The logistic curve as a tool to describe the daily ruminal pH pattern and its link with milk fatty acids

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ABSTRACT

Daily ruminal pH variation can be summarized by a cumulative logistic curve based on the amount of time below multiple pH points and characterized by 2 parameters (β_0 and β_1). Moreover, rumen pH variation affects the rumen microbiome as well as the biohydrogenation pathways resulting in a modified secretion of milk fatty acids (FA). The aims of this study were to assess the shifts in milk FA due to rumen pH changes and to estimate the relationship between milk FA and the 2 parameters of the logistic curve. The data consisted of milk samples of 2 experiments. In experiment 1, 3 cows were subjected to 5 treatments in which the type and amount of concentrate were changed during 33 d: (1) control diet 1, (2) stepwise replacement of a standard concentrate (CONC) by a CONC rich in rapidly fermentable carbohydrates, (3) increase in the total amount of CONC, (4) treatment with a buffer solution, and (5) control diet 2. A 3×3 Latin square design with 3 cows was used in the second experiment. During the first 14 d of each period, the cows received a control diet with a standard CONC, whereas in the last 7 d the standard CONC was replaced step-by-step by a CONC rich in rapidly fermentable carbohydrates and the amount of CONC was increased. During each period, a different buffer treatment was added to the diet. Milk FA and pH reacted similarly in both experiments: decreasing proportions of *iso* FA and increasing proportions of odd-chain FA were observed. However, an abrupt change to a 76% CONC diet as for one cow of experiment 1 led to almost a 10-fold increase in C18:1 *trans*-10 (0.79 vs. 6.75 g/100 g of FA). In experiment 2, the stepwise approach of adding CONC and the continuous supplementation of buffer led to minimal increases in C18:1 *trans*-10 and decreases in rumen pH compared with the diet with standard CONC only.

Fatty acid proportions were influenced by the level of rumen pH (β_1) or the rumen pH variation (β_0), or both. High proportions of C18:1 *trans*-10 (above 4 g/100 g of FA) occurred with low and largely fluctuating pH (low β_1 , low β_0), whereas situations with low, stable pH (low β_1 , great β_0) did not induce a shift toward the secondary biohydrogenation pathway. C18:1 *trans*-11 and C18:2 *cis*-9, *trans*-11 were only influenced by the pH variation and not by the average pH, whereas *iso* C14:0 and *iso* C16:0 FA were only dependent on the average pH and not influenced by diurnal pH variation. Overall, milk FA changes were related to pH changes; however, this relationship is not straightforward and needs further research.

Key words: milk fatty acid, rumen pH, acidosis

INTRODUCTION

Ruminal acidosis is a nutritional disorder generally resulting from feeding diets rich in rapidly fermentable carbohydrates (**RFCH**) or low in digestible fiber, particularly when animals are not adapted to these feeds (e.g., insufficient rumen buffering and nonadapted rumen papillae). It is caused by an increase in the production of VFA, which lowers ruminal pH to nonphysiological levels (Krause and Oetzel, 2006; Bramley et al., 2008; Aschenbach et al., 2011). As such, rumen pH parameters are the only reliable tool to diagnose acute ruminal acidosis and SARA.

Depending on the method used to measure ruminal pH, different pH parameters have been used to diagnose SARA. Garrett et al. (1999) performed rumenocentesis to measure pH and suggested that a mean ruminal pH of 5.5 is a useful threshold for the identification of cows that are suffering from SARA. However, the diurnal pH variation is not taken into account by this threshold value. This is necessary as the rumen pH may decrease several times during the day within the SARA range without any sign of acidosis (Aikman et al., 2011). However, diurnal pH variation cannot be taken into account when rumenocentesis is applied. As such, measurements of pH over time are introduced by

Received November 8, 2011.

Accepted June 10, 2012.

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implementing indwelling pH probes in rumen-fistulated animals. New parameters, such as the time rumen pH is below 5.8, have been proposed, together with various threshold values (Beauchemin et al., 2003; Gozho et al., 2005). Nevertheless, these threshold values still do not provide information on the rumen pH range covered throughout the day. To avoid this problem, AlZahal et al. (2007) compared 4 different models and considered the logistic curve as the optimal choice to fit cumulative pH data.

Ongoing research aims to develop noninvasive methods that can be used to determine rumen pH without the need for rumenocentesis (Nordlund et al., 1995) or rumen-fistulated animals (Kilic, 2011). A possible method is the analysis of the milk FA profile, as milk fatty acids are affected by rumen pH. A decrease in rumen pH leads to a change in the biohydrogenation of unsaturated FA, resulting in the formation of C18:1 *trans*-10 and C18:2 *trans*-10, *cis*-12 (Figure 1; Bauman and Griinari, 2003). Further, the composition of the rumen microbial system is affected by rumen pH and, because bacteria are the major source of odd- and branched-chain FA (**OBCFA**) in milk (Vlaeminck et al., 2006), changes in the ruminal population are reflected in the milk FA profile. As such, it is hypothesized that not only the diet but also the rumen environment has an effect on the milk FA profile and specifically, the OBCFA and C18 biohydrogenation intermediates.

The aims of this study were to assess the shifts in milk FA due to rumen pH changes and to estimate the relationship between milk OBCFA and C18 biohydrogenation intermediates (e.g., C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *trans*-11, *cis*-15, C 18:2 *cis*-9, *trans*-11, and C18:2 *trans*-10, *cis*-12) and parameters describing logistic rumen pH curves.

MATERIALS AND METHODS

Experimental Design and Animals

Two acidosis-induction experiments were performed at Schothorst Feed Research (Lelystad, the Netherlands). In each experiment, 3 multiparous rumen-fistulated Holstein-Friesian dairy cows were used, of which 2 were common across the experiments. Although a larger number of animals would be opportune, this was not feasible, as rumen-fistulated animals were necessary to measure rumen pH. Average DIM and milk yield (kg/d; mean $\pm$ SD) at the beginning of experiment 1 were 127 ± 61.9 and 33.0 ± 6.77 , respectively. Average (mean $\pm$ SD) DIM and milk yield (kg/d) at the beginning of experiment 2 were 170 ± 30.3 and 43.6 ± 4.42 , respectively. In the first experiment (33 d), 3 cows were

subjected to a sequential design consisting of 5 different blocks (8 rations; Table 1):

- (1) Diet consisting of 65% forage (50% corn silage + 50% grass silage) and 35% standard concentrate (**S-CONC**) for 7 d (ration 1);
- (2) Stepwise daily replacement of the S-CONC by a concentrate rich in wheat (**W-CONC**) until 100% W-CONC [forage:concentrate ratio (**F:C**) = 65/35] for 10 to 13 d (number of days dependent on the response of the cow's rumen pH to the dietary shifts). The 4 diets selected from this period were
 - (2.1) S-CONC/W-CONC/forage: 29/9/65 (ration 2-1);
 - (2.2) S-CONC/W-CONC/forage: 17/17/66 (ration 2-2);
 - (2.3) S-CONC/W-CONC/forage: 9/26/66 (ration 2-3);
 - (2.4) S-CONC/W-CONC/forage: 0/34/66 (ration 2-4);
- (3) Increase in the total amount of concentrate for 4 to 8 d, dependent on the response of the cow's rumen pH to the dietary shifts (F:C = 48/52, 42/58, and 24/76; ration 3);
- (4) Increase in the forage amount due to signs of sickness of the cows (low DMI, foamy feces, or reduced rumen motility) + addition of a buffer solution [sodium bicarbonate (cow X, 1 portion of 250 g/d mixed through forage), dried alfalfa (cow Y, 2 portions of 375 g/d mixed through forage), and rapeseed straw (cow Z, 2 portions of 150 g/d mixed through forage)] for 2 d (ration 4);
- (5) Same diet as in block 1 with an F:C ratio of 65:35 and the concentrate fraction consisting of

Figure 1. Main intermediates of the primary (full arrows) and a secondary biohydrogenation (dotted arrows) pathway as affected by rumen pH. Adapted from Jenkins et al. (2008) and Bauman and Griinari (2003).

Table 1. Information on dietary composition (g/kg of DM), nutritional values, and number of milk samples selected per cow in experiment 1

Ration	Cow (no. of milk samples)	S-CONC ¹	W-CONC ¹	Forage	VEM ²	DVE ³	OEB ⁴
1	X (1), Y (1), Z (1)	346	0	654	999	85	12
2-1	X (1), Y (1), Z (1)	256	86	656	982	82	11
2-2	X (1), Y (1), Z (1)	171	172	657	984	81	9
2-3	X (1), Y (1), Z (1)	88	256	656	987	79	7
2-4	X (1), Y (1), Z (1)	0	341	659	990	77	5
3	X (4)	260	320	420	1,026	88	9
	Y (4)	196	324	480	1,017	85	8
	Z (4)	452	308	240	1,053	96	11
4	X (2)	259	319	422	1,026	88	9
	Y (2)	0–209	0	791–1,000	926–958	69–78	8–11
	Z (2)	209–452	0–308	240–791	958–1,053	78–96	11
5	X (7), Y (4), Z (5)	352	0	658	979	84	13

¹S-CONC = standard concentrate; W-CONC = wheat-based concentrate.

²Fodder unit milk (1 VEM = 6.9 MJ of NE_L; CVB, 1995).

³Protein digestible in the small intestine (Tamminga et al., 1994).

⁴Rumen-degradable protein balance (Tamminga et al., 1994).

S-CONC only for 4 to 7 d, depending on the cow (ration 5).

In the second experiment (Table 2), a 3 × 3 Latin square design with 3 cows was used. The experiment consisted of 3 periods and each period lasted 21 d. During the first 14 d of the period, the cows received a diet with an F:C of 68:32 and S-CONC only, whereas in the last 7 d, the S-CONC was replaced step-by-step by W-CONC (from 100% S-CONC to 44% S-CONC and 56% W-CONC). The wash-out period consisted of only 14 d, which could affect the reported results. Simultaneously, the amount of concentrate was increased to an F:C up to 46:54. The 3 dietary treatments of the Latin square design included 3 different buffering solutions [sodium bicarbonate (1 portion of 250 g/d mixed through forage), dried alfalfa (2 portions of 375 g/d mixed through forage), or rapeseed straw (2 portions of 150 g/d mixed through forage)].

The barn was illuminated by fluorescent lamps from 6 to 22 h during the experiments. Both experiments were approved by the ethical review committee on experimental animals of the Livestock Science Group (Lelystad, the Netherlands; code 2008-20).

Experimental Treatments and Diets

As the absolute amount of concentrate in the ration increased, the energy level increased. During the first period of the second experiment, the ration was balanced based on the protein digestible in the small intestine/fodder unit milk (DVE/VEM) ratio, which was estimated from the expected mean milk production. A mixture of corn silage and grass silage (50/50) was offered as forage. Feeding was twice per day (around 0830 and 1630 h) and feed refusals were collected once daily before morning feeding. Cows were fed ad libitum with a small proportion of feed residues to prevent selection

Table 2. Information on dietary composition (g/kg of DM) and nutritional values and number of milk samples selected per cow in experiment 2

Day	No. of milk samples per cow	S-CONC ¹	W-CONC ¹	Forage	VEM ²	DVE ³	OEB ⁴
13	3	327	0	673	973	84	13
14	3	314	39	646	979	84	12
15	3	305	79	616	986	85	12
16	3	303	110	587	992	86	11
17	3	289	160	551	998	86	11
18	3	275	197	528	1,004	87	10
19	3	266	238	496	1,010	87	10
20	3	254	276	469	1,016	88	9
21	3	242	313	445	1,022	88	9

¹S-CONC = standard concentrate; W-CONC = wheat-based concentrate.

²Fodder unit milk (1 VEM = 6.9 MJ of NE_L; CVB, 1995).

³Protein digestible in the small intestine (Tamminga et al., 1994).

⁴Rumen-degradable protein balance (Tamminga et al., 1994).

Table 3. Ingredients, chemical composition, and nutritive values of the 2 concentrates

Item	Standard concentrate	Wheat-based concentrate
Composition (g/kg of DM)		
Rapeseed meal (formaldehyde treated)	42.0	—
Corn	204	—
Peas	79.0	226
Soybean meal	30.0	—
Corn gluten feed meal	30.0	—
Wheat	163	700
Wheat middlings	83.0	—
Palm kernel expeller	100	—
Rapeseed meal	130	—
Citrus pulp	50	—
Molasses, cane	55	60.0
Limestone	15	—
Urea	—	14.0
Salt	11.5	—
Mineral-vitamin mixture	7.5	—
Analysis (g/kg of product)		
DM (g/kg of fresh material)	860	862
Ash	71	28
CP	162	169
Crude fat	33	20
Crude fiber	64	32
Starch	271	462
Sugar	62	48
NDF	185	96

(approximately 2.5 to 5% of feed refused). Cows were fed a TMR with the concentrate mixed through the basal diet.

Sample Collection and Measurements

Feed Analysis and Cow Performance. Both forages and concentrates were sampled weekly. At the end of the experiment, a pooled sample of the forages and concentrates was analyzed separately by wet chemical analysis (Schothorst Feed Research). Results included contents of DM by oven drying at 103°C (ISO, 1999a), crude ash by incineration at 550°C (ISO, 2003), CP by the Dumas method (ISO, 2004), crude fiber (NEN, 1988), crude fat by acid hydrolysis (ISO, 1999b), NDF

assayed with a heat-stable amylase and expressed exclusive of residual ash (ISO, 2005), ADL by solubilization of cellulose with sulfuric acid (ISO, 2008), and sugar by 40% ethanol extraction (NEN, 1974). The composition and chemical analysis of the S-CONC and W-CONC are given in Table 3. The chemical compositions of the grass and corn silage are given in Table 4. Dry matter intake and milk yield were recorded daily.

Rumen Function. In both experiments, rumen pH was measured every day for 22 to 24 h with an indwelling pH probe (HI 6101405; Hanna Instruments, BV IJsselstein, the Netherlands). Probes were calibrated daily at pH 4 and 7 and allowed simultaneous registration of temperature, time, and pH, which were recorded every minute (MadgeTech pHTemp101 data logger; MadgeTech, Warner, NH). To exclude erroneous data, only pH measurements associated with a temperature registration greater than 32°C and a pH value between 5 and 7.2 were used in further calculations, whereas other registrations were discarded. Daily (24-h) pH values were assessed proportionally from the 22- to 24-h registration period.

Milk Analysis. Milk samples of the evening of and the morning after the pH registration day were sampled and pooled, based on the evening and morning milk yield, for further analysis. In the first experiment, milk from each ration presented in the section Experimental Design and Animals was analyzed ($n = 49$; Table 1), whereas milk samples from d 13 to 21 of each period were analyzed in the second experiment ($n = 81$; Table 2). To assess how quickly milk FA returned to original values after a dietary acidosis challenge, all samples of the fifth block of experiment 1 were selected for analysis. As milk FA proportions returned within a day back to their normal range, the averages of the samples of block 5 are presented.

Milk fat, protein, and lactose contents were determined according to the International Organization for Standardization (ISO, 1999c) by Qlip (Zutphen, the Netherlands). Milk samples were stored at -20°C until being analyzed for FA composition. Milk FA were

Table 4. Chemical composition and nutritive values of corn and grass silage of experiments 1 and 2

Analysis (/kg of DM)	Corn silage, experiment 1, ration 1	Corn silage, experiment 1, rations 2–5	Grass silage, experiment 1	Corn silage, experiment 2	Grass silage, experiment 2, period 1	Grass silage, experiment 2, periods 2–3
DM (g/kg of fresh material)	309	277	447	277	447	518
Ash (g)	44	59	106	59	106	115
CP (g)	74	75	171	75	171	179
Crude fat (g)	36	34	39	34	39	39
Crude fiber (g)	174	219	265	219	265	257
Starch (g)	347	262	—	262	—	—
Sugar (g)	<12	12	76	12	76	87
NDF (g)	354	422	505	422	505	504

quantified by GLC after extraction (Chouinard et al., 1997) and methylation (Stefanov et al., 2010) and were expressed as g/100 g of FA methyl esters. Tridecanoic acid (as triacylglyceride; Sigma, Bornem, Belgium) was added as internal standard.

GC Analysis. All samples were injected on a GC CP-Sil88 column for FA methyl esters (100 m $\times$ 0.25 mm $\times$ 0.2 μ m; Chrompack Inc., Middelburg, the Netherlands) at 2 temperature programs, as described by Stefanov et al. (2010). These temperature programs are needed to quantify FA that are co-eluting in 1 of the 2 programs (e.g., *anteiso* C17:0 with C16:1 *cis*-10). Two microliters of the sample was injected with a split ratio of 1/50. Most FA peaks were identified through mixtures of methyl ester standards (BR2 and BR3, Larodan Fine Chemicals AB, Malmö, Sweden; Supelco 37, Supelco Analytical, Bellefonte, PA; PUFA-3, Matreya LLC, Pleasant Gap, PA), based on retention time.

Statistical Analysis

All statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL) or R version 2.12 (R Foundation for Statistical Computing, Vienna, Austria). Cow performance, rumen pH, and milk composition variables of experiment 1 were compared using the linear mixed model $Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}$, where Y_{ijk} = independent variable, μ = mean, A_i = fixed effect of ration, B_j = random effect of cow, and ε_{ijk} = residual error term.

Cow performance variables, rumen pH variables and milk composition variables of experiment 2 were compared using the linear mixed model $Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijklm}$, where Y_{ijklm} = independent variable, μ = mean, A_i = fixed effect of day (dietary change), B_j = random effect of period, C_k = fixed effect of treatment (adding 1 of the 3 buffer treatments), D_l = random effect of cow, and ε_{ijklm} = residual error term. The Bonferroni post hoc test was performed on both mixed models. This test is based on Student's *t* statistics and adjusts the observed significance level based on the fact that multiple comparisons are made.

Three samples of ration 4 in experiment 1 were discarded from the data analyses described below due to clinical signs of acidosis such as an extremely low DMI of 0.5 kg/d. One hundred twenty-six samples were kept for data analyses. For each sample, the time that pH was <5.0 until the time that pH was <7.2 , with steps of 0.1, were calculated in minutes per day, which led to 23 new pH-related data points for each sample. Based on these data points, a logistic curve was fitted for each sample by R version 2.12 with package *drc*, function *drm*. A logistic curve depends on 3 parameters that

describe the slope (β_0), the upper limit (β_2), and the inflection point of the curve (β_1):

$$y = \frac{\beta_2}{1 + \exp[-\beta_0 \times (x - \beta_1)]}$$

The y-axis represents the accumulated time (min/d) spent below each corresponding pH point on the x-axis. Values on the x-axis represent the ruminal pH. As the upper limit of the curve was kept constant at 1,440 min/d for all curves, the logistic curve is described by 2 parameters: the slope of the curve (β_0), and the inflection point of the curve (β_1). The advantage of the logistic curve compared with the regularly used threshold parameters to define acidosis is that it integrates the information of the daily pH pattern in 2 parameters only. To evaluate the logistic curve fitted to the data points of each day of each cow, the coefficient of determination was calculated for each logistic curve and evaluated. A coefficient of determination of 1 means that the curve fits the data exactly.

Pearson correlation coefficients (τ_p) between the parameters of the logistic curve and milk FA were assessed by SPSS 15.0. Principal component analysis (PCA) was performed based on the logistic parameters (β_0 and β_1), milk FA related to the microbial community, and rumen biohydrogenation (g/100 g of FA; *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *anteiso* C15:0, *iso* C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12, and C18:2 *trans*-11, *cis*-15) by SPSS 15.0 for Windows (SPSS Inc.) and rumen parameters [pH minimum, average pH, area under the curve (AUC) of pH <5.8 or 6.0, and time with pH <5.8 or 6.0].

A multiple linear regression has been performed by SPSS 15.0 with the logistic parameters of the rumen pH curve as independent variable and milk FA as dependent variables (C5:0, C7:0, C9:0, C11:0, *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *anteiso* C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12, and C18:2 *trans*-11, *cis*-15). The final variables of the linear model were determined based on a stepwise selection method.

Finally, several scatterplots were prepared with Excel 2010 (Microsoft Office 2010; Microsoft Corp., Redmond, WA). These plots gave more insight into the combination of β_0 and β_1 on one hand and the milk FA on the other hand. Parameters β_0 and β_1 was plotted on the x- and y-axis, respectively. Milk FA (*anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *anteiso* C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, C17:0, C18:1 *trans*-10,

C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, and C18:2 *trans*-11,*cis*-15) were used as a third variable and were plotted on the figure using a color code.

RESULTS

Generally, cow or period (experiment 2 only) effects were not significant either in experiment 1 or experiment 2 (results not shown). For confidentiality reasons, treatment effects (experiment 2) were not disclosed and hence are not discussed further in this paper.

Feed Intake and Milk Production

Tables 5 and 6 show the results of the mixed model for DMI, milk yield, milk fat, milk protein content, and the milk fat:protein ratio for experiment 1 and 2, respectively. In experiment 1, the lowest DMI and milk yield were recorded when a buffer solution was added to the diet (ration 4), although it is uncertain whether this is at the origin of the lower intake and yield. Milk fat or protein content was not affected by ration; however, milk fat was numerically lowest when the absolute intake of concentrate reached its maximum level. In experiment 2, no effect on DMI, milk yield, and milk protein was recorded. Milk fat gradually decreased during the experiment and reached the lowest value at d 20 of each period.

Rumen pH and Milk FA Changes

In experiment 1, rumen pH (Table 5) and milk FA were both affected by ration (Appendix Tables A1–A6). When the absolute intake of concentrate in the ration reached its maximum (ration 3), the lowest average pH and minimum pH were recorded. All samples of ration 3 were classified as suffering from acidosis based on the threshold time with pH <5.8 (475 or more min/d; AlZahal et al., 2007). At the same time, increasing proportions of some biohydrogenation FA intermediates (C18:1 *trans*-10 and C18:1 *trans*-11) and odd-chain FA (C15:0 and C17:0) were observed, whereas the proportions of saturated (C4:0, C6:0, C8:0, C10:0, and C12:0) and *iso* FA (*iso* C14:0, *iso* C15:0, *iso* C16:0, and *iso* C17:0) in the milk fat decreased (Figure 2). Maximum levels of C18:1 *trans*-10 were reached with an average value of 2.75 g/100 g of FA.

In experiment 2, a more gradual increase in the concentrate in the ration was offered to the cows in combination with 1 of the 3 treatments aiming at buffering rumen pH. At d 21, 4 out of 9 milk samples were classified as subacute acidotic; however, on aver-

age, time with pH <5.8 did not reach the threshold value of AlZahal et al. (2007). Milk OBCFA and SFA showed the same pattern as in experiment 1 but less pronounced (Appendix Tables A2 and A6). The SFA C4:0 and C18:0 decreased in experiment 2, whereas C10:0 and C12:0 increased compared with the control diet; C18:1 *trans*-10 did not reach the maximum level as in experiment 1, notwithstanding a minimal increase of C18:1 *trans*-10 was observed (0.26 vs. 0.43 g/100 g of FA).

Logistic Curve

To quantify the relationship between rumen pH and milk FA, a method to describe the daily pH pattern consists of fitting a logistic curve for each sample of each cow of each day (Figure 3). In our data set, the coefficient of determination of the logistic curve for each sample was greater than 0.96.

The overall average (SD) of parameters β_0 and β_1 was 7.15 (2.10) and 6.07 (0.18; based on data from both experiments 1 and 2, $n = 126$). Parameters β_0 and β_1 ranged from 3.83 to 16.4 and from 5.7 to 6.5, respectively. The higher the β_0 value, the more stable the rumen pH was throughout the day. A higher β_1 value indicates a higher average rumen pH. Curves with a lower β_1 value are linked to acidotic cows, whereas curves with a higher β_1 value are linked to healthy cows. Rumen conditions characterized by low β_1 and β_0 values indicate a low average rumen pH associated with a large diurnal pH range, whereas rumen conditions characterized by a low β_1 but high β_0 value indicate a low average rumen pH that hardly varies throughout the day (Figure 3). The opposite, a high β_1 value associated with both low and high β_0 values also occurs, as illustrated in Figure 3.

Link Between Milk FA and Parameters of the Logistic Curve

PCA and Correlations. As parameters β_0 and β_1 reflect the daily pH pattern and this pH pattern might be reflected in the milk FA profile, a PCA (Figure 4) was performed to assess whether these parameters were linked with milk FA (more specifically, OBCFA: *iso* C13:0, *iso* C14:0, *iso* C15:0, *iso* C16:0, *iso* C17:0, *iso* C18:0, *anteiso* C13:0, *anteiso* C15:0, *anteiso* C17:0, C15:0, and C17:0) and C18 biohydrogenation intermediates (C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, and C18:2 *cis*-11,*trans*-15).

Principal component analysis was used to assess whether the parameters of the logistic curve were re-

Table 5. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on DMI, cow performance variables, and rumen parameters of experiment 1

Variable	n ¹	Mean	SE	Ration								P-value (ration) ²
				1	2-1	2-2	2-3	2-4	3	4	5	
S-CONC (g/kg of DM)				346	256	171	88	0	196-452	0-452	342	
W-CONC (g/kg of DM)				0	86	172	256	341	308-324	0-319	0	
n				3	3	3	3	3	12	6	16	
DMI (kg/d)	46	22.1	1.168	26.7 ^a	24.1 ^a	24.2 ^a	22.8 ^{ab}	21.2 ^{ab}	23.8 ^a	13.5 ^{b,3}	20.8 ^a	**
Milk yield (kg/d)	35	31.9	2.531	33.7 ^a	34.3 ^a	34.3 ^a	35.1 ^a	33.2 ^{ab}	32.2 ^a	22.1 ^b	30.2 ^{ab}	**
Milk fat (g/100 g)	35	3.90	0.230	4.07	4.08	3.94	3.85	4.00	3.56	4.31	3.45	0.49
Milk protein (g/100 g)	35	3.66	0.172	3.61	3.61	3.64	3.66	3.65	3.69	3.74	3.70	0.66
Milk fat/milk protein	35	1.07	0.105	1.13	1.14	1.08	1.06	1.10	0.98	1.18	0.93	0.46
Mean pH	49	6.08	0.030	6.05 ^{ab}	6.04 ^{ab}	6.07 ^{ab}	6.09 ^{ab}	6.01 ^{ab}	5.89 ^a	6.29 ^b	6.22 ^b	***
Minimum pH	49	5.54	0.039	5.44 ^{ab}	5.51 ^{ab}	5.55 ^{ab}	5.49 ^{ab}	5.52 ^{ab}	5.24 ^a	5.86 ^b	5.72 ^b	***
Maximum pH	49	6.94	0.042	7.08	6.84	7.00	6.94	6.80	6.95	6.99	6.90	0.60
Time pH <5.8 (min/d)	49	281	39.8	391 ^{ab}	262 ^{ab}	188 ^{ab}	185 ^{ab}	307 ^{ab}	608 ^b	253 ^{ab}	50.1 ^a	***
AUC ⁴ pH <5.8 (min/d × pH)	49	47.4	9.16	58.1 ^{ab}	40.7 ^{ab}	21.6 ^a	21.8 ^a	36.8 ^{ab}	149 ^b	45.9 ^a	5.5 ^a	***

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).¹Number of samples lower than 49 is due to missing measurements.²P-values according to the linear mixed model $Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}$, where Y_{ijk} = independent variable, μ = mean, A_i = fixed effect of ration, B_j = random effect of cow, and ε_{ijk} = residual error term.³Low average DMI due to 2 extremely low DMI (0.5 kg/d) measurements for cow Y.⁴AUC = area under the curve.**0.001 < $P < 0.01$; *** $P < 0.001$.

Table 6. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on cow performance variables and rumen parameters of experiment 2

Variable	n ¹	Mean	SEM	13	Day										P-value ³	
					14	15	16	17	18	19	20	21	SE ²	Trt	Day	Day
S-CONC (g/kg of DM)				327	314	305	303	289	275	266	254	242				
W-CONC (g/kg of DM)				0	39	79	110	160	197	238	276	469				
n				9	9	9	9	9	9	9	9	9				
DMI (kg/d)	81	20.5	0.81	19.4	19.8	20.3	20.5	20.8	20.6	20.4	21.6	20.8	0.96	0.33	0.26	
Milk yield (kg/d)	81	22.8	5.19	22.7	23.2	22.4	23.3	22.8	22.5	22.9	22.8	22.6	5.21	***	0.96	
Milk fat (g/100 g)	80	4.28	0.135	4.67 ^a	4.52 ^{ab}	4.48 ^{ab}	4.46 ^{ab}	4.04 ^b	4.21 ^{ab}	4.05 ^b	4.05 ^b	4.04 ^b	0.182	0.88	**	
Milk protein (g/100 g)	80	3.81	0.121	3.85	3.78	3.77	3.80	3.75	3.81	3.82	3.83	3.86	0.124	0.25	0.09	
Milk fat/milk protein	80	1.12	0.022	1.21 ^a	1.20 ^{ab}	1.19 ^{abc}	1.17 ^{abc}	1.08 ^{abc}	1.11 ^{abc}	1.06 ^{bc}	1.06 ^{bc}	1.05	0.036	0.79	***	
Mean pH	80	6.10	0.080	6.18	6.14	6.13	6.08	6.07	6.04	6.08	6.09	6.10	0.089	0.82	0.42	
Minimum pH	80	5.61	0.050	5.76	5.65	5.66	5.53	5.56	5.56	5.64	5.57	5.55	0.069	0.19	*	
Maximum pH	80	6.91	0.117	6.95	6.92	6.95	6.89	6.92	7.01	6.87	6.87	6.84	0.132	0.71	0.72	
Time pH <5.8 (min/d)	80	225	87.2	70	155	166	262	228	283	285	295	282	109.5	0.64	0.30	
AUC ⁴ pH <5.8 (pH × min/d)	80	26.4	11.09	40.5	14.7	11.6	27.8	27.8	43.4	44.8	26.4	37.0	15.87	0.27	0.24	

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).
¹Number of samples lower than 81 due to missing measurements.
²SE = weighted SE of d 13 to 21.
³P-values according to the linear mixed model $Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijklm}$, where Y_{ijklm} = independent variable, μ = mean, A_i = fixed effect of day, B_j = random effect of period, C_k = fixed effect of treatment (Trt), D_l = random effect of cow, and ε_{ijklm} = residual error term.
⁴Area under the curve.
*0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001.

Ration	Standard concentrate (g/kg of DM)	Wheat-based concentrate (g/kg of DM)
1	346	0
2-1	256	86
2-2	171	172
2-3	88	256
2-4	0	341
3	196 - 452	308 - 324
4	0 - 452	0 - 319
5	342	0

Figure 2. Graphical representation of the effect of gradual replacement of a standard concentrate by a wheat-based concentrate (rations 2-1 to 2-4), increase in the amount of concentrate (ration 3), and supplementation of buffer (ration 4) in experiment 1 on time with pH < 5.8 (min/d), C18:1 *trans*-10, the sum of *iso* FA (*iso* C13:0, *iso* C14:0, *iso* C15:0, *iso* C16:0, *iso* C17:0, and *iso* C18:0), the sum of *anteiso* FA (*anteiso* C15:0 and *anteiso* C17:0), and the sum of odd-chain FA (C15:0 and C17:0) in milk.

lated to parameters that are regularly used to identify acidosis, such as pH minimum, average pH, AUC of pH < 5.8 or 6.0 and time with pH < 5.8 or 6.0. Principal

component (PC) 1 and PC 2 are both linear regressions of the variables used in the analysis and as such, they both explain part of the variance in the current

Figure 3. Four examples from the current data set of logistic curves with different slope (β_0) and inflection point (β_1) values ($\diamond$: $\beta_0 = 12.5$, $\beta_1 = 5.9$; $\blacksquare$: $\beta_0 = 4.8$, $\beta_1 = 6.3$; $\blacktriangle$: $\beta_0 = 4.8$, $\beta_1 = 5.9$; $\circ$: $\beta_0 = 16.4$, $\beta_1 = 6.3$). The cow with pH measurements corresponding to curve $\blacktriangle$ was classified as acidotic based on the threshold value of AlZahal et al. (2007; time with pH < 5.8 = 475 min/d).

data set. Principal component 1 and PC 2 explain 44.6 and 17.5% of the variance in the data set, respectively. Principal component 1 is positively correlated with the odd-chain FA and negatively with most *iso* FA. Principal component 2 is positively correlated with the biohydrogenation intermediates, particularly C18:1 *trans*-11, C18:2 *cis*-9, *trans*-11, and C18:2 *cis*-1, *trans*-15. Parameters β_0 and β_1 have a PC 1 score of -0.07 and -0.82 , respectively, and a PC 2 score of -0.50 and 0.18 , respectively.

Both logistic parameters are linked with one of the PC and, thus, are relatively independent of each other ($\tau_p = 0.02$, $P = 0.85$). Rumen parameters minimum pH and average pH were positively correlated with parameter β_1 , whereas AUC of pH < 5.8 and 6.0 and time with pH < 5.8 and 6.0 showed a negative relationship with this parameter.

In accordance with the associations between logistic curve parameters and milk FA, as illustrated by the PCA loading plot (Figure 4), Pearson correlations revealed a negative correlation of parameter β_0 with *anteiso* C15:0 ($\tau_p = -0.22$, $P < 0.05$), *anteiso* C17:0 ($\tau_p = -0.29$, $P < 0.01$), C15:0 ($\tau_p = -0.22$, $P < 0.05$), and C17:0 ($\tau_p = -0.24$, $P < 0.01$; (Figure 5). Parameter β_1 is positively correlated with all *iso* FA, *anteiso* C13:0 ($\tau_p = 0.43$, $P < 0.01$), and *anteiso* C15:0 ($\tau_p = 0.22$, $P < 0.05$), and negatively correlated with C15:0 ($\tau_p = -0.50$, $P < 0.001$) and C17:0 ($\tau_p = -0.24$, $P < 0.01$), respectively. A negative correlation with parameter β_1

was found for C18:1 *trans*-10 ($\tau_p = -0.26$, $P < 0.01$; Figure 5). Parameter β_0 was negatively correlated with C18:1 *trans*-11 ($\tau_p = -0.26$, $P < 0.01$) and C18:2 *cis*-9, *trans*-11 ($\tau_p = -0.28$, $P < 0.01$).

Linear Regression. Variation in individual milk FA (R^2) reflected 8.24 and 35.3% of the variation in parameters β_0 and β_1 (respectively) at the most. Hence, a multiple linear regression was performed. The regression consisted of either the parameters β_0 and β_1 as dependent variables and the OBCFA (C5:0, C7:0, C9:0, C11:0, *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *anteiso* C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, and C17:0) and C18 biohydrogenation intermediates (C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12, and C18:2 *trans*-11, *cis*-15) as independent variables. The independent variables retained after the stepwise regression were C9:0, C15:0, and *iso* C16:0 in the case of parameter β_0 ($\beta_0 = 8.62 - 82.21 \times \text{C9:0} + 4.35 \times \text{C15:0} - 9.81 \times \text{iso C16:0}$) and *iso* C14:0 and *iso* C16:0 in the case of parameter β_1 ($\beta_1 = 5.80 - 7.23 \times \text{iso C14:0} - 1.13 \times \text{iso C16:0}$). The coefficient of determination of both regressions was 0.26 ($\tau = 0.51$) and 0.38 ($\tau = 0.62$) for parameter β_0 and β_1 , respectively.

Scatterplots. As parameters β_0 and β_1 are independent from each other, 4 extreme combinations of both parameters exist, as shown in Figure 3. To assess the correlation between milk FA and combined variation in β_0 and β_1 , changes were visualized by scatterplots

Figure 4. Results of a principal component (PC) analysis based on rumen parameters [pH minimum (min pH), average pH (Avg pH), area under the curve (AUC) of pH <5.8 or 6.0, and time with pH <5.8 or 6.0], milk fat % (Fat %), FA proportions in milk fat (g/100 g of FA; *anteiso* C13:0, *iso* C13:0, *iso* C14:0, *iso* C15:0, *anteiso* C15:0, C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, C17:0, *iso* C18:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, and C18:2 *trans*-11,*cis*-15), and the parameters of the logistic curve [slope (β_0) and inflection point (β_1)]. c = *cis*; t = *trans*.

for each OBCFA and C18 biohydrogenation intermediate. Figures 6 to 8 show the scatterplots for *iso* C14:0, C18:1 *trans*-11, and C15:0, respectively.

Low proportions of *iso* C14:0 (Figure 6) and *iso* C16:0 were found when β_1 was low, independent of the value of β_0 . The highest proportions of C15:0 (Figure 7) and C17:0 were associated with low β_1 values (low average pH), but only when simultaneously associated with low β_0 (relatively large pH range). Samples with low C18:1 *trans*-11 (Figure 8) and C18:2 *cis*-9,*trans*-11 proportions were linked with a large rumen pH range (low β_0 values), independent of the average rumen pH. High C18:1 *trans*-10 (above 6 g/100 g of FA) proportions only occurred in situations with low, but variable rumen pH (results not shown). High proportions of *anteiso* C13:0 were only observed for the samples with a low β_0 and a high β_1 value.

DISCUSSION

Differences in rumen pH parameters and milk FA between cows were not observed in both experiments. Absolute values of both rumen pH parameters and milk FA concentrations obviously differed between cows in experiment 1, but shifts through the acidosis induction protocol of both experiments followed the same trend. In experiment 2, no effect of period was observed on pH and milk FA, as the shifts through the gradual acidosis induction followed the same trend in each period.

Both experiments were effective in inducing SARA (all samples of ration 3 in experiment 1 and 4 out of 9 samples at d 21 of experiment 2 were classified as acidotic); however, on average, no effect on rumen parameters was observed in experiment 2. An effect on DMI and milk yield was observed in experiment 1

Figure 5. Pearson bivariate correlations between parameters slope (β_0) and inflection point (β_1) of the logistic curve and milk odd- and branched-chain FA (OBCFA) and biohydrogenation intermediates [g/100 g of FA; *iso* C13:0, *iso* C14:0, *iso* C15:0, *iso* C16:0, *iso* C17:0, *iso* C18:0, *anteiso* C13:0, *anteiso* C15:0, *anteiso* C17:0, C15:0, C17:0, C18:1 *trans*-10 (C18:1t10), C18:1 *trans*-11 (C18:1t11), C18:2 *trans*-10,*cis*-12 (C18:2t10c12), C18:2 *cis*-9,*trans*-11 (C18:2c9t11), and C18:2 *trans*-11,*cis*-15 (C18:2t11c15)]. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

only. When a buffer was supplemented (ration 4) to the ration after increasing the total amount of concentrate (ration 3), the lowest DMI and milk yield were recorded. This was due to rumen acidotic conditions, induced during former periods, which led to intake refusal of 2 cows (DMI < 0.5 kg/d).

Milk OBCFA and C18 biohydrogenation intermediates reacted similarly as in 2 grain-based acidosis induction experiments (Enjalbert et al., 2008; Colman et al., 2010). Whereas odd-chain FA increased as the supply of rapidly fermentable carbohydrates increased, *iso* FA decreased. Vlaeminck et al. (2006) discussed the positive relationship between odd-chain FA and the abundance of amylolytic bacteria and between *iso* FA and the abundance of cellulolytic bacteria in the rumen. As such, the abundance of the cellulolytic bacteria decreased and the abundance of the amylolytic bacteria increased with increasing supply of RFCH.

Changes in the milk FA profile not only occurred in the OBCFA, but also in C18:0 and C18:2 n-6, which decreased and increased, respectively, with increasing

supply of RFCH. As the amount of RFCH increased, the pH decreased, which could have been associated with a decrease in the lipolytic or biohydrogenation activity (or both) of the rumen microbial population, thus leading to a decrease in C18:0 and an increase in C18:2 n-6 (Van Nevel and Demeyer, 1996).

Milk FA and pH changes, particularly time with pH < 5.8 and C18:1 *trans*-10, were more prominent in the first experiment. To a large extent, this was caused by shifts in 1 cow in experiment 1 receiving a diet with the lowest F:C ratio (24:76). In this situation, C18:1 *trans*-10 proportions increased almost 10 fold compared with the control diet (0.79 vs. 6.75 g/100 g of FA) and the longest amount of time pH below 5.8 was recorded (857 min/d). This abrupt change to a major proportion of W-CONC, in combination with a very limited amount of effective fiber from roughage in the rumen of this cow, seemed to induce a shift in the biohydrogenation pathway, resulting in a 10-fold increase in C18:1 *trans*-10. This is supported by the fact the cellulolytic bacteria are the main ruminal biohydrogenating bac-

Figure 6. Scatterplot with the logistic parameters slope (β_0) and inflection point (β_1) on the x- and y-axis. The color code represents the proportion of *iso* C14:0 (g/100 g of FA). The dashed line marks the separation between samples with mainly low and high proportions of *iso* C14:0. Color version available in the online PDF.

Figure 7. Scatterplot with the logistic parameters slope (β_0) and inflection point (β_1) on the x- and y-axis. The color code represents the proportion of C15:0 (g/100 g of FA). The dashed line marks the separation between samples with mainly low and high proportions of C15:0. Color version available in the online PDF.

Figure 8. Scatterplot with the logistic parameters slope (β_0) and inflection point (β_1) on the x- and y-axis. The color code represents the proportion of C18:1 *trans*-11 (g/100 g of FA). The dashed line marks the separation of high proportions of C18:1 *trans*-11. Color version available in the online PDF.

teria (Martin and Jenkins, 2002; Jenkins et al., 2008) and their abundance decreases as pH lowers (Russell and Dombrowski, 1980). On the other hand, Choi et al. (2005) concluded that C18:2 *trans*-10,*cis*-12-producing bacteria may be more aero- and acid-tolerant than C18:2 *cis*-9,*trans*-11-producing bacteria, which is consistent with our results.

To quantify the link between milk FA and rumen pH, parameters that describe the daily rumen pH pattern were used. Commonly used parameters to describe pH are average rumen pH and the amount of time pH is below 5.8. However, studies of Oba and Allen (2000, 2003) showed that increasing the concentration of dietary starch also increases the pH variation. Because the commonly used rumen pH parameters do not include daily pH variation, researchers looked for new methods to describe the daily pH pattern including both average pH and pH range (Palmonari et al., 2010). AlZahal et al. (2007) compared 4 different candidate functions to describe rumen pH, of which the logistic curve was considered the optimal choice for this purpose. In the current data set, the coefficient of determination for each sample ($n = 126$) was higher than 0.96, meaning that the logistic curve was

a good fit to the data of each day of each experiment. The logistic curve contains 2 parameters: β_0 and β_1 . Values for parameter β_1 in this data set are consistent with the results obtained by AlZahal et al. (2007). However, AlZahal et al. (2007) did not cover the same range of β_0 values in their experiment because all of their samples had a bout of rumen pH below 5.8, whereas in our data set, in some situations, rumen pH stayed above 6.0 and, thus, covered a very small range (i.e., high β_0).

Based on the PCA results, only β_1 is correlated significantly with the commonly used pH parameters. None of these parameters had a good correlation with β_0 , meaning that these parameters do not contain information on the ruminal pH range. Parameter β_0 is not only related to the pH range, but also to the number of bouts of low rumen pH (<5.8) during the day. Indeed, situations with the same amount of time with rumen pH <5.8 could show different rumen pH patterns and, thus, different β_0 and β_1 values. More bouts of low rumen pH occurred as the pH range was smaller (high β_0 ; Figure 9). As such, the number of bouts of low rumen pH has been taken into account by determination of the parameters of the logistic curve.

Figure 9. Rumen pH profile of 2 situations showing the same amount of time that pH is below 5.8 (762 min/d) but with a different number of bouts of low rumen pH (gray: a single long period of low rumen pH vs. black: several shorter bouts of pH below the threshold of 5.8), which is reflected in large variation in slope (β_0), representing the diurnal pH range. β_1 = inflection point; AM = morning; PM = afternoon.

From a rumen physiological and microbial point view, stable low pH situations (high β_0 and low β_1) might be quite different from low average pH but associated with considerable daily pH variation (low β_0 and low β_1 ; Palmonari et al., 2010). Scatterplots are a way to qualitatively assess the relation between both rumen pH parameters and milk FA. Here, we give examples of 3 of such scatterplots that illustrate ranges of 3 variables (β_0 , β_1 , and milk FA concentration) in a 2-dimensional figure. This is done by using a color code for the third variable, which was, in this case, a milk FA.

The scatterplots for *iso* C14:0 (Figure 6) and *iso* C16:0 (results not shown) showed that low proportions of these FA were linked with low pH, independent of their rates of variation (β_0). This could indicate that cellulolytic bacteria are always suppressed at low pH (pH < 6.0). Some FA proportions were influenced by rumen pH variation only and not by rumen pH level (e.g., C18:1 *trans*-11; Figure 8) and C18:2 *cis*-9, *trans*-11 (results not shown), both belonging to the primary biohydrogenation pathway of C18:2 n-6 or C18:3 n-3, or both. Greater concentrations of these milk FA were associated with fluctuating rumen pH.

The scatterplot for milk C18:1 *trans*-10 illustrated that high values of this FA (>6 g/100 g of FA) only occurred with low and largely fluctuating pH (small β_1 and β_0), whereas situations with low, stable pH (low β_1 and great β_0) did not induce a shift toward the secondary biohydrogenation pathway (Bauman and Grinari, 2003; Figure 1). Similarly, animals that had a stable but low pH did not show an increase in odd-chain FA (C15:0 and C17:0; Figure 7). Apparently, this low pH seemed a rather normal situation for such cows, as the odd-chain FA proportions in a control and acidosis induction period were similar. A combination of low

and largely fluctuating rumen pH induced greater milk odd-chain FA concentrations, which might indicate a greater abundance of amylolytic bacteria in the rumen (Vlaeminck et al., 2006).

The dependence of *iso* FA on β_1 , irrespective of β_0 (as illustrated in the scatterplot for *iso* C14:0), is reflected in the positive bivariate correlations between *iso* FA and β_1 and the low correlations between *iso* FA and β_0 . Negative correlations of β_0 with some biohydrogenation intermediates (C18:1 *trans*-11 and C18:2 *cis*-9, *trans*-11) showed the effect of fluctuating rumen pH on the proportions of these FA in milk, whereas the level of the average rumen pH did not play a role, as correlations of these FA with β_1 were negligible; C15:0 and C17:0 showed negative correlations with both β_0 and β_1 , reflecting the results of the scatterplots.

The positive correlation between β_1 and milk *iso* FA are in accordance with the greater abundance of cellulolytic bacteria as the rumen pH increases as observed in goats and sheep by Sun et al. (2010) and Mosoni et al. (2007), respectively. On the other hand, decreasing β_0 and β_1 were associated with lower milk C15:0 and C17:0, which is in agreement with the increasing abundance of the amylolytic bacteria, especially *Streptococcus bovis*, as pH decreases (Nagaraja and Titgemeyer, 2007; Sun et al., 2010). Nevertheless, these single correlations only explained 8.24 and 35.3% of the variation in parameters β_0 and β_1 , respectively. Hence, a multiple linear regression model was tested to assess the potential of combining milk FA to predict β_0 and β_1 . This regression has been performed with either of the 2 parameters (β_0 and β_1) as dependent variable and the OBCFA and biohydrogenation intermediates as independent variables. Only 38% of the variance of parameter β_1 was explained by the linear model with *iso*

C14:0 and *iso* C16:0 as independent variables. In the case of parameter β_0 , the linear regression model with C9:0, C15:0, and *iso* C16:0 as independent variables explained only 26% of the total variance of parameter β_0 . Based on these linear regressions, milk FA do not seem to effectively predict rumen pH (pattern). Eventually, nonlinear regression methods could improve these results and have to be evaluated to describe the relationship between rumen pH (pattern) and milk FA as well as possible.

CONCLUSIONS

Both parameters of the logistic curve were related with OBCFA and C18 biohydrogenation intermediates. Different combinations of these 2 parameters led to different milk FA profiles, which suggests that prediction of both parameters at the same time based on the milk FA profile would be opportune. Hence, to describe the relationship between pH and milk FA as well as possible, different methods, such as multiple output regression and support vector regression, have to be evaluated.

ACKNOWLEDGMENTS

W. Waegeman is supported by the Research Foundation of Flanders (Brussels, Belgium). The PhD research of E. Colman is financed by the Institute for the Promotion of Innovation by Science and Technology (IWT) in Flanders, Belgium, and the Commission of the European Communities [FP7, KBB-2007-1 (RedNEx)].

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APPENDIX

Table A1. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on milk short- and medium-chain FA proportions (g/100 g of FA) of experiment 1 (n = 49)

Variable	Mean	SE	Ration								P-value (ration) ¹
			1	2-1	2-2	2-3	2-4	3	4	5	
S-CONC (g/kg of DM)			346	256	171	88	0	196-452	0-452	342	
W-CONC (g/kg of DM)			0	86	172	256	341	308-324	0-319	0	
n			3	3	3	3	3	12	6	16	
C4:0	4.01	0.368	4.35 ^{bc}	4.18 ^{bc}	4.11 ^{bc}	4.07 ^{abc}	4.18 ^{bc}	3.28 ^a	3.60 ^{ab}	4.34 ^c	***
C6:0	2.25	0.164	2.44 ^{bc}	2.34 ^{abc}	2.35 ^{abc}	2.35 ^{abc}	2.42 ^{bc}	1.95 ^a	1.92 ^{ac}	2.20 ^c	***
C8:0	1.40	0.094	1.51 ^{bc}	1.45 ^{abc}	1.47 ^{abc}	1.48 ^{abc}	1.54 ^c	1.29 ^{abc}	1.22 ^a	1.28 ^{ab}	***
C10:0	3.53	0.248	3.68 ^{bc}	3.58 ^{bc}	3.75 ^c	3.76 ^c	3.95 ^c	3.51 ^{bc}	3.09 ^{ab}	2.96 ^a	***
C12:0	4.75	0.389	4.86 ^{bc}	4.76 ^{bc}	4.99 ^c	5.03 ^c	5.20 ^c	5.03 ^c	4.24 ^{ab}	3.85 ^a	***
C14:0	12.3	0.642	12.4 ^{abc}	12.5 ^{bc}	12.8 ^c	12.8 ^c	13.0 ^c	12.2 ^{bc}	11.2 ^a	11.6 ^{ab}	***
C14:1 <i>cis</i> -9	1.45	0.180	1.30 ^{ab}	1.37 ^{ab}	1.41 ^{ab}	1.42 ^{ab}	1.41 ^{ab}	1.90 ^b	1.47 ^{ab}	1.29 ^a	**
C16:0	28.9	0.913	28.0 ^{ab}	28.6 ^{ab}	29.9 ^{ab}	30.4 ^b	30.6 ^b	28.8 ^{ab}	27.0 ^a	28.1 ^{ab}	**
C16:1 <i>cis</i> -9	1.80	0.410	1.45 ^a	1.56 ^{ab}	1.62 ^{ab}	1.65 ^{ab}	1.64 ^{ab}	2.56 ^b	2.19 ^{ab}	1.74 ^a	**

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹P-values according to the linear mixed model $Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}$, where Y_{ijk} = independent variable, μ = mean, A_i = fixed effect of ration, B_j = random effect of cow, and ε_{ijk} = residual error term.

0.001 < $P < 0.01$; * $P < 0.001$.

Table A2. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on milk short- and medium-chain FA proportions (g/100 g of FA) of experiment 2 (n = 81)

Variable	Mean	SEM	Day										P-value ²	
			13	14	15	16	17	18	19	20	21	SE ¹	Trt	Day
S-CONC (g/kg of DM)			327	314	305	303	289	275	266	254	242			
W-CONC (g/kg of DM)			0	39	79	110	160	197	238	276	469			
n			9	9	9	9	9	9	9	9	9			
C4:0	4.06	0.049	4.45 ^c	4.16 ^{abc}	4.17 ^{bc}	4.11 ^{abc}	4.02 ^{ab}	4.02 ^{ab}	3.92 ^{ab}	3.86 ^{ab}	3.82 ^a	0.086	0.14	***
C6:0	2.36	0.022	2.45	2.36	2.39	2.38	2.35	2.36	2.32	2.30	2.32	0.047	0.51	0.41
C8:0	1.46	0.029	1.45	1.41	1.46	1.46	1.46	1.47	1.47	1.47	1.48	0.039	0.99	0.86
C10:0	3.65	0.099	3.44 ^{ab}	3.41 ^a	3.58 ^{abc}	3.60 ^{abc}	3.68 ^{abc}	3.74 ^{bc}	3.77 ^c	3.82 ^c	3.83 ^c	0.117	0.32	***
C12:0	4.85	0.160	4.45 ^a	4.48 ^a	4.66 ^{ab}	4.72 ^{abc}	4.90 ^{bcd}	4.98 ^{bcd}	5.07 ^{cd}	5.18 ^d	5.20 ^d	0.178	**	***
C14:0	12.2	0.12	12.2	12.0	12.2	12.3	12.3	12.3	12.3	12.3	12.1	0.16	*	0.41
C14:1 <i>cis</i> -9	1.48	0.086	1.41 ^a	1.45 ^{ab}	1.43 ^{ab}	1.46 ^{ab}	1.49 ^{ab}	1.48 ^{ab}	1.50 ^{ab}	1.54 ^{ab}	1.57 ^b	0.091	***	*
C16:0	28.8	1.92	29.1	28.8	29.0	28.8	28.8	28.7	28.6	28.6	28.3	1.95	***	0.89
C16:1 <i>cis</i> -9	1.80	0.163	1.75	1.81	1.77	1.76	1.77	1.76	1.78	1.86	1.97	0.170	**	†

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹SE = weighted SE of d 13 to 21.

²P-values according to the linear mixed model $Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijklm}$, where Y_{ijklm} = independent variable, μ = mean, A_i = fixed effect of day, B_j = random effect of period, C_k = fixed effect of treatment (Trt), D_l = random effect of cow, and ε_{ijklm} = residual error term.

*0.01 < $P < 0.05$; **0.001 < $P < 0.01$; *** $P < 0.001$; †0.05 < $P < 0.10$.

Table A3. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on milk C18 SFA and unsaturated FA proportions (g/100 g of FA) of experiment 1 (n = 49)

Variable	Mean	SE	Ration								P-value (ration) ¹
			1	2-1	2-2	2-3	2-4	3	4	5	
S-CONC (g/kg of DM)			346	256	171	88	0	196-452	0-452	342	
W-CONC (g/kg of DM)			0	86	172	256	341	308-324	0-319	0	
n			3	3	3	3	3	12	6	16	
C18:0	7.22	0.980	8.00 ^b	7.94 ^b	7.19 ^{ab}	7.09 ^{ab}	6.99 ^{ab}	5.23 ^a	6.95 ^b	8.37 ^b	***
C18:1 <i>trans</i> -6/7/8	0.251	0.010	0.319	0.288	0.243	0.202	0.182	0.252	0.296	0.226	*
C18:1 <i>trans</i> -9	0.232	0.010	0.284	0.266	0.219	0.191	0.177	0.240	0.256	0.222	0.12
C18:1 <i>trans</i> -10	1.06	0.850	0.570	0.558	0.435	0.357	0.339	2.64	2.75	0.810	*
C18:1 <i>trans</i> -11	0.849	0.053	0.960	0.886	0.758	0.687	0.624	0.92	1.15	0.803	†
C18:1 <i>trans</i> -12	0.399	0.011	0.499 ^c	0.459 ^{abc}	0.383 ^{abc}	0.336 ^{abc}	0.297 ^a	0.430 ^{bc}	0.436 ^{abc}	0.354 ^{ab}	***
C18:1 <i>trans</i> -16	0.458	0.027	0.513	0.501	0.445	0.417	0.423	0.452	0.422	0.487	0.39
C18:1 <i>cis</i> -9	15.6	0.46	15.8 ^{ab}	15.7 ^{ab}	15.1 ^{ab}	15.0 ^{ab}	14.6 ^{ab}	13.3 ^a	16.7 ^{ab}	18.4 ^b	***
C18:1 <i>cis</i> -11	0.848	0.081	0.774 ^a	0.760 ^a	0.747 ^a	0.736 ^a	0.698 ^a	1.11 ^b	1.16 ^b	0.803 ^a	***
C18:1 <i>cis</i> -12	0.343	0.036	0.448 ^b	0.402 ^b	0.348 ^b	0.307 ^b	0.281 ^b	0.350 ^b	0.345 ^b	0.261 ^a	*
C18:1 <i>cis</i> -13	0.101	0.026	0.081	0.077	0.071	0.067	0.068	0.158	0.174	0.109	**
C18:1 <i>cis</i> -15	0.157	0.010	0.158	0.161	0.156	0.144	0.145	0.167	0.170	0.154	0.79
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.490	0.037	0.519	0.500	0.450	0.410	0.366	0.564	0.632	0.479	0.21
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.006	0.000	0.005 ^{ab}	0.003 ^{ab}	0.008 ^{ab}	0.006 ^{ab}	0.006 ^{ab}	0.009 ^b	0.008 ^{ab}	0.005 ^a	*
C18:2 <i>trans</i> -11, <i>cis</i> -15	0.102	0.014	0.103	0.098	0.081	0.077	0.080	0.130	0.135	0.114	0.14
C18:3 n-3	0.464	0.012	0.399 ^a	0.393 ^a	0.413 ^a	0.425 ^{ab}	0.426 ^{ab}	0.559 ^c	0.545 ^{bc}	0.548 ^c	***
C18:3 n-6	0.022	0.004	0.020 ^{ab}	0.022 ^{ab}	0.021 ^{ab}	0.024 ^{ab}	0.023 ^{ab}	0.024 ^b	0.025 ^b	0.018 ^a	†
C18:2 n-6	1.67	0.046	1.53 ^b	1.49 ^b	1.52 ^b	1.52 ^b	1.48 ^b	2.06 ^a	2.09 ^a	1.70 ^b	***

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).¹P-values according to the linear mixed model $Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}$, where Y_{ijk} = independent variable, μ = mean, A_i = fixed effect of ration, B_j = random effect of cow, and ε_{ijk} = residual error term.* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$; † $0.05 < P < 0.10$.

Table A4. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on milk C18 SFA and unsaturated FA proportions (g/100 g of FA) of experiment 2 (n = 81)

Variable	Mean	SEM	Day											SE ¹	Trt	Day	P-value ²
			13	14	15	16	17	18	19	20	21						
S-CONC (g/kg of DM)			327	314	305	303	289	275	266	254	242						
W-CONC (g/kg of DM)			0	39	79	110	160	197	238	276	469						
n			9	9	9	9	9	9	9	9	9						
C18:0	7.66	0.582	8.09 ^a	8.00 ^{ab}	7.99 ^{ab}	7.87 ^{ab}	7.71 ^{abc}	7.68 ^{abc}	7.52 ^{abc}	7.16 ^{bc}	6.94 ^c	0.607	***	***	***	***	***
C18:1 <i>trans</i> -6/7/8	0.185	0.019	0.187	0.188	0.183	0.185	0.181	0.179	0.182	0.190	0.195	0.019	0.73	0.73	0.22	0.22	0.22
C18:1 <i>trans</i> -9	0.177	0.016	0.177	0.181	0.178	0.173	0.178	0.174	0.171	0.179	0.181	0.016	0.97	0.97	0.91	0.91	0.91
C18:1 <i>trans</i> -10	0.310	0.043	0.266 ^a	0.274 ^{ab}	0.279 ^{ab}	0.283 ^{ab}	0.294 ^{ab}	0.294 ^{ab}	0.306 ^{ab}	0.367 ^{bc}	0.428 ^c	0.047	0.10	***	***	***	***
C18:1 <i>trans</i> -11	0.615	0.092	0.685 ^a	0.676 ^{ab}	0.645 ^{abc}	0.642 ^{abc}	0.600 ^{abc}	0.572 ^c	0.564 ^c	0.576 ^{bc}	0.573 ^c	0.094	**	***	***	***	***
C18:1 <i>trans</i> -12	0.306	0.038	0.303	0.310	0.309	0.303	0.302	0.293	0.295	0.315	0.320	0.039	0.71	0.52	0.71	0.52	0.52
C18:1 <i>trans</i> -16	0.410	0.040	0.424	0.420	0.414	0.403	0.421	0.412	0.401	0.400	0.390	0.042	0.98	0.58	0.98	0.58	0.58
C18:1 <i>cis</i> -9	17.0	0.95	17.2	17.7	17.1	17.2	16.9	16.8	16.7	16.5	16.6	0.98	***	†	***	†	†
C18:1 <i>cis</i> -11	0.787	0.020	0.638 ^a	0.737 ^{ab}	0.727 ^{ab}	0.737 ^{ab}	0.760 ^{abc}	0.795 ^{bc}	0.844 ^{bcd}	0.891 ^{cd}	0.951 ^d	0.035	0.43	***	0.43	***	***
C18:1 <i>cis</i> -12	0.268	0.028	0.250	0.267	0.273	0.269	0.266	0.262	0.261	0.280	0.281	0.029	0.33	0.27	0.33	0.27	0.27
C18:1 <i>cis</i> -13	0.077	0.012	0.080	0.077	0.079	0.073	0.074	0.073	0.074	0.081	0.085	0.013	***	0.23	***	0.23	0.23
C18:1 <i>cis</i> -15	0.139	0.009	0.144	0.144	0.137	0.141	0.141	0.136	0.136	0.132	0.140	0.010	*	0.54	*	0.54	0.54
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.380	0.054	0.403	0.406	0.377	0.391	0.372	0.356	0.355	0.369	0.387	0.055	0.13	0.15	0.13	0.15	0.15
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.005	0.000	0.006	0.006	0.005	0.004	0.004	0.005	0.004	0.005	0.005	0.001	0.14	0.95	0.14	0.95	0.95
C18:2 <i>trans</i> -11, <i>cis</i> -15	0.078	0.014	0.099 ^a	0.091 ^{ab}	0.085 ^{ab}	0.085 ^{ab}	0.082 ^{abc}	0.075 ^{abc}	0.066 ^{bc}	0.060 ^c	0.063 ^c	0.015	0.13	***	0.13	***	***
C18:3 n-3	0.521	0.067	0.549	0.535	0.525	0.529	0.528	0.524	0.509	0.495	0.495	0.068	0.37	*	0.37	*	*
C18:3 n-6	0.021	0.003	0.022	0.021	0.021	0.020	0.020	0.021	0.023	0.022	0.022	0.003	**	0.25	**	0.25	0.25
C18:2 n-6	1.44	0.122	1.33 ^a	1.36 ^{ab}	1.38 ^{ab}	1.38 ^{ab}	1.41 ^{abc}	1.44 ^{abc}	1.48 ^{bc}	1.53 ^{cd}	1.62 ^d	0.125	0.75	***	0.75	***	***

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹SE = weighted SE of d 13 to 21.

²P-values according to the linear mixed model $Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijklm}$, where Y_{ijklm} = independent variable, μ = mean, A_i = fixed effect of day, B_j = random effect of period, C_k = fixed effect of treatment (Trt), D_l = random effect of cow, and ε_{ijklm} = residual error term.

*0.01 < $P < 0.05$; **0.001 < $P < 0.01$; *** $P < 0.001$; †0.05 < $P < 0.10$.

Table A5. Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on milk odd- and branched-chain FA proportions (g/100 g of FA) of experiment 1 (n = 49)

Variable	Mean	SE	Ration								P-value (ration) ¹
			1	2-1	2-2	2-3	2-4	3	4	5	
S-CONC (g/kg of DM)			346	256	171	88	0	196–452	0–452	342	
W-CONC (g/kg of DM)			0	86	172	256	341	308–324	0–319	0	
n			346	256	171	88	0	196–452	0–452	342	
<i>iso</i> C13:0	0.026	0.002	0.027 ^{ab}	0.027 ^{ab}	0.024 ^a	0.024 ^a	0.028 ^{ab}	0.022 ^{ab}	0.022 ^{ab}	0.032 ^b	***
<i>iso</i> C14:0	0.065	0.007	0.066 ^{bc}	0.067 ^{bc}	0.064 ^{ab}	0.068 ^{bc}	0.071 ^{bc}	0.045 ^a	0.054 ^{ab}	0.082 ^c	***
<i>iso</i> C15:0	0.201	0.022	0.202 ^{abcd}	0.198 ^{abcd}	0.191 ^{abcd}	0.212 ^{bcd}	0.231 ^{cd}	0.156 ^a	0.177 ^{ab}	0.241 ^d	***
<i>iso</i> C16:0	0.183	0.021	0.182 ^{ab}	0.190 ^{ab}	0.183 ^{ab}	0.185 ^{ab}	0.199 ^{ab}	0.148 ^a	0.160 ^a	0.221 ^b	***
<i>iso</i> C17:0	0.287	0.020	0.269 ^{ab}	0.255 ^{ab}	0.262 ^{ab}	0.286 ^{abc}	0.304 ^{abc}	0.262 ^a	0.315 ^{bc}	0.340 ^c	***
<i>iso</i> C18:0	0.036	0.001	0.030 ^{ab}	0.032 ^{ab}	0.030 ^{ab}	0.029 ^a	0.030 ^{ab}	0.040 ^{abc}	0.051 ^c	0.044 ^{bc}	***
C15:0	1.49	0.170	1.30 ^a	1.43 ^a	1.50 ^{ab}	1.52 ^{ab}	1.49 ^{ab}	2.12 ^b	1.53 ^a	1.04 ^a	***
C17:0	0.536	0.011	0.467 ^a	0.489 ^a	0.497 ^a	0.524 ^{ab}	0.542 ^{abc}	0.630 ^c	0.632 ^{bc}	0.507 ^a	***
<i>anteiso</i> C13:0	0.011	0.001	0.012	0.011	0.010	0.010	0.012	0.010	0.010	0.011	0.27
<i>anteiso</i> C15:0	0.442	0.022	0.441	0.420	0.414	0.443	0.449	0.462	0.439	0.469	0.73
<i>anteiso</i> C17:0	0.353	0.012	0.315 ^{ab}	0.300 ^a	0.303 ^a	0.343 ^{abc}	0.347 ^{abc}	0.408 ^{bc}	0.423 ^c	0.383 ^{abc}	***

^{a–d}Means within a row with different superscripts differ ($P < 0.05$).¹P-values according to the linear mixed model $Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}$, where Y_{ijk} = independent variable, μ = mean, A_i = fixed effect of ration, B_j = random effect of cow, and ε_{ijk} = residual error term.*** $P < 0.001$.**Table A6.** Effect of gradual replacement of a standard concentrate (S-CONC) with a wheat-based concentrate (W-CONC) and increase in the amount of concentrate in the diet on milk odd- and branched-chain FA proportions (g/100 g of FA) of experiment 2 (n = 49)

Variable	Mean	SEM	Day										P-value ²	
			13	14	15	16	17	18	19	20	21	SE ¹	Trt	Day
S-CONC (g/kg of DM)			327	314	305	303	289	275	266	254	242			
W-CONC (g/kg of DM)			0	39	79	110	160	197	238	276	469			
n			9	9	9	9	9	9	9	9	9			
<i>iso</i> C13:0	0.028	0.004	0.030 ^a	0.029 ^{ab}	0.028 ^{ab}	0.029 ^a	0.030 ^a	0.028 ^{ab}	0.028 ^{ab}	0.026 ^{ab}	0.025 ^b	0.004	**	**
<i>iso</i> C14:0	0.075	0.016	0.087 ^a	0.083 ^{ab}	0.077 ^{ab}	0.083 ^{ab}	0.080 ^{ab}	0.076 ^{abc}	0.071 ^{bcd}	0.062 ^{cd}	0.059 ^d	0.016	*	***
<i>iso</i> C15:0	0.229	0.023	0.255 ^a	0.244 ^{ab}	0.238 ^{ab}	0.239 ^{ab}	0.230 ^{abc}	0.224 ^{bcd}	0.221 ^{bcd}	0.208 ^{cd}	0.199 ^d	0.024	*	***
<i>iso</i> C16:0	0.227	0.044	0.238	0.239	0.229	0.239	0.241	0.232	0.220	0.204	0.199	0.045	*	*
<i>iso</i> C17:0	0.308	0.015	0.303	0.309	0.311	0.313	0.311	0.313	0.310	0.304	0.303	0.016	***	0.90
<i>iso</i> C18:0	0.033	0.002	0.040 ^b	0.032 ^a	0.032 ^a	0.031 ^a	0.032 ^a	0.034 ^{ab}	0.033 ^a	0.034 ^{ab}	0.034 ^{ab}	0.003	†	**
C15:0	1.25	0.161	1.05 ^a	1.06 ^a	1.07 ^{ab}	1.13 ^{ab}	1.21 ^{ab}	1.30 ^{abc}	1.41 ^{bc}	1.51 ^c	1.54 ^c	0.175	***	***
C17:0	0.494	0.022	0.493 ^{abc}	0.456 ^a	0.456 ^a	0.461 ^a	0.474 ^{ab}	0.500 ^{ab}	0.519 ^{abc}	0.535 ^{bc}	0.551 ^c	0.026	0.13	***
<i>anteiso</i> C13:0	0.012	0.001	0.012 ^{ab}	0.013 ^a	0.012 ^{ab}	0.012 ^{ab}	0.012 ^{ab}	0.012 ^{ab}	0.012 ^{ab}	0.011 ^{ab}	0.011 ^b	0.001	**	***
<i>anteiso</i> C15:0	0.485	0.026	0.463	0.468	0.470	0.486	0.491	0.502	0.503	0.490	0.489	0.028	*	†
<i>anteiso</i> C17:0	0.361	0.020	0.348 ^{ab}	0.328 ^a	0.333 ^{ab}	0.346 ^{ab}	0.360 ^{ab}	0.381 ^{ab}	0.379 ^{ab}	0.388 ^b	0.391 ^b	0.022	0.17	***

^{a–d}Means within a row with different superscripts differ ($P < 0.05$).¹SE = weighted SE of d 13 to 21.²P-values according to the linear mixed model $Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijklm}$, where Y_{ijklm} = independent variable, μ = mean, A_i = fixed effect of day, B_j = random effect of period, C_k = fixed effect of treatment (Trt), D_l = random effect of cow, and ε_{ijklm} = residual error term.* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$; † $0.05 < P < 0.10$.