

Orally Administrable Wireless Activity and pH Probe for Cattle Reticulum

Lars Mattias Andersson,¹ Shozo Arai,² and Hironao Okada^{1*}

¹Research Center for Ubiquitous MEMS and Micro Engineering (UMEMSME),
National Institute of Advanced Industrial Science and Technology (AIST),
1-2-1 Namiki, Tsukuba, Ibaraki 305-8564, Japan

²National Institute of Animal Health
National Agriculture and Food Research Organization (NARO),
3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan

(Received July 2, 2018; accepted November 2, 2018)

Keywords: wearable sensor, wireless sensor, rumen probe, pH sensing, movement sensing

High-energy diets can have a negative impact on the function of the digestive system of cattle. Nevertheless, in modern farming, there are many reasons why reinforced diets are desirable, and it is therefore of interest to be able to continuously monitor their impact. Here, an orally administrable indwelling wireless probe that can track pH and movement is presented. It is designed to maintain its position in the reticulum and to provide measurements for several years without maintenance. An in situ test of the probe reveals that daily variations in pH, as well as changes induced by the diet, can be detected by the probe. It is also shown that the probe can detect reticulum contractions and that probe data can be used to verify that the probe is in its proper location.

1. Introduction

Each animal in cattle farming represents a significant monetary investment for the farmer. In order to maximize the returns on this investment, it is necessary to ensure that animals are kept healthy and in good condition while being as productive as possible with respect to, for example, milk yield. A large part of this involves ensuring proper nutrition. Natural food sources, such as grasses, are low in energy content, and thus, there are both economical and practical reasons for cattle farmers to include concentrates in the daily diet of their animals. However, the digestive system of ruminants such as cattle is quite sensitive. It relies on specialized enzymes, symbiotic bacteria, and rumination movements for the difficult task of digesting cellulose from grasses. Large deviations in the conditions of the stomach can negatively impact the function of these enzymes and bacteria. Concentrates contain a high level of rapidly digestible carbohydrates and cause a drop in rumen pH when consumed in very large quantities. A reduction in rumen pH leads to a decreased biochemical function and can eventually even lead to a cessation of the rumen contractions necessary to control the flow of

*Corresponding author: e-mail: hironao.okada@aist.go.jp
<https://doi.org/10.18494/SAM.2018.2046>

the digesta. When this happens, the animal loses its ability to digest food and the condition can therefore be fatal. An animal that exhibits prolonged periods of low rumen pH is said to suffer from subacute ruminal acidosis (SARA).

By continuously monitoring the pH and rumen movements, it is possible to ensure that the animal has an optimized diet for the maintenance of perfect health even during periods of varying nutritional needs such as in lactation. However, doing so can be very costly and resource intensive, and is currently not a common practice. Under normal circumstances, relevant measurements cannot be taken from the outside of the animal without surgery.⁽¹⁾ The solution is to use an indwelling probe. Such a probe must have no negative health impact on the animal and must be able to perform the desired measurements and subsequent wireless data transfer to the outside for an extended period of time without maintenance. Both indwelling ruminal pH probes⁽²⁾ and indwelling rumen movement probes⁽³⁾ have been reported, and both types can provide viable data.

The first stomach of cattle is called the reticulorumen, which consists of one large compartment towards the rear, the rumen, and a smaller compartment, the reticulum, in the front. Although the rumen pH has traditionally been used to diagnose SARA,⁽⁴⁾ foreign objects in the rumen tend to eventually get transferred to the reticulum because of the rumen movements and then remain there indefinitely.⁽⁵⁾ It has been shown that, although the pHs in the rumen and reticulum correlate well, they differ with that in the reticulum exhibiting a smaller pH drop in relation to SARA.⁽⁶⁾ However, the reticulum is smaller than the rumen and there is evidence suggesting that this leads to a more stable pH,⁽⁷⁾ so the smaller pH drop might be manageable. Considering the natural predisposition for foreign objects to end up in the reticulum, it makes sense to target the reticulum for indwelling probes despite the smaller pH drop associated with SARA. Here, an orally administered wireless reticulum probe, which has been improved in terms of being smaller and capable of both high-resolution pH measurements and movement measurements at a high sampling rate, is presented. The smaller form factor, which can easily be further miniaturized using the same hardware platform, is a great advantage for oral administration, and the high sampling rate for the movement measurements allows the probe location to be verified from data alone.

2. Probe Appearance and Function

2.1 Construction

There are several constraints in the physical construction of the probe. It must be made entirely of benign materials having no negative impact on the health of the animal. Since it is intended for oral administration, its shape and outer dimensions must be kept appropriate. It also must be strong enough to withstand inadvertent chewing that can occur during oral administration, and to remain intact and waterproof for its intended 2-year lifetime. Finally, its density must be high enough so that it sinks to the bottom of the reticulum in order to maintain its position and provide reliable data.

The probe housing is made from biocompatible polytetrafluorethylene (PTFE) with a stainless-steel lid and sealed with o-rings and silicon sealant. Its diameter is 30 mm, its length 100 mm, and its weight 124 g. A three-dimensional (3D)-printed washer is used to anchor the electronic circuits to the pH sensing assembly. The whole pH electrode assembly is encased in the PTFE housing with ruminal liquid access through 10 5-mm-diameter holes placed in a pattern matching the shape of the pH sensing assembly so as to provide maximum protection for the glass sensing electrode. Figure 1 shows the disassembled and assembled views of the probe.

2.2 Hardware and architecture

The probe contains a commercially available pH and reference electrode assembly from EUTECH Instruments, PHSENSOR03DJ, controlled through a custom-built interface. This assembly has a medically safe PEEK body and contains a conventional pH sensing glass electrode and a double-junction Ag/AgCl reference electrode. Movement is monitored using an ADXL362 accelerometer from Analog Devices integrated into a custom-built microcontroller (MCU) and a transmitter circuit. The microcontroller is C8051F930 from Silicon Labs and the transmitter is Si4461-B1, also from Silicon Labs. Power is provided by a heavy-duty 3 V lithium battery, and all active components are designed for sub-3 V operation to ensure functionality also when the battery potential decreases during discharge. Figure 2 shows the basic architecture of the system. It is a flexible system that can be configured for arbitrary measurement and transmission schemes. The microcontroller turns off every function that is not in use and enters the sleep mode itself when the probe is inactive, making the idle power consumption extremely small. A reed switch allows the system to be turned on or off when inside its sealed housing. Figure 3 shows the microcontroller/transmitter and pH sensor interface printed circuit boards (PCBs).

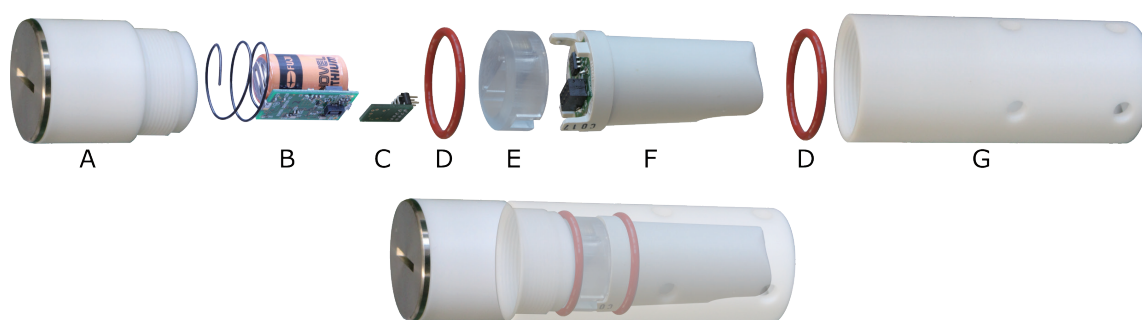


Fig. 1. (Color online) (top) Disassembled and (bottom) assembled views of the probe. A shows the electronics compartment. B shows the main PCB with an MCU, a transmitter, a temperature sensor, an accelerometer, an RF antenna, and a battery. C shows the pH sensor interface PCB with reference voltage generation. D shows o-rings. E shows a 3D-printed washer to accommodate the pH sensor assembly. F shows the pH and sensor assembly. G shows the pH sensing compartment with 10 5-mm-diameter access holes.

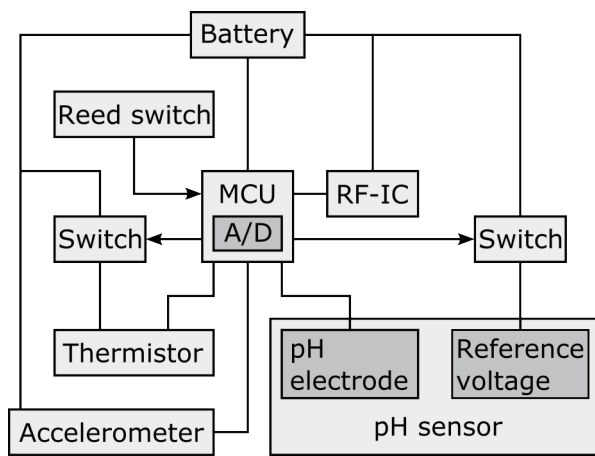


Fig. 2. (Color online) System architecture.

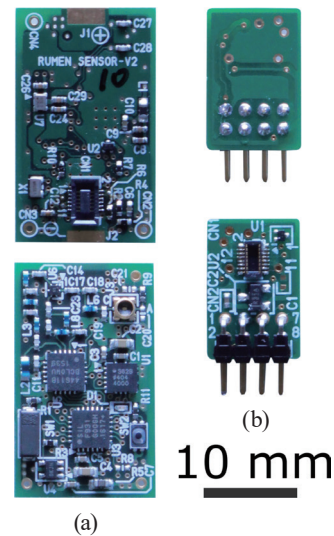


Fig. 3. (Color online) (a) Microcontroller/transmitter PCB, and (b) pH sensor interface PCB. The pH sensor interface PCB also contains circuitry for reference voltage generation.

3. System Performance

3.1 Measurement resolution, range, and sampling rate

There is great flexibility in the measurements using this system. It is possible to independently measure and transmit data from any of the sensors contained in the probe. Although the sampling rates and measurement resolutions should be minimized to conserve power for long-term operation, they are kept high during this short-term trial to allow the prototype probe to capture detailed data that can be used to develop more optimized measurement schemes for future iterations. The acceleration is probed at 1 s intervals and in a ± 2 g range with a 10 bit resolution, while pH is sampled once per minute in a pH range of 2–10 and a resolution of 0.03 pH units. All measurements are transmitted to the receiver in real time. As will be evident in Sect. 4, these sampling rates and measurement resolutions are much higher than necessary for the intended functions. At this point, they are chosen simply on the basis of the characteristic behaviors of the measurables; the rumen contractions occur roughly once per minute and have a duration of approximately 8 s, while the pH fluctuations are much slower with significant changes occurring over several minutes.

3.2 Data transfer

Transmissions are in the 429 MHz band. This frequency band does not require any license, but the maximum effective isotropic radiated power (EIRP) is restricted to 12.14 dBm. Since the radiation power of the sensor is attenuated considerably by body tissue and reticulas

juice when the probe is in its intended location, the maximum allowed transmission power is insufficient to maintain a viable reception range for a regular farm environment. In order to overcome this, the system utilizes a collar repeater to relay the transmissions to a receiver that stores the data on a cloud connected server where it is accessible for manual or automated evaluation on any viable internet-enabled device. The transmission speed is 4800 bps. Figure 4 shows an overview of the system in operation.

3.3 Power consumption and operational lifetime

A typical measurement and transmission cycle consumes less than 350 nAh, almost all of which is attributed to transmission. Given the 1000 mAh capacity of the battery and the negligible standby current ($<1 \mu\text{A}$), this means that the probe in the configuration used here has a calculated lifetime of slightly over one month. Real tests confirm that the lifetime is well over one month. There are several ways to increase the lifetime of the probe, and the target of a two-year lifetime without maintenance can be easily met by modifying the measurement protocol. Ways to achieve this include reducing the sampling rate and number of transmission events, as well as reducing the measurement resolution. Another alternative is to use more elaborate measurement schemes where sampling rates are relatively high, but measurements are performed only intermittently. Moreover, the probe can easily be configured for any desired measurement protocol and the target lifetime can easily be achieved.

4. Experimental Conditions

All animal-related procedures followed in this study were approved by the Institutional Care and Use Committee for Laboratory Animals of the National Institute of Animal Health (Protocol No. 16-010). A rumen-fistulated (see Fig. 4), primiparous nonlactating Holstein cow weighing

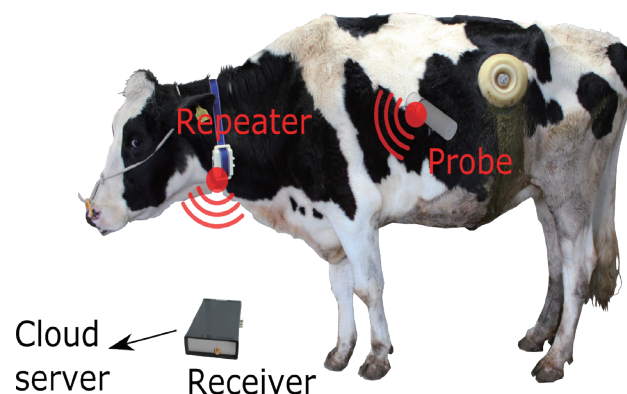


Fig. 4. (Color online) System operation. The cow in the picture is fistulated, i.e., it has a permanent surgically created opening into the rumen through which the probe can be easily inserted and extracted.

approximately 540 kg was used in this study. It was housed in a freestall barn at the National Institute of Animal Health in Ibaraki, Japan. Before the experiment, it was maintained on a basal diet consisting of 8 kg of hay and 2 kg of commercial cattle concentrate fed twice a day (at 09:00 and 15:00). The experimental diet used to induce a SARA-like response was 2 kg of hay and 8 kg of commercial cattle concentrate for one day (two meals). The cow had free access to water at all times.

5. Results

5.1 Probe insertion and location verification

The intended location of the probe is the reticulum, which is a small compartment at the front of the rumen. For these trials, it was possible to insert the probe and manually verify its location through the fistula. During the trial, the probe was extracted once per week in order to check the drift of the pH sensor and it was thus inserted 4 times. Depending on the contents of the reticulorumen, primarily the amount of dry matter, it can take a significant amount of time for the probe to sink to the bottom where it needs to be in order to deliver reliable measurements. When inserted into the rumen, the probe will eventually (within a few days) spontaneously transfer from the rumen to the reticulum where it remains indefinitely.

Although it is not possible to say for certain on the basis of this very limited trial, it seems possible to determine the location of the probe from the measurement data alone. The average pH in the rumen is lower than that in the reticulum. Literature data suggest that the difference is about 0.2 pH units in healthy cows,^(6,7) which is consistent with the data obtained during this trial by comparing the data collected after the probe was inserted directly into the reticulum and after it was inserted into the rumen before its spontaneous transfer to the reticulum. There also appears to be a discernible difference in the movement pattern between the rumen and the reticulum. As can be seen from Fig. 5, the latter exhibits more feature-rich movements, where each movement generally displays three local maximums, while the former displays smoother movements with fewer maximums and a slightly different rhythm. According to the results obtained in this trial (see Fig. 6), it can take 5 d for the probe to start generating stable reticulum data when it is initially inserted into the rumen.

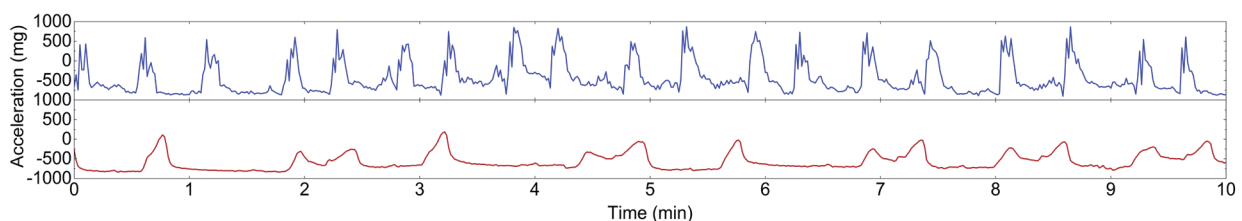


Fig. 5. (Color online) Acceleration data obtained from the (top) reticulum and (bottom) main rumen compartments.

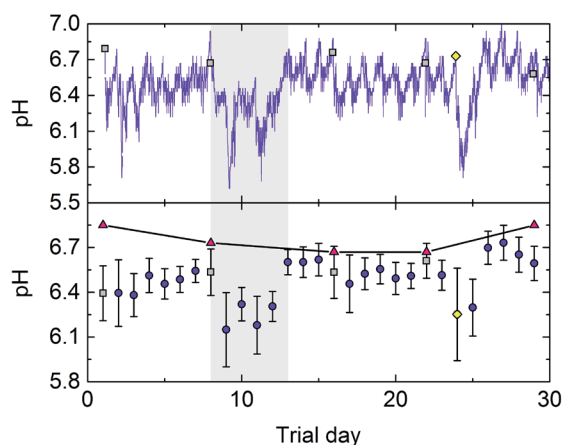


Fig. 6. (Color online) Reticulum pH. The top panel shows the raw data from the complete trial, while the bottom panel shows the daily averages together with the standard deviation. Gray squares indicate when the probe was extracted for calibration checks and pink triangles show the probe response to pH 6.86 buffer solution during those calibration checks. The shaded area is when the probe was located in the rumen and yellow diamonds indicate the day of the experimental diet.

Since the accelerometer is subject to Earth gravity, it is possible to monitor its orientation. Once in its intended location in the reticulum, the accelerometer data reveal that most of the time, the probe is oriented at a roughly 10° angle versus the horizontal position with the pH sensor slightly elevated compared with the stainless-steel lid. The probe sometimes shifts horizontally or vertically, but almost always with the pH sensor end pointing up. This is consistent with its center of gravity, which is slightly towards the stainless-steel lid. Although not experimentally verified, this might be a good thing since the bottom of the reticulum contains a significant amount of debris such as small stones and it might be beneficial to keep the pH sensor away from this sediment in order to reduce the risk of physical damage to the delicate sensing electrode.

5.2 Rumen pH

One of the most challenging aspects of the probe design is to ensure stability in the pH measurements. Long-term tests of the pH sensor assembly in buffer solution reveal a linear drift consistent with the expected out-diffusion of ions from the KCl solution in the reference electrode. On the basis of the test results, a drift corresponding to an increase of roughly 0.1 pH units is expected for a one-month trial like the current one. To monitor the state of the pH sensor, the probe was extracted once per week and the pH response tested using buffer solutions. Figure 6 shows the pH data collected during the trial and also includes the sensor output in pH 6.86 buffer solution during the calibration checks. The data from the calibration checks are inconsistent with the out-diffusion of ions and instead show a nonmonotonic behavior with a maximum deviation of about -0.2 pH units towards the midpoint of the trial and roughly no net drift over the duration of the experiment. Although the amount of data is

clearly insufficient, this implies that contamination effects, for example, might be an important factor to consider with regard to the accuracy of the sensor. It is possible to choose whether to put the probe in the reticulum or rumen when it is inserted through the fistula since both compartments are accessible. If it is inserted into the rumen, it will eventually get transferred to the reticulum because of the rumen movements. After the first extraction for calibration check, the probe was reinserted into the rumen instead of the reticulum. The pH data collected from the rumen is distinctly different from that collected from the reticulum and does indeed show a higher volatility as well as a lower average value. In this particular case, it took about 5 d for the probe to spontaneously transfer to the reticulum. The time spent in the rumen by the probe is indicated by the shaded area in Fig. 6.

The cow on which this study was performed is fed regularly at 09:00 and 15:00, and the reticulum pH reflects this in its systematic variations during the course of the day. Figure 7 shows that concomitant with feeding, the pH drops by roughly 0.15 units when under the regular diet. The recovery is slow enough so that equilibrium is never reached and pH thus varies constantly throughout the day. In order to test the SARA detection capability of the probe, the cow was fed a high-energy experimental diet for 1 d, which increased the feed-related pH drop to about 0.4 pH units per meal (Fig. 7). Figure 6 shows that the increased drop in pH from the experimental diet is also visible in the daily average pH.

Fast changes are clearly possible to detect, and short-term (one month in this trial) monitoring for acidosis appears to be possible using this probe. However, slow long-term changes in pH due to less extreme deliberate dietary changes might be more problematic because of the drift of the pH sensor, and it is presently unclear if the probe can perform its intended function for two years using this pH sensor.

Although the rumen pH varies throughout the day and conceivably also between populations and individuals, there have been attempts to define thresholds for diagnosing SARA. An example of such a threshold is when the rumen pH is more than 0.9 pH units below normal for

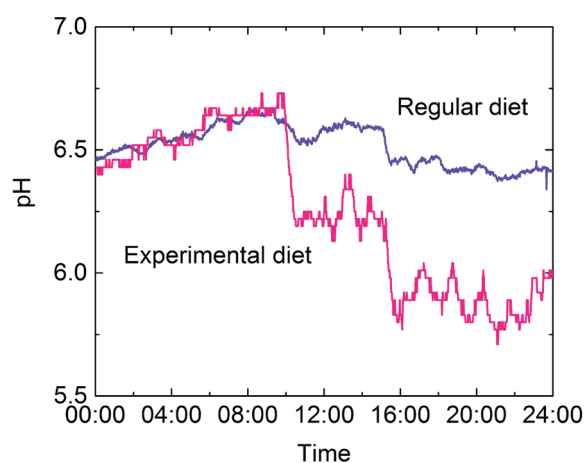


Fig. 7. (Color online) Daily reticulum pH variations during regular and high-energy diets. The regular diet data are trial averages, while the experimental diet data are for a single day.

an extended period of time.⁽⁸⁾ As is evident from Fig. 7, the maximum decrease in pH due to the experimental diet in this trial is less than that, but this is consistent with other studies that suggest that the SARA-related drop in pH is smaller in the reticulum than in the rumen.⁽⁶⁾ On the basis of the relative stability and consistency of the reticulum pH when on a regular diet and the response to the experimental diet observed in this trial, it appears possible to define such a threshold also for the reticulum and this probe.

5.3 Reticulum movement

Acceleration measurements are influenced by factors such as the movements of the cow and the flow of the contents of the reticulorumen. Because of this, the fidelity of the collected data vis-à-vis the reticulorumen movements varies over time. There are, however, many periods in which relatively clear data dominate the signal and thus it is possible to analyze the behavior of the probe and the reticulorumen movements more closely. The cylindrical shape of the probe allows it to rotate freely around its longitudinal axis and this is reflected in the collected data. Probe rotations occur with varying frequency and tend to be concomitant with the reticulum or rumen movements. Figure 8 shows acceleration data from the reticulum for the radial axes of the probe while it was in an almost horizontal position. During the 10 min, as shown in the figure, the probe rotates slightly over 90° in total divided between 5 major rotations. The basic reticulum movements appear to have a very distinct vertical pattern and, when a radial axis is aligned vertically, the patterns are clearly visible. They occur roughly 1.5 times per minute, have a duration of approximately 8 s, and display three local maximums at around 1 g. The 1 Hz sampling rate of the probe allows the local maximums to be resolved, and as per the discussion in Sect. 5.1, resolving the shape might be useful for verifying the probe location but is unnecessarily high for simply detecting reticulum movements, which can be performed with a lower sampling rate. Since reticulum movements can also be detected indirectly through probe rotations, it is likely possible to detect, for example, atony even at very low sampling rates provided that the probe, such as in the case here, is cylindrical and horizontally aligned. In any event, the probe can clearly fulfill its intended function in terms of movement sensing.

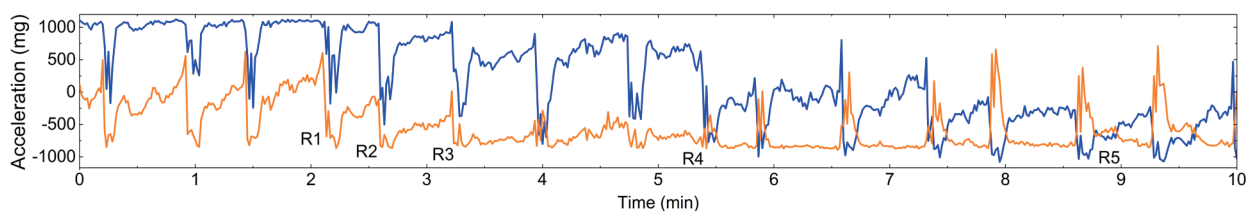


Fig. 8. (Color online) Reticulum movements as registered along the two radial axes of the accelerometer when the probe is in an almost horizontal position with respect to the longitudinal axis. 5 major rotations, labelled R1-5 in the figure, occur concomitantly with the reticulum movements.

6. Conclusions

Although the probe can perform its intended tasks well in a short term, as demonstrated by this one-month trial, there are two primary concerns that should be addressed before it can be commercially available. Firstly, its size should be further reduced. It is possible to administer this probe orally, but it is too large and may be uncomfortable for the animal. The size-limiting part is the pH sensing assembly and it is thus necessary to find a smaller alternative or to develop a customized solution. Secondly, again related to the pH sensor, are the concerns regarding the long-term stability of pH measurements. As it stands, the probe can likely detect rapid changes in pH for the duration of its lifetime, but it might not be possible to detect slow changes such as those that would presumably occur if the diet is optimized over the course of several weeks. It is therefore desirable to increase the stability of the pH sensor.

With respect to the general functionality of the electronic hardware, it can be concluded that it is fit for the intended purpose and can easily perform its desired tasks in a very flexible manner.

Acknowledgments

This research was supported by grants from the Project of the Bio-oriented Technology Research Advancement Institution, NARO (the research project for future agricultural production utilizing artificial intelligence).

References

- 1 E. Bramley, I. J. Lean, W. J. Fulkerson, M. A. Stevenson, A. R. Rabiee, and N. D. Costa: *J. Dairy Sci.* **91** (2008) 308. <https://doi.org/10.3168/jds.2006-601>
- 2 S. Sato, H. Mizuguchi, K. Ito, K. Ikuta, A. Kimura, and K. Okada: *Prev. Vet. Med.* **103** (2012) 274. <https://doi.org/10.1016/j.prevetmed.2011.09.004>
- 3 H. Nogami, S. Arai, H. Okada, L. Zhan, and T. Itoh: *Sensors* **17** (2017) 687. <https://doi.org/10.3390/s17040687>
- 4 J. C. Plaizier, D. O. Krause, G. N. Gozho, and B. W. McBride: *Vet. J.* **176** (2009) 21. <https://doi.org/10.1016/j.tvjl.2018.10.002>
- 5 J. Gasteiner, T. Guggenberger, J. Häusler, and A. Steinwidder: *Vet. Med. Int.* **2012** (2012) 236956. <https://doi.org/10.1155/2012/236956>
- 6 S. Sato, A. Ikeda, Y. Tsuchiya, K. Ikuta, I. Murayama, M. Kanehira, K. Okada, and H. Mizuguchi: *Vet. Res. Commun.* **36** (2012) 201. <https://doi.org/10.1007/s11259-012-9528-8>
- 7 M. Falk, A. Münger, and F. Dohme-Meier: *J. Dairy Sci.* **99** (2016) 1951. <https://doi.org/10.3168/jds.2015-9725>
- 8 T. Duffield, J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride: *J. Dairy Sci.* **87** (2004) 59. [https://doi.org/10.3168/jds.S0022-0302\(04\)73142-2](https://doi.org/10.3168/jds.S0022-0302(04)73142-2)