Original Article

Development of wireless device prototype for measurement of rumen pH and temperature continuously in cattle

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Abstract

This study aims to develop a prototype rumen pH and rumen temperature measuring device for continuous measurement. The benefit is for the monitoring and early diagnosis of metabolic diseases in cattle, especially rumen acidosis and subacute ruminal acidosis. The developed prototype device was approximately 50 millimeters in diameter, 145 millimeters in length and weighed 400 grams. The outer structure of the prototype was made from abs plastic made by a 3D printer, while the internal device contained pH and temperature sensors, a microcontroller and battery. The prototype was placed in the ventral sac of the rumen of fistulated cattle for three days to test the transfer of pH and temperature data. It was found that the pH drifted over time while the temperature remained stable. The pH and temperature signals showed a fast response after feeding. The rumen pH and rumen temperature measured with the prototype were 5.3 - 7.0 and 37.25 - 38.75, respectively. The pH drift over 48 and 72 hours was around 0.15 and 0.28, respectively.

Keywords: dairy cattle, intra-rumen, pH, temperature, wireless

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Introduction

The collection of data continuously is very important to illustrate the daily fluctuation of rumen pH for diagnosis of important metabolic diseases in dairy cows such as subacute ruminal acidosis (SARA). The optimal rumen pH in the cattle is in a range of 6.0 – 7.0 (Yang *et al*., 2001). A cow is considered SARA when the rumen pH is between 5.5 – 5.8 (Garrett *et al*., 1999). The consequences of SARA include a reduction of feed intake (Krajcarski-Hunt *et al*., 2002), laminitis (Nocek, 1997), rumenitis (Krehbiel *et al*., 1995), abomasal displacement (Shaver, 1997), loss in milk production (Stone, 1999; Krause and Oetzel, 2006) and lower in reproductive performance (Inchaisri *et al*., 2014). The prevalence of SARA at the cow level in small dairy herds in Nakorn-Pathom province, Thailand has been reported to be about 14% (Inchaisri *et al*., 2013).

The rumen fluid is collected by a specific method, for instance by using a stomach tube and ruminocentesis techniques. However, collecting ruminal fluid by stomach tube or ruminocentesis technique are not able to measure the pH continuously and are impractical. In addition, the application of these techniques may result in trauma with the complication especially of abscesses and peritonitis after the ruminocentesis (Duffield *et al*., 2004). In addition, the reliability of data from these techniques should be considered because the intra-rumen localization of stomach tube influences the pH value and the contamination of saliva in the rumen fluid in the stomach tube interferes with the rumen pH value (Duffield *et al*., 2004; Enemark *et al*., 2004). Moreover, the skills of the operator can also influence the pH value (Duffield *et al*., 2004).

Since spot sampling techniques cannot collect the rumen content from cattle continuously, since 2004, wired devices for rumen pH measurement have been developed (Duffield *et al*., 2004; Marden *et al*., 2005; Penner *et al*., 2006; AlZahal *et al*., 2007). Although they can generate continuous data at the time, wired devices have limitations. For instance, the device can only be placed in the rumen of a fistulated cow. More recently, novel techniques, such as digital wireless devices have been developed (Mottram *et al*., 2008; AlZahal *et al*., 2009; Zosel *et al*., 2010; Gasteiner *et al*., 2012; Sato *et al*., 2012). These can measure rumen pH continuously and directly inside the dairy cow without sampling the rumen fluid and all data is transferred by a wireless system, including radio frequency (Mottram *et al*., 2008; AlZahal *et al*., 2009; Zosel *et al*., 2010; Gasteiner *et al*., 2012; Sato *et al*., 2012).

In many countries, commercial and prototype device systems to monitor and to illustrate rumen pH continuously have been applied in dairy farms (Mottram *et al*., 2008; Gasteiner *et al*., 2012; Sato *et al*., 2012). However, using commercial devices is expensive and they have a short lifespan due to battery life and sensor drift. In addition, nowadays, electronic devices are much cheaper. Therefore, developing these devices for small scale farmers within the country should be worthwhile and suitable for Thai dairy farming. Therefore, the objective of the present study is to develop the prototype of a digital wireless device for continuous rumen pH measurement and to perform functional tests of the device in pH and temperature transmission from the rumen.

Materials and Methods

The present experiment was carried out according to the guidelines for the ethical principles and the use of animals for scientific purposes, edited by the National Research Council of Thailand. The research proposal was approved by the institutional animal care and use committee in accordance with Chulalongkorn University regulations and policies governing the care and use of experimental animals (animal use protocol no. 1731044).

System configuration: The wireless device will be developed and enclosed with several parts of a sensor system consisting of a wireless module, pH sensor, temperature sensor, microcontroller, battery, external antenna, Wi-Fi router and personal computer (PC) with installed phpMyAdmin software. (phpMyAdmin project, www.phpmyadmin.net). This software will be used to handle the administration of Structured Query Language (SQL) over the Website to operate all data received from the wireless device. The wireless system utilizes a specified low-power radio station system (433 MHz band), which can be operated without a license in accordance with Thai radio law.

ISFET pH and temperature sensor (WINSENSE®): In this experiment, the ISFET pH sensor will be used. The Si_3N_4 insulating gate ISFET devices measure the pH value in a wide range from basic to acidic solutions. The pH and temperature senor specifications are shown in Table 1. For stable measurement, an Ag/AgCl reference electrode is required. Submerged together with the packaged ISFET pH sensor, this acts as a metal gate electrode and provides a stable potential reference.

Wireless device. The ISFET pH sensor (WINSENSE®) will be housed in a cylinder-shaped bolus, which also encloses a pH amplifier circuit, a microcontroller (CPU) circuit, a radio frequency (RF) circuit and a battery. The expected device's dimensions are around 400 g in weight, 50 millimeters in diameter and 145 millimeters in length (Figure 1a). The device can be administered orally into the cow's rumen using a bolus gun without any surgical procedure. The housing of the pH sensor is made of plastic. Plastic materials of acrylonitrile butadiene styrene (ABS) type are superb because of their inexpensive price and being easily molded by 3D printer (daVinci 1.0A, XYZprinting®, Taiwan). With a suitable quality of plastic, ABS housing has been used in several products being inserted into rumen such as rumen pH measurements, rumen magnets and electronic identification rumen boluses. An ISFET sensor measures the ruminal pH with temperature compensation thus the degree of accuracy is at a high level. A lithium-ion battery (3.6 V, 1700 mA) will be used as an internal power source. A block diagram of the device is shown in Fig.1b.

Data measurement receiver and relay unit: The block diagram and communication diagram of the wireless device system is shown in Figs. 2-3 The rumen pH measurement receiver receives data from the pH sensor by radio waves. The functions of receivers are operating and correcting the pH sensor. The receiver with RF and CPU circuit inside will be assembled in a plastic housing. It operates using phpMyAdmin software version 4.8.1 (phpMyAdmin project, www.phpmyadmin.net) which is installed in a PC. Since the radio waves transmitted from the wireless module are greatly attenuated when passing through the cow's body, the data measurement relay unit will be placed in the vicinity of the wireless pH sensor and signal to the receiver, which is enclosed in the CPU circuit, the RF circuit, and the power source. A commercial power source will be used at the locations with an electric power supply.

Animals: A rumen-fistulated 87.5% Holstein steer with 400 kg body weight. Throughout this study, the experiment will be performed under the usual experimental cattle management at the Farm Animal Hospital Nakorn Pathom, Faculty of Veterinary Science, Chulalongkorn University. Cattle are raised in a free stall and have free access to fresh water. The cattle will be fed by commercial concentrate first, followed by Corn silage (components feeding; 2.5% of body weight with 70:30 ratio of R:C) at 10.00 a.m. daily. The prototype device will be given to the cattle through rumen cannula before meals on the first day of the experiment.

Ruminal pH measurement using continuous recording system: The pH sensor will be 3 points calibrated with pH 4.01, pH 7.01 and pH 10.01 standard buffer solution (TRM®, National Institute of Metrology, Thailand) at the start of the experiment. In the experiment for the evaluation of the ruminal pH, the wireless device will be placed in the ventral sac of the rumen or reticulum through the rumen-fistula. Rumen pH will be continuously measured every 10 minutes for 24 hours. The continuous rumen pH data will be obtained for 3 days after the start of the experiment. The location of the wireless device will be examined in the morning using a metal detector which confirms that the wireless device remains in the ventral sac of the rumen or reticulum throughout the experimental period (3 days).

Table 1 ISFET pH and temperature sensor specifications.

Parameters	pH	Temperature
Sensitivity	50 mV/pH	-2.93 mV/C
Range	pH 2 – 12	0 – 100
Accuracy	0.01 pH	0.01 C
Response time	10 sec	1 sec

Table 2 Descriptive of rumen pH and temperature.

Rumen pH and temperature (°C) signal	Day 1		Day 2		Day 3	
	pH	Temp	pH	Temp	pH	Temp
Max	6.30	38.31	6.60	38.75	6.80	38.63
Min	5.56	37.38	5.30	37.75	5.35	37.25
Average	5.85	37.85	6.00	38.27	6.99	37.90
SD	0.18	0.26	0.35	0.30	0.37	0.36
N	338		413		233	

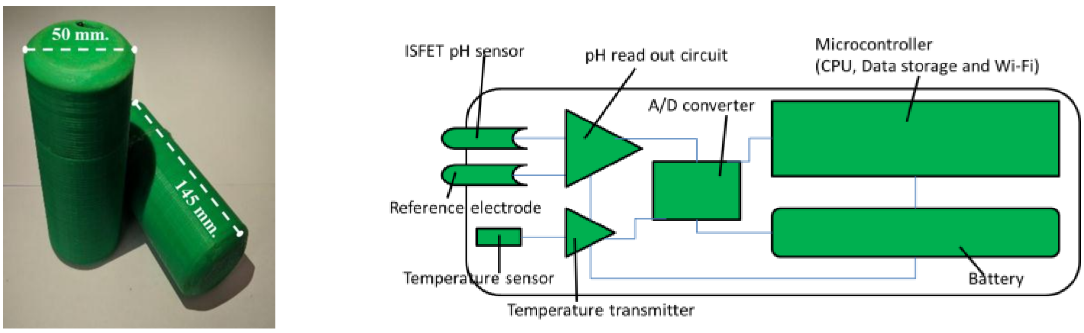

Figure 1 Photograph of device and block diagram of device.

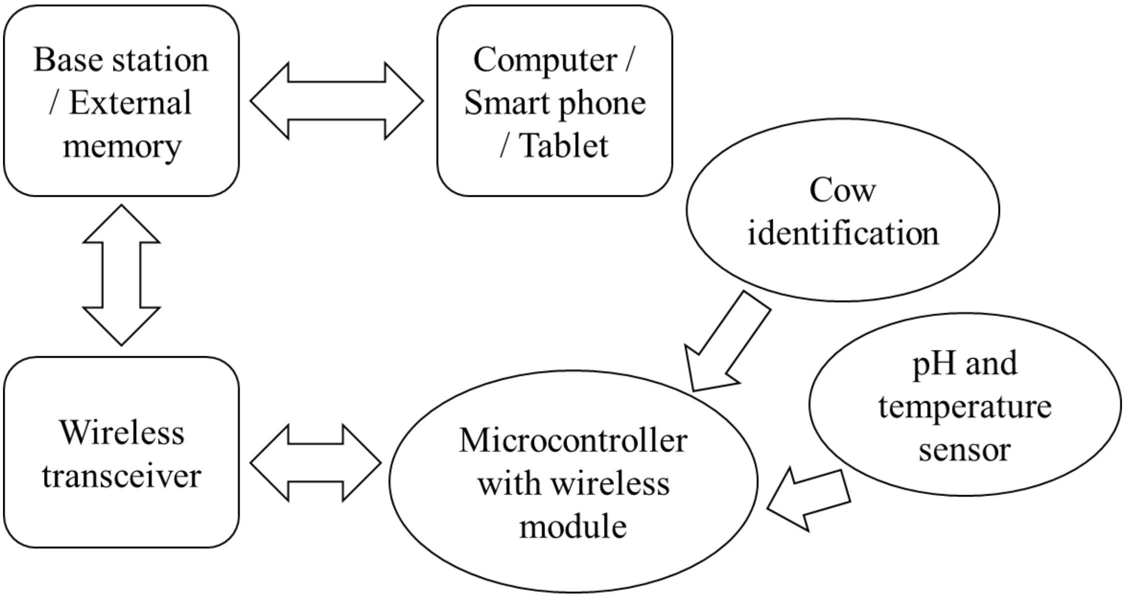

Figure 2 Block diagram of system unit. Square boxes represent outside cow units and oval boxes represent inside cow units.

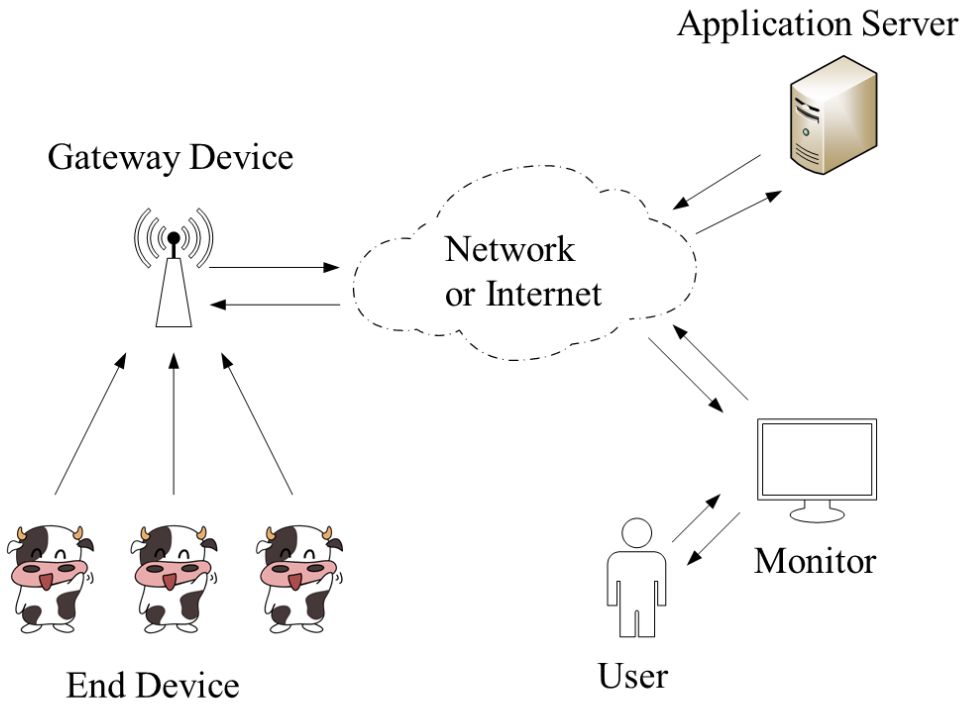

Figure 3 Communication diagram of the pH wireless sensor system..

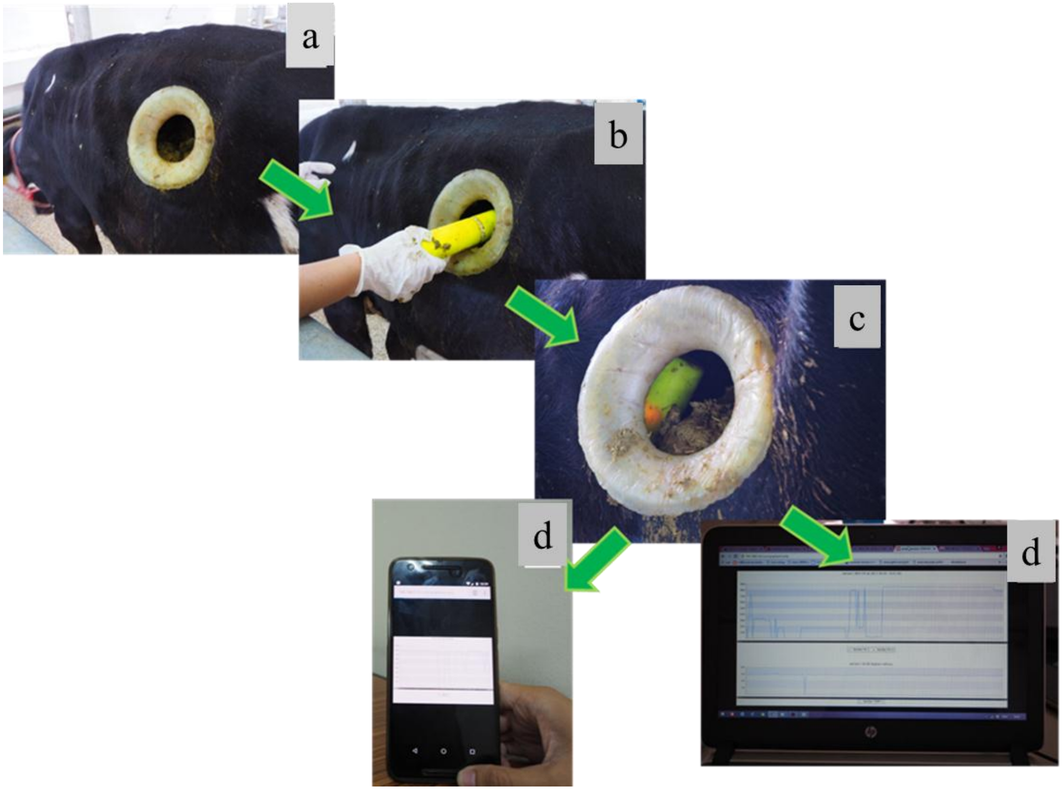

Figure 4 Step of measurement, prepare a fistulated cow (a), insert a prototype into rumen (b), wait for exact location (c) and transfer data to devices (d).

Results and Discussion

After giving the device to the cow (Figure 4a-c), the location of the device will be in rumen 24 hours later, Although the preferred location is retention in the rumen, it may be in the reticulum. 24 hours after the device is given to the cow, it is most likely to fall into the reticulum due to its weight and the rumen's motility (Gasteiner *et al*., 2009). In addition, installation time is also important for device retention. The device should be given to the cows before meals because the descent to the bottom of the rumen will not interfered with by floating mat. It has been shown that the pH at the bottom of the rumen positively correlates with the pH in the reticulum of dairy cows (Kimura *et al*., 2012). Moreover, the reticular pH values measured with a wireless device has also been recommended as a suitable SARA diagnosis (Neubeuer *et al*., 2017). However, the location of the wireless device can be confirmed by a metal detector (Sato *et al*., 2012).

From all 1091 signals of both rumen pH and rumen temperature they were completely calibrated and illustrated as shown in Fig. 5 and descriptive statistics of selected signals in each day are shown in Table 2. The pH and temperature signal show a fast response after feeding time. As known from the literature (Duffield *et al*., 2004, Enemark *et al*., 2003, Mottram *et al*., 2008) the rumen pH may vary in a relatively wide range. Due to the feeding conditions in this experiment the pH ranged between 5.3 and 7.0. The pH drift of the pH sensor was tested by calibration with a standard buffer solution. We found that the pH drifts were increased over time (Fig. 5) whereas the rumen temperature was stable. The pH drift in Day 2 and Day 3 was 0.15 and 0.28 pH unit, respectively. The pH drift in this study was most probably caused by the decrease of the battery of the prototype.

In Figure 2 the rumen temperature graph shows the pattern probably caused by water intake. Rumen temperature is heavily influenced by drinking behavior and it is easily possible to tell the number of drinks per day for the monitored animal by the temperature dipping to 35°C (Mottram *et al*., 2008). Temperature decreased rapidly and needed more time for recovery. The rumen pH decreased after its peak at t = 250 min indicating the high buffer capacity of the rumen fluid. It was noticed that the temperature drops were followed by a pH increase. In contrast, the continuous measurement for 3 days also showed the same temperature graph pattern. The correlation of these graph patterns with feeding required control of water and feed intake. Those experiments were not carried out but are planned as the next stage of our further study.

Due to no pathognomonic signs of SARA in affected cows (Krause and Oetzel, 2005) and clinical signs of SARA still not well defined (Danscher *et al*., 2015), the rumen pH measurement is the most reliable method to identify this health problem. Experts recommend that the rumen pH measurement gives the direct information on rumen condition (Kleen *et al*., 2003). Although there is no agreement on the rumen pH threshold to diagnose SARA, the accepted guidelines have been reported. The guidelines using continuous data in the rumen for SARA identification including when the rumen pH drops below 5.6 for more than 3 hours for a 24 hour period (Plaizier *et al*., 2008), below 5.8 for more than 5–6 hours for a 24 hour

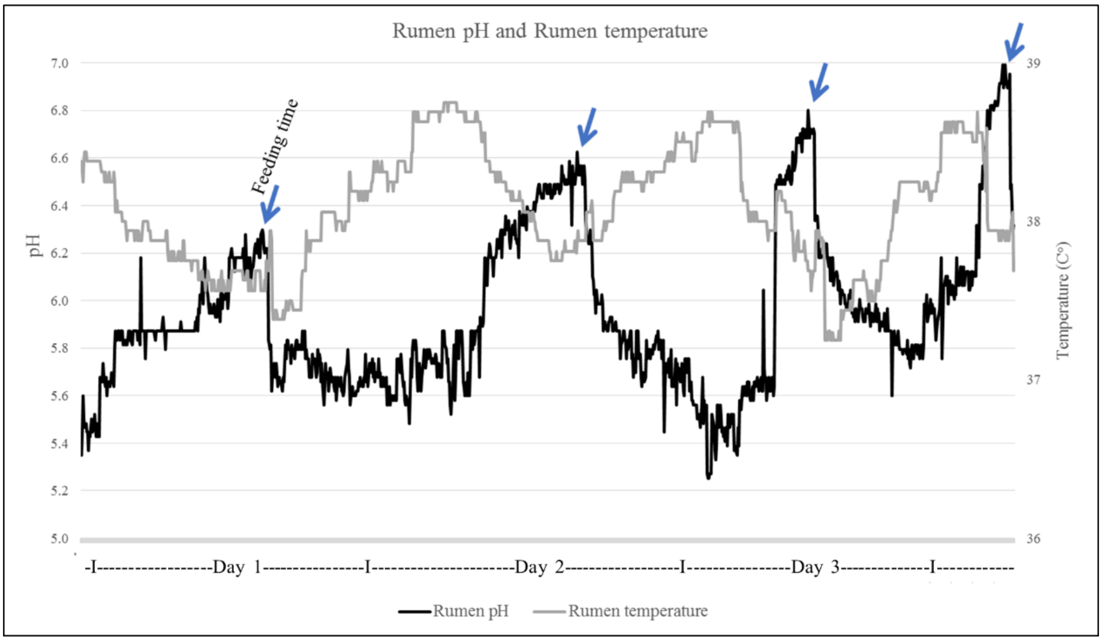

Figure 5 Rumen pH and rumen temperature during around 3 days of experiment, arrow is feeding time.

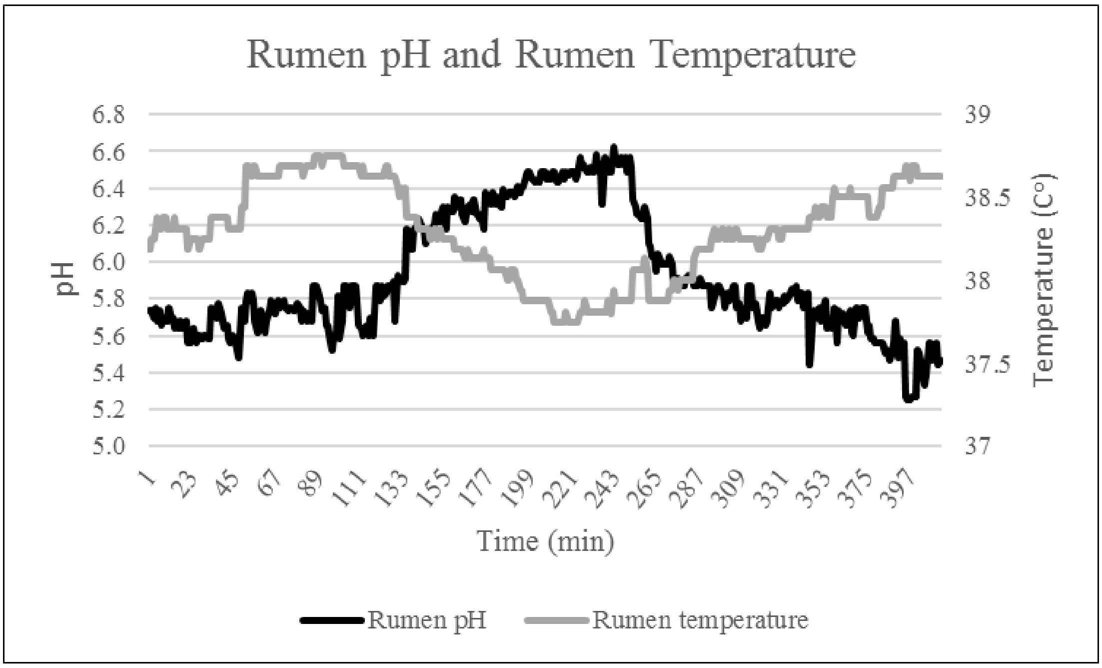

Figure 6 Detail of the course of rumen pH and temperature in Day 2.

period (Zebeli *et al*., 2008) or when the rumen pH is lower than 5.8 for 3-5 hours for a 24 hour period (Enemark, 2008). Moreover, a reticular pH of 6.0 has been recommended as a suitable SARA threshold (Neubauer *et al*., 2017).

Several methods of collecting rumen fluid for the rumen pH measurement include stomach tube, rumen canulation and rumenocentesis. Stomach tubing is not a reliable technique in the diagnosis of SARA because the pH may vary due to the intra-rumen localization of the stomach tube, saliva contamination and the time of sampling in relation to feeding (Enemark *et al*., 2002). Rumen cannulation obtains quite reliable samples of rumen fluid because it can determine the intra rumen localization properly (Nocek, 1997). Due to repeat removal the rumen fluid in this method disturbs the cow, is not practical method and it is limited to research purposes. Duffield *et al*. (2004), considers that rumenocentesis was a practical field test and better than the stomach tube for ruminal pH measurement. Rumen pH of rumen fluid that was collected by rumenocentesis had a positive linear relationship with the rumen pH of that collected by a rumen cannula. The time after feeding is very important for rumen fluid sampling by ruminocentesis. Furthermore, it depends on the type of feed and feeding management. In separate feeding herds, the samples should be obtained 2 to 4 hours after concentrate feeding while ruminal fluid should be collected 4-8 h after meals in TMR feeding herds (Kleen *et al*., 2003; Nordlund *et al*., 1995). Although rumenocentesis is the most practical method for SARA diagnosis in dairy herds, it still has some disadvantages, including being an invasive technique and not being able to collect rumen content continuously. Moreover, it affects the health and productivity of collected cows (Kleen *et al*., 2004).

Using a wired indwelling pH electrode, rumen pH has obvious changes and continuous monitoring of the rumen pH by the wired indwelling pH electrode provides a lot of information in ruminal pH changes. Due to putting fistulation in a cow before measurement, this method is only used in research studies. Moreover, the contamination and clogging of the electrodes is a major problem with prolonged use (Enemark *et al*., 2003).

The indwelling wireless device, the data transmitting unit allows real-time monitoring of the rumen pH, which can help in the early detection of abnormal patterns of rumen pH. It is less invasive compared to other methods of rumen pH measurement. Several studies have found a relationship between the pH measurement by wireless system and the actual data recorded by reference method (Gasteiner *et al*., 2009; Lin, 2010). Mottram *et al*., 2008) reported the use of a wireless device to measure rumen pH and rumen temperature. They can detect low rumen pH levels induced by heavy feeding of concentrates continuously in housed herds. Moreover, some bolus records data in the reticulum which is approximately 0.26 pH units higher than pH in the ventral sac of rumen which is traditionally used to diagnose SARA (Mottram *et al*., 2008). Thus, this issue will be considered. Moreover, major problems remain in big data management, analysis and interpretation and in developing systems and algorithms particularly with a low power supply for the long life of a battery.

There are many techniques or equipment that can be used to detect pH values, such as pH image sensors (Hizawa *et al*., 2006), conductimetric pH sensors (Sheppard Jr. *et al*., 1995), cantilevers (Bashir *et al*., 2002), optical fiber pH sensors (Hashemi and Zarjani, 2008), glass electrodes (Baucke, 1975) and ion-sensitive field-effect transistor (ISFET)-based pH sensors (Oelβner *et al*., 1995). The most popular technique is glass electrode and most of ruminal pH measurement wireless devices also use the same technique. In many pH applications, the fragile glass electrode needs to be replaced by an alternative non-glass pH electrode. For example, in food processing, a glass breakage can bring the whole process to a stop, no matter how small the glass fragments and in the application in bodies of animals including rumen of the cows. Among all pH measurement techniques, the ISFET is a non-glass electrode and it is one of the most popular electrical biosensors (Lee *et al*., 2009).

After the introduction of the ISFET biosensor by Bergveld (1970) and the first report by Caras and Janata (1980) regarding the use of an enzymatically modified ISFET for the direct detection of penicillin, numerous biosensors were established based on the theoretical development of ISFET technology (Lee *et al*., 2009). Currently, the use of ISFET technology encompasses a wide range of applications in a variety of areas, including the biomedical, environmental and livestock monitoring areas. ISFET pH sensors have been used in research on the development device for ruminal pH measurement in beef cattle (Zhang *et al*., 2016). In summary, the ISFETs have several advantages over the conventional ion-selective glass electrode (Yuqing *et al*., 2003; Dzyadvych *et al*., 2006), including ISFETs are very robust and durable. Unlike many conventional sensors, ISFET probes withstand cleaning with a toothbrush. The ISFETs are mass-produced by integrated circuit (IC) group technology, which makes them very small and cost effective. ISFETs can be stored dry and require little routine maintenance and ISFETs can be used over an extremely wide temperature range and are stabilizable.

In conclusion, a prototype for rumen pH and rumen temperature measurement in dairy cattle was developed and tested the functionality in pH and temperature signal transmission from inside the rumen. The prototype facilitates data transmission. However, the prototype shows more rumen pH drift overtime whereas rumen temperature is shown to be more stable. The prototype was in the ventral sac of the rumen during around 3 days of experiment. For further study, it is necessary to test for accuracy in comparison with rumen content collected at the same time from the same fistulated cattle. In addition, the pH drift requires further study to find a solution. This may start with optimization of the power consumption of the prototype and designing a battery life to last if possible. Moreover, there needs to be control of water and feed intake.

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