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Modeling reticular and ventral ruminal pH of lactating dairy cows using ingestion and rumination behavior

A. Mensching,[1,2]* K. Bünemann,[3] U. Meyer,[3] D. von Soosten,[3] J. Hummel,[4] A. O. Schmitt,[2,5] A. R. Sharifi,[1,2] and S. Dänicke[3]

[1]Animal Breeding and Genetics Group, Department of Animal Sciences, University of Goettingen, Albrecht-Thaer-Weg 3, 37075 Goettingen, Germany
[2]Center for Integrated Breeding Research (CiBreed), University of Goettingen, Albrecht-Thaer-Weg 3, 37075 Goettingen, Germany
[3]Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Bundesallee 37, 38116 Brunswick, Germany
[4]Ruminant Nutrition Group, Department of Animal Sciences, University of Goettingen, Kellnerweg 6, 37077 Goettingen, Germany
[5]Breeding Informatics Group, Department of Animal Sciences, University of Goettingen, Margarethe von Wrangell-Weg 7, 37075 Goettingen, Germany

ABSTRACT

The prevention and control of metabolic and digestive diseases is an enormous challenge in dairy farming. Subacute ruminal acidosis (SARA) is assumed to be the most severe feed-related disorder and it impairs both animal health and economic efficiency. Currently, ruminal pH as well as variables derived from the daily pH curve are the main indicators for SARA. The objective of this study was to explain the daily pH course in the ventral rumen and reticulum of dairy cows using ingestion pattern and rumination behavior data gathered by automated data recording systems. The data of 13 ruminally fistulated lactating cows were collected at the experimental station of the Friedrich-Loeffler-Institut (Brunswick, Germany). The data included continuous pH measurements, which were recorded simultaneously in the reticulum by pH-measuring boluses and in the ventral rumen by a separate data logger. In addition, rumination behavior was measured using jaw movement sensors, and feed and water intakes were recorded by transponder-assisted systems. Milk yield and body weight were determined during and after each milking, respectively. For statistical evaluation, the data were analyzed using time-series modeling with multiple linear mixed regressions. Before applying the developed mathematical statistical modeling, we performed a plausibility assessment to ensure data quality. The major part of the mathematical statistical modeling consisted of data preparation, where all variables were transformed into a uniform 1-min resolution. Signal transformations were used to model individual feed and water intakes as well as rumination behavior events over time. Our results indicated that diurnal pH curves of both the reticulum and ventral rumen could be predicted by the transformed feed and water intake rates. Rumination events were associated with a marginal temporal increase in pH. We observed that the pH of the ventral rumen was delayed by approximately 37 min compared with that of the reticulum, which was therefore considered in the modeling. With the models developed in this study, 67.0% of the variance of the reticular pH curves and 37.8% of the variance of the ruminal pH curves could be explained by fixed effects. We deduced that the diurnal pH course is, to a large extent, associated with the animal's individual feed intake and rumination behavior.

Key words: ruminal pH, time series, statistical modeling, behavior

INTRODUCTION

In dairy farming, the prevention and control of metabolic and digestive disorders is an enormous challenge. In particular, subclinical conditions such as SARA are hard to diagnose but can severely impair both animal health and efficiency over the long term. Therefore, adequate feeding of lactating cows is of particular importance, especially for high-yielding animals. The 2 most relevant adjustable parameters of a diet are the energy content and the amount of physically effective fiber (Nocek, 1997). However, the energetic upgrading of the diet by concentrates rich in rapidly fermentable carbohydrates can predispose an animal to SARA, particularly in the first 3 mo of lactation when the need for increased energy concentration in the diet is high (Gröhn and Bruss, 1990). At the subacute stage, no distinct clinical signs can be detected at the individual

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*Corresponding author: andre.mensching@uni-goettingen.de

animal level in the short term. Nevertheless, associations with various clinical signs at the herd level can be observed, including reduced feed intake, lower efficiency of milk production, loose stools (reduced DM content of the feces), and claw diseases, as well as an overall higher culling rate (Kleen et al., 2003). Even if the same diet is fed, high interanimal variability in pH parameters can be observed, suggesting that susceptibility to SARA varies among individuals (Humer et al., 2015). In particular, the high interanimal variance, which is enhanced by factors such as lactation stage or parity, makes the herd-level variables more meaningful because of the aggregation of data from several animals.

The major challenge with the early diagnosis of SARA is therefore the identification of a suitable and practical diagnostic parameter. pH thresholds are currently the most appropriate measure for diagnosing SARA. As already noted by Zebeli et al. (2008), various thresholds for different pH parameters can be found in the literature. In a meta-analysis, those authors determined that the daily mean ruminal pH should be >6.16 and the time at pH <5.8 should not exceed 5.24 h/d to reduce the risk of SARA. Additionally, orally applied measuring boluses have recently been used for continuous measurement of the intrareticular pH value (Denwood et al., 2018; Humer et al., 2018). However, these devices are prone to considerable drifts over time (Villot et al., 2018) and it is not yet clear to what extent their measurements reflect the ventral ruminal pH. Initial approaches have been made to characterize the relationship between reticular and ruminal pH measurements. Falk et al. (2016) studied the reticular pH measured with eCow boluses (eCow Ltd., Exeter, UK) in association with the ruminal pH measured with LRCpH loggers (Dascor Inc., Escondido, CA) of fistulated lactating cows. The authors concluded that no clear relationship between the reticular and the ruminal pH can be provided. In contrast, Neubauer et al. (2018) analyzed the relationship between measured pH by eCow boluses and 3 daily spot samples collected from the ventral free-rumen liquid using a rumen pump. They concluded that a pH of 5.8 of the free-rumen liquid corresponds to a pH of 6.0 in the reticulum.

Generally, the daily pH course can be associated with the individual feed intake behavior and daily feeding frequency. Le Liboux and Peyraud (1999) observed a smaller daily pH range if the feeding frequency was increased from 2 to 6 times per day. Milking frequency and time of milking are also reflected in the pH curve and contribute to a farm-specific pH profile (Denwood et al., 2018). Another influencing factor is the composition of the feed. Jiang et al. (2017) showed that increasing dietary roughage from 40 to 70% led to longer feed intake and higher daily average pH values. Feeding twice a day, the authors observed a decrease in pH immediately after each feeding, in which a sinusoidal pH progression with 2 periods per day was determined independently of the roughage level.

In a meta-analysis, Mensching et al. (2020) examined the associations between 3 ruminal pH variables and milk- and diet-specific variables and determined high between-study heterogeneity, even if the 4 most explanatory variables were considered together in a multiple regression model. Consequently, precise prediction of pH variables remains challenging. The newest approaches focus increasingly on indicators; for example, the fatty acid composition of the milk fat, which is known to be associated with ruminal fermentation characteristics (Vlaeminck et al., 2006).

The main objective of this study was to explain temporal pH progression in the reticulum and ventral rumen with high-resolution sensor data of animal-specific feed and water intakes, as well as rumination behavior, using time-series analysis methods. We strived to gain a deeper insight into the causality of the daily pH progression to identify eventually reliable indicators for SARA.

MATERIALS AND METHODS

The experiment was conducted in accordance with the German legislation on animal protection (Animal Welfare Act). It was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, Oldenburg, Germany) in consultation with an independent ethics committee (AZ 33.19-42502-04-15/1858). Further results of this experiment have been published by Bünemann et al. (2019), who investigated the effects of BCS and concentrated feed on energy metabolism of dairy cows.

To give an overview on the extensive Material and Methods section, Figure 1 provides a summary of all essential steps in a flowchart. This figure was created using the yEd graph editor (yWorks GmbH, 2019).

Data Recording

Data from 13 ruminally fistulated cows were collected between November 9, 2016, and March 17, 2017, at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), in Brunswick, Germany. They were kept in a loose-housing stable with resting pens in a group of 60 cows in total. Fresh feed was provided once a day between 1100 and 1200 h. Information on the diets is given in Supplemental Table S1 (https://doi.org/10.3168/jds.2020-18195). Further experimental details can be found in Bünemann et al. (2019).

The data examined in this study include pH and temperature data, which were recorded simultaneously with pH measurement boluses (eCow Ltd., Exeter, UK) in the reticulum and LRCpH loggers (type 4, Lethbridge Research Center ruminal pH measurement system, Dascor Inc., Escondido, CA) in the ventral rumen. Seven LRCpH loggers were available for the 13 fistulated cows, so devices had to be interchanged between animals. Before and after each use, the electrode of the LRCpH logger was calibrated at 39°C in pH 4 and pH 7 buffer solutions. Assuming a linear drift, this 2-point calibration was used to correct the measured values in millivolts, which then were converted to pH values as described by Penner et al. (2006). All 13 eCow boluses, which were activated in a water bath at 39°C before insertion, were calibrated in pH 4 and pH 7 buffer solutions. A feature of the data collection was that, because the animals were ruminally fistulated, the eCow boluses were also exchanged between animals. As a result, bolus was not confounded with cow, so that bolus effects could be determined.

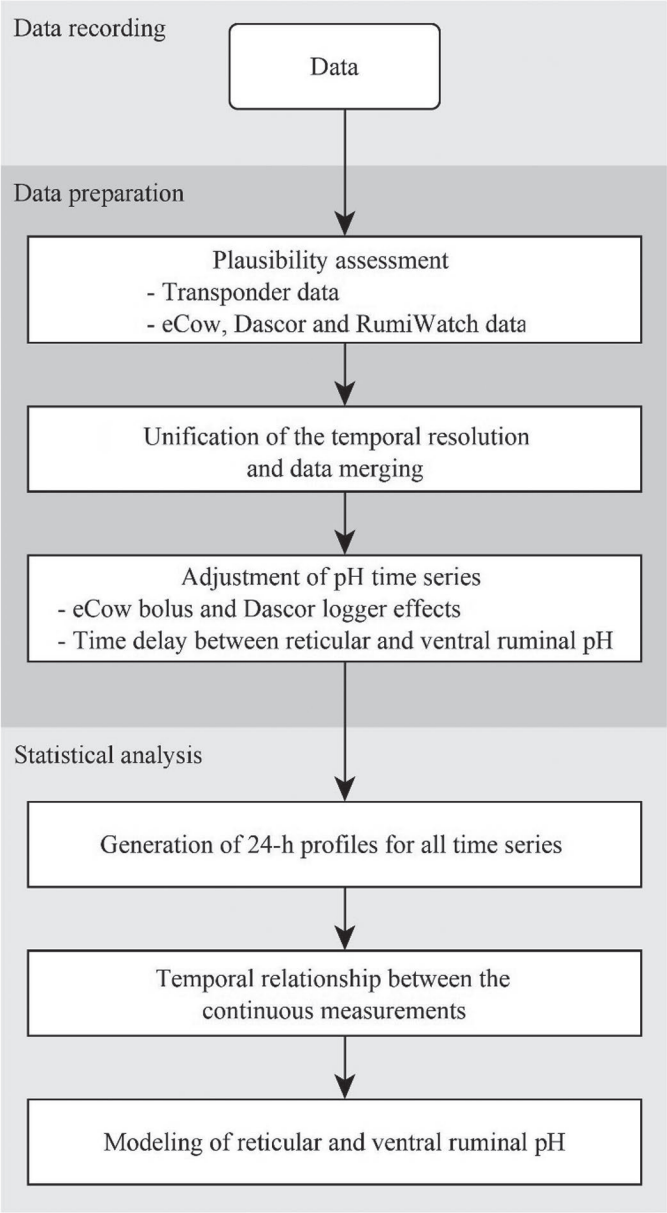

Figure 1. A flowchart showing all steps, as described in detail in the Materials and Methods section. eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA); RumiWatch = RumiWatch noseband halter (ITIN + HOCH GmbH, Liestal, Switzerland).

To measure rumination behavior, RumiWatch (ITIN + HOCH GmbH, Liestal, Switzerland) noseband halters were used. Again, 7 devices were available so the devices had to be interchanged between the animals and used simultaneously with the LRCpH logger. The raw data of the RumiWatch noseband sensor were converted using RumiWatch Converter V0.7.3.36 (ITIN + HOCH GmbH) into a 1-min resolution. Thereby, every observation was assigned to 1 of 6 classifications: 0 = other, 1 = ruminating, 2 = eating with head position down, 3 = eating with head position up, 4 = drinking, 5 = jaw movement for ripping of grass.

For each animal, the individual feed intake of the partially mixed ration (**PMR**) and the amount of water were recorded using transponder-assisted weighing troughs (RIC, Insentec B.V., Marknesse, the Netherlands). For each single observation of feed or water intake (i.e., the visit to the trough), the start and end times and start and end weights of available feed and water were recorded. With this information, the duration of the visit and the amount of PMR or water consumed by an animal during its visit were calculated. The weighing troughs were equipped with a pneumatic hatch that opened and closed at the beginning and end of each visit. The PMR was offered ad libitum and contained 30% concentrate on a DM basis. To create 2 feeding treatments, additional transponder-based concentrate feeders (Insentec B.V.) were used to offer supplementary concentrate feed so that 6 cows were fed 35% ($\mathbf{C_{35}}$) and 7 cows were fed 60% concentrate feed ($\mathbf{C_{60}}$) on a DM basis. The amount of concentrated feed in the C_{60} group was increased from 35 to 60% in the first 3 wk postpartum. To attain the targeted concentrate feed proportions while simultaneously offering the PMR for ad libitum consumption, the amount of concentrate feed required was adjusted weekly depending on the amount of PMR consumed and provided restrictively. For all concentrated feed observations, the start and end times and quantity of concentrates distributed were recorded. Milk yield was determined twice daily during milking, starting at 0530 and 1500 h, using an automatic milk counter (Lemmer Fullwood

GmbH, Lohmar, Germany). After each cow left the milking parlor (twice a day), BW was measured using a walkover live weight measurement system (Insentec B.V.).

Data Preparation

Plausibility Assessment of Transponder Data. It is generally known that transponder-based acquisition systems are susceptible to technical errors. This has mainly been investigated in pig farming; for example, Eissen et al. (1998) and Casey et al. (2005). After visual examination of the data of our study, typical technical errors were found, especially in the case of PMR intake. This included observations in which the visit duration at the trough was very long or visits when implausibly large amounts of feed disappeared in a short time. This circumstance is represented graphically in Supplemental Figure S1 (https://doi.org/10.3168/jds.2020-18195), which was created with the *heatscatter* function from the LSD (Schwalb et al., 2018) package. In addition, overlapping visits of the same animal and of different animals on one weighing trough were identified. Relatively long visits and time overlaps were found in particular at 4 of the 60 weighing troughs for PMR intake and were caused by technical errors of the antenna. This malfunction concerned the logout of the transponder from the transponder station. The login, however, was found to be valid.

In the analysis of high-resolution time series data in which the cumulative effect of ingestion over time is considered, ignoring or excluding these technically invalid observations could lead to bias in the results. Therefore, we decided to identify and correct implausible observations caused by systematic technical errors.

The problem of overlapping visits was treated as follows. Because the login was considered valid, the preceding affected visit was cut off so that its end fell at the beginning of the subsequent visit. The further invalid records were then examined individually for each animal with statistical models. For this purpose, an approximately linear relationship between visit duration and the amount of feed consumed was estimated. First, both the PMR amounts and visit durations had to be transformed due to right-skewed distributions. A Box-Cox transformation was used to determine the square root as an appropriate transformation for both variables. Based on transformed data, a robust standardized major axis (**SMA**) regression (Warton et al., 2006) was applied to describe the relationship between PMR amount and time per visit at the transponder station. In this SMA regression model, a linear relationship between the transformed variables was assumed as follows:

$$\sqrt{PMR_i} = b_0 + b_1\sqrt{VD_i}, \quad [1]$$

where PMR_i is the amount of PMR intake i, VD_i is the corresponding visit duration, b_0 is the intercept, and b_1 is the slope. The analysis with SMA models was carried out using the *sma* function from the smatr (Warton et al., 2018) package in R. Observations with standardized residuals >3 (i.e., eating rate too high) or <−3 (i.e., visit duration too long) were classified as outliers. In a further step, these implausible observations were corrected using the SMA model; that is, if the visit duration was too long, the expected visit duration was calculated based upon the plausible eating quantity. If the eating rate was too high and the visit duration was plausible, the expected amount of feed was calculated using the given visit duration. Using this procedure, 0.627% of PMR >0 records with a visit duration too long and 0.517% of PMR >0 records with a feeding rate too high were identified and corrected (see Supplemental Figures S2 and S3 for examples; https://doi.org/10.3168/jds.2020-18195).

Plausibility Assessment of eCow, Dascor, and RumiWatch Data. To assess the plausibility of the records, values on a daily basis (i.e., daily average values for the reticular and ruminal pH) as well as total daily rumination time were calculated for all animals. Observations were regarded as outliers if the value was >1.5 times the interquartile distance away from the lower or upper quartile. With regard to the eCow data, observations of 3 boluses were identified that showed extreme drift after approximately 80 d of measurements and recorded daily average pH values >7.1. Using this procedure, 109 of 786 d were classified as outliers in the eCow data and only 2 of 339 d in the Dascor data. Regarding the RumiWatch data, 12 of 212 d were classified as outliers. Among them were cases where the cows temporarily dropped the halter, which led to measuring gaps. If one day of data was detected as an outlier, data from the previous and following days were also excluded.

Unification of the Temporal Resolution of the Data. Because all measured variables had different temporal resolution depending on the respective technique, the data were transformed to a 1-min resolution and had to be merged for further statistical analysis. This required different strategies depending on the type of data.

Based on the amounts of PMR, concentrate, and water consumed and the time required for this intake, the average intake rates per minute were calculated using the plausibility checked transponder data. For example, if 1 kg (DM) of the PMR was consumed during a 10-min visit, an average intake rate of 0.1 kg/min was

assumed for these 10 time periods. Because the start times of the feed and water intakes were recorded at a 1-s resolution, observations of an animal within the same minute were combined. All starting times of the visits as well as visit duration were then rounded to whole minutes. For observations with a visit duration of <30 s, a duration of 1 min per visit was assumed. With this transformation, we ensured that the sum of all intake rates corresponded exactly to the recorded quantities (see Supplemental Figure S4 for an example; https://doi.org/10.3168/jds.2020-18195).

The raw pH and temperature data of the eCow boluses were provided as 15-min summaries presented as a mean value by the devices. To transform these data into a 1-min resolution, the time recorded in hours, minutes, and seconds was rounded to whole minutes. Then, the eCow pH and temperature mean values of the 15-min summaries were assumed for these time intervals. The resulting step-shaped pH and temperature curves were then smoothed with a moving average of a window length of 15 min (see Supplemental Figure S5 for an example; https://doi.org/10.3168/jds.2020-18195).

The pH and temperature data collected with the LRCpH logger already had the required 1-min data resolution. For the sake of comparability, the data were treated like the eCow data and first aggregated to 15-min summaries and then smoothed again with a moving average with a window length of 15 min.

RumiWatch data converted to a 1-min resolution were also prepared. Thereby, a new rumination variable was created, which was set to 1 for "rumination" and else to 0.

The total amounts of PMR, concentrate, and water consumed per animal and day were calculated by aggregating the individual transponder observations. The resulting observations with a daily resolution were also included in the 1-min resolution data set, together with daily milk yields and daily average BW.

Adjustment of pH Time Series. It is known that in vivo pH measurement of ventral ruminal pH can underlie a random drift after a certain amount of time, which is why a calibration before and after the measurement with a LRCpH logger is common (Penner et al., 2006). However, calibration of the intrareticular eCow pH measurement boluses is not possible for the use intended by the manufacturer because they remain in the animal for the rest of its life. Because the cows examined here were ruminally fistulated experimental animals, the eCow boluses could be exchanged between animals, so the data could be adjusted for bolus-specific effects using linear mixed regression models to obtain adjusted pH values. In this procedure, we took into account further information on intake and thus the feeding group (C_{35} and C_{60}), DIM, milk yield, and BW of the individual animal. Body weight was used as a covariate because it represents animal-individual information and is highly correlated with parity. To adjust eCow pH measurements, the following multiple linear mixed regression model was applied:

$$pH_{ijkt} = \beta_0 + \beta_1 \Sigma PMR_{it} + \beta_2 \Sigma C_{it} + \beta_3 \Sigma W_{it} + \beta_4 DIM_{it} + \beta_5 BW_{it} + \beta_6 MY_{it} + A_i + \gamma_{0jk} + \gamma_{1jk} TD_{it} + e_{ijkt}, \quad [2]$$

where pH_{ijkt} is the continuously measured reticular pH of animal i at time point t; β_0 is the intercept; $\beta_1, \ldots, \beta_6$ are the fixed regression coefficients; ΣPMR_{it}, ΣC_{it}, and ΣW_{it} are the daily consumed amounts of PMR, concentrate, and water, respectively; DIM_{it} is the days in milk; BW_{it} is the average daily BW; and MY_{it} is the daily milk yield. The individual cow with repeated measurements was considered with a random normally distributed effect A_i. For the interaction of bolus j and month k, a random intercept, γ_{0jk}, and a random slope, γ_{1jk}, for time in days (TD) from the beginning of data recording were also considered in the model. Further, e_{ijkt} represents a normally distributed random error. After parameter estimation, the adjusted pH values were determined by removing bolus effects from the raw observations, leaving the variance attributed to the fixed effects, the random animal effects, and the residuals. Because the co-variable TD_{it} was taken into account on a daily basis, the curve remains unchanged over 1 d so that the adjustment results in only a slight change of the daily mean pH value.

Because the pH value measurements for each individual animal were made using different LRCpH loggers, these values could be adjusted by applying the following model:

$$pH_{ijt} = \beta_0 + \beta_1 \Sigma PMR_{it} + \beta_2 \Sigma C_{it} + \beta_3 \Sigma W_{it} + \beta_4 DIM_{it} + \beta_5 BW_{it} + \beta_6 MY_{it} + A_i + \gamma_{0j} + TD_{it} + e_{ijt}. \quad [3]$$

This model basically corresponds to the model from Equation [2], considering the jth LRCpH logger as a random intercept γ_{0j}. The determination of the adjusted values was done in the same way as for the eCow data, so that only the variability was left, which was covered by the fixed effects, the random animal effects and residuals.

In this procedure, no LRCpH logger effect could be determined, which is attributable to the before and after calibrations. In contrast, the eCow measurements showed considerable bolus effects, with drift also play-

ing a significant role. Supplemental Figure S6 (https://doi.org/10.3168/jds.2020-18195) compares raw and adjusted pH values. The adjustment did not cause any visible change in the data of the LRCpH loggers. In the eCow data, however, the drift of some boluses could be fixed by the adjustment.

Cross-correlation analysis was used to analyze the temporal relationship between reticular and ventral ruminal pH. The cross-correlation function ρ_{xy} between 2 time series x_t and y_t as a function of the time lag τ is defined as

$$\rho_{xy}(\tau) = \frac{\gamma_{xy}(\tau)}{\sqrt{\gamma_x(0)\gamma_y(0)}},$$

$$\text{with } -1 \leq \rho_{xy}(\tau) \leq 1, \quad [4]$$

where

$$\gamma_{xy}(\tau) = \text{cov}(x_{t+\tau}, y_t) \quad [5]$$

is the cross-covariance function considering a time lag τ (Shumway and Stoffer, 2017). In addition to analyzing the general relationship between 2 time series, the cross-correlation function is able to analyze lagged relations. Thus, time lags between time series can be determined. Cross-correlation analysis was performed using the *ccf* function from the R basic package (https://www.R-project.org/). As the pH and temperature respond more quickly to an animal's ingestion behavior in the reticulum than in the ventral rumen, the pH measurements in the ventral rumen were individually adjusted for the identified time lag.

Description of Continuous Measurements in the Course of the Day and in Relation to Each Other

To describe the general pH progressions in the reticulum and ventral rumen, 24-h pH profiles averaged over all days were calculated separately for each feeding regimen (C_{35} and C_{60}) as mean and standard deviation for each minute of the day, considering the subset of the data in which all measured variables were available. Furthermore, the relative frequency of observed PMR, concentrate, and water recordings as well as rumination behavior were determined per minute of the day to create 24-h profiles. To this end, feed and water intakes were transformed into a 1/0 coding to reduce the time series to whether an activity has taken place or not. Depending on the feeding group and the time of day, the mean of each minute of the day was calculated over all test days. For comparability, these profiles were then scaled over both feeding groups with the following maximum-minimum transformation to the value range from 0 to 1:

$$\text{T}[x] = \frac{x - x_{\min}}{x_{\max} - x_{\min}}. \quad [6]$$

The previously described cross-correlation analysis in Equations [4] and [5] was also used to investigate the general temporal relationships between continuous measurements. To do so, the animal-specific cross-correlation functions for the pH and temperature in the reticulum and ventral rumen, the PMR, concentrate, and water intakes, and the rumination behavior were calculated.

Modeling of Reticular and Ventral Ruminal pH

Because the diurnal pH course is continuous and smooth, the time series with spike data-like observations of feed and water intake rates, as well as the rumination behavior, had to be transformed. With regard to the amount of PMR, concentrate, and water consumed, we assumed that these not only influence pH immediately during or after intake but also have a sustained and delayed effect over a period of time. Further, we supposed that the effects of individual intakes accumulate over time and overlap in their effects on the ruminal fermentation and thus the pH course. To account for these complex interrelations, the animal's individual intake rates were transformed using recursive time-series filters as described by Shumway and Stoffer (2017). This recursive filter, which corresponds to a combination of an accumulation and exponential decrease of a time series, can be described as follows:

$$\text{T}[x]_t = x_t + \sqrt[60]{\alpha} \times \text{T}[x]_{t-1}, \quad [7]$$

where $\text{T}[x]_t$ is the transformed time series of the original intake rate x_t, and $\alpha \in (0,1)$ corresponds to a rate of change per hour resulting in an exponential decrease. The cumulative part of this filter captures the accumulation of further intakes over time, and the exponential decrease represents the decreasing contribution to the overall fermentation process over time. The latter will thus include both the decrease in fermentation activity as well as a passage rate effect. For better interpretation, α was considered on an hourly basis, which re-

quires the formation of the 60th root to meet the 1-min data resolution. For example, $\alpha = 0.96$ corresponds to a rate at which the effect of the transformed PMR intake rate would decrease by 4% per hour. For better understanding, Figure 2A illustrates simulated PMR recording rates and the result of 3 filters ($\alpha = 0.90$, $\alpha = 0.85$, and $\alpha = 0.80$) for a period of 8 h. We can see that the larger α is, the higher the curve tends to be. Additionally, the transformed intake rate increases in phases of numerous intake events and decreases exponentially between such events. This type of filter was used to transform PMR, concentrate, and water intake rates. The units of the transformed variables were not changed by the procedures described above. Because the rates of change will be determined so that they best explain the pH, the resulting transformed variables are artificially generated variables. Therefore, the units of these transformed variables are not further specified.

The graphical examination of the intake, pH, and rumination time series showed that rumination (**RB**) had an immediate effect on the pH, expressed as a temporal increase. Therefore, RB coded as 1/0 was transformed to T[RB] using a convolutional moving-average smoother with a Gaussian kernel (Shumway and Stoffer, 2017) and a window length of 61 min, which caused the best fit in later analyses. This transformation can be described as follows:

$$\mathrm{T}\left[RB\right]_t = \sum_{i=0}^{b} w_i \times RB_{t-\frac{b}{2}+i}, \quad [8]$$

where $\mathrm{T}[RB]_t$ is the transformed rumination behavior time series, RB_t is the raw rumination behavior time series, b is the window length of the symmetric kernel, and w_i are the weights. The weights correspond to the scaled function values of a Gaussian curve from −3 to +3 standard deviations (**SD**) so that $\sum_{i=0}^{b} w_i = 1$. This transformation is illustrated in Figure 2B using a simulated example.

The reticular and ventral ruminal pH time series was modeled using the following linear mixed random intercept model:

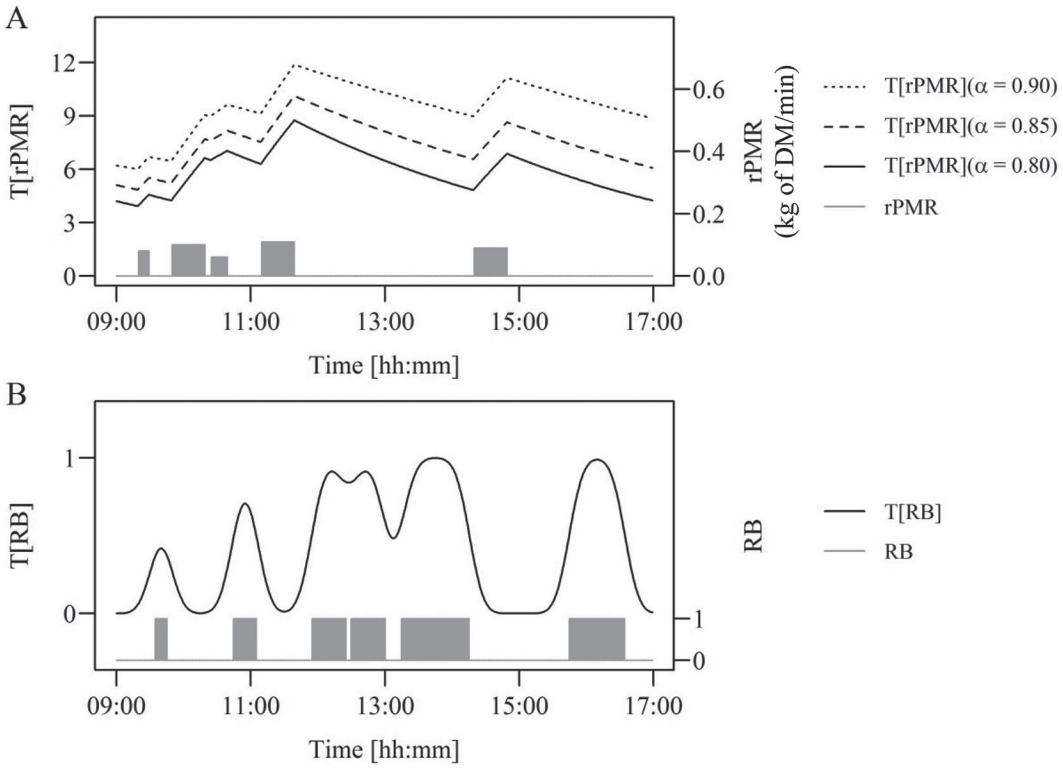

Figure 2. Examples for the signal transformation with recursive filters (A) and a symmetric Gaussian filter smoothing (B). Panel A shows the partial mixed ration (PMR) intake rates (rPMR, light gray bars) and the result of 3 recursive filters (T[rPMR]) with α as rate of change for the exponential decrease. Panel B illustrates the rumination observations (RB; light gray bars) and the result of filtering with the symmetric Gaussian filter with a window length of 61 min (T[RB]).

$$pH_{it} = \beta_0 + \beta_1 \mathrm{T}[rPMR]_{it}(\alpha_{PMR}) + \beta_2 \mathrm{T}[rC]_{it}(\alpha_C) + \beta_3 \mathrm{T}[rW]_{it}(\alpha_W) + \beta_4 \mathrm{T}[RB]_{it} + \beta_5 \Sigma PMR_{it} + \beta_6 \Sigma C_{it} + \beta_7 \Sigma W_{it} + \beta_8 DIM_{it} + \beta_9 BW_{it} + \beta_{10} MY_{it} + A_i + e_{it}, \quad [9]$$

where pH_{it} is the adjusted continuously measured pH (eCow or Dascor) of animal i at time point t; β_0 is the intercept and $\beta_1, \ldots, \beta_{10}$ are the fixed regression coefficients; $\mathrm{T}[rPMR]_{it}$, $\mathrm{T}[rC]_{it}$, and $\mathrm{T}[rW]_{it}$ are the transformed intake rates of PMR, concentrate, and water depending on the decreasing rates α_{PMR}, α_{C}, and α_{W}; and $\mathrm{T}[RB]_{it}$ is the transformed rumination behavior. Further, ΣPMR_{it}, ΣC_{it}, and ΣW_{it} correspond to the total consumed amount of PMR, concentrate, and water per day; DIM_{it} are the days in milk; BW_{it} is the mean BW per day; and MY_{it} is the daily milk yield. The individual cow is considered with a random effect A_i with $A_i \sim N(0, \sigma_A)$, and e_{it} is a random error with $e_{it} \sim N(0, \sigma_e)$. In this model, the transformed feed and water intake rates, which are considered independent variables, depend on the respective rate of change α. To determine α_{PMR}, α_{C}, and α_{W} so that the transformed time series best explained the pH time series, all combinations between 0 and 1 in 0.01 steps were tested via grid search. As a criterion for the selection of the optimal parameters, the root mean square error (**RMSE**) of the model was used. The combination of α_{PMR}, α_{C}, and α_{W} leading to the smallest RMSE was considered to be the best fitting.

For the final models, the marginal R^2 $\left(\mathrm{R}^2_{\mathrm{m}}\right)$ and conditional R^2 $\left(\mathrm{R}^2_{\mathrm{c}}\right)$ according to Nakagawa et al. (2017) were calculated using the estimated variance components; $\mathrm{R}^2_{\mathrm{m}}$ represents the explained variance of the fixed effects, and $\mathrm{R}^2_{\mathrm{c}}$ represents the explained variance of the entire model.

Although observations of continuous measurements during 21.8 ± 5.8 d with RumiWatch data, 34.5 ± 7.9 d with Dascor data, and 58.1 ± 27.8 d with eCow data were available after the plausibility check per cow on average, only observations for which all time series were available were used in the final modeling. As shown in the Venn diagram in Figure 3, a total of 145 d of continuous and parallel records of all considered variables from the 13 cows were available and included in the final analysis. The Venn diagram was created using the *draw.quad.venn* function from the VennDiagram (Chenn et al., 2018) package in R.

Unless otherwise stated, the entire data preparation, creation of figures, and statistical analysis was done within the software environment R (https://www.R-project.org/).

RESULTS AND DISCUSSION

Description of Continuous Measurements in the Course of the Day

To obtain a general overview of daily pH progressions in the reticulum and ventral rumen, and of ingestion and rumination behavior, the time series were first visualized as 24-h profiles (Figure 4). The graphical representation was done separately for the 2 feeding regimens (C_{35} and C_{60}); time windows of the morning (0530 to 0730 h) and evening (1500 to 1700 h) milkings, as well as the daily supply of fresh feed between 1100 and 1200 h, are marked with vertical lines. The pH curves represent the adjusted pH values averaged as a function of the time of the day. It can be seen that the pH curves at both measuring sites are essentially comparable for the C_{35} and C_{60} groups. The averaged curves for both groups were about 0.4 pH units higher for reticular pH values than for those of the ventral rumen. This tends to be in line with the results of Neubauer et al. (2018), who found a difference of about 0.2 pH units at a similar pH range between the reticular pH and the pH of the free-rumen liquid. In addition, it can be seen that the progression of the ruminal pH has a higher variance compared with the reticular pH, which is also consistent with previous results (Falk et al., 2016).

The profiles of PMR, concentrate, and water intakes had common characteristics for both groups. The PMR intake increased from the onset of feeding to shortly

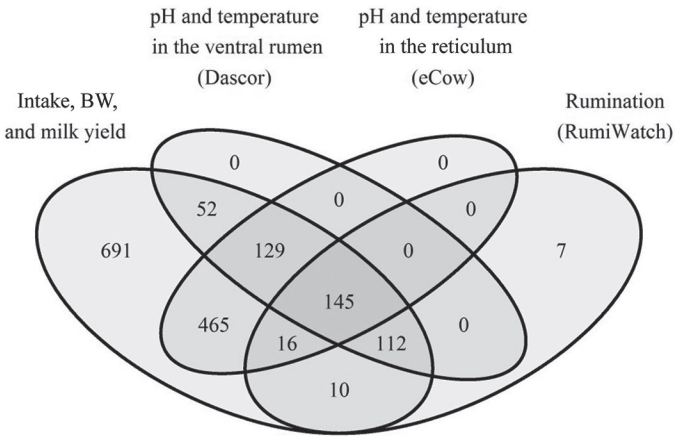

Figure 3. Venn diagram (Chenn et al., 2018) of available data quantity from various automated acquisition systems in days. eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA); RumiWatch = RumiWatch noseband halter (ITIN + HOCH GmbH, Liestal, Switzerland).

after evening milking. This is to be expected because freshly presented feed is preferred and stimulates feed intake (DeVries et al., 2003; Oberschätzl-Kopp et al., 2016). With concentrated feed, intake during the day seemed to decrease progressively in the C_{60} group in particular. Cows of the C_{35} group had consumed their entitled amount of additional concentrated feed by noon. In comparison, the probability of visits in which concentrate was consumed decreased during the day in the C_{60} group. However, this was partly due to a restriction for release so that an equal supply of concentrate feed supply is provided during the day. Nevertheless, it appears that the permitted amount of concentrated feed is consumed as early in the day as possible, which corresponds to a preference of concentrate over the PMR and is associated with the displacement of roughage by additional concentrated feed (Lawrence et al., 2015). The peaks of water intake were similar to those of PMR intake and, in the case of the C_{60} group, to those of concentrate intake. This implies that feed and water intake events are often combined. On average, rumination behavior minima can be observed, especially during milking times and fresh feed offering. The phases of highest rumination activity were observed between the evening and early morning hours.

Ingestion behavior showed a local maximum shortly after 0000 h. This could be explained by the calibration of the weighing troughs, which took place between

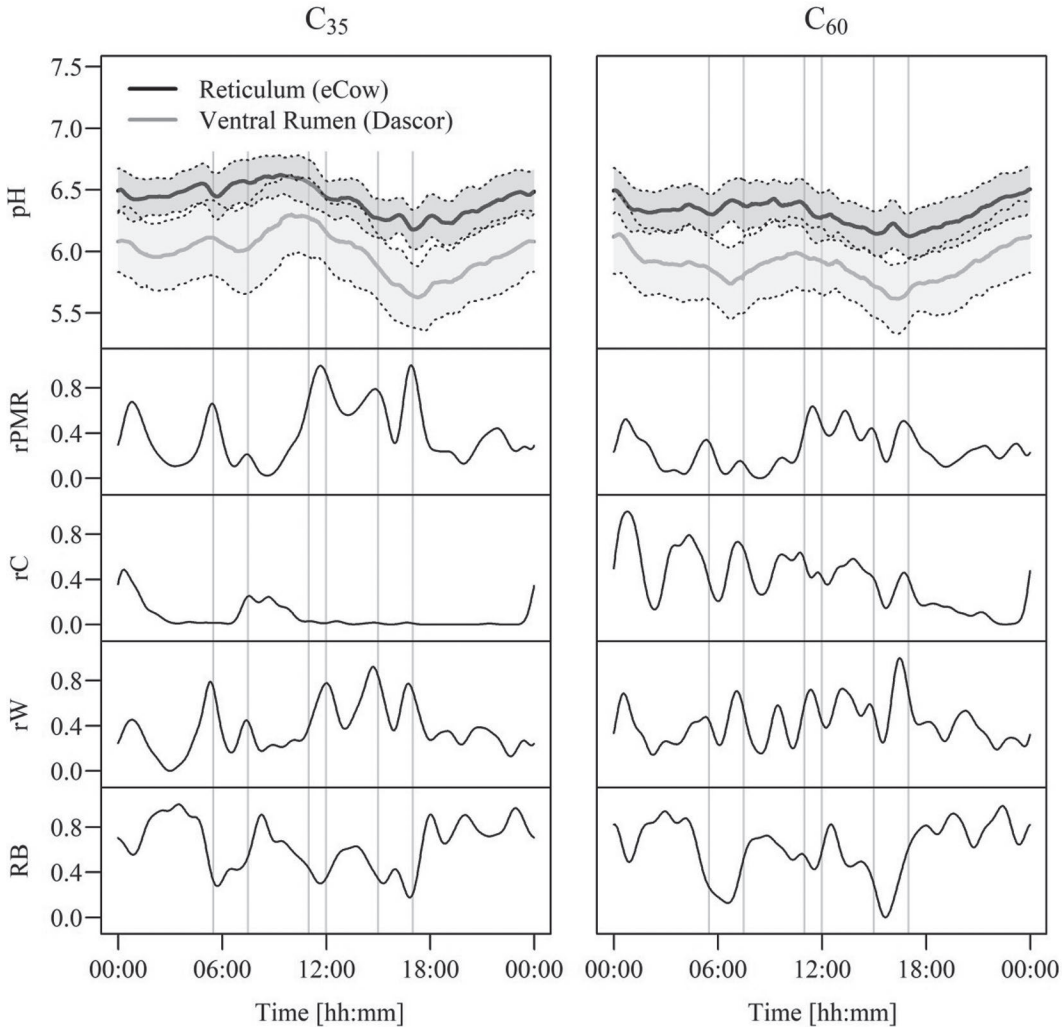

Figure 4. Averaged 24-h profiles of reticular and ruminal pH and behavior for 2 feeding groups (C_{35} = 35% concentrate; C_{60} = 60% concentrate) from the periods in which all measured values were available. The light gray areas around the curves correspond to the ±1 SD environment. The other 4 time series [rPMR = partial mixed ration (PMR) intake, rC = concentrate intake, rW = water intake, RB = rumination] correspond to the relative frequencies scaled from 0 to 1 for the respective activity depending on the time of day. The vertical lines indicate the time spans of morning (0530 to 0730 h) and evening milking (1500 to 1700 h) as well as of the fresh feed supply (1100 to 1200 h). eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA).

2345 and 0000 h. In the course of this calibration, the pneumatic hatches make noise, which presumably triggers the cows' behavior. In this context, the peak before milking in the morning should also be noted. Because the experiment took place in the winter months, lights were switched on every morning at 0500 h, about 30 min before the morning milking. This seemed to influence the behavior of animals as well as the pH progressions.

From Figure 4, we can see that the intake of PMR in particular affected the pH value. During the phases with the highest probability of PMR intake (shortly after midnight, before a.m. milking, between fresh feed supply, and until shortly after p.m. milking), the pH value in both the reticulum and ventral rumen tended to decrease in both feeding groups, whereas it tended to increase in phases with a low probability of intake. This is in line with the effect of concentrate intake and can be observed in the C_{60} group in the first half of the day. Comparing the pH progressions in the reticulum and ventral rumen for the 2 feeding groups, it is noticeable that the pH progressions in the reticulum are similar but the pH tended to decrease slightly more in the morning hours in the C_{60} group than in the C_{35} group, which might be due to the different amounts of concentrate feed intake at this time.

Temporal Relationship Between Continuous Measurements

The calculated cross-correlation functions of animals between the time series are shown in Figure 5 for -120 min $\leq \tau \leq 120$ min. The cross-correlation functions of the 2 pH time series show that they were generally positively correlated in the observed range of τ [pH (ecow) vs. pH (Dascor)]. In addition, for all functions a local maximum at -37 ± 24 min can be determined. This means that the reticular pH of 37 min prior is maximally correlated with the current ruminal pH or that the ruminal pH value is delayed by this time lag. A comparable time delay can be observed in the results of Falk et al. (2016; Figure 1). Consequently, adjusting the ruminal pH values regarding the time lag determined individually for each animal is justifiable.

Nevertheless, it is not clear to what extent the identified time lag can be attributed to the fact that ruminally fistulated cows were used, in which the fistula could partially affect the motility of the rumen due to its fixation to the rumen wall. Furthermore, gas exchange between the reticulorumen and the atmosphere caused by general leakage and opening and closing of the fistula is expected. The resulting reduction of the CO_2 partial pressure in the forestomach system could lead to an increase of the reticuloruminal pH and therefore affect the bicarbonate-based buffer system (Kohn and Dunlap, 1998). Strictly speaking, the results generated here are therefore only valid for ruminally fistulated cows. However, past studies (e.g., Hayes et al., 1964; MacRae and Wilson, 1977) comparing fistulated and nonfistulated ruminants have shown that there are only minor differences, if at all, in behavior or digestibility. Thus, we can assume that the results are transferable to nonfistulated cows.

Further, the cross-correlation functions showed that both the reticulum and the ventral rumen experienced a drop in temperature after water intake for both feeding groups [T (eCow) vs. rW, T (Dascor) vs. rW]. The temperature drop was almost immediate in the reticulum and slightly delayed in the ventral rumen and can be explained by the consumed water, the temperature of which (approximately 8 to 15°C) is far below the body temperature of 39°C. The temporal difference and the temperature change can be explained by the anatomy of the cow: the rumen of lactating cows, with a volume of 102 to 148 L (Budras and Wünsche, 2002), accounts for the largest proportion of the forestomach system, whereas the reticulum is considerably smaller. This difference in size, therefore, makes the rumen a more inert system for changes in pH and temperature. In addition, the reticulum is closer to the esophagus, which is why the eCow boluses remain there after oral insertion. Bewley et al. (2008) determined that reticular temperature decreases depending on the amount and temperature of the water, especially in the first 15 min after water intake.

A similar relationship could also be observed between temperature and PMR and concentrate intakes [T (eCow) vs. rPMR; T (Dascor) vs. rPMR; T (eCow) vs. rC; T (Dascor) vs. rC]. Because the experiment was carried out in winter, the temperature of the PMR and concentrate was presumably much lower than body temperature and therefore led to a temperature drop in the reticulum and ventral rumen immediately after ingestion.

The analysis with cross-correlations also showed that rumination led directly to an increase in reticular pH and a delayed increase in ventral ruminal pH [pH (eCow) vs. RB; and pH (Dascor) vs. RB]. This was to be expected and was due to the increased swallowing of saliva with a pH value of ~8.2 during rumination (Aschenbach et al., 2011).

Furthermore, behavior patterns can be derived. Water intake often followed a previous concentrate intake (rC vs. rW). With regard to PMR and water intake events, water intakes were generally accompanied by PMR intakes; especially, PMR intakes in the C_{60} group more often followed water intakes (rPMR vs. rW).

Intake of PMR and concentrate feed also tended to coincide, with concentrate feed being consumed more frequently before PMR (rPMR vs. rC).

Modeling of Reticular and Ventral Ruminal pH Progressions

The final analysis of the reticular and ruminal pH course was done using the model presented in Equation [9]. The results of the grid search for the determination of α_{PMR}, α_C, and α_W are illustrated in Figure 6. For the sake of illustration and better comparability, the ratio between the SD of the pH measurements and the RMSE of the respective model are plotted in 3-dimensional wireframe surface plots (Sarkar, 2008), each dependent on 2 rates of change. The higher the ratio, the better the fit of the model. Figure 6A and C show that there were unique local maxima at $\alpha_{PMR} = 0.85$, $\alpha_C = 0.91$, and $\alpha_W = 0.86$ for the modeling of the reticular pH. Furthermore, we can infer that the change in α_{PMR} had the greatest impact on the fit statistic and therefore the entire modeling. The second largest influence was exerted by α_C. The modeling of the ruminal pH value showed a slightly different relationship (Figure 6B and D). The local maxima were at $\alpha_{PMR} = 0.87$, $\alpha_C = 0.89$, and $\alpha_W = 0.42$. The change of α_{PMR} again showed the greatest effect on the fit statistic but the variation of α_W had almost no influence. Furthermore, the SD/RMSE was generally lower than in the grid search for the reticular pH.

The interpretation of the rates of change (α_{PMR}, α_C, and α_W) is not trivial. As explained in the Material and Methods, this is an abstract quantity that is presumably associated with fermentation properties and the passage rate of feed through the rumen. By subtracting the estimated values of α_{PMR} with 0.85 and 0.87 and α_C with 0.91 and 0.89 from 1 (i.e., $1 - \alpha$), hourly decreasing rates of 0.15 and 0.13 as well as 0.09 and 0.11

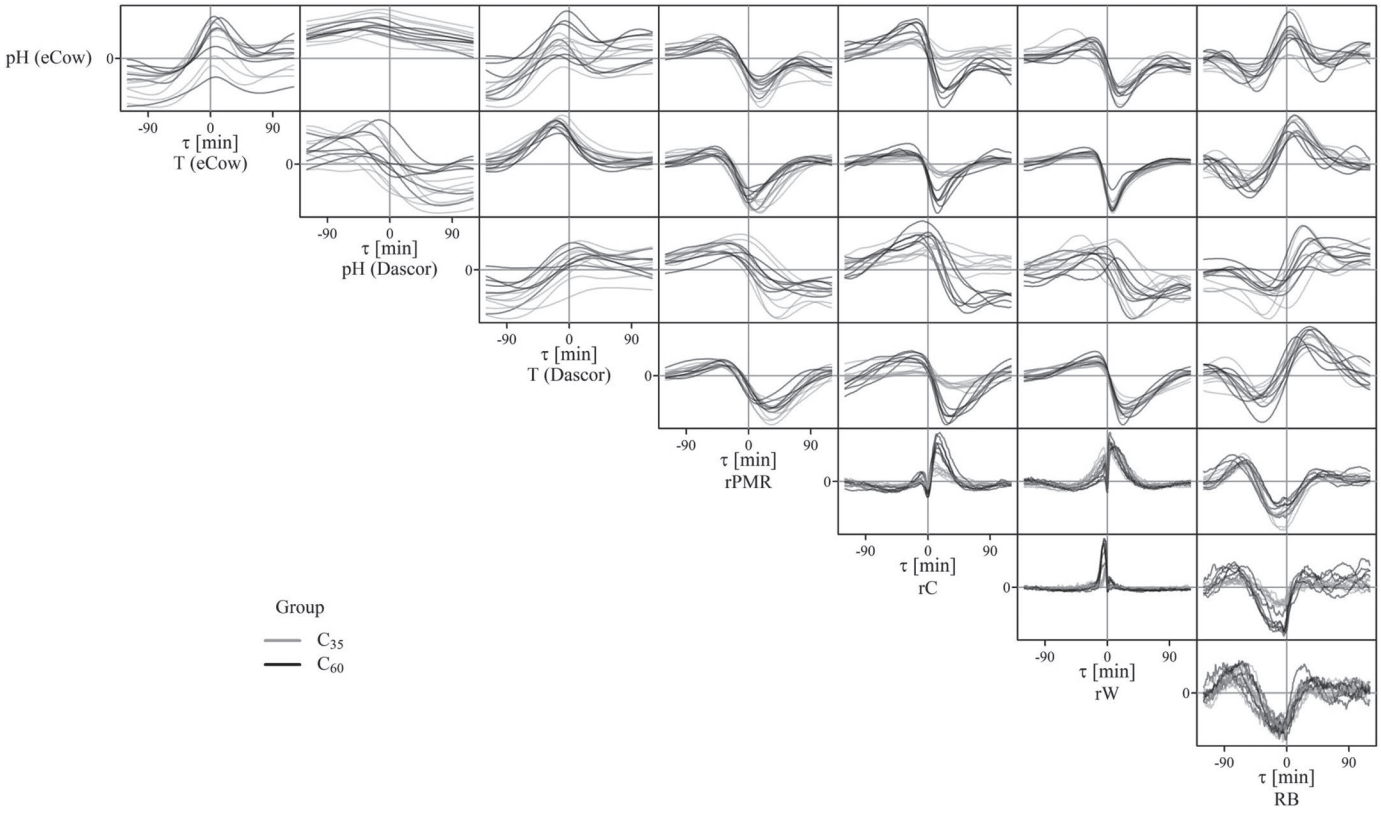

Figure 5. Pairwise visualization of cow-specific cross-correlation functions of 8 time series for a time lag of -120 min $\leq \tau \leq 120$ min with the feeding group affiliation being illustrated by 2 different gray scales ($C_{35} = 35\%$ concentrate; $C_{60} = 60\%$ concentrate). The cow-specific curves display the correlation between 2 different time series x (variable name to the left of the row) and y (variable name at the bottom of the column) when the time series x is shifted by the time interval τ (see Equation [4]). Interpretation example for x = T(eCow) and y = T(Dascor): From the uniform local maximum of the 13 curves at approximately $\tau = -20$ min, it can be seen that the temperature in the reticulum recorded with the eCow bolus 20 min ago is maximally correlated with the current temperature recorded with the Dascor in the ventral rumen. TpH (eCow) and pH (Dascor) = reticular and ruminal pH, T(eCow) and T(Dascor) = reticular and ruminal temperature, rPMR = intake rate of partial mixed ration (PMR), rC = intake rate of concentrate, rW = intake rate of water, RB = rumination behavior. eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA).

result. Interestingly, these values are in the same order of magnitude as estimated ruminal degradation rates of dietary DM. For example, Hatew et al. (2015) estimated fractional degradation rates between 0.043 and 0.139 per hour for the OM part of the ration. Maxin et al. (2013) determined similar degradation rates of DM for different protein-containing dietary supplements, with values between 0.05 and 0.09.

The estimates of the regression coefficients for the transformed time series of PMR and concentrate intake had negative signs in both models (Table 1). The sign of the transformed water intake was also negative for reticular pH in the modeling, but positive for ruminal pH. In both cases, the sign for the transformed rumination behavior was positive, from which we can conclude that rumination leads to a temporary increase in pH values, which is in line with the results presented in Figure 5. These complex relationships are illustrated in Figure 7A and B by showing the pH curves, intake rates, and rumination behavior as well as their transformations for 2 cows (one from each feeding group; panel A = C_{35}, panel B = C_{60}) over a period of 48 h. Both cows were in the sixth parity. The figures show that the pH curves essentially correspond to a combination of the transformed PMR and concentrate intake rates mirrored on the horizontal axis, which is in line with the signs of the regression coefficients from the models. This means that pH during feed intake decreases and increases progressively more slowly in the time after intakes (see Figure 7A and B).

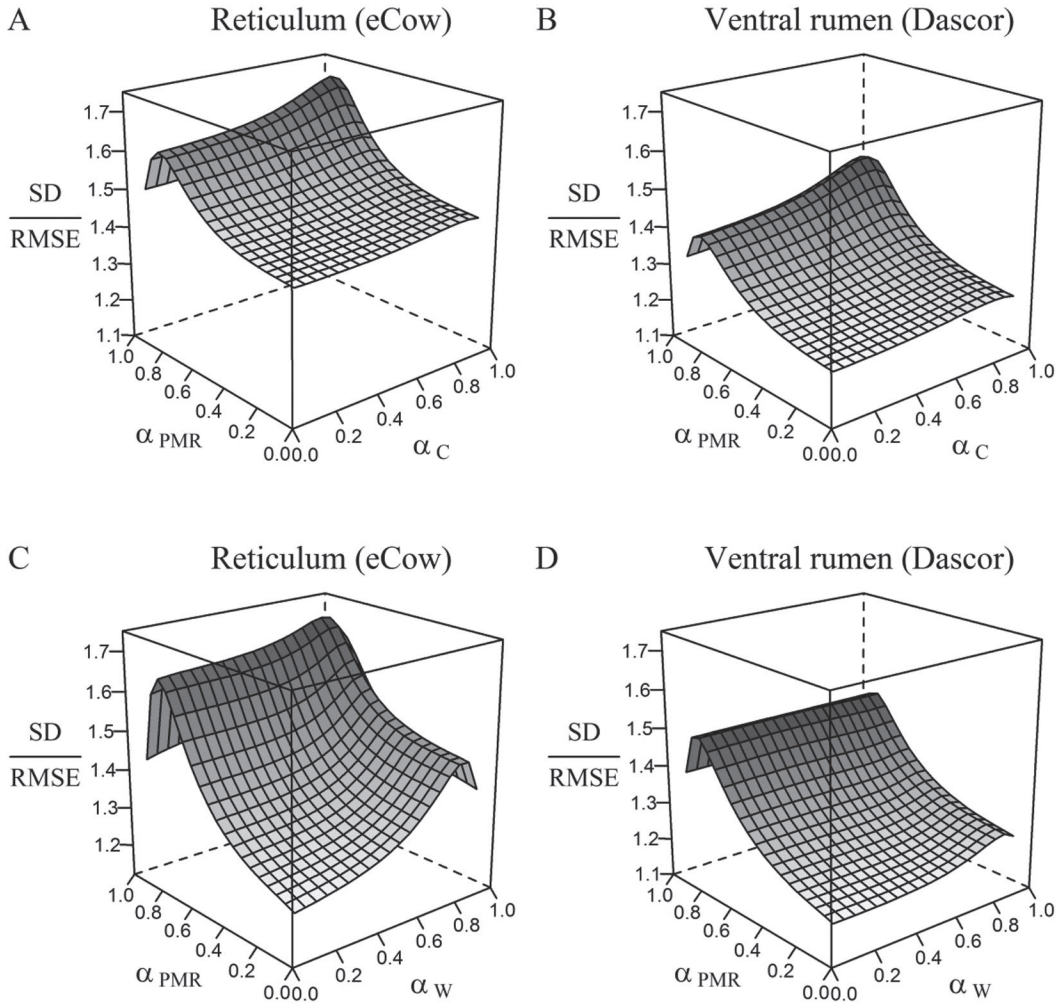

Figure 6. Results of the grid search to determine the optimal rates of change (α_{PMR}, α_C, and α_W, respectively) for the recursive filtering of partial mixed ration (PMR), concentrate and water intake for the modeling of the reticular (panels A + C) and ruminal (panels B + D) pH development. Visualized are the ratios between the standard deviation (SD) and root mean square error (RMSE) of the linear mixed models depending on the rate of change for α_{PMR}, α_C, and α_W with 3-dimensional wireframe surface plots (Sarkar, 2008). eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA).

The significance and *F*-values must be interpreted with caution. Due to the very large sample size caused by the temporal resolution, the *F*-values are extremely large, so that even the smallest effects become significant. It is therefore helpful to consider the ratio between the *F*-values of the covariables. The *F*-values showed that T[rPMR] and T[rC], in particular, contributed substantially to the explanation of the variance for both pH measurements. In contrast to the modeling of the ventral ruminal pH, T[rW] and T[RB] also explained a considerable amount of the variance in reticular pH. Furthermore, lactation stage affects reticular pH. The other variables measured daily showed only small effects, except for daily consumed amount of PMR in the modeling of the ruminal pH. The 3 regression coefficients for ΣPMR, ΣC, and ΣW are generally difficult to interpret, because the covariables were also partly represented in the transformed time series, T[rPMR], T[rC] and T[rW], and were only included in the model for correction purposes.

The proportion of explained variance of the fixed effects was high for reticular pH $\left(R^2_m = 67.0\%\right)$ and moderate for ruminal pH $\left(R^2_m = 37.8\%\right)$. The variance explained by the entire model had relatively high values, with $R^2_c = 76.0\%$ and $R^2_c = 71.4\%$ for both models, indicating good fitting of the models to the data. This was also evident when comparing observed with predicted values by the model in Figure 7A and B. The reason why more variance of the reticular pH can be explained by the fixed effects may also be due to the fact that the reticulum, due to its smaller size and proximity to the esophagus, reacts faster and is less inert than the ventral rumen. Further, the individual-associated variance was higher in ruminal pH than in reticular pH, making the absolute pH level (and therefore the ruminal daily mean) difficult to predict. Nevertheless, ruminal pH is decisive for the digestive disorder SARA.

Table 1. Estimates of regression coefficients (b), transformation traits, and model statistics of linear mixed models for reticular and ruminal pH (n = 13 animals, in total 145.3 d of continuous measurements)[1]

	Reticulum (eCow)				Ventral rumen (Dascor)			
Variable[2]	b	SE	Significance	*F*-value	b	SE	Significance	*F*-value
Intercept	6.87437	0.02681	***		6.40964	0.07168	***	
T[rPMR] (kg)	−0.07508	0.00025	***	86,982.6	−0.13304	0.00037	***	127,390.3
T[rC] (kg)	−0.09562	0.00056	***	29,343.1	−0.19145	0.00102	***	35,374.5
T[rW] (kg)	−0.00877	0.00006	***	23,870.2	0.00340	0.00013	***	642.2
T[RB]	0.09829	0.00082	***	14,439.7	0.06733	0.00147	***	2,090.2
ΣPMR (kg/d)	0.00930	0.00013	***	5,082.2	0.02694	0.00022	***	14,357.8
ΣC (kg/d)	0.01093	0.00054	***	413.6	0.02982	0.00092	***	1,049.6
ΣW (kg/d)	0.00095	0.00003	***	1,042.0	−0.00187	0.00005	***	1,555.2
DIM (d)	0.00407	0.00002	***	48,739.7	0.00123	0.00003	***	1,430.1
BW (100 kg)	−0.08243	0.00254	***	1,052.6	−0.07939	0.00448	***	313.6
MY (kg/d)	0.00749	0.00011	***	4,360.5	0.01433	0.00020	***	5,156.8
Rate of change								
α_{PMR}	0.85				0.87			
α_C	0.91				0.89			
α_W	0.86				0.42			
Model statistics								
RMSE	0.124				0.217			
SD	0.212				0.326			
SD/RMSE	1.719				1.501			
σ_A	0.075				0.235			
σ_e	0.124				0.217			
R^2_m (%)	67.0				37.8			
R^2_c (%)	76.0				71.4			

[1]eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA).

[2]T[rPMR], T[rC], and T[rW] = transformed intake rates of partial mixed ration (PMR), concentrate, and water; T[RB] = transformed rumination behavior; ΣPMR, ΣC, and ΣW = daily amounts of PMR, concentrate, and water; MY = daily milk yield; α_{PMR}, α_C, and α_W = rates of change for the recursive filtering of PMR, concentrate, and water intake rates; RMSE = root mean square error; SD/RMSE = ratio between SD and RMSE; σ_A = standard deviation of random animal effects; σ_e = standard deviation of random errors; R^2_m = marginal R-squared, R^2_c = conditional R-squared.

***$P < 0.001$.

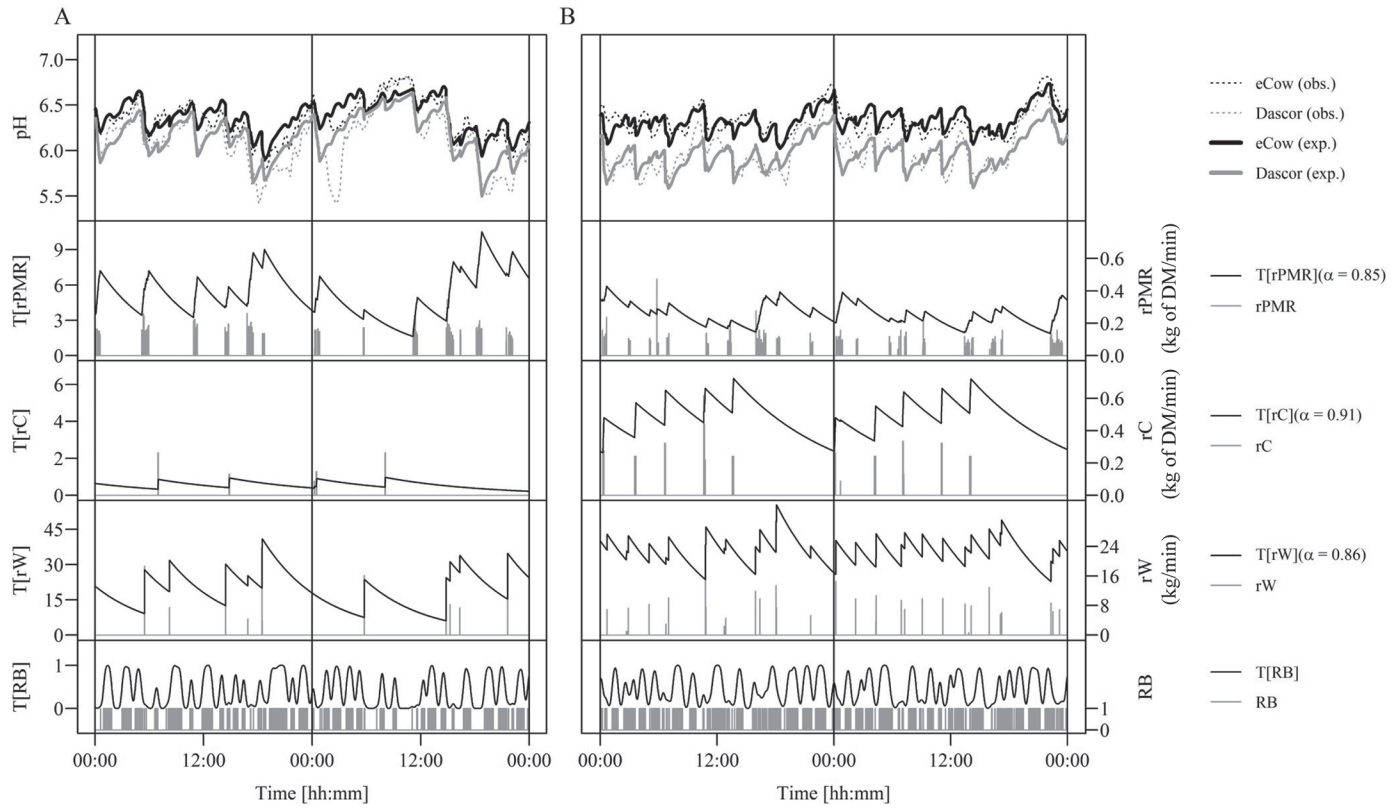

Figure 7. Examples for the observed time-series data of a cow from the C_{35} group (A, 35% concentrate) and from the C_{60} group (B, 60% concentrate) for 48 h. Shown are the reticular and ruminal pH progressions, the partial mixed ration (PMR), concentrate, and water intake rates (rPMR, rC, and rW) as well as rumination behavior (RB). In addition, the transformed intake rates T[rPMR], T[rC], T[rW] and the transformed rumination behavior T[RB] were added as black lines. The pH values predicted by the linear mixed models are included as bold lines. α = rate of change for the recursive filtering. eCow = eCow pH bolus (eCow Ltd., Exeter, UK); Dascor = LRCpH logger (Dascor Inc., Escondido, CA).

The results show that behavior patterns during the entire day substantially determine the individual reticular and ruminal diurnal pH course of the animal. Behavior is influenced by management-related factors, such as different milking and feeding times and presumably by other factors that either stimulate or suppress feed intake behavior (e.g., claw disorders, mastitis, or other diseases). The fact that the behavior of the animal is directly reflected in the pH is also in line with the results of Macmillan et al. (2017), who found that cows classified as having a higher risk for SARA ingested feed less evenly during the day than did low-risk cows. Cows at lower and higher SARA risk also differ in sorting behavior (Coon et al., 2019): low-risk cows sorted less feed and thus ate a more balanced diet than did high-risk cows.

A further goal of modeling was to split the variance in pH caused by feed intake and rumination. However, this was not possible with the modeling applied here. The reason for this is that there is, to a certain extent, confounding between feed intake and rumination, because at a certain time point only one of the 2 activities is possible. As can be seen in Figure 7A and B, the probability of rumination was very high when there is no ingestion. For this reason, the transformed feed intake, which is included in the model with a negative coefficient and therefore increases between intake events, will also capture the increase in pH caused by rumination.

The neutralization of the pH by saliva and the release of short-chain fatty acids by fermentation were, to a certain extent, captured by our modeling. However, it is not yet clear to what extent the animal-specific mechanisms of absorption and buffering by bicarbonate secreted via the rumen wall contribute to pH development. These 2 mechanisms are difficult to quantify and could vary from animal to animal, which makes ruminal pH such a complex variable and hard to estimate.

Although the pH progression in the cow's forestomach system is the result of a complex and dynamic regulation by endogenous and exogenous factors, this modeling shows enormous potential to explain pH development over the course of the day. Because the pH values in the reticulum and ventral rumen can essen-

tially be modeled using feed intake behavior, a causal relationship between the 2 measurement locations is confirmed.

CONCLUSIONS

The rumen is a complex system in which a multitude of physiological processes take place. The present work indicates that methods that are uncommon in animal sciences, such as high-resolution time-series data or the application of signal transformations, can offer new insights into complex physiological processes. In the current study, the collected data were not reduced to variables on a daily basis, but were considered to the full extent to elucidate reticular and ruminal pH development. We demonstrated that both pH progressions over a day can be modeled using animal transponder-based individual ingestion and sensor-based rumination behavior. By this approach, 67.0 and 37.8% of the variance of the reticular and ventral ruminal pH, respectively, could be explained by applying linear mixed models on 1-min resolution time-series data. Although all animals were subject to the same daily management routine, the different pH profiles could be attributed to the individual temporal distribution of feed and water intake and rumination during the day. The investigated physiological relationships in this study were quantified for the first time with a high degree of precision by using these innovative approaches. These methods could also be used to investigate the relationship between the 2 pH measurement sites (reticulum and ventral rumen) in more detail. The use of time-series data is a powerful approach because time delays between different measures can be captured and quantified. With regard to precision livestock farming, which increasingly relies on automated data acquisition (e.g., with automated milking or heat detection systems), the enhanced consideration of the longitudinal nature of these data could improve herd management.

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ORCIDS

A. Mensching https://orcid.org/0000-0002-7302-9010
J. Hummel https://orcid.org/0000-0002-8876-7745
A. O. Schmitt https://orcid.org/0000-0002-4910-9467