P < 0.050, ***P < 0.010, ****P < 0.001, significance of difference in LSM of RAP or GRAD for the same sampling day and variable.
Morphological Adaptation of Rumen Papillae

Daily milk production is shown in Figure 2A and weekly milk production by week in Figure 2B. Weekly milk production increased during the treatment period ($P < 0.001$) and tended to be less ($P = 0.067$) in group RAP (33.2 ± 1.3 kg/d) than group GRAD (37.6 ± 1.3 kg/d). No interaction between concentrate treatment and week was observed for milk production ($P = 0.416$). Weekly average milk production, expressed as fat- and protein-corrected milk (kg/d), was less ($P = 0.033$) in group RAP (34.0 ± 1.1 kg/d) than group GRAD (38.3 ± 1.1 kg/d; data not shown), whereas no interaction between concentrate treatment and week was observed.

Both milk protein and milk fat content (Figure 2B) were affected by week and decreased in wk 1 through 4 ($P < 0.001$). During wk 5 through 12, milk protein and milk fat contents did not vary ($P \geq 0.161$). Milk protein content was similar between treatments ($P = 0.174$), averaging 3.52 ± 0.07%, and an interaction between concentrate treatment and week for milk protein content was observed ($P = 0.034$). Milk protein content in group RAP (3.71%) was greater ($P = 0.024$) in wk 3 compared with group GRAD (3.35%). Milk fat content (Figure 2B) averaged 4.12 ± 0.12% and was neither affected by concentrate treatment nor by its interaction with week ($P \geq 0.590$).

Rumen content mass (solids and liquid) was affected by sampling day, increasing from 64.5 ± 2.8 kg during the pretreatment to 77.2 ± 2.8 kg during the treatment period ($P < 0.001$), but was not affected by concentrate treatment ($P = 0.826$) nor its interaction with sampling day ($P = 0.154$). Total VFA ($\text{mM}$) and rumen fluid pH (Figure 3A) were affected by sampling day ($P < 0.001$). Total rumen VFA concentration increased during the pretreatment period ($P = 0.011$), whereas pH decreased ($P < 0.001$). Total VFA averaged 79.5 ± 5.2 mM during the dry period, −50 to −10 d ap, increasing to 96.4 ± 5.2 mM at 3 d pp ($P = 0.002$), whereas fluid pH averaged 6.71 ± 0.09 during the dry period and decreased to 6.27 ± 0.09 at 3 d pp ($P < 0.001$). In the treatment period, total VFA increased to 120 ± 5.2 mM at 44 d pp ($P < 0.001$), whereas pH tended to decrease to 6.07 ± 0.09 at 44 d pp ($P = 0.097$); thereafter both remained similar up to 80 d pp. Neither total VFA nor pH were affected by concentrate treatment ($P \geq 0.538$) nor its interaction with sampling day ($P \geq 0.289$).

The molar proportions of acetic, propionic, and butyric acid in the rumen fluid (Figure 3B) were affected by sampling day ($P < 0.001$). Molar proportion of ace-
tic acid was larger \((P < 0.001)\) during the pretreatment period compared with the treatment period \((718 \pm 7 \text{ vs. } 645 \pm 7 \text{ mmol/mol, respectively})\), whereas the molar proportions of propionate and butyrate were smaller \((P < 0.001)\) during the pretreatment period \((181 \pm 7 \text{ and } 101 \pm 3 \text{ mmol/mol, respectively})\) compared with the treatment period \((236 \pm 7 \text{ and } 119 \pm 3 \text{ mmol/mol, respectively})\). An interaction was observed between concentrate treatment and sampling day for molar proportions of acetic and propionic acid \((P \leq 0.003)\), but not for butyric acid \((P = 0.501)\). The molar proportion of acetic acid in group RAP was 4.5 and 8.7% lower at 9 and 16 d pp, respectively, compared with group GRAD, with an opposite effect for molar proportion of propionic acid.

**Macroscopic Rumen Papillae Assessment**

Papilla surface area (Table 3) was affected by sampling day \((P < 0.001)\). During the pretreatment period, papilla surface area decreased by 18.6% \((P = 0.018)\), caused by a 17.8% decrease \((P < 0.001)\) in papilla width. The future RAP and GRAD groups showed neither a difference in papilla surface area \((P = 0.520)\) nor in length or width \((P \geq 0.268; \text{ data not shown})\) in the pretreatment period. More specifically, at 3 d pp, the day before starting the concentrate treatment, papilla surface area was similar for RAP and GRAD \((P = 0.306)\), but papilla surface area increased by 125% from 28.0 mm² at 3 d pp to 63.0 mm² at 80 d pp \((P < 0.001)\). Concentrate treatment interacted with sampling day for papilla surface area \((P = 0.011)\) and from 16 through 44 d pp papilla surface area was larger in group RAP \((P \leq 0.013)\) compared with GRAD.

Papilla length increased by 39% \((P < 0.001)\) and width by 50% \((P < 0.001)\) from 3 to 80 d pp. Papilla length tended to be affected by concentrate treatment \((P = 0.058)\) without concentrate treatment interacting with sampling day, whereas papilla width was affected by concentrate treatment \((P = 0.041)\) and by an interaction between concentrate treatment and sampling day \((P = 0.020)\). Group RAP had wider papillae at 16 and 30 d pp \((P \leq 0.035)\) and tended to have wider papillae at 44 d pp \((P = 0.053)\) when compared with GRAD.

Between sampling sites, papilla surface area differed (Figure 4; \(P = 0.001)\). Papillae from the ventral rumen sac \((46.0 \pm 1.9 \text{ mm}^2)\) had a larger surface area \((P \leq 0.017)\) compared with the ventral blind \((39.3 \pm 1.9 \text{ mm}^2)\) and dorsal blind \((41.8 \pm 1.9 \text{ mm}^2)\) rumen sacs, which did not differ \((P = 0.160)\). All sites were affected by sampling day \((P < 0.001)\) and showed a similar development of papilla surface area. Concentrate treatment affected the papilla surface area in the ventral rumen sac and the dorsal blind rumen sac \((P \leq 0.031)\), but did not affect the area in the ventral blind rumen sac \((P = 0.105)\). A tendency for an interaction between concentrate treatment and sampling day was observed for papilla surface area in the ventral rumen sac \((P = 0.068)\), but not in the ventral blind rumen sac \((P = 0.140)\) and the dorsal blind rumen sac \((P = 0.159)\).
Microscopic Rumen Papillae Assessment

Thickness of the rumen papillae at the tip and in the middle, thickness of the epithelium in the middle, and stratum basale-to-stratum corneum ratio (Table 4) were affected by sampling day ($P \leq 0.002$). During the pretreatment period, papilla thickness at the middle, stratum basale-to-stratum corneum ratio increased ($P \leq 0.027$); the future RAP and GRAD groups showed no differences for any of the microscopic papilla variables ($P \geq 0.289$; data not shown). In the treatment period, the change in daily FOM intake was greatest between 3 to 9 d pp for RAP and GRAD ($P \leq 0.004$). Thereafter, the change in daily FOM intake decreased and was approximately zero between 44 to 60 d pp and onwards. Change in daily FOM intake was not affected by concentrate treatment ($P = 0.124$), but an interaction between concentrate treatment and sampling day existed ($P < 0.001$). The change in daily FOM intake was greater in group RAP than group GRAD between 3 to 9 d pp (0.59 vs. 0.46 ± 0.03 kg/d, respectively; $P < 0.001$), and between 9 to 16 d pp (0.42 vs. 0.17 ± 0.03 kg/d, respectively; $P = 0.018$). Between 16 to 30 d pp, the change in daily FOM intake decreased to 0.23 ± 0.03 kg/d for group GRAD, and was lower than that for group GRAD with 0.23 ± 0.03 kg/d ($P = 0.018$). In group GRAD, the change in daily FOM intake decreased from 0.23 ± 0.03 kg/d at 16 to 30 d to 0.02 ± 0.03 kg/d between 44 and 60 d pp ($P < 0.001$), remaining similar thereafter ($P = 0.619$).

Change in papilla surface area (Figure 5B) was affected by sampling day ($P < 0.001$). During the pretreatment period, change in papilla surface area averaged $-0.13 \pm 0.13 \text{mm}^2$/d, and the future treatment groups did not reveal differences in change in papilla surface area ($P = 0.335$). The treatment period showed an interaction between concentrate treatment and sampling day ($P = 0.013$), but overall change in papilla surface area was not affected by concentrate treatment ($P = 0.169$). Change in papilla surface area was greater ($P < 0.001$) between 9 to 16 d pp in group RAP compared with GRAD, at 1.51 versus 0.66 ± 0.13 mm$^2$/d,

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**Table 3.** Least square means of papilla surface area, length, and width for rapid rate of increase (1.0 kg of DM/d) of concentrate allowance (RAP; n = 6) and gradual rate of increase (0.25 kg of DM/d) of concentrate allowance (GRAD; n = 6) treatments

<table>
<thead>
<tr>
<th>Item</th>
<th>Pretreatment period</th>
<th>Treatment period</th>
<th>Fixed effects$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D$^1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>−50 −30 −10$^4$ 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papilla surface area,$^5$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAP</td>
<td>34.4 31.7 30.2 28.0</td>
<td>35.2 46.0 55.4 60.5 64.7 66.1</td>
<td>203 0.015 &lt;0.001 0.001 0.011</td>
</tr>
<tr>
<td>GRAD</td>
<td>28.6 33.2 41.2 49.7 58.4 59.9</td>
<td>0.25 0.058 &lt;0.001 &lt;0.001 0.199</td>
<td></td>
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<tr>
<td>Papilla length, mm</td>
<td>7.8 7.5 7.8 7.5</td>
<td>8.4 9.1 9.7 10.1 10.5 10.6</td>
<td></td>
</tr>
<tr>
<td>RAP</td>
<td>7.4 7.8 8.7 9.2 10.3 10.3</td>
<td>0.25 0.058 &lt;0.001 &lt;0.001 0.199</td>
<td></td>
</tr>
<tr>
<td>GRAD</td>
<td>7.4 7.8 8.7 9.2 10.3 10.3</td>
<td>0.25 0.058 &lt;0.001 &lt;0.001 0.199</td>
<td></td>
</tr>
<tr>
<td>Papilla width, mm</td>
<td>2.8 2.5 2.4 2.3</td>
<td>2.7 3.0 3.4 3.6 3.6 3.4</td>
<td>0.10 0.041 &lt;0.001 0.183 0.020</td>
</tr>
</tbody>
</table>

$^1$D = sampling day relative to calving.

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

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

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

$^5$Average number of papillae collected per site per cow per sampling day was 11.2 (SD = 2.9). Mean of coefficients of variation were 0.22, 0.14, and 0.17 for papilla surface area, length, and width, respectively.

†$P < 0.100$, *$P < 0.050$, **$P < 0.010$, significance of difference in LSM of RAP or GRAD for the same sampling day and variable.
respectively. After this peak for group RAP, change in papilla surface area gradually declined to 0.09 mm$^2$/d between 60 to 80 d pp. In group GRAD, change in papilla surface area was similar ($P = 0.868$) from 3 to 9 d pp through the 44 to 60 d pp interval, averaging $0.57 \pm 0.13$ mm$^2$/d, and growth declined to $0.06 \pm 0.13$ mm$^2$/d between 60 to 80 d pp ($P = 0.001$).

**DISCUSSION**

The present study provides an overview of macroscopic and microscopic morphological changes of the rumen papillae in adult dairy cows during the dry period and subsequent 80 d of lactation and presents the first data showing the effect of postpartum concentrate build-up on rumen morphology. During the dry period, papilla surface area decreased and then increased rapidly again from d 3 pp onwards. In line with our hypothesis, the rapid rate of increase of concentrate allowance created a temporary difference in FOM intake, affecting rumen papilla development. Rumen papilla surface area and width increased at a higher rate in the group with the rapid rate of increase of concentrate allowance. In contrast to our expectations, neither the thickness of the papillae nor the thickness of the epithelium were affected by the concentrate treatment.

In the present experiment, rumen papillae surface area and papilla width showed a 19% reduction in some 50 d during the pretreatment period, whereas DM and FOM intake did not change. Earlier work suggests rapid rumen papilla atrophy is possible, with an approximate 65% reduction in surface area over 14 d (Dirksen et al., 1984). However, the 65% reduction coincided with an approximate 50% decrease in NE$_{l}$ intake (86 to 41 MJ/d) suggesting the rate of atrophy is influenced by the magnitude in ration change. In contrast to the limited papilla atrophy during the dry period, large and rapid changes in papilla surface area occur during early lactation. Depending on concentrate treatment, papilla surface area increased by 9 to 16 d to approximately the surface area at 50 d ap and increased by a further 85% by 80 d pp. The time required for rumen papilla adaptation to reach a steady state after transition to a higher plane of nutrition varies. Six to 8 wk are often cited, based on the work of Dirksen et al. (1984) and Liebich et al. (1987), whereas Bannink et al. (2008) concluded less than 4 wk of adaptation is required, although these studies base their conclusions on different measurements and experimental conditions. In the present study, papilla surface area, length, and width appear to reach a steady state between 60 and 80 d pp. However, it is evident that papilla surface area increases rapidly when daily FOM intake increases rapidly. Therefore, discussing rumen adaptation in dairy cattle

![Figure 4](image-url) Papilla surface area (mm$^2$) at the 3 rumen sampling sites for a rapid (1.0 kg of DM/d; RAP, ■, n = 6) and gradual (0.25 kg of DM/d; GRAD, □, n = 6) rate of increase of concentrate allowance postpartum. Day = day relative to calving. Values represent LSM ± SE.
in terms of change per day in morphological variables under study compared with change per day in ration variables (such as FOM) seems more appropriate. The initial increase in change of daily FOM intake between 3 and 9 d pp coincided with growth of the rumen papillae. This implies a capacity for a quick initiation of a response by the rumen papillae to an increase in FOM intake. However, in group RAP, daily change in surface area almost doubled between 9 and 16 d pp compared with 3 to 9 d pp, whereas the change in daily FOM intake peaked between 3 to 9 d pp, indicating a lag effect in the maximum response of papilla surface area to ration changes. In group GRAD, the initial peak in change of daily FOM intake between 3 to 9 d pp was not followed by a peak in change in papilla surface area, but the papilla surface area showed almost linear growth up to 60 d pp. Daily change in FOM intake from 30 to 44 and 44 to 60 d pp, however, was much smaller than that from 9 to 16 and from 16 to 30 d pp, also indicating a lag effect in the response of the rumen papillae in group GRAD. Obviously, the rumen papillae show a capacity for rapid growth, but the maximum response is delayed when compared with the changes in FOM. It remains unclear, however, why the capacity for rapid growth is not used later in lactation (especially in GRAD and to lesser extent in RAP) to reach a steady state in rumen papilla surface area in relation to the FOM intake earlier, as FOM intake was similar from 44 d pp onwards.

The lack of effect of rate of increase of concentrate allowance on histological variables suggests that rumen papillae proliferate in a similar manner when faced with a rapid or gradual rate of increase of concentrate allowance (Bannink et al., 2012) and, arguably, maintain a similar functional capacity per unit of surface area. In line with results of Steele et al. (2011) and Bannink et al. (2012), papilla thickness and the thickness of the epithelial layer in the middle decreased during the treatment period, although Steele et al. (2015) did not report a difference in epithelium thickness in the middle of the papillae when cows transitioned to a lactation ration. The thinner epithelium was maintained at the end of the experimental period, whereas the papilla surface area remained similar from 60 d onward. This suggests a thinner epithelium is also advantageous after absorption of VFA into the epithelium at the luminal side, either passively or by mediated transport (Aschenbach et al., 2011). Rumen papilla epithelium might be regarded as a functional syncytium, with cell-cell gap junctions allowing rapid diffusion between the apical and basal cell layers aided by a distinct distribution of pumps on the luminal and basal side of the epithelium (Graham and Simmons, 2005; Graham et al., 2007). A thinner epithelium reduces the distance VFA have to diffuse across before transfer from the epithelium into the blood, arguably enhancing the VFA absorption capacity.

A higher ratio between the stratum basale and stratum corneum indicates more extensive folding of the basal membrane and reflects the extent of rete-peg formation in the epithelium. The increase in this ratio at 9 d pp is in accordance with Dirksen et al. (1984), Liebich

Table 4. Least square means of total papilla thickness at the tip and at the middle, epithelium thickness at the papilla middle, and ratio of stratum basale to stratum corneum for rapid rate of increase (1.0 kg of DM/d) of concentrate allowance (RAP; n = 6) and gradual rate of increase (0.25 kg of DM/d) of concentrate allowance (GRAD; n = 6) treatments

<table>
<thead>
<tr>
<th>Item</th>
<th>Pretreatment period</th>
<th>Treatment period</th>
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<tr>
<td></td>
<td>D1</td>
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</tr>
<tr>
<td></td>
<td>−50</td>
<td>−30</td>
<td>−10⁴</td>
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<tr>
<td>Total papilla thickness, mm</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RAP</td>
<td>0.33 0.34 0.35 0.35</td>
<td>0.36 0.34 0.33 0.31 0.32 0.30</td>
<td>0.013</td>
</tr>
<tr>
<td>GRAD</td>
<td>0.39 0.37 0.37 0.33 0.33 0.32</td>
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<td></td>
</tr>
<tr>
<td>Papilla thickness middle, mm</td>
<td>0.29 0.28 0.27 0.23 0.22 0.22</td>
<td>0.009</td>
<td>0.306</td>
</tr>
<tr>
<td>站点厚度, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAP</td>
<td>0.25 0.26 0.28 0.27</td>
<td>0.27 0.25 0.23 0.23 0.22 0.22</td>
<td>0.009</td>
</tr>
<tr>
<td>GRAD</td>
<td>0.29 0.28 0.27 0.23 0.22 0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial thickness, μm</td>
<td>66 68 74 72</td>
<td>73 71 69 65 68 66</td>
<td>0.048</td>
</tr>
<tr>
<td>Ratio of length stratum basale to stratum corneum</td>
<td>1.71 1.79 1.84 1.76</td>
<td>1.63 1.68 1.58 1.60 1.63 1.58</td>
<td>0.035</td>
</tr>
</tbody>
</table>

³D = sampling 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); S = sampling site; 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.

et al. (1987), and Steele et al. (2015), who showed similar changes after switching cows to a higher plane of nutrition. Arguably, this increase is associated with the onset of papilla growth seen at this time (Figure 5B) and might reflect a high rate of cellular proliferation at the papilla tip, as suggested by Steele et al. (2015). The process responsible for the later decrease in the ratio remains unclear but might be associated with restructuring of the epithelium. The temporary larger stratum basale-to-stratum corneum ratio at 9 d pp might also have functional consequences. It can be speculated that more extensive folding of the stratum basale increases the capacity to maintain a large concentration gradient of VFA across the epithelium by increasing the capacity for efflux of VFA over the basal membrane to the capillaries with which it is closely associated (Dobson et al., 1956).

In the present study, manipulating the rate of increase of concentrate allowance created a difference in daily FOM intake as well as a greater intake of starch, sugar, and NE_{L} at 16 d pp in group RAP. In general, higher supply of nutrients in early lactation increases daily milk production (Kokkonen et al., 2004). Therefore, in the present experiment we expected, but did not observe, an interaction between sampling day and concentrate treatment for milk production, with a greater milk production and earlier peak in production for cows in group RAP than in group GRAD. We fed the basal diet and concentrate separately, and intake of the basal diet (mainly silage) was greater in the GRAD than the RAP group. Similarly, Ingvartsen et al. (2001) did not observe positive effects of faster concentrate supply in early lactation on milk yield with separate feeding of silage and concentrate. They attributed this lack of response to a decline in silage intake with fast concentrate supply, whereas a complete diet feeding resulted in highest milk production in their trial. It should also be stressed that unintended systematic differences in milk production capacity between treatment groups, despite the random assignment of cows, cannot be ruled out because treatment groups were relatively small (n = 6) and cows were blocked on expected calving date before random assignment to either one of the treatment groups.

The effects of the dry period ration, basal lactation ration, and concentrate treatment on rumen fluid affected total VFA concentration, VFA molar proportions, and pH of the rumen fluid, which is in agreement with earlier work (Dijkstra, 1994; Sutton et al., 2003; Bannink et al., 2008). All 3 major VFA likely affect rumen papillae development (Sander et al., 1959; Sakata and Tamate, 1979; Suarez et al., 2006), with butyric acid probably having the most pronounced effect (Sakata and Tamate, 1978; Shen et al., 2005; Malhi et al., 2013). Although the production rate of the different VFA influences their molar proportions in the rumen fluid (Dijkstra, 1994), no conclusions on proliferative effects of any specific VFA can be drawn based on VFA concentration and molar proportion alone.

In conclusion, our study clearly shows the relation between changes in FOM intake and papilla morphology (papilla length, width, thickness, surface area, and epithelium) in Holstein Friesian dairy cows, and these
results were obtained under experimental conditions and with rations that reflect common dairy practice. During the 8-wk dry period, papilla surface area decreased, but, within 1 to 2 wk pp, surface area returned to the level at the start of the dry period and continued to increase up to 8 to 9 wk pp. Rate of increase of concentrate allowance after calving affected rate of increase of papilla surface area, indicating rumen papillae can mount a variable response to the level of increase of daily FOM intake.

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