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A New Device for Measuring Kinetics of Ruminal pH and Redox Potential in Dairy Cattle

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ABSTRACT

A sampling and measuring device was set up to measure continuously the pH and the redox potential (E_h) of ruminal content in absence of any gaseous contamination (method 1). It was compared with a conventional suction device in which no precaution was taken to prevent air from coming into contact with the surface of collected samples (method 2). Two fistulated dry cows were used and fed a total mixed ration. Redox potential and pH measurements were performed repeatedly on collected samples using these 2 methods during a 9-h period; each period started 1 h before feeding. The partial pressure of oxygen (log $f(O_2)$) was calculated from Nernst's equation using pH and E_h values. Results indicated that pH, E_h, and log $f(O_2)$ were affected by sampling method. In method 1, pH values ranged from 6.7 to 6.37 and E_h from –173.5 to –216.8 mV. In method 2, pH and E_h values varied, respectively, from 6.93 to 6.49 and from –111.3 to –139.5 mV. The partial pressure of oxygen was 10^6 times lower in samples that were continuously collected than in hand-samples. As a result, method 1 could make accurate measurements of pH and E_h of ruminal content.
(**Key words:** redox potential, method of measurement, rumen, dairy cow)

Abbreviation key: $\mathbf{E_h}$ = redox potential, $\mathbf{\log f\,(O_2)}$ = partial pressure of oxygen.

INTRODUCTION

Measurements of pH and redox potential ($\mathbf{E_h}$) in rumen content can give a basis for the understanding of the microbiological activity and dynamics of fermentation (Broberg, 1957a). These measures were usually performed by potentiometry on rumen fluid samples collected by an oral probe or by percutaneous puncture of the caudo-ventral ruminal sac or from a ruminally cannulated animal using a suction-strainer device (Duffield et al., 2004). In some cases, measurements were performed directly in the rumen through a well-closed rumen fistula (Broberg, 1958; Müller and Kirchner, 1969; Barry et al., 1977).

In normal conditions, the ruminal milieu is anaerobic with a redox potential markedly negative, reflecting the absence of oxygen and a strong reducing power. Nevertheless, the interpretation of E_h values reported in the literature is confusing because the redox potential is not always expressed in uniform terms. Some authors expressed E_h as a potential difference (E) between a platinum electrode and a reference electrode, i.e., calomel or silver:silver chloride. Under these conditions, "E_h" of rumen medium varied from –302 to –340 mV in sheep (Mathieu et al., 1996), from –335 to –370 mV in wether (Broudiscou et al., 2001), and from –327 to –352 mV in goat (Andrade et al., 2002). However, by definition, the redox potential is the potential difference between a platinum electrode and a standard hydrogen electrode (The International Hydrogen Zero). Because, in common practice, the hydrogen electrode is not used, all records must be corrected for $E_h = E_0 + C$, where E_0 is the potential of the platinum electrode, and C is the potential of the reference electrode used relative to the standard hydrogen electrode (Kjaergaard, 1977; Sauer and Teather, 1987). After this correction, E_h values of ruminal fluid were much higher: –150 to –260 mV on average in sheep (Broberg, 1957b; Barry et al., 1977) and between –145 and –190 mV in goat (Marounek et al., 1982). Thus, the discrepancy between reported values can most likely be attributed to the fact that Mathieu et al. (1996), Broudiscou et al. (2001), and Andrade et al. (2002) did not correct the raw E_h data.

However, sampling and measuring techniques that alter the strict conditions of the ruminal milieu can also lead to sources of considerable error. The chemical composition of the ruminal liquid phase is in equilibrium with the gas mixture in the rumen headspace, composed approximately of 52 to 63% CO_2, 27% CH_4, 7 to 18% N_2, with traces of H_2 and H_2S (Barry et al.,

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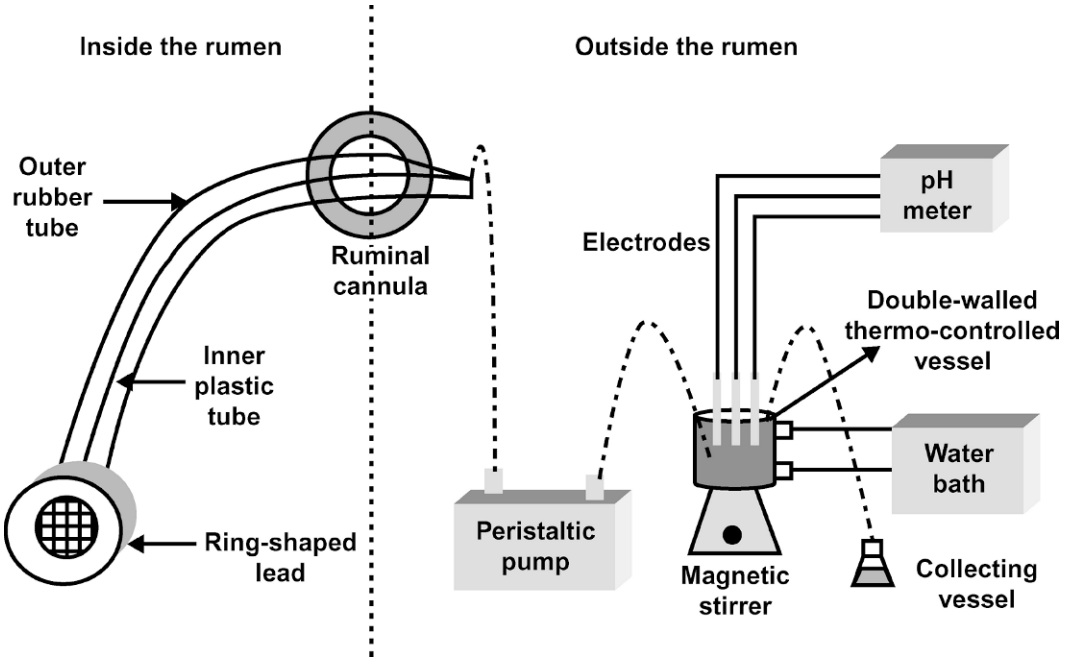

Figure 1. Method of continuous sampling and measurements with elimination of gaseous contamination.

1977; Silley, 2000). If the rumen liquor is in contact with air, O_2, CO_2, or another gaseous atmosphere different from the ruminal gas mixture, then a new equilibrium will be established that can modify the original ruminal physico-chemical characteristics (Nordstrom and Wilde, 1998).

Therefore, the aims of this work were 1) to test a new method (method 1) of measuring ruminal pH and E_h in absence of any oxygen contamination, by continuous pumping of ruminal fluid and 2) to compare this method to a conventional approach (method 2) which consisted of a manual suction-strainer device that pumped out ruminal fluid from a cannulated animal to measure pH and E_h of collected hand-samples in contact with atmospheric air.

MATERIALS AND METHODS

Sampling Devices

In method 1, the sampling device (Figure 1) was adapted from Corley et al. (1999). One part, inside the rumen, consisted of a ring-shaped lead covered on both sides with a sieve cloth of pore size 46 —m, one outer rubber tube (L = 650 mm; o.d. = 18 mm; i.d. = 6 mm), one inner plastic tube (L = 750 mm; o.d. = 2 mm; i.d. = 1.5 mm), and a perforated ruminal cannula cover. The other part, outside the rumen, was composed of a peristaltic pump (Gilson, Minipuls 2, Viliers Le Bel, France) and pumping tygon tubes (Bioblock, Illkirch, France). The rubber tube was fused on the outside of the lead ring, and the plastic tube was connected directly to the inner part of the ring where suction of rumen fluid took place. The outer tube strengthened the small inner plastic tube during rumen contractions, and the weight of the lead ring (1.1 kg) maintained the filter in the middle-ventral region of the rumen. To immobilize the rubber tube outside the rumen, a perforated cannula cover of the same diameter was used. The emerging plastic tube was then connected to the peristaltic pump. Fluid that passively entered the filter was removed continuously, traveled through the plastic tube into a 50-mL double-walled thermocontrolled vessel via the peristaltic pump. The animals rapidly became accustomed to the instrument and ate, ruminated, and stayed completely undisturbed so that measurements could be taken after introduction of the device.

In method 2, ruminal fluid samples were collected at 1-h intervals from 1 h before feeding to 8 h after feeding, using a water suction pump which consisted of a polyvinyl chloride tube (L = 700 mm; o.d. = 22 mm; i.d. = 17 mm), a polyvinyl chloride flask, and a vacuum pump. A composite sample of the middle-ventral region of the rumen was made. Immediately after collection, all samples were filtered through a metal sieve (1-mm mesh).

Measuring Device

The pH and E_h measurements were made with 3 electrodes connected to a digital pH meter (Metrohm

model 713 CH-9101, Herisau, Switzerland): a glass pH electrode (combined electrode with diaphragm DG SC), an E_h platinum electrode (Pt SC with Ag:AgCl as reference), and a platinum thermo-electrode (Pt 100 RNEA911 – Pt100). Electrodes were dipped in the collected rumen fluid to a depth of 4 cm. To obtain accurate values, the measuring device in method 1 was an airtight flowthrough system. In method 2, the collecting vessel containing hand-samples of ruminal fluid and electrodes used was in contact with air, and measurements were performed after a 25-min stabilization period as recommended by Andrade et al. (2002).

In both methods, the vessel was maintained at 39°C in a water bath (Exatherm U3 Electronic, Julabo, Germany). The vessel was wrapped in aluminum paper to prevent any measure fluctuations due to light intensity and placed on a magnetic stirrer (728 Stirrer, Metrohm, Switzerland) to establish equilibrium between the electrodes and ruminal fluid. After each record, the electrodes were rinsed with distilled water, dipped in NaOH (1 *N*), and washed again with distilled water.

Experimental Procedure

Two nonlactating Holstein cows, fitted with permanent ruminal cannulas, were used in a 2 × 2 Latin Square design. For each method, 2 kinetics of pH and E_h were established per cow and per period. The cows were fed (8 kg of DM/cow per day) with TMR offered twice daily (0900 and 1700 h). The TMR consisted of 43.2% corn silage, 35.5% hay (orchardgrass and fescue), and 21.3% concentrate mixture of ground corn, soybean meal, and minerals, on a DM basis.

Calculations

Because in the actual measurements, the reference electrode was not a hydrogen electrode, all records of the potential difference were corrected using the formula:

$$\mathbf{E_h = E_0 + C}$$

where E_0 is the potential of the platinum electrode, and C is the potential of the reference electrode relative to the standard hydrogen electrode (i.e., +199 mV at 39°C) (Nordstrom, 1977).

Oxygen partial pressure, also known as oxygen fugacity [$\mathbf{\log f(O_2)}$] was calculated from Nernst's equation (Valsaraj, 2000) with E_h and pH values:

$$\mathbf{\log f(O_2) = 64.59\ E_h + 4\ pH - 78.60\ (at\ 39°C).}$$

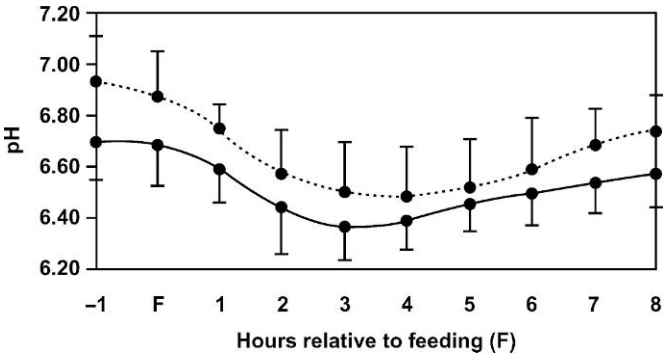

Figure 2. Dynamics of the pH in rumen fluid with 2 sampling methods: hand-sampling (– – –) and continuous sampling (——).

Statistical Analyses

The data were processed with the repeated measures ANOVA procedure of SYSTAT (Version 5.03 for Windows, SYSTAT Inc., Evanston, IL), which takes into account the nonindependence of the measurements between times of sampling. The statistical model was as follows:

$$Y_{ijk} \text{ for sampling hour 0 to 9} = \mu + M_i + C_j + P_k + e_{ijk}$$

where Y is the dependent variable, μ is the overall mean, M_i is the mean effect of sampling method i, C_j is the mean effect of cow j, P_k is the mean effect of period k, and e_{ijk} is random residual. All tests were carried out with the level of significance at 5%.

RESULTS AND DISCUSSION

The main objective of this study was to demonstrate how values of pH and E_h vary with time when exposed to air. A sampling and measuring system was set up allowing these measures to be taken without air contamination. The results (Figure 2) showed that rumen pH varied from 6.7 to 6.37, and from 6.93 to 6.49 using methods 1 and 2, respectively. With both methods, rumen pH dropped rapidly during the first hours following feeding, reached a minimum 3 h after the meal, and then increased progressively. Mathieu et al. (1996) and Duffield et al. (2004) obtained similar curves. A significant difference was found between the 2 methods, and values obtained in method 2 were higher ($P = 0.034$) than in method 1. Table 1 showed that the difference between the methods was not systematic during the entire period of measurement. Moreover, no correlation (R = 0.08; n = 80) was found to exist between the methods and consequently, no correction could be made on samples exposed to air. This difference in pH could be attributed to the exposure of ruminal fluid to air. As

Table 1. Mean values and pH range between methods of sampling.[1]

	Method 1		Method 2
Mean	6.52		6.67
SE	0.11		0.15
Difference between initial values		0.23	
Difference between nadir values		0.12	
Difference between final values		0.17	

[1]Method 1 = continuous pumping of ruminal fluid; method 2 = hand-sampling.

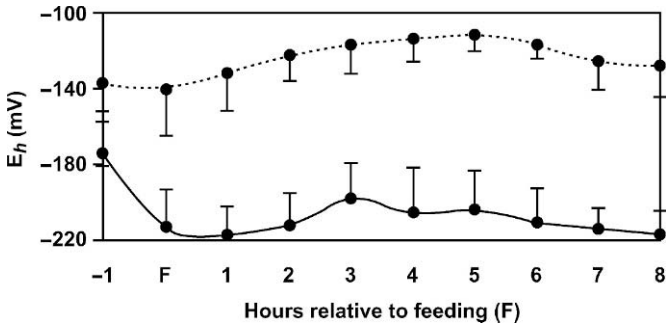

Figure 3. Dynamics of the redox potential (E_h) in rumen fluid with 2 sampling methods: hand-sampling (– – –) and continuous sampling (——).

reported by Turner and Hodgetts (1954), leaving samples in contact with air permits spontaneous losses of gases, particularly CO_2, from the medium. The participation of CO_2 in the fluid is determinant because when dissolved in aqueous medium, CO_2 produces carbonic acid buffer (H_2CO_3), responsible for the establishment of the correct pH. The equation below illustrates the equilibrium between dissolved CO_2 and amount of H^+ ions via the production of carbonic acid.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^-$$

Variations in the amount of CO_2 dissolved in an aqueous medium will automatically change the amount of H^+ ions. For example, when CO_2 is added, the equilibrium shifts to the right leading to the production of carbonic acid and ultimately, release of H^+ ions. On the other hand, when CO_2 is allowed to escape, the reverse reaction occurs, i.e., production of H^+ ions decreases and pH rises. This explanation confirms that of Kohn and Dunlap (1998), who demonstrated the strong relationship between the partial pressure of CO_2 and the pH by the Hendersen–Hasselbach equation:

$$pH_{rumen} = 7.74 + \log ([HCO_3^-] / pCO_2)$$

As the ruminal pH is dependent upon the partial pressure of carbon dioxide, vigorous precautions must be taken to maintain the equilibrium between the ruminal gaseous mixture and the ruminal liquid phase so that correct pH measurements can be taken.

In the present study, method 1 provided all the necessary precautions to prevent any gaseous contamination from contacting the sample thereby maintaining the latter in its natural gaseous environment.

A highly significant difference ($P = 0.001$) was found when considering the effect of sampling and measuring techniques on rumen redox potential values. The E_h measured in rumen contents by method 1 was lower than that observed in method 2 and moved toward more reducing values over time (Figure 3). With method 1, E_h ranged from –173.5 to –216.8 mV. It increased 3 h after feeding and then slowly declined until the end of the measurement period. Marounek et al. (1982), Mathieu et al. (1996), and Andrade et al. (2002) obtained similar curves. The slow increase in E_h after the meal would be mainly due to the supply of oxygen directed toward the rumen during feed intake, mastication, and water intake. The subsequent decline in E_h could be explained by the rapid uptake of O_2 by microorganisms (Broberg, 1957b) to maintain anaerobic conditions of the rumen. These assumptions were confirmed by Barry et al. (1977), who found higher concentrations of N_2 and O_2 in the gas mixture of rumen headspace during feeding than between consecutive meals.

In method 2, E_h did not decrease after feeding and at no time dropped below –140 mV. Compared with method 1, the range of E_h values was smaller, from –111.3 to –139.5 mV. High oxidizing E_h values were observed by Broberg (1957c), who showed that bubbling air through the rumen contents resulted in an elevation of the E_h, whose magnitude was directly proportional to the amount of air. The difference in E_h measurements between the methods used in our study agrees with the results of Broberg (1957b), who measured E_h in samples freely exposed to air and in samples taken and kept under CO_2. It was performed to establish the effect of oxygen in air upon the E_h. He showed that in presence of atmospheric oxygen, a continuous change in the E_h toward more positive values took place. On the contrary, in an atmosphere of CO_2, no change in E_h could be observed and values were less than in samples exposed to air. However, as proved earlier, CO_2 will modify the pH of the medium by shifting the equilibrium to form more H^+ ions. Consequently, a change in E_h would be also observed.

Therefore, we first concluded from our experiment that atmospheric oxygen was responsible for this change in E_h curve and that the absence of E_h fluctuations observed with method 2 was probably influenced by large amounts of O_2, which smoothed sampling time

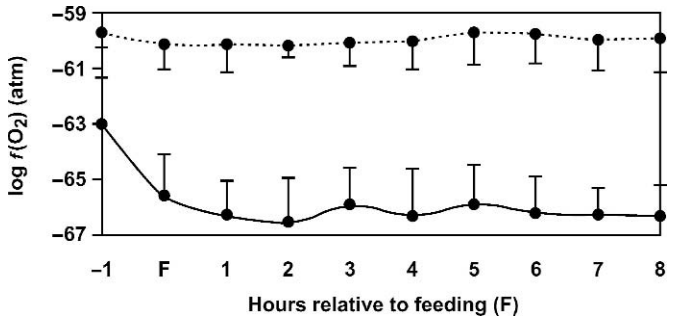

Figure 4. Dynamics of the log $f(O_2)$ (atm) in rumen fluid with 2 sampling methods: hand-sampling (– – –) and continuous sampling (——).

dependent variations as shown by the significant time–method interaction ($P = 0.0005$). To verify the presence of oxygen, its fugacity or partial pressure was calculated [log $f(O_2)$] from Nernst's equation. The results (Figure 4) showed that different log $f(O_2)$ curves were obtained ($P = 0.0012$) depending on whether the rumen samples were allowed to stand with the surface exposed to atmospheric air or were protected against gaseous contamination. The log $f(O_2)$ which resulted before feeding remained unchanged for several hours postfeeding. When rumen fluid was continuously sampled, an immediate change toward lower values was observed. During the whole measurement period, the average log $f(O_2)$ were 10^{-66} and 10^{-60} atm for methods 1 and 2, respectively. Thus, the lower values of log $f(O_2)$ obtained with method 1 showed that the environment of collecting samples remains strictly anaerobic, corresponding to the natural environment of the rumen.

CONCLUSIONS

The metabolic activity of ruminal anaerobic bacteria depends largely upon the main physico-chemical parameters, pH and redox potential. If these are not correctly measured using the appropriate methodology, they can be a source of considerable error. Only measures made under strict anaerobic conditions, such as those in method 1, would permit accurate diagnosis of metabolic disorders.

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