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An Evaluation of the Accuracy and Precision of a Stand-Alone Submersible Continuous Ruminal pH Measurement System[1]

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ABSTRACT

The objectives of this study were 1) to develop and evaluate the accuracy and precision of a new stand-alone submersible continuous ruminal pH measurement system called the Lethbridge Research Centre ruminal pH measurement system (LRCpH; Experiment 1); 2) to establish the accuracy and precision of a well-documented, previously used continuous indwelling ruminal pH system (CIpH) to ensure that the new system (LRCpH) was as accurate and precise as the previous system (CIpH; Experiment 2); and 3) to determine the required frequency for pH electrode standardization by comparing baseline millivolt readings of pH electrodes in pH buffers 4 and 7 after 0, 24, 48, and 72 h of ruminal incubation (Experiment 3). In Experiment 1, 6 pregnant Holstein heifers, 3 lactating, primiparous Holstein cows, and 2 Black Angus heifers were used. All experimental animals were fitted with permanent ruminal cannulas. In Experiment 2, the 3 cannulated, lactating, primiparous Holstein cows were used. In both experiments, ruminal pH was determined continuously using indwelling pH electrodes. Subsequently, mean pH values were then compared with ruminal pH values obtained using spot samples of ruminal fluid (MANpH) obtained at the same time. A correlation coefficient accounting for repeated measures was calculated and results were used to calculate the concordance correlation to examine the relationships between the LRCpH-derived values and MANpH, and the CIpH-derived values and MANpH. In Experiment 3, the 6 pregnant Holstein heifers were used along with 6 new submersible pH electrodes. In Experiments 1 and 2, the comparison of the LRCpH output (1- and 5-min averages) to MANpH had higher correlation coefficients after accounting for repeated measures (0.98 and 0.97 for 1- and 5-min averages, respectively) and concordance correlation coefficients (0.96 and 0.97 for 1- and 5-min averages, respectively) than the comparison of CIpH to MANpH (0.88 and 0.87, correlation coefficient and concordance correlation coefficient, respectively). The concordance correlation analysis indicated that the ruminal pH data for LRCpH (1- and 5-min averages) vs. MANpH had location shifts that were smaller than those of the CIpH vs. MANpH. However, the scale shift was similar between the LRCpH and the CIpH. The plotted data from both systems closely resembled the line y = x, indicating that both systems were accurate and precise. In Experiment 3, changes in baseline millivolt readings for pH readings after 24, 48, or 72 h of ruminal incubation were not significantly different than zero, indicating that daily standardization of new electrodes was not essential. Results from this study indicate that the LRCpH system can accurately and precisely measure ruminal pH; thus, it provides increased opportunity for researchers to measure ruminal pH and the occurrence of ruminal acidosis in unrestrained cattle.

Key words: ruminal pH, indwelling ruminal pH probe, acidosis, dairy cow

INTRODUCTION

Ruminal acidosis is a common problem in modern ruminant production, particularly in high-producing dairy herds (Nocek, 1997) as the demands for increased milk production have resulted in high-grain, low-fiber diets being the norm to maximize energy intake. Feeding high-grain diets can result in a decrease in ruminal pH due to the ruminal accumulation of VFA and, to a smaller extent, lactate; thus, resulting in acute or subacute ruminal acidosis (Owens et al., 1998). Dairy cows experiencing ruminal acidosis will exhibit a range of clinical symptoms including decreased milk production, intermittent diarrhea, and laminitis (Underwood, 1992; Nocek, 1997). Measurement of ruminal fluid pH is a reliable and accurate diagnostic test for ruminal acidosis. For this reason, various techniques are available for measuring ruminal pH under both experimental and field conditions.

Rumenocentesis and oro-ruminal probes have been used to collect ruminal fluid samples for measurement of ruminal pH under both experimental and field conditions (Oetzel and Nordlund, 1998; Garrett et al., 1999;

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Duffield et al., 2004). However, the usefulness of rumenocentesis in research studies is limited. Furthermore, the risk of peritonitis even with adequate surgical preparation may discourage the use of this technique (Keefe and Ogilvie, 1997; Duffield et al., 2004). Alternatively, various oro-ruminal probes are available, notably the one designed by Geishauser (1993); however, ruminal fluid samples collected through oro-ruminal probes are prone to salivary contamination leading to inaccurate ruminal pH values (Keefe and Ogilvie, 1997; Duffield et al., 2004). Direct ruminal fluid sampling via a ruminal cannula is still a common method for ruminal pH measurement in controlled research studies (Reis and Combs, 2000; Kononoff et al., 2003; Duffield et al., 2004). However, spot sampling techniques (rumenocentesis, oro-ruminal probes, direct sampling via a ruminal cannula) all have inherent limitations as they only indicate ruminal pH at one point in time. Additionally, characterizing ruminal pH variation over time using spot sampling techniques requires regular sampling with short intervals between samples, making it tedious and labor intensive.

Monitoring ruminal pH has been automated with the advent of continuous indwelling pH systems (Dado and Allen, 1993). Continuous indwelling systems have provided data allowing improved characterization of postfeeding ruminal pH variation, which has increased our understanding of the interactions between diet fermentability, meal size, eating behavior, and ruminal pH (Maekawa et al., 2002; Krause and Combs, 2003). Most continuous indwelling pH systems have inherent limitations because they restrict animal mobility and, thus, application is limited to tethered animals (Dado and Allen, 1993; Krause and Combs, 2003; Bevans et al., 2005). Recently, several stand-alone systems have been developed (Enemark et al., 2003; Graf et al., 2005). These stand-alone systems continuously measure reticular or ruminal pH without the use of external cables, thereby allowing the measurement of ruminal pH in grazing or loose-housed animals. Although stand-alone ruminal pH measurement systems have been developed, they are in limited use and a thorough validation of these systems is lacking. Therefore, the objectives of the present study were 1) to develop a stand-alone submersible ruminal pH measurement system for use in cattle and to evaluate its accuracy and precision by comparing its output to pH measurement of ruminal fluid samples collected via a ruminal cannula (Experiment 1); 2) to compare the stand-alone system to an older, well-documented indwelling ruminal pH measurement system (Experiment 2); and 3) to determine the required frequency of electrode standardization necessary to minimize changes in baseline millivolt readings between the start and end of the measurement period (Experiment 3).

MATERIALS AND METHODS

Experiment 1: Design and Evaluation of the Lethbridge Research Centre pH Measurement System

Animals and Management. Three lactating, primiparous Holstein cows (606 ± 34 kg of BW; 25 ± 1 DIM), 6 pregnant Holstein heifers (634 ± 92 kg of BW), and 2 Black Angus, nonpregnant heifers (512 ± 7 kg of BW) were used in this experiment. All animals were fitted with permanent ruminal cannulas. The 3 lactating Holstein cows were offered ad libitum a diet consisting of (DM basis) barley silage (42%), barley grain (29%), a protein, mineral, and vitamin supplement (16%), and alfalfa hay (4%). The 6 pregnant Holstein heifers were fed ad libitum a diet consisting of (DM basis) barley silage (69%), a protein, mineral, and vitamin supplement (17%), grass hay (12%), and barley grain (7%). All diets were fed as a TMR at 1330 h daily. The 2 Black Angus heifers were offered ad libitum a diet consisting of (DM basis) barley grain (88%), barley silage (9%), and a supplement containing minerals and vitamins (3%). This TMR was fed at 1630 h daily. The rationale for using 11 experimental animals at different physiological states and fed a wide range of diets was to determine the sensitivity of the Lethbridge Research Centre pH measurement system (**LRCpH**) under a wide range of pH values and ruminal conditions (i.e., rumen size, ruminal mat structure, etc.). Experimental animals were housed in individual tie stalls at the Lethbridge Research Centre. In all experiments, experimental animals were cared for according to the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada) and the Lethbridge Research Centre Animal Care Committee approved their use for this study.

Design of the LRCpH. The LRCpH system was developed using a data logger (model M1b-pH-1KRTD, Dascor, Escondido, CA), a 9-V battery, and an electrode cable (model S653-ATC-20-BNC, Sensorex, Garden Grove, CA), which were housed in a watertight capsule constructed of polyvinyl chloride material (Figure 1). The pH electrode (model S650-CDHF, Sensorex) was covered by a 38-mm diameter shroud with four 25-mm holes, which was designed to allow particle and liquid passage while preventing the electrode from contacting the ruminal epithelium. Two 900-g weights were fastened to the bottom of the electrode shroud to maintain the electrode in the ventral sac of the rumen. A 30-cm polyester cable was connected to the capsule and the ruminal cannula plug to aid in system location within

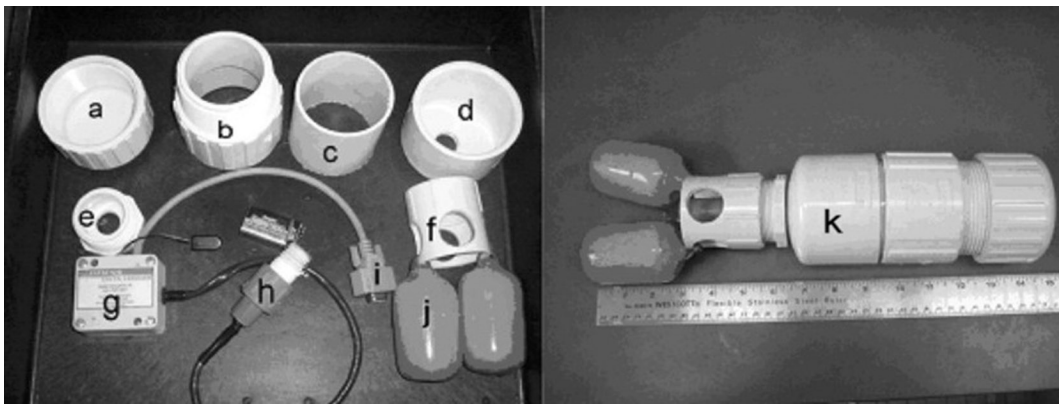

Figure 1. Illustration of the Lethbridge Research Centre ruminal pH measurement system (LRCpH) in unassembled and assembled forms: a = threaded cap; b = male adapter; c = pipe; d = slip cap; e = adapter; f = electrode shroud; g = data logger and pH meter; h = electrode sensor cable; i = computer interface cable; j = 900-g weight; and k = assembled LRCpH system.

the rumen and to help maintain the electrode in a vertical position.

Measurement of Ruminal pH. With the LRCpH system, ruminal pH was monitored continuously for 24 h during each collection period. Ruminal pH readings were taken every 30 s and readings were averaged over 1- and 5-min intervals, and then stored. A 30-s reading interval was used for the LRCpH system to reduce the amount of memory required for data storage because battery power usage by the data logger increases with memory use. Before measuring ruminal pH, readings from the electrodes were recorded in standard buffers (pH 4 and 7). Data transfer from the data logger to a computer and standardization of the pH electrodes were conducted daily around 1230 h. Briefly, the LRCpH was removed from the rumen, and the pH electrode was then washed in water at 39°C, and millivolt readings were recorded in pH 4 and 7 buffer solutions. During this time, pH electrodes and buffers were maintained at 39°C until the data were downloaded and millivolt recordings were taken. The electrodes used have a high thermal mass and maintaining them at 39°C reduces the requirement for temperature compensation. The shift in millivolt readings from the electrodes between the start and end standardizations was assumed to be linear and was used to convert millivolt readings to pH units.

For manual pH measurement (**MANpH**), the ruminal cannula plug was opened and a covered 20-mL container was submersed in the rumen contents. Upon submersion, the LRCpH electrode was located within the rumen and ruminal fluid was allowed to fill the container from the immediate location of the LRCpH electrode. The open end of the container was again covered, removed from the rumen, the ruminal cannula plug was replaced, and ruminal fluid pH was immediately measured using a portable pH meter (model IQ150, IQ Scientific Instruments Inc., San Diego, CA) with a glass electrode (model PHE-1411, Omega Engineering, Stamford, CT). The portable pH meter was calibrated with a 2-point calibration once daily using pH 4 and 7 buffer solutions. Recalibration occurred if readings obtained with pH buffer solutions were not within 0.02 pH units. The time of ruminal fluid collection was recorded to compare the MANpH method to results obtained from corresponding LRCpH measurement. During ruminal fluid collection, positioning of the LRCpH was noted. Ruminal fluid samples were collected on 4 d over a 2-wk duration. On d 1, ruminal fluid samples were collected from the 6 pregnant Holstein heifers every 30 min starting 1 h postfeeding until 3 h postfeeding, and sampling commenced again 8 h postfeeding and ending 10 h postfeeding. Thus, 66 ruminal fluid samples were collected in total on d 1. On d 2, the 3 lactating Holstein cows were sampled every 30 min starting 2.5 h postfeeding and ending 6.5 h postfeeding. In total, 21 ruminal fluid samples were collected. On d 3, ruminal fluid was again collected from the 3 lactating Holstein cows. However, 1 cow was omitted from this collection period because her ruminal contents were very dry, which prohibited adequate LRCpH placement and ruminal pH measurement. Ruminal fluid sampling began 6 h prefeeding and ended at 0.5 h prefeeding with samples collected every 30 min, resulting in a total of 24 samples. On d 4, ruminal fluid samples were obtained every 30 min from the 2 Black Angus heifers, starting at 2 h prefeeding with sampling ending at feeding. Sampling commenced again starting at 2.5 h postfeeding until 5 h postfeeding. In total, 20 ruminal fluid samples were collected. Across all experimental animals, a total of 131 ruminal fluid samples were obtained for determination of ruminal pH.

Experiment 2: Evaluation of a Continuous Indwelling Ruminal pH Measurement System

Animals and Management. The 3 cannulated, lactating primiparous Holstein cows used in Experiment 1 were used in this experiment. These cows were selected for this experiment because a wide range of ruminal pH values was measured in these animals during Experiment 1. Diets and feeding management were the same as in Experiment 1.

Design of the Continuous Indwelling Ruminal pH Measurement System. The design and use of the continuous indwelling ruminal pH measurement system (**CIpH**) system has been previously documented (see Maekawa et al., 2002; Beauchemin and Yang, 2005; Bevans et al., 2005). Briefly, the CIpH system comprised an industrial microprocessor-based pH controller (model PHCN-37, Omega Engineering). The pH electrodes (S650-CDHF, Sensorex) were connected to the pH controller with a 9-m cable (PHEH-65-30-ATC, Omega Engineering) suspended above the cows. The cable passed through a ruminal cannula plug and extended approximately 50 cm into the rumen. The cable was protected from the ruminal environment with a plastic hose. A shroud was constructed around the pH electrode with four 25-mm holes, which allowed material to percolate through but prevented the electrode from contacting the ruminal epithelium. Two 900-g weights were attached to the electrode shroud to maintain positioning within the ventral sac.

Measurement of Ruminal pH. Continuous measurements of ruminal pH were collected over 2 consecutive 24-h collection periods using the CIpH. Ruminal pH was measured every 5 s. For the CIpH system, the 5-s ruminal pH readings were averaged over 5-min intervals and recorded by a data logger (model CR10, Campbell Scientific, Logan, UT). Averaging the 5-s pH readings over 5-min intervals has previously been reported in our laboratory (Beauchemin and Yang, 2005). The 5-min averages corresponded to actual ruminal fluid sampling times used for MANpH (i.e., when the rumen cannula plug was open) and were used to determine the relationship between CIpH and MANpH. In this experiment, pH electrodes were standardized as described for the LRCpH system in Experiment 1.

For manual pH measurement, ruminal fluid samples were collected using procedures already described in Experiment 1. During ruminal fluid collection, positioning of the CIpH was noted. Ruminal fluid samples were collected at 30-min intervals over 48 h. In the first 24 h, ruminal fluid samples were collected starting at 5.5 h prefeeding, ending at 3.5 h postfeeding, and recommencing at 7 h postfeeding, and ending at 10 h postfeeding. During the second 24-h interval, ruminal fluid samples were collected starting at 4.5 h prefeeding and ending at 1 h prefeeding. Thus, 32 ruminal fluid samples were collected from each of the 3 cows resulting in a total of 96 data pairs.

Experiment 3: Frequency of Electrode Standardization

Animals and Management. The 6 cannulated, pregnant Holstein heifers used in Experiment 1 were selected for use in this experiment. Animals were housed individually in tie-stalls. Diets and feeding management were the same as in Experiment 1.

Continuous Measurement of Ruminal pH and Electrode Standardization. Continuous ruminal pH measurement was conducted using the LRCpH system as already described. At the beginning of the experiment, 6 new pH electrodes (model S650CD-HF, Sensorex) were installed. In this experiment, pH electrodes were assigned to a random sequence of 3 treatments: baseline millivolt readings after 24, 48, or 72 h in the rumen. Millivolt readings in pH 4 and 7 buffer solutions for each pH electrode were recorded immediately before pH electrodes were placed into the rumens of experimental animals. Corresponding pH electrodes were removed from the rumen at 24, 48, or 72 h, and millivolt readings in pH 4 and 7 buffer solutions were again recorded for each pH electrode. Baseline reading difference was defined as the difference in millivolt readings between the start and end of each treatment duration.

Statistical Analyses

Experiments 1 and 2. Paired data for ruminal pH from the LRCpH (1- and 5-min averages) and MANpH and from the CIpH (5-min averages) and MANpH were analyzed using the MIXED procedure of SAS (version 9.13, SAS Institute, Inc., Cary, NC) with repeated measures to calculate the correlation coefficient (Hamlett et al., 2004). The correlation coefficient was then used to calculate the concordance correlation (Lin, 1989, 1992). Also used in this calculation were the overall mean and variance for each ruminal pH measurement method. The correlation coefficient calculated to account for repeated measures was used to determine precision by determining the deviation of the data from the best-fit linear line. The concordance correlation coefficient was used to determine accuracy by determining how much the best-fit line deviated from the line y = x. The data from the experimental method (LRCpH, 1- and 5-min averages and CIpH, 5-min averages) were plotted on the y-axis and the data obtained from MANpH were plotted on the x-axis. Data were also analyzed separately for 2 cows (1 with very dry and 1 with very liquid

ruminal contents) using the same procedures as above. This was conducted to determine if consistency of ruminal contents (due to DM content) had an effect on accuracy and precision.

Experiment 3. To evaluate the frequency required for electrode standardization, the change in baseline millivolt readings between the start and end of each treatment duration was calculated for each electrode. These data were analyzed as a double Latin square design using the MIXED procedure of SAS. The model included the fixed effect of treatment with period and electrode considered random effects. Differences among treatments were compared using Fisher's protected LSD test adjusted with the Tukey-Kramer option. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Experiments 1 and 2: Comparison of the LRCpH and CIpH Systems to MANpH

The primary objective of the present study was to develop a stand-alone submersible ruminal pH measurement system for use in unrestrained cattle and to evaluate its accuracy and precision by comparing its output to pH measurement of ruminal fluid samples collected via a ruminal cannula. Several other direct methods, including oro-ruminal probes and rumenocentesis, are currently in use for the measurement of ruminal pH; however, the major drawback of these techniques is that they involve spot sampling of ruminal fluid at various intervals and do not yield comprehensive data on postfeeding profiles of ruminal pH. Dado and Allen (1993) originally developed a system for performing continuous measurements of ruminal pH; the major drawback of this system is that the indwelling pH electrode is directly connected by a cable to a pH transmitter or computer located next to the cow; thus, animal mobility is restricted and application is limited to tethered animals. The LRCpH system developed in this study does not require animals to be tethered and provides the capability to measure comprehensive ruminal pH data.

Relationships between the LRCpH and CIpH continuous ruminal pH measurement systems and manual sampling via the ruminal cannula are shown in Table 1. Because the CIpH averaged ruminal pH measurements over 5-min intervals, the LRCpH ruminal pH measurements were also averaged over 5-min intervals. This allowed direct comparison of the 2 systems. In addition, ruminal pH measurements from the LRCpH were averaged over 1-min intervals to determine if averaging readings over a shortened interval affects accuracy and precision. Overall, mean ruminal pH values obtained using the LRCpH (1- and 5-min averages) and the CIpH systems were numerically lower (0.03 and 0.05 pH units, respectively) when compared with mean ruminal pH values obtained using the MANpH method (Table 1). In other studies, mean ruminal pH values were 0.11 pH units (Dado and Allen, 1993) and 0.06 to 0.18 pH units (Graf et al., 2005) lower when measured using an indwelling pH electrode system that continuously monitored ruminal pH compared with direct measurement in ruminal fluid samples obtained via ruminal cannula. With manual sampling of ruminal fluid through the ruminal cannula, there is usually a delay until ruminal fluid pH is actually measured. Smith (1941) postulated that this delay might allow the escape of CO_2 from ruminal fluid samples, thus elevating ruminal fluid pH. In the current study, continuous ruminal pH measurement occurred while the ruminal cannula plug was open. Thus, the release of carbon dioxide and disruption of the ruminal mat during manual sampling may have increased the values of the continuous measurements. In the current study, the effects of opening the ruminal cannula plug on ruminal fluid pH were not of concern, because we did not intend to characterize ruminal pH as a function of diet. Rather, the objective was to evaluate the accuracy and precision of 2 continuous ruminal pH measurement systems by comparing the output to MANpH. This study confirms results from other laboratories (Dado and Allen, 1993; Graf et al., 2005) indicating that mean pH from manual sampling methods are higher than mean pH values obtained from

Table 1. Relationship between continuous ruminal pH measurement and manual ruminal pH measurement using two different continuous ruminal pH measurement systems (LRCpH and CIpH)[1]

Variable	LRCpH vs. MANpH		CIpH vs. MANpH
	1-min[2]	5-min[3]	
Number of data pairs	131	131	96
Mean pH (MANpH)	6.14	6.14	6.14
Variance	0.171	0.171	0.126
Mean pH (continuous pH system)	6.11	6.11	6.09
Variance	0.188	0.194	0.111
Correlation coefficient[4]	0.98	0.97	0.88
Concordance correlation	0.96	0.97	0.87
Location shift[5]	0.07	0.09	0.16
Scale shift[6]	0.95	0.93	1.04

[1]LRCpH = Lethbridge Research Centre ruminal pH measurement system; MANpH = manual pH measurement of rumen fluid samples obtained via a rumen cannula; and CIpH = continuous indwelling ruminal pH measurement system.

[2]LRCpH values were averaged over 1-min intervals corresponding to MANpH.

[3]LRCpH values were averaged over 5-min intervals corresponding to MANpH.

[4]Correlation coefficient accounting for repeated measures as described by Hamlett et al. (2004).

[5]Would equal 0 if y = x.

[6]Would equal 1 if y = x.

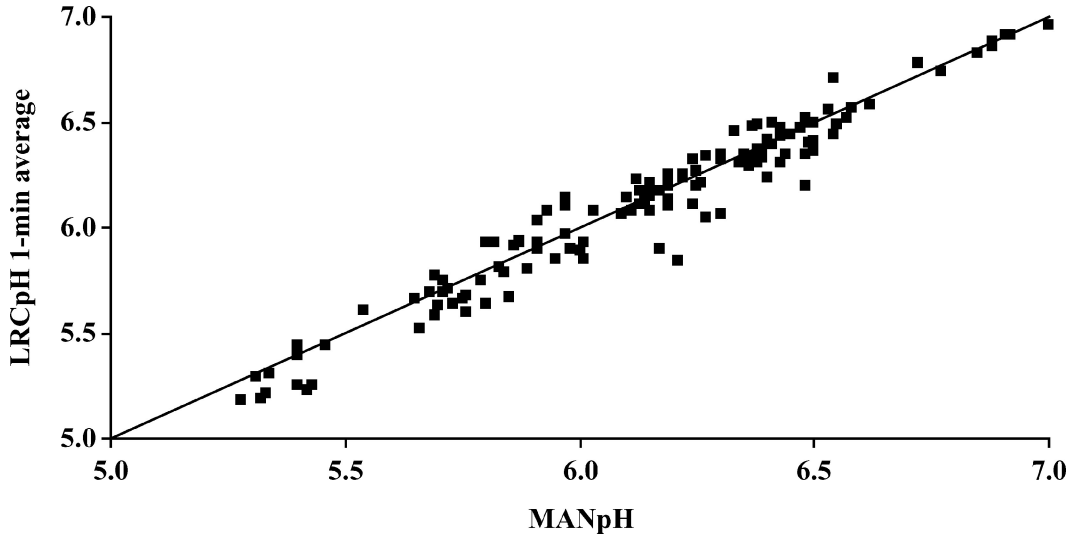

Figure 2. Relationship between ruminal pH determined using ruminal fluid obtained via a ruminal cannula (MANpH) and a submersible continuous ruminal pH measurement system (LRCpH) when output was averaged over 1-min intervals. The solid line represents the line y = x.

continuous pH systems although our mean differences were lower than previously reported values. However, caution should be used when evaluating mean ruminal pH differences between 2 systems because means indicate very little about the relationship between the 2 methods.

Averaging the LRCpH output over 1- or 5-min intervals did not affect mean pH (Table 1) and, consequently, the correlation coefficient that accounted for repeated measures and concordance correlation coefficients were similar between the LRCpH and MANpH when LRCpH output was averaged over 1 or 5 min. The LRCpH (1- and 5-min averages) correlation coefficients that accounted for repeated measures (0.98 and 0.97, respectively) were numerically higher than that calculated for the CIpH (0.88; Table 1). The concordance correlation coefficient was numerically higher for the LRCpH when compared with the CIpH. Dado and Allen (1993) previously reported a Pearson correlation coefficient of 0.85 for the relationship between a continuous indwelling ruminal pH measurement system and manual ruminal pH measurement. The Pearson correlation coefficient indicates how closely the results are related between 2 methods but does not indicate how the plotted data deviates from the line y = x (Lin, 1992). Thus, in our study, a correlation coefficient that accounted for repeated measures was used to replace the Pearson correlation coefficient and was also used to calculate the concordance correlation. The repeated measures correlation is used to evaluate precision because it determines the deviation of the data set from the best-fit linear line whereas the concordance correlation is used to evaluate accuracy by determining the deviation of the best-fit linear line to the line y = x.

To our knowledge, no other studies have reported the correlation coefficient, accounting for repeated measures, or the concordance correlation coefficient when evaluating the accuracy and precision of a continuous ruminal pH measurement system. The correlation coefficient results from this study suggest that the LRCpH more closely reflected the MANpH results than did the CIpH, thus indicating higher accuracy and precision. The improvement in accuracy and precision can be attributed to the design of the system and not the pH recording interval because the LRCpH had similar correlation coefficients and concordance correlation coefficients regardless of whether the data were averaged over 1- or 5-min intervals corresponding to ruminal fluid collection time for MANpH (Table 1).

The location shift for the relationship between LRCpH and MANpH (0.07 and 0.09 for the 1- and 5-min averages, respectively) was lower than that of CIpH and MANpH (0.16). The line y = x would have a location shift of zero and the location shift indicates how the y-intercept of the plotted data differs from the y-intercept of the line y = x. Thus, for both systems the y-intercept was less than zero indicating that MANpH results are slightly higher than results from the LRCpH or CIpH. The scale shift was similar for the relationship between the LRCpH and MANpH and between the CIpH and MANpH; however, the shift occurred in opposite directions. The scale shift indicates a discrepancy in slope between the plotted data and the line y = x. The plotted data for the LRCpH (1- and 5-min averages) vs.

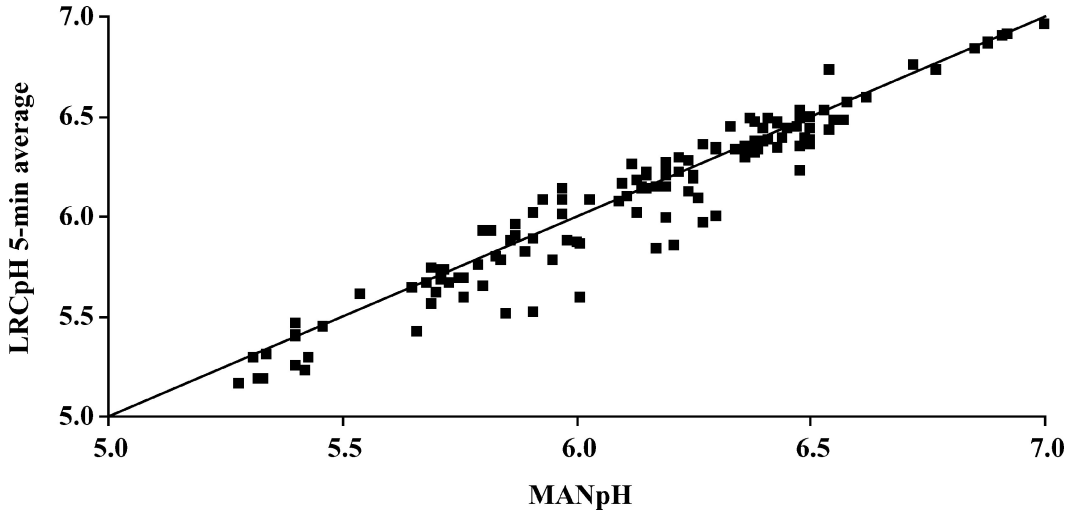

Figure 3. Relationship between ruminal pH determined using ruminal fluid obtained via a ruminal cannula (MANpH) and a submersible continuous ruminal pH measurement system (LRCpH) when output was averaged over 5-min intervals. The solid line represents the line y = x.

MANpH, and CIpH vs. MANpH appear in Figures 2, 3, and 4, respectively.

Other General Observations. It was noted during manual ruminal fluid collection that the LRCpH did not migrate within the rumen to the same extent as the CIpH likely because of the design elements; rigid PVC encapsulation, flexible cable fastening to the rumen cannula plug and heavier total weight (two 900-g weights + data logger + PVC capsule). Ruminal conditions can affect the accuracy and precision of the data collected from continuous pH measurement systems as the pH electrodes are designed for submersible application and require movement of liquid over the sensor for reliable measurement. We observed that accuracy and precision were numerically reduced when ruminal pH was measured in an animal observed to have relatively dry ruminal contents when compared with measurement in an animal observed to have relatively fluid ruminal contents. The correlation coefficient, which accounted for repeated measures, and concordance correlation coefficient were 0.75 and 0.74, respectively, in the animal with dry ruminal contents, and were 0.96

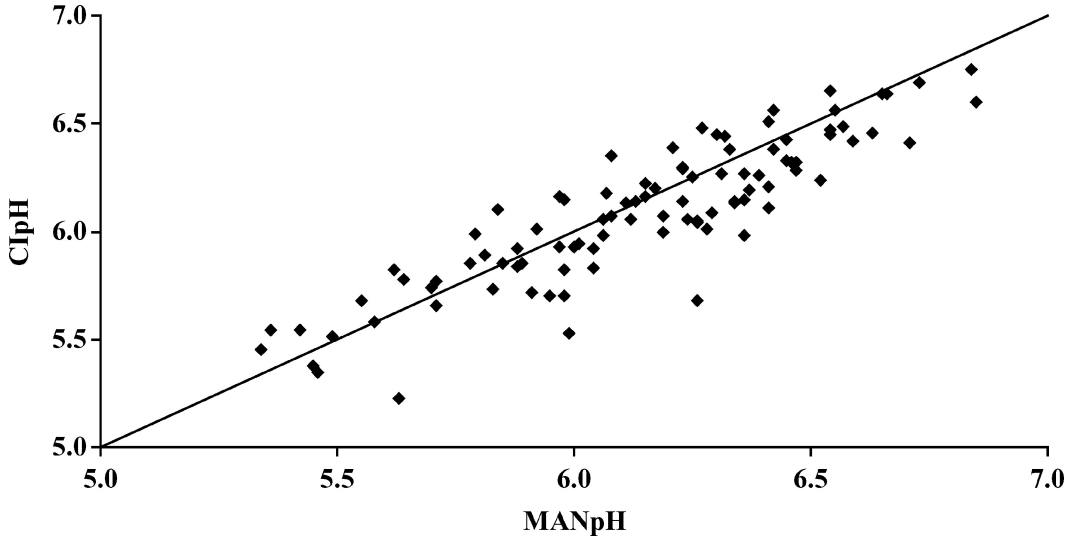

Figure 4. Relationship between ruminal pH determined using ruminal fluid obtained via a ruminal cannula (MANpH) and using a continuous indwelling pH measurement system (CIpH). The solid line represents the line y = x.

Table 2. Effect of electrode standardization frequency on electrode readings in standard buffers at pH 4 and 7

	Treatment[1]				
Variable	R24	R48	R72	SEM	*P*-value
Mean baseline reading difference in buffer 4, mV	2.70	4.38	4.67	4.365	0.9425
Difference from zero in buffer 4, *P*-value	0.55	0.33	0.30		
Mean baseline reading difference in buffer 7, mV	–8.40	4.90	3.78	5.827	0.2341
Difference from zero in buffer 7, *P*-value	0.17	0.41	0.53		

[1]R24, R48, and R72 represent electrodes maintained in the rumen for 24, 48, and 72 h, respectively.

and 0.96, respectively, in the animal with more fluid ruminal contents. This indicates that the system may function less well when used in animals with a lower proportion of ruminal fluid. However, results generated from the entire data set indicate that there were no significant differences in accuracy and precision among animals ($P = 0.12$) or time relative to feeding ($P = 0.25$).

Experiment 3: Frequency of Electrode Standardization

No electrode failure occurred in the current study. Mean baseline millivolt readings in pH 4 ($P = 0.94$) and 7 ($P = 0.23$) buffer solutions were not different for pH electrodes after 24, 48, or 72 h of ruminal incubation (Table 2). Furthermore, the changes in baseline millivolt readings for all treatments did not differ ($P > 0.05$) from zero (Table 2). However, electrode drift for individual probes did not always occur in the same direction for any of the treatment durations (data not shown). Previously, Nocek et al. (2002) noted a requirement for electrode standardization; however, that study only examined the effect of probe cleansing and standardization on pH readings after 4, 6, 8, 12, and 24 h in the rumen. Enemark et al. (2003) maintained electrodes in the reticulum for 8 d with minimal electrode drift; however, only 2 electrodes were used. Results from the current study suggest that new electrodes can be maintained in the rumen for at least 72 h without having a significant impact on millivolt readings. Using millivolt readings to calculate pH values indicated that the mean error that occurred by not recalibrating electrodes and not correcting data for changes in baseline millivolt readings within a 72-h period was 0.03 pH units. The maximum possible error found in this study between consecutive standardizations was 0.13, 0.18, and 0.10 pH units after 24, 48, and 72 h in the rumen, respectively, within a pH range of 4.5 to 7.0. Based on the results of this study, there is no requirement for daily removal and standardization of new electrodes; however, there may be a requirement for regular inspection of electrode function, because malfunction would result in a loss of data. Unlike the CIpH, the LRCpH does not have a visual display indicating millivolt or pH readings and electrode failure would not be diagnosed until the time of electrode standardization. More research is required to determine how electrode usage over time influences baseline millivolt reading stability between standardizations. It can be concluded that daily standardization of new electrodes is not required and the duration between consecutive standardizations could be extended to 72 h.

CONCLUSIONS

Indwelling systems for continually monitoring ruminal pH provide an accurate means of measuring changes in pH over time. A new submersible system called the LRCpH that can be used in feedlots, freestall dairies, or in grazing applications was developed and shown to be highly accurate and precise. Based on results of this study, new electrodes can be continuously maintained in the rumen for at least 72 h without adverse effects on measurement accuracy. The LRCpH provides an increased opportunity for researchers to accurately and precisely measure ruminal pH and the occurrence of ruminal acidosis in unrestrained cattle.

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