Stability Evaluation of Reference and Indicating Electrodes of pH Sensor during Monitoring of Cow's Rumen

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Drift has been a problem for rumen pH sensors, but it is not known whether the reference electrode or the indicating electrode of the pH sensor is the main cause of the drift. In this study, the drift of each electrode was evaluated by placing pH sensors in a cow's rumen and temporarily removing them at intervals over a four-month period. Our experiment revealed that the drift of the indicating electrode caused by changes in sensitivity is the critical drift issue in realizing a stable rumen pH sensor, although the drift is caused by both the reference electrode and the indicating electrode. The sensitivity of the indicating electrode was around 57.0 mV/pH unit at the start of the experiment but dropped to 37.1 and 49.0 mV/pH unit for Cow1 and Cow2, respectively, after placing the pH sensors in the rumen for four months. By assuming a change in the rumen's pH of around 2 pH units, the maximum drift caused by the indicating electrode appears to be 0.68 pH unit. From the observation of the indicating electrode, we found that the drift was caused by impurities on the glass surfaces, and the pH sensitivity was recovered by removing these impurities. Because the glass electrode cannot be cleaned when in the cow's rumen, the development of a glass electrode with resistance to impurities is required for rumen pH sensors.

1. Introduction

In recent years, the health monitoring of ruminants such as cows and sheep has become increasingly important.[1–6] In particular, pH monitoring of the cow's rumen has attracted attention because of the strong relationship between the pH and the deadly disease of rumen acidosis.[7–9] Giving cows rapidly digestible carbohydrates, which is effective for the stable production of rich milk and marbled beef, can cause rumen acidosis. The pH of a cow's rumen

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decreases from approximately 7.0 under normal conditions to below 5.5 during rumen acidosis.[10] To prevent rumen acidosis, continuous measurement of the pH of a cow's rumen is important.

In recent years, wireless rumen pH sensors have been developed to make it easier to measure the pH of a cow's rumen continuously.[11–18] For example, a wireless rumen pH sensor using a glass electrode was first developed in 2008.[12] Also, an orally administered rumen sensor was developed in 2012.[13,14] Furthermore, the development of orally administered pH sensors using semiconductor sensors is also in progress.[15–18]

On the other hand, it has been reported that the characteristics of rumen pH sensors change during rumen measurements, causing measurement errors. This is mainly due to the drift of the rumen pH sensor. For example, a drift of 0.03 pH unit at 72 h and a maximum drift of 0.18 pH unit have been reported.[19] In other cases, a drift of 0.20 pH unit after 14 days,[20] a drift of 0.03 pH unit per week,[21] and a drift of 0.5 pH unit after 34 days[22] have been reported. Such drift results in measurement errors that cannot be corrected because of the difficulty of calibrating sensors placed inside a cow's body.

A pH sensor usually has two electrodes: a reference electrode and an indicating electrode. The reference electrode always shows a constant potential, whereas the potential of the indicating electrode changes according to the pH of the target. By measuring the potential between the reference electrode and indicating electrode, the pH of the target can be measured. Although many types of reference electrodes, such as the reference electrode field-effect transistor (REFET)[23,24] and solid-state Ag/AgCl reference electrode,[25,26] have been studied, a conventional Ag/AgCl reference electrode with saturated potassium chloride (KCl) aqueous solution has been found to be the most stable and accurate[23,27] and is widely used.[28,29] Typically, a saturated KCl aqueous solution with a concentration of 3.3 M is used. However, chloride ions inside the reference electrode are consumed during the measurement, and if there is a shortage of chloride ions during the measurement, the measured pH value will not be constant. The operation of the reference electrode may also be unstable when the contents of the rumen flow into the internal fluid. For the indicating electrode, various types of electrodes, such as a glass electrode,[30] metal oxide electrode,[31] and polymer-based electrode,[32] have been studied, and the glass electrode is the most stable and commonly used. In the case of a glass electrode, a potential difference is generated by the pH difference between the target solution and the solution stored inside the glass, and the pH is measured on the basis of this potential difference. However, there is a possibility of drift due to the deterioration of the glass and other factors.

Both the reference electrode and indicating electrode of a pH sensor may cause drift. However, despite the above-mentioned reports of the amount of drift in previous studies on rumen pH sensors, it was not determined whether the drift was caused by the reference electrode or the indicating electrode. To develop more stable rumen pH sensors, it is important to clarify which electrode causes the drift.

In this study, pH sensors were placed in the rumen of fistulated cows and were temporarily removed after 1 week, 2 weeks, 1 month, and 4 months to individually evaluate the changes in the characteristics of the reference electrode and indicating electrode. The effect of each reference electrode and indicating electrode on the drift was thus estimated.

2.   Materials and Methods

The measurement principle of the pH sensor is shown in Fig. 1. Both the reference electrode and the indicating electrode are placed in the solution to be measured. The reference electrode should always show a constant potential, whereas the potential of the indicating electrode changes according to the pH of the target because of its sensitivity to the pH. By measuring the potential between the two electrodes, the pH of the target can be calculated.

The experimental flow of this study is shown in Fig. 2, and one of the devices used in this study is shown in Fig. 3. The devices were chained to the fistula for easy pickup and placed in the rumen of fistulated cows. The devices were temporarily removed after 1 week (7 days), 2 weeks (14 days), 1 month (28 days), and 4 months (119 days) to evaluate their characteristics.
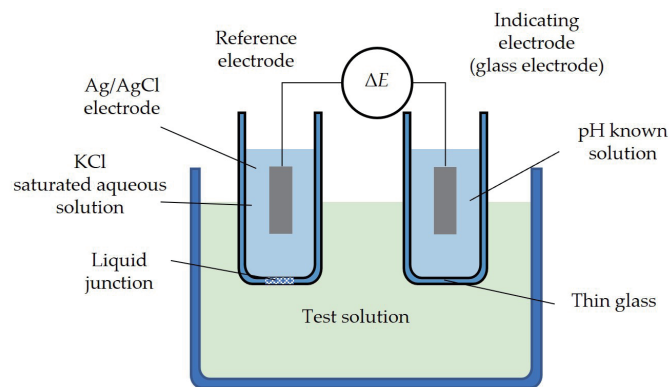

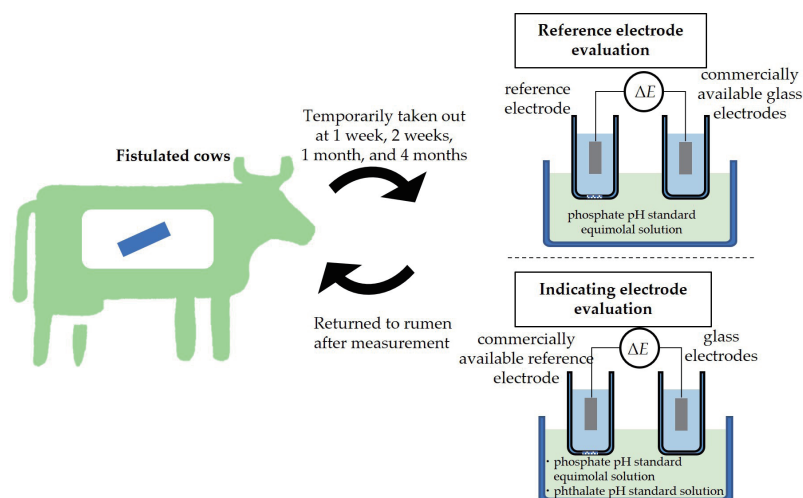Fig. 1.   (Color online) Measurement principle of the pH sensor.

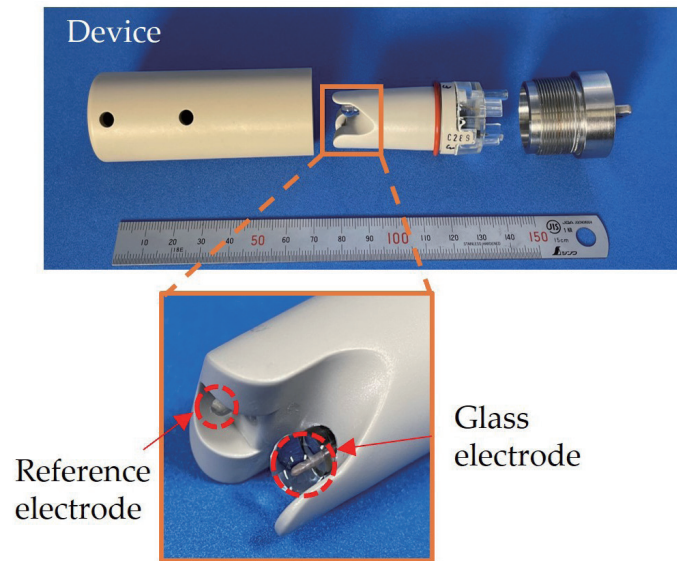Fig. 2.   (Color online) Experimental flow of this study.

Fig. 3.   (Color online) Optical image of the device used in this study.

Two identical devices and two fistulated cows were used, and the same measurements were performed for each cow. A reference sample not placed in rumen fluid was also prepared for comparison. Note that the devices removed from the rumen were rinsed several times in water before each measurement. The handling of the animals used in this study was approved by the Institutional Care and Use Committee for Laboratory Animals of the National Institute of Animal Health (Protocol No. 2020-053).

The device was prepared on the basis of previous research by Andersson *et al.*,[18] and unnecessary parts such as circuits were removed. A PHSENSOR03DJ pH sensor (Eutech Instruments Pte Ltd., Singapore) was embedded in the device. The pH sensor has a common glass indicating electrode of about 6 mm diameter and a Ag/AgCl reference electrode with double junctions.

For the evaluation of the reference electrode, the commercially available glass electrode that was not placed in the rumen was used as an indicating electrode. The reference electrodes removed from the rumen and the indicating electrode were dipped in a phosphate pH standard equimolal solution (DKK-TOA Corp., Japan) whose pH was 6.86 at 25 °C, and the potential between the electrodes was measured with a digital multimeter (DMM7510, Keithley Instruments Inc., USA) through a voltage follower. The potential of the Ag/AgCl electrode E depends on the chloride ion concentration as follows:

$$E = E^\circ - \frac{RT}{F} \ln a_{Cl^-}, \tag{1}$$

where E°, R, T, F, and a_{Cl^-} are the standard electrode potential, gas constant, temperature, Faraday constant, and chloride ion activity, respectively. During measurement, the internal

liquid is released outside through the liquid junction, and the amount of chloride ions decreases with the exchange of liquids and ions. Furthermore, the amount of chloride ions may decrease with the inflow of rumen fluid. If there is a change in the chloride ion concentration during the measurement, the measured pH value will not be constant, i.e., drift will occur. In this study, the effect of rumen fluid mixing with the internal solution was also evaluated by adding rumen fluid to the internal solution and measuring the potential difference from the initial condition.

To evaluate the indicating electrode, the potential compared with that of a reference electrode (RE-1CP, BAS Inc., Japan) not placed in the rumen was measured in a phosphate pH standard equimolal solution and in a phthalate pH standard solution whose pH was 4.01 at 25 °C. The method of potential measurement was the same as that for the reference electrode. The sensitivity of the indicating electrode was obtained by calculating the slope.

3. Results

3.1 Evaluation of reference electrode

The measured potential of the reference electrode is shown in Fig. 4(a). Figure 4(a) indicates that the measured value drifted by a maximum of about +37 mV when the reference electrode was placed in the cow's rumen, even though no drift should have occurred. Figure 4(b) shows the drift of the pH value calculated from the change in potential. The amount of drift increased with time, and the maximum drift was about 0.63 pH unit for Cow2.

Considering the operating principle of the pH sensor, the cause of the drift of the reference electrode seems to be either the decrease in the amount of chloride ions in the internal solution or the effect of rumen fluid mixing with the internal solution. The amount of chloride ions in the
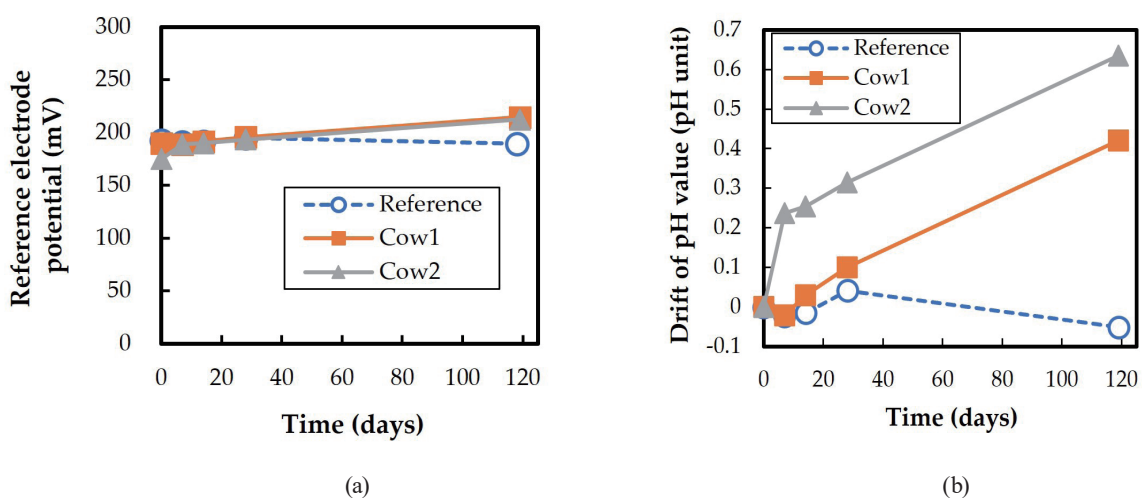

(a)

(b)

Fig. 4.   (Color online) Measurement results for the reference electrode. (a) Measured potential of the reference electrode. (b) Calculated drift of the pH value.

internal liquid can be calculated from the measured potential using Eq. (1). The calculated change in the chloride ion activity of the inner liquid is shown in Fig. 5; the chloride ion activity of the inner liquid gradually decreased over time, which may have been the cause of the drift.

Furthermore, to investigate the effect of rumen fluid mixing, a reference electrode with rumen fluid mixed with the internal solution was prepared, and the potential difference from the initial condition was measured. Note that a sufficient amount of KCl crystals was added to ensure sufficient chloride ion activity. Figure 6 shows that the difference in potential from the initial state was 3.8 mV even when the concentration of rumen fluid was 100%. The change in the potential of 3.8 mV corresponds to a change of 0.06 pH unit. This suggests that the effect of the rumen fluid mixing with the internal liquid is small, especially when a sufficient amount of KCl crystals is added.

3.2 Evaluation of indicating electrode

Figure 7(a) shows the potential measured in a phosphate pH standard equimolal solution (pH: 6.86 at 25 °C) and in a phthalate pH standard solution (pH: 4.01 at 25 °C) after 1 week (7 days), 2 weeks (14 days), 1 month (28 days), and 4 months (119 days) for the device placed in the rumen. Figure 7(b) shows the change in the pH sensitivity of the indicating electrode calculated from the measurement result. The initial pH sensitivity was around 57 mV/pH unit, and the pH sensitivity decreased significantly from the first month to the fourth month, falling to 37.1 and 49.0 mV/pH unit for Cow1 and Cow2, respectively. This decrease corresponds to a drift of 0.34 or 0.14 pH unit for a change of 1 pH unit in the rumen's pH. Considering that there is a variation of about 2 pH units in the rumen, this corresponds to a maximum drift of 0.68 pH unit.

To investigate the cause of the decrease in the pH sensitivity, the glass electrode placed in the rumen for four months was observed using a microscope. The optical image shown in Fig. 8(a) indicates that the surface of the glass electrode became impure after being stored in the rumen for a long time. These impurities can be removed by wetting with water and rubbing. An optical image obtained after the removal of the impurities is shown in Fig. 8(b). The pH sensitivity was measured again after the removal of the impurities, and the measurement result is shown in Fig. 9. The pH sensitivity was greatly recovered by removing the impurities, reaching 56.4 and
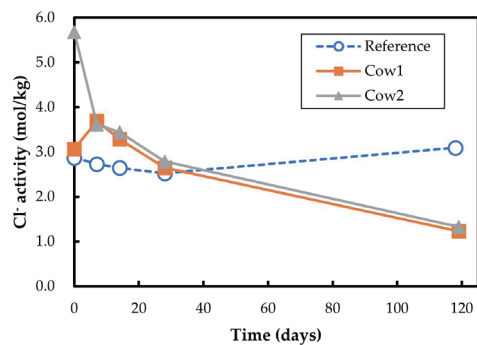
Fig. 5.   (Color online) Calculated change in the chloride ion activity of the inner liquid.
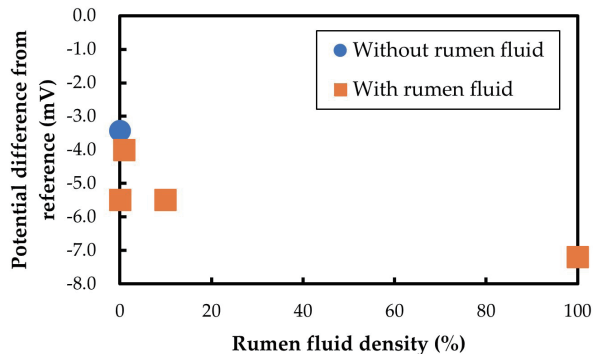
Fig. 6.   (Color online) Potential difference with and without rumen fluid added to the internal solution.
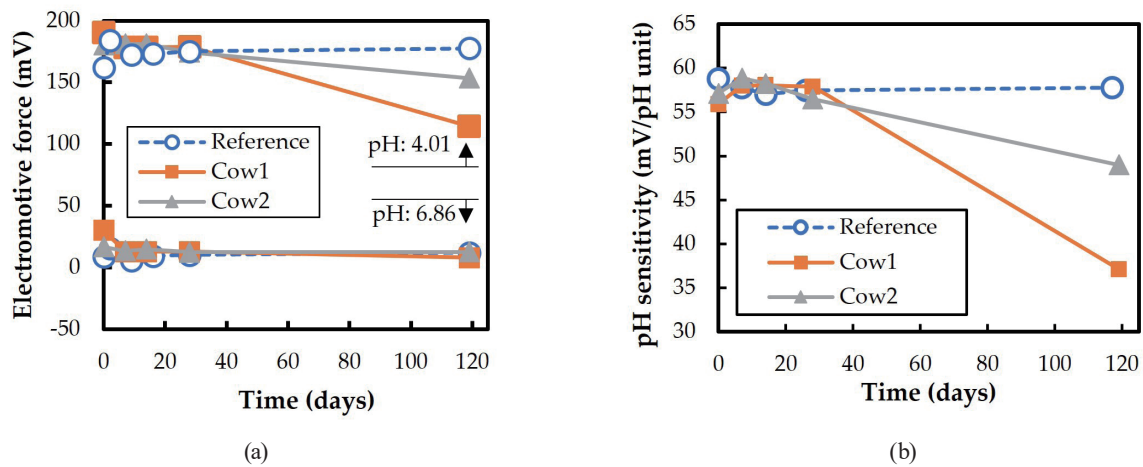
Fig. 7.   (Color online) Measurement results for the indicating electrode. (a) Potential measured in phosphate pH standard equimolal solution (pH: 6.86 at 25 °C) and in phthalate pH standard solution (pH: 4.01 at 25 °C) after 1 week (7 days), 2 weeks (14 days), 1 month (28 days), and 4 months (119 days) for the device placed in the rumen. (b) Change in pH sensitivity of the indicating electrode calculated from the measured potential.
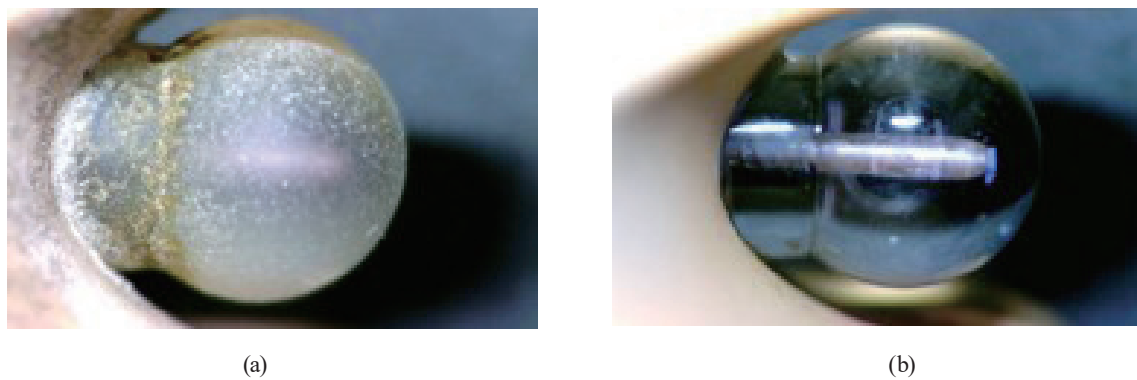

Fig. 8.   (Color online) Optical image of the glass electrode. (a) Glass electrode placed in the rumen for 4 months. (b) After removal of the impurities.
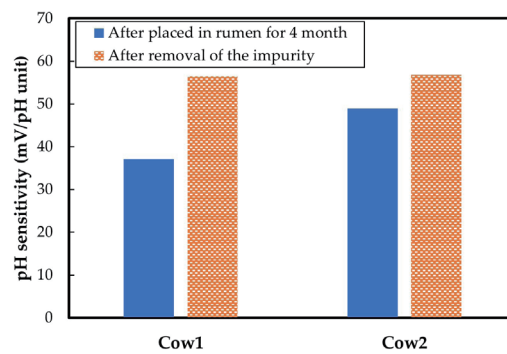

Fig. 9.   (Color online) Measured pH sensitivity before and after removal of the impurities.

56.8 mV/pH unit for Cow1 and Cow2, respectively, which were comparable to the initial values. It is considered that the impurities were the main cause of the pH sensitivity degradation and thus the cause of the drift of the indicating electrode.

4. Discussion

In this study, the drift of the reference and indicating electrodes during long-term storage in a cow's rumen was evaluated. In an actual pH sensor, the drift of both the electrodes will have an effect on the measurement accuracy. Therefore, we estimated the amounts of drift of both the reference electrode and the indicating electrode from the experimental results shown in Figs. 4(a) and 7(a). The estimated drift in the case of measuring the pH of phosphate pH standard equimolal solution (pH: 6.86 at 25 °C) is shown in Fig. 10; there was a change in the reference value of about 0.2 pH unit even though it should have been constant. Such a change may have been due to an error in the experimental setup. As shown in Fig. 10, the maximum drift considering both the reference and indicating electrodes appears to be 0.63 pH unit for Cow2, and the results for Cow1 and Cow2 showed a change in pH in the same direction after a certain amount of time had elapsed. This indicates the possibility of compensating for this change by calculation or other means.

The experimental result of the reference electrode revealed that the decrease in the chloride ion activity of the inner liquid caused the drift of the reference electrode. To maintain a constant ion activity, KCl crystals should be added in advance. Assuming that the ionic activity consumption is 2.89 mol/(kg · month) based on Fig. 5, 5.17 g of KCl crystals, which is equivalent to a volume of 2.61 cm^3, should be provided for 1 mL of the internal solution to maintain a constant ionic activity for 2 years. This volume can be fitted inside the rumen pH sensor. The effect of mixing rumen fluid with the internal liquid was also evaluated and revealed to be small from the rumen fluid mixing experiment. Thus, the drift of the reference electrode is expected to be suppressed by controlling the amount of KCl crystals.

On the other hand, the drift of the indicating electrode was due to the degradation of the pH sensitivity, which was caused by the impurities on the glass surfaces. Because the glass electrode cannot be cleaned when in the cow's rumen, the development of a glass electrode with resistance to impurities is required for rumen pH sensors.
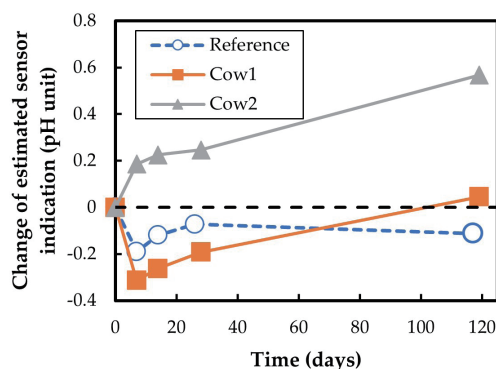

Fig. 10.   (Color online) Estimated amount of drift of the entire device considering both the reference electrode and the indicating electrode.

5.   Conclusions

In this study, pH sensors placed in the rumen of fistulated cows were temporarily removed after 1 week, 2 weeks, 1 month, and 4 months to evaluate their characteristics. The results showed that the drift of the indicating electrode caused by the sensitivity changes was the critical drift issue of the rumen pH sensor, and its sensitivity decreased significantly from the start to the fourth month, with the pH sensitivity dropping from around 57 mV/pH to 37.1 and 49.0 mV/pH unit for Cow1 and Cow2, respectively. Assuming a change in the rumen's pH of around 2 pH units, the maximum drift appears to be 0.68 pH unit. From the observation of the indicating electrode, we found that the drift was caused by impurities on the glass surfaces, and the pH sensitivity was recovered by removing the impurities. Note that although drift caused by the reference electrode was also observed, the drift is expected to be suppressed by adding sufficient KCl crystals because the effect of mixing rumen fluid with the inner liquid was small. To realize a stable rumen pH sensor, the sensitivity change of the indicating electrode is the more important issue that cannot be avoided, and the development of a glass electrode with resistance to impurities is required.

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