Simultaneous Estimation of the pH of Rumen and Reticulum Fluids of Cows Using a Radio-Transmission pH-Measurement System

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ABSTRACT. Circadian pH changes in the fluid of the rumen (bottom and middle) and reticulum were assessed simultaneously using wireless and wired radio-transmission pH-measurement systems in cows fed a control diet (C diet) or rumen-acidosis-inducing diet (RAI diet). The pH in the three sites decreased following the morning and evening feedings. In cows fed the C diet, the bottom-rumen and reticular pH reverted to the basal level by the next morning, while the middle-rumen pH did not recover completely, suggesting that active fermentation occurred in the middle of the rumen. The mean pH at 1 hr intervals was higher in the reticulum than at the bottom and in the middle of the rumen. The relatively stable reticular pH may result from dilution due to salivation. In cows fed the RAI diet, the bottom-rumen pH fell to approximately 5.2 after the evening feeding, but returned to the basal level by the next morning. In contrast, the middle-rumen pH did not return to the basal level (6.5) within 24 hr, presumably owing to continuous, vigorous fermentation. There were positive correlations between the pH at the bottom and in the middle of the rumen and at the bottom of the reticulum. These findings indicate that our radio-transmission pH-measurement system may be suitable tool for simultaneous measurement of pH in the rumen and reticulum fluid.

KEY WORDS: circadian change, dairy cows, pH measurement system, rumen and reticulum fluids, subacute ruminal acidosis.


NOTE Internal Medicine

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Subacute ruminal acidosis (SARA) affects the health and production of dairy herds [20], decreasing feed intake, milk production, and rumen digestion, while causing diarrhea and rumen mucosal damage [2, 14]. Moreover, SARA provokes various diseases and their preconditions, including laminitis, reduced and erratic feed intake, a low body condition score (BCS), low milk fat syndrome, abomasal displacement and ulceration, and rumenitis [8, 23].

SARA is characterized by the presence of an abnormally low rumen pH [7, 8, 24] of less than 5.5; a pH of 5.8 is the marginal threshold for SARA [9–11, 14, 21]. Repeated collections of rumen fluid are needed to measure pH, which can lead to peritonitis following rumenocentesis with a trocar and esophageal injuries induced by oro-ruminal probe suction. Moreover, the reliability of the rumen pH likely depend on the operator’s skill, the sampling site (bottom vs. middle of the rumen and rumen vs. reticulum), and sampling procedure (rumenocentesis vs. an oro-ruminal probe) [3, 6, 9, 17, 25]. An indwelling wire system has been used to measure the pH at the bottom of the rumen [2, 18, 19, 22, 23], but there are no reports on simultaneous pH measurements of fluids at the bottom and in the middle of the rumen and in the reticulum (i.e., three sites) of cows. The objective of this study was to assess the possible use of radio-transmission pH-measurement system for measurement of pH in the rumen (bottom and middle) and reticulum. First, we compared the circadian pH changes in the three sites for cows fed either a control diet (C diet) or a rumen-acidosis-inducing diet (RAI diet) using a wireless and wired systems. Then we examined the relationship between the pH in the rumen and reticulum in cows fed the C and RAI diets.

The experimental design was approved by the Iwate University Laboratory Animal Care and Use Committee. Four non-lactating, rumen-fistulated Holstein cows of first parity, weighing 620–720 kg and housed in a stanchion barn, were used in this study. The cows were offered orchardgrass and timothy mixed hay (dry matter [DM] basis; 8.8% crude protein [CP], 67% neutral detergent fiber [NDF], 14% non-fiber carbohydrates [NFC]) daily at 9:00 and 16:30, and had free access to fresh water. Then, for 2 weeks before commencing the experiment, the cows were fed a new diet consisting of orchardgrass and timothy mixed hay, and concentrate including equivalent amount of flaked barley and corn (Table 1). Each cow was then offered orchardgrass and timothy mixed hay (dry matter [DM] basis; 9:1 and 3:7, and the percentage of starch in the DM was adjusted to 7 and 37% (the level for inducing ruminal acidosis) for the C and RAI diets, respectively.

The wireless radio-transmission pH-measurement system consisted of a wireless pH sensor, data receiver, relay unit, and personal computer (PC) with special soft-
A lithium-ion battery (3.6 V, 1,700 mA·hr) was administered orally into the cow’s rumen using a catheter, radio frequency (RF) circuit, and battery. The device was housed in a bullet-shaped bolus, which weighs 184 g, 30 mm in diameter and 145 mm in length, and enclosed a pH-amplifier circuit, central processing unit (CPU) circuit, and battery. The device was administered orally into the cow’s rumen using a catheter, without surgery. A lithium-ion battery (3.6 V, 1,700 mA·hr) was used as the internal power source; this supplied sufficient current and power for steady operation over a long period.

The wired pH-measurement system consisted of a pH sensor, data receiver, and PC with special software. The wired pH sensor, which had the same configuration as the wireless pH sensor, was connected to the receiver. The measured pH value by the wired pH sensor was same as that by the wireless pH sensor in preliminary experiment, which used the same ruminal fluid.

The pH was measured separately at the bottom and in the middle of the rumen by the wireless and wired pH sensors, respectively. The pH sensor was calibrated at the start of each experiment with pH 4 and 7 buffer solutions. The wireless pH sensor was placed in the rear ventral sac at the bottom of the rumen and in the reticulum. The wired pH sensor was fixed 10–20 cm below the ruminal mat surface (middle of the rumen) by a stout wire through a rumen cannula via the fistula, because the middle-rumen pH cannot be measured without fixing the sensor in the ruminal mat. The pH of the three sites was recorded continuously (every 10 min) for 24 hr from 9:00 to 9:00 the next day. The locations of the pH sensors in the rumen and reticulum were confirmed every 12 hr by palpation through rumen cannula; they remained in location throughout the trial, and the pH sensor at the ventral sac of rumen was rolled on the ruminal mucosa.

Four cows were assigned randomly to a 2 × 2 Latin square for each feeding protocol. Two cows were fed the C diet and the other two cows were fed RAI diet for 2 weeks, followed by the opposite diet for 2 weeks after a 2-week interval. The rumen and reticulum pH were measured at the 14 days after commence of each feeding protocol.

The continuous rumen and reticular pH data were summarized for each hour, and the mean, minimum, and maximum pH, and the duration time of the pH was <5.8 and <5.5, were calculated for 24 hr in all cows. Quantitative data were expressed as the means ± standard error (SEM) of the cows fed C and RAI diet. One-way repeated measures analysis of variance (ANOVA) followed by Dunnett’s multiple comparison method were used to determine the significance of the difference in pH from that at the morning feeding (9:00). Student’s t-test was used to compare the difference in the circadian pH changes, the differences in the average, minimum, and maximum pH, and the duration time of the pH was <5.8 and <5.5 for 24 hr for the rumen and reticulum pH. Pearson’s correlation coefficient (r) was generated between the pH of the each three sites. P-values<0.05 were considered significant.

The bottom- and middle-rumen and reticular pH decreased gradually following the morning or evening feeding compared to the corresponding basal pH (taken at 9:00). After reaching the lowest values, the bottom-rumen and reticular pH increased gradually by the next morning, while the middle-rumen pH did not return to the basal level by the next morning. In cows fed the C diet, the mean pH at 1 hr intervals after the morning feeding decreased from 6.95 (9:00) to 6.45 (13:00) in the bottom of the rumen, from 6.87 (9:00) to 6.00 (1:00) in the middle of the rumen, and from 6.87 (9:00) to 6.80 (14:00) in the reticulum (Fig. 1). The mean pH in the middle and at the bottom of the rumen and in the reticulum of cows fed the C diet was significantly lower at 12:00–2:00, 11:00–21:00, and 12:00–21:00 (1:00), respectively, compared to that at the morning feeding. Furthermore, the mean pH was significantly (P<0.01) higher at the bottom of the rumen than in the middle of the rumen, and was significantly (P<0.01) lower than that in the reticulum at 9:00–8:00. The mean pH in the middle of the rumen was significantly (P<0.01) lower than that in the reticulum at 9:00–8:00.

In cows fed the RAI diet, the mean pH at 1 hr intervals after the morning feeding decreased from 6.50 (9:00) to 5.59 (23:00) in the middle of the rumen, 6.75 (9:00) to 5.23 (2:00) at the bottom of the rumen, and 7.26 (9:00) to 6.25 (22:00) in the reticulum (Fig. 1). The mean pH at the bottom and in the middle of the rumen and in the reticulum of cows fed the C diet was significantly lower at 15:00–21:00, 18:00–2:00, and 15:00–23:00, respectively, compared to that at the morning feeding. The mean pH was significantly (P<0.01) higher at the bottom of the rumen than in the middle of the rumen, and was significantly (P<0.01) lower than that in the reticulum at 9:00–8:00. The mean pH in the middle of the rumen was significantly (P<0.01) lower than that in the reticulum at 9:00–8:00.

The continuous rumen and reticular pH data were summarized for each hour, and the mean, minimum, and maximum pH, and the duration time of the pH was <5.8 and <5.5, were calculated for 24 hr in all cows. Quantitative data were expressed as the means ± standard error (SEM) of the cows fed C and RAI diet. One-way repeated measures analysis of variance (ANOVA) followed by Dunnett’s multiple comparison method were used to determine the significance of the difference in pH from that at the morning feeding (9:00). Student’s t-test was used to compare the difference in the circadian pH changes, the differences in the average, minimum, and maximum pH, and the duration time of the pH was <5.8 and <5.5 for 24 hr for the rumen and reticulum pH. Pearson’s correlation coefficient (r) was generated between the pH of the each three sites. P-values<0.05 were considered significant.

The bottom- and middle-rumen and reticular pH decreased gradually following the morning or evening feeding compared to the corresponding basal pH (taken at 9:00). After reaching the lowest values, the bottom-rumen and reticular pH increased gradually by the next morning, while the middle-rumen pH did not return to the basal level by the next morning. In cows fed the C diet, the mean pH at 1 hr intervals after the morning feeding decreased from 6.95 (9:00) to 6.45 (13:00) in the bottom of the rumen, from 6.87 (9:00) to 6.00 (1:00) in the middle of the rumen, and from 6.87 (9:00) to 6.80 (14:00) in the reticulum (Fig. 1). The mean pH in the middle and at the bottom of the rumen and in the reticulum of cows fed the C diet was significantly lower at 12:00–2:00, 11:00–21:00, and 12:00–21:00 (1:00), respectively, compared to that at the morning feeding. Furthermore, the mean pH was significantly (P<0.01) higher at the bottom of the rumen than in the middle of the rumen, and was significantly (P<0.01) lower than that in the reticulum at 9:00–8:00. The mean pH in the middle of the rumen was significantly (P<0.01) lower than that in the reticulum at 9:00–8:00.

The 24-hr mean and minimum pH values at the bottom
of the rumen and in the reticulum of cows fed the C diet were significantly (P<0.01 and P<0.05 in 24-hr mean, P<0.01 in 24-hr minimum) higher than those in the middle of the rumen (Fig. 2). Similarly, the 24-hr mean and maximum pH values in the reticulum of cows fed the RAI diet, which were significantly (P<0.01) lower than those of cows fed the C diet, were significantly (P<0.01, P<0.05) higher than those in the middle of the rumen. There was no significant difference between the cows fed C and RAI diet regarding the duration time of the pH was <5.8 (555, 278, and 5 min in the middle and at the bottom of the rumen and in the reticulum of cows fed the RAI diet, respectively) and <5.5 (238, 200, and 0 min in the middle and at the bottom of the rumen and in the reticulum of cows fed the RAI diet, respectively). Furthermore, significant (P<0.01) positive correlations were observed in the pH of cows fed the C diet between in the middle and at the bottom of the rumen (r=0.409) and at the bottom of the rumen and in the reticulum (r=0.826; Table 2). Similarly, significant (P<0.01) positive correlations were observed in the pH of cows fed the RAI diet between in the middle and at the bottom of the rumen (r=0.894), in the middle of the rumen and in the reticulum (r=0.764), and at the bottom of the rumen and in the reticulum (r=0.797).

The pH of rumen samples taken immediately after the morning feeding was significantly higher than that of samples taken in the afternoon without feeding [6]. Furthermore, the rumen pH was lowest when the samples were collected 2–5 hr after feeding a concentrate in component feeding [19, 20]. In the present study, the pH at the bottom and in the middle of the rumen of cows fed C diet decreased following the morning feeding. Compared to the pH at the morning feeding (9:00), the mean pH in the middle of the rumen was significantly lower at 12:00–2:00 (cows fed the C diet) and 18:00–0:00 (cows fed the RAI diet). The changes in the rumen pH after the morning feeding observed in the present study were consistent with those of previous studies [13, 16]. The lower pH in the middle of the rumen of cows fed the C diet, and at the bottom and in the middle of the rumen of cows fed the RAI diet may have persisted long after the morning feeding, and the pH may have decreased further after the evening feeding, as described previously [13, 16].

The concentrations of bicarbonate, potassium, and chloride in the rumen significantly contributed to the rumen pH [6]. When the levels of these compounds were considered, sampling time had no effect on the rumen pH [6], suggesting that the diurnal pH changes were due, at least in part, to salivation and the presence of strong ions in the rumen. In the SARA model, lactic acid is not considered a cause of pH reduction in cows [13, 21]. Most cows diagnosed with SARA have normal ruminal lactate concentrations, indicating that an elevated total volatile fatty acid (VFA) concentration is one of the main causes of low rumen pH in cows with SARA [21]. However, we did not examine the concentrations of bicarbonate, potassium, chloride, lactic acid, or VFA in the rumen fluid. Therefore, the factors related to the circadian pH changes in the rumen could not be determined.

The rumen pH varies considerably with the site where the sample is collected [6, 25]. Tafaj et al. [25] collected digesta samples from three layers of the rumen digesta; the top and middle were from 5–10 and 25–35 cm below the ruminal mat surface, respectively, and the bottom sample was 5–10 cm above the rumen bottom. They reported that the higher concentration of VFA and lower pH in the dorsal rumen reflected higher fermentation activity in the solid digesta. In our study, the middle- and bottom-rumen sites corresponded to the middle and bottom regions described in Tafaj et al. [25], and the mean pH in the middle of the rumen was significantly lower than that at the bottom of the rumen of cows fed the C and RAI diets. Significant positive correlations were also observed in the pH in the middle and at the bottom of the rumen. It is not known why the middle of the rumen had a lower pH, but it may be a site of active fermentation, and may have a higher concentration of VFA [6, 25]. Furthermore, the extremely low pH (at the bottom and in the middle of the rumen) of cows fed the RAI diet may be related to increased VFA concentrations in their rumens [21].

Few reports have examined the pH of reticulum fluid [4, 17]. Lane et al. [17] found the highest pH in the reticulum and the lowest pH at the top or middle of the rumen. These differences were still apparent 3 hr after feeding, although
the pH had decreased [17]. In the present study, the reticular pH decreased following the morning feeding, and increased gradually by the next morning feeding, similar to the change in the rumen. The 24-hr mean, minimum, and maximum pH values were significantly higher in the reticulum than in the rumen, as described previously [17]. Hummel et al. [12] found similar liquid contents in the ventral rumen and reticulum. Stratification of the reticulorumen contents likely indicates further spread of the ruminal mat into the ventral rumen [1, 12, 15]. These reports suggest that the pH patterns in the rumen and reticulum are similar. We observed a significant positive correlation between rumen and reticulum pH values, and the pH was lower in the middle and at the bottom of the rumen than in the reticulum. The higher pH in the reticulum may be caused by the dilution of fluid by salivation and drinking water [5, 6].

Throughout the present study, it was indicated that our radio-transmission pH-measurement system may be suitable tool for simultaneous measurement of pH in the rumen and reticulum fluid. Further studies are needed to elucidate the differences between the pH in the rumen and reticulum, and the characteristics of circadian pH changes in the rumen and reticulum.

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![Fig. 2. Comparison of the pH of fluid at the bottom and in the middle of the rumen and in the reticulum of cows fed the C diet (A) or RAI diet (B). The mean (24-hr Mean), minimum (24-hr Min), and maximum (24-hr Max) pH values are represented as the means ± SEM (n=4). a: P<0.05; b: P<0.01 (significant difference compared to the corresponding middle-rumen pH).](image-url)


