Technical note: A comparison of reticular and ruminal pH monitored continuously with 2 measurement systems at different weeks of early lactation

M. Falk,*† A. Münger,* and F. Dohme-Meier*[1]
*Agroscope, Institute for Livestock Sciences ILS, 1725 Posieux, Switzerland
†Veterinary Physiology, Vetsuisse Faculty, University of Bern, CH-3001 Bern, Switzerland

ABSTRACT

Subacute ruminal acidosis is one of the most important digestive disorders in high-yielding dairy cows fed highly fermentable diets. Monitoring of forestomach pH has been suggested as a potentially valuable tool for diagnosing subacute ruminal acidosis. The objective of the present study was to compare continuously recorded measurements of an indwelling telemetric pH sensor inserted orally in the reticulum with those obtained from a measurement system placed in the ventral sac of the rumen through a cannula. The experiment was conducted with 6 ruminally cannulated Holstein cows kept in a freestall barn. Equal numbers of cows were assigned to 2 treatment groups based on their previous lactation milk yield. Cows in treatment CON− were offered a diet consisting of only fresh herbage cut once daily, and cows in treatment CON+ got fresh herbage plus a concentrate supplement according to the individual milk yield of each cow to meet their predicted nutrient requirements. The experiment lasted from 2 wk before the predicted calving date until wk 8 of lactation. During the whole experiment, a pH value was recorded every 10 min in the reticulum using a wireless telemetry bolus including a pH sensor (eBolus, eCow Ltd., Exeter, Devon, UK), which had been applied orally using a balling gun. Simultaneously, in wk 2, before the estimated calving date and in wk 2, 4, 6, and 8 of lactation, the ruminal pH was measured every 30 s for 48 h with the LRCpH measurement system (Dascor Inc., Escondido, CA) placed in the ventral sac of the rumen through the cannula. The readings of the LRCpH measurement system were summarized as an average over 10 min for statistical analysis. The recorded pH values were on average 0.24 pH units higher in the reticulum than in the rumen. The reticular pH also showed less fluctuation (overall SD 0.19 pH units) than pH profiles recorded in the rumen (overall SD 0.51 pH units). Regardless of measurement system, pH was not influenced by treatment, but varied across week of lactation and decreased with advancing lactation. The difference between ruminal and reticular pH varied across week of lactation. Due to this variation, no fixed conversion factor can be provided to make pH measurements in the reticulum comparable with those in the rumen.

Key words: dairy cow, reticular pH, ruminal pH, herbage

Technical Note

Subacute ruminal acidosis is a disorder of ruminal fermentation that is characterized by extended periods of depressed ruminal pH (Plaizier et al., 2008). This disease is a widespread problem of high-yielding dairy cows that receive highly rumen fermentable diets (Duffield et al., 2004; Plaizier et al., 2008). Cows suffering from SARA produce less milk and milk fat because of irregular feed intake and lower ruminal fermentation efficiency and they show increased incidences of further diseases such as caudal vena cava syndrome and laminitis (Kleen et al., 2003; Krause and Oetzel, 2006). Therefore, SARA can cause important economic losses for dairy farmers due to decreased efficiency of milk production, impaired cow health and cow welfare, and high rates of involuntary culling (Krause and Oetzel, 2005).

The symptoms of SARA are difficult to detect (Enemark, 2008); nevertheless, an early diagnosis of SARA is very important for the therapy and the adjustment of feeding (Enemark, 2008). Monitoring of the ruminal pH is suggested as a potentially useful tool for the diagnosis of SARA because it would be the most meaningful and direct response parameter relating to variation in ruminal fermentation (Kleen et al., 2003). On a herd level, methods such as stomach tubing and rumenocentesis are available for obtaining spot measurements of

pH obtained from rumen fluid samples. However, these methods have limitations [e.g., pH variations according to intraruminal localization of the stomach tube and saliva contamination (Enemark, 2008); health problems and injuries caused by the invasive sampling procedure (Sato et al., 2012a)] and usually do not account for diurnal pH fluctuations. This aspect has been considered thus far by continuous measurements using indwelling probes in ruminally cannulated dairy cows (e.g., Graf et al., 2005; Penner et al., 2006).

Recently, various measurement systems have become available for continuous monitoring of the ruminal and reticular pH in noncannulated cows. One of these is a wireless telemetry bolus that includes a pH sensor (eBolus, eCow Ltd., Exeter, Devon, UK); it is applied orally into the reticulum using a balling gun. The objective of this study was to compare continuously recorded pH profiles of the eBolus with those obtained using a pH measurement system (Lethbridge Research Center ruminal pH measurement system, LRCpH, Dascor Inc., Escondido, CA) placed in the rumen through a cannula at different weeks of lactation (2 wk prepartum until 8 wk postpartum). Because this comparison was part of a larger study in which different diets were fed, the effect of diet was taken into account. The variation in pH differences (**ΔpH**) between measurement systems was also evaluated across week of lactation.

The experiment was carried out with 6 ruminally cannulated multiparous Holstein dairy cows kept in a freestall barn at Agroscope, Institute for Livestock Sciences ILS, Posieux, Switzerland. All procedures were in accordance with the Swiss guidelines for animal welfare and were approved (No. 2012_12_FR) by the Animal Care Committee of the Canton Fribourg. The cows were divided into 2 homogeneous groups based on their previous lactation milk yield [**CON−**, 7,347 (SD 420) kg; **CON+**, 7,083 (SD 107) kg]. Cows in group CON− received fresh herbage cut once daily ad libitum without concentrate. Cows in group CON+ were also offered fresh herbage ad libitum and were supplemented with a cereal/corn gluten-based concentrate to meet their predicted nutrient requirements. All cows received a mineral mix, and water was available at all times. The experiment was started individually for each cow at 2 wk [14 (SD 5) d] before the predicted calving date (**LW−2**) and lasted until wk 8 of lactation (**LW8**). Each cow was equipped with the 2 measurement systems, as detailed below. Herbage samples were taken daily and pooled across week of lactation. Concentrate was sampled once per production batch. The analysis of chemical composition was carried out as described by Thanner et al. (2014). The herbage offered to group CON− contained per kilogram of DM: ADF, 285 (SD 18.9) g; NDF, 451 (SD 32.0) g; NFC, 280 (SD 37.1) g; NE$_L$, 5.7 (SD 0.4) MJ; absorbable protein in the small intestine (**APD**), 95.2 (SD 8.5) g. The herbage fed to group CON+ contained per kilogram of DM: ADF, 290 (SD 16.3) g; NDF, 461 (SD 27.6) g; NFC, 270 (SD 34.5) g; NE$_L$, 5.6 (SD 0.3) MJ; APD, 93.3 (SD 7.4) g. The concentrate contained per kilogram of DM: ADF, 62 (SD 4) g; NDF, 137 (SD 17) g; starch, 500 (SD 21) g; NE$_L$, 8.1 MJ; APD, 202 g).

The eBolus, described functionally by Mottram et al. (2008), is a wireless telemetry device that records pH and temperature continuously for up to 6 mo, according to the manufacturer's manual. The eBolus is 115 mm long, has a diameter of 27 mm, and weighs 200 g (Mottram et al. 2013). It has a perforated stainless-steel end cap that contains the sensors and protects them from damage in the reticulum, but allows the free flow of reticular fluid. The remaining part of the body is composed mostly of resin surrounding the electronics. This construction ensures that the device stays in an essentially upright position in the reticulum. The eBolus provides a real-time monitoring of the reticular pH. It is factory set to determine pH every min and record averaged data at selectable intervals (set to 10 min in the present experiment). Setup of the eBolus, as well as transfer of logged data, is made by telemetric communication. The eBoluses are factory calibrated. Nevertheless, before use in the present study they were checked and adjusted, if necessary, with calibration solution of pH 4 and pH 7. For setup and data download, the manufacturer provides a tablet computer (Samsung NP-Q1, Samsung, Seoul, South Korea) running the Windows XP operating system and an antenna that plugs into the USB port. The included software allows data downloads from the bolus and storage as .csv-type files.

The eBolus automatically turns off if the ambient temperature is below 32°C. Therefore, in the present study, the eBolus was activated by warming up to 39°C in a water bath for about 10 min. Subsequently, the eBolus was placed in the reticulum orally using a balling gun in LW−2. Data were downloaded once per week using the provided tablet computer. The quality and stability of the radio connection depended on the cow's position and presumably on the position and orientation of the eBolus in the reticulum. The best signal reception was achieved at a distance of 10 to 30 cm from the cow, ventral to the breastbone or on the cow's left side caudal to the elbow. In a few cases, the signals could be better received on the right side of the cow. If the cow was lying down, the signal reception was more difficult, regardless of the side of the cow. The download time varied considerably, from 2 min up to 30 min,

depending on the intensity of the signal. After LW8, the eBoluses still located in the reticula were removed through the ruminal cannula.

The LRCpH was used to record the ruminal pH. The system, which was described in detail by Penner et al. (2006), was equipped with 2 weights fastened to the bottom of the electrode shroud to maintain the LRCpH in the ventral sac of the rumen. The system was connected with a cord to the stopper of the ruminal cannula to help maintain the electrode in a vertical position. Before inserting into and after removing from the rumen, the electrode of the LRCpH system was calibrated in pH 4 and pH 7 buffer solutions. The drift occurring between start and end calibration was assumed to be linear over time and was used in the conversion of the recorded readings measured in millivolt to pH units (Penner et al., 2006).

The LRCpH system was inserted into the ventral sac of the rumen of each cow at LW$-$2, 2, 4, 6, and 8. Ruminal pH was monitored continuously for 48 h after each insertion. Readings were taken every 30 s. After 48 h, the LRCpH system was removed from the rumen. In doing this, the system was noted several times to no longer be situated in the ventral sac of the rumen. After downloading and transformation of the readings, the ruminal pH data were averaged over 10 min.

The measurements of the eBolus were compared with those of the LRCpH by taking into account the corresponding 48 h within the respective week of lactation (LW$-$2, 2, 4, 6, and 8). The statistical procedures were conducted with Systat 13 (Systat Software, Chicago, IL) and R version 3.1.0 (The R Foundation for Statistical Computing, Vienna, Austria) using Package 'metRology' (version 0.9–17). The pH and ΔpH [= pH (LRCpH) − pH (eBolus)] were analyzed by linear mixed models with autoregressive AR(1) error variance-covariance matrix (Verbeke and Molenberghs, 2000; Davis, 2002). The error correlation [AR(1)] was 0.676 for pH as response variable, and 0.720 for ΔpH, respectively. Autocorrelation estimates of the full set of >21,000 data points revealed very highly correlated observations. This indicated considerable redundancy within the pH data as well as within the ΔpH differences. Redundancy reduction was applied by a random sampling of approximately 5% of the observations.

The pH data were analyzed according to the following model:

$$pH = \mu + s_i + g_j + w_k + \beta t_{(k)} + A_m + T_q + e_{ijkmqr},$$

where μ = general mean; s_i = fixed effect of measurement system i, i = {LRCpH, eBolus}; g_i = fixed effect

of treatment group j, j = {CON+, CON−}; w_k = fixed effect of wk of lactation k, k = {LW$-$2, 2, 4, 6, 8}; β = regression coefficient for time $t_{(k)}$ within lactation week k, $t_{(k)}$ = [0, 3.12639 d]; A_m = random effect of animal m, m = {Cow1, Cow2 Cow3, Cow4, Cow5, Cow6}, with variance σ_A^2; T_q = random effect of time difference q between measurements, q = {−16, −15, −14, −13, −11, −8, −6, −3, −1, 0, 1, 2 s}, with variance σ_T^2; e_{ijkmqr} = random (residual) error with variance-covariance $\rho^{|v-u|}\sigma^2$, autoregressive AR(1) within animals, measurement system, and week of lactation, with error correlation ρ and time indices v and u.

The ΔpH data were analyzed with the same model used for the pH data but without the fixed effect of the measurement system.

Reduced models (without random factors with near-zero variance components) of the random subsets were finally considered for inference on the difference between measurement systems and time dependence of pH and ΔpH. The model reduction was based on the numeric values of the variance components for the random effects (s_A^2 and s_T^2). A robust estimation of the overall difference between the measurement systems was obtained for the trimmed ΔpH means of the 25 combinations of measurement system, animal, and week of lactation using Huber's proposal 2 robust estimator of location and scale (Huber, 1981). A one-sample t-test (H$_0$:Δ-pH = 0) was computed from the robust estimate of the standard error, assuming (approximate) independence between the robust means. Effects were considered significant at $P < 0.05$. The results are presented as LSM together with the SEM.

The measured pH was higher ($P < 0.001$) with the eBolus in the reticulum (pH 6.35) than with the LRCpH in the rumen (pH 6.11). The mean difference was 0.24 ($\pm$0.08) pH units. The higher reticular pH could be caused by the dilution of the reticulum content with fresh and less fermented feed or with saliva (Duffield et al., 2004; Sato et al., 2012b), or both. The averaged pH profiles for all 6 cows at all weeks of lactation of measurement (Figure 1) also showed less fluctuation over 48 h and fewer variations within the reading time points for the reticular pH compared with the ruminal pH. The overall SD for the reticular and ruminal pH was 0.19 and 0.51 pH units, respectively. A reason for the more stable reticular pH might be the smaller volume of the reticulum with a more homogenous content compared with the rumen, thereby reducing the dislocation of eBolus and the variation in the measurements. In contrast, the LRCpH seems to have moved within the rumen in the present study, resulting in varying pH readings (Duffield et al., 2004). In this con-

Table 1. Variations in the difference between ruminal[1] and reticular[2] pH (ΔpH) in different weeks of lactation

Week of lactation[3]	LW2		LW4		LW6		LW8	
	ΔpH	SEM	ΔpH	SEM	ΔpH	SEM	ΔpH	SEM
LW−2	−0.145	0.1509	0.324	0.1377	0.092	0.1370	0.299	0.1407
LW2			0.469*	0.1318	0.237	0.1318	0.444*	0.1339
LW4					−0.232	0.1149	−0.025	0.1171
LW6							0.207	0.1170

[1]pH recorded with the LRCpH (Dascor Inc., Escondido, CA).

[2]pH recorded with the eBolus (eCow Ltd., Exeter, Devon, UK).

[3]LW−2, 2 wk before predicted calving; LW2, 2 wk postpartum; LW4, 4 wk postpartum; LW6, 6 wk postpartum; LW8, 8 wk postpartum.

*$P < 0.01$.

text, Enemark et al. (2003) decided to use the reticular pH to follow the pH fluctuations occurring when diets with varying forage to concentrate ratio were fed and the daily distribution of concentrate changed. However, the treatment had no effect ($P = 0.29$, data not shown) on reticular or ruminal pH, so the lower fluctuation of the reticular pH over the 48-h measurement period compared with the ruminal pH might also indicate a lower sensitivity to changes in feeding.

Diagnosis of SARA in the past has been made using defined thresholds of ruminal pH [e.g., 5.5 by Duffield et al. (2004)]. Therefore, the variation in the difference between the reticular and ruminal pH hinders the detection of SARA based on measurements in the reticulum. Sato et al. (2012b) previously suggested a higher threshold of reticular pH for the diagnosis of SARA. They found differences between reticular and ruminal

pH of up to 0.7 pH units in acidotic cows; this was much higher than the mean difference in the present study (0.24 ± 0.08 pH units). However, the variations in ΔpH among week of lactation (Table 1) indicate that the difference between reticular and ruminal pH might depend on diet composition or DMI, or both. Therefore, no fixed conversion factor can be offered to correct the pH measurements from the reticulum to predict ruminal pH.

ACKNOWLEDGMENTS

REFERENCES

Davis, C. S. 2002. Normal-theory methods: Linear mixed models. Pages 130–133 in Statistical Methods for the Analysis of Repeated Measurements. Springer-Verlag, New York, NY.

Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87:59–66.

Enemark, J. M. D. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Vet. J. 176:32–43.

Enemark, J. M. D., G. Peters, and R. J. Jorgensen. 2003. Continuous monitoring of rumen pH—A case study with cattle. J. Vet. Med. Ser. A Physiol. Pathol. Clin. Med. 50:62–66.

Graf, C. M., M. Kreuzer, and F. Dohme. 2005. Effects of supplemental hay and corn silage versus full-time grazing on ruminal pH and chewing activity of dairy cows. J. Dairy Sci. 88:711–725.

Huber, P. J. 1981. Robust Statistics. John Wiley and Sons, New York, NY.

Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): A review. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50:406–414.

Krause, K. M., and G. R. Oetzel. 2005. Inducing subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 88:3633–3639.

Krause, K. M., and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Technol. 126:215–236.

Mottram, T., J. Lowe, M. McGowan, and N. Phillips. 2008. Technical note: A wireless telemetric method of monitoring clinical acidosis in dairy cows. Comput. Electron. Agric. 64:45–48.

Mottram, T. T., J. Mc Cubbine, and S. B. Nimmo. 2013. A novel method of calculating a SARA index by wireless rumen pH telemetry. Pages 793–798 in D. Berckmans and J. Vandermeulen, ed.
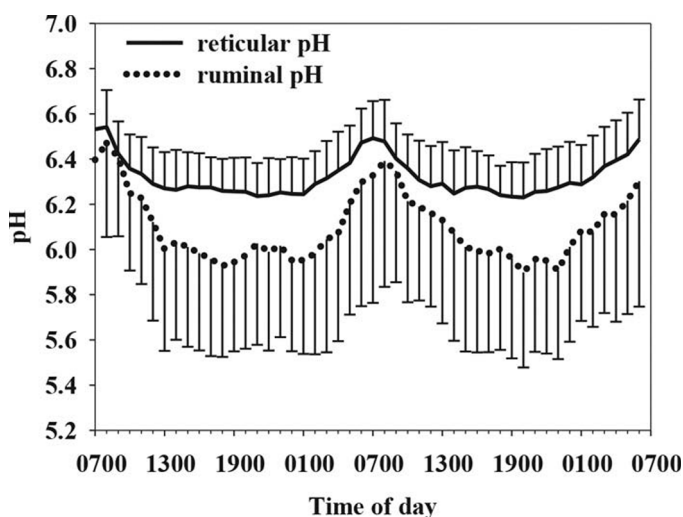
Figure 1. Averaged 48-h pH profiles for all 6 cows at all weeks of lactation recorded simultaneously in the reticulum (eBolus, eCow Ltd., Exeter, Devon, UK) and in the rumen (LRCpH, Dascor Inc., Escondido, CA). The bars represent SD of the arithmetic means.

Proc. 6th European Conference on Precision Livestock Farming: Vol. 1. Precision Livestock Farming '13, Leuven, Belgium.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132–2140.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176:21–31.

Sato, S., A. Ikeda, Y. Tsuchiya, K. Ikuta, I. Murayama, M. Kanehira, K. Okada, and H. Mizuguchi. 2012b. Diagnosis of subacute ruminal acidosis (SARA) by continuous reticular pH measurements in cows. Vet. Res. Commun. 36:201–205.

Sato, S., A. Kimura, T. Anan, N. Yamagishi, K. Okada, H. Mizuguchi, and K. Ito. 2012a. A radio transmission pH measurement system for continuous evaluation of fluid pH in the rumen of cows. Vet. Res. Commun. 36:85–89.

Thanner, S., F. Dohme-Meier, S. Görs, C. C. Metges, R. M. Bruckmaier, and F. Schori. 2014. The energy expenditure of 2 Holstein cow strains in an organic grazing system. J. Dairy Sci. 97:2789–2799.

Verbeke, G., and G. Molenberghs. 2000. General guidelines for model building. Pages 121–132 in Linear Mixed Models for Longitudinal Data. Springer-Verlag, New York, NY.