

Continuous monitoring of ruminal pH using wireless telemetry

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Abstract. This paper describes the performance of a prototype telemetric intraruminal bolus that measures and records pH continuously, can be delivered orally to the reticulum via bolting gun, has no external attachments and allows unrestricted activity of the animal. When interrogated by wireless the bolus transmits the recorded data to an operator standing beside the animal with a handheld receiving station. Boluses were placed in fistulated animals to enable direct comparison with samples obtained directly from the rumen and measured with a laboratory instrument. Overall, the mean (\pm s.d.) pH recorded on the manually collected samples (pH 6.64 ± 0.67) was generally less than that of the continuously measured telemetric system (pH 7.03 ± 0.54) with a correlation of $r = 0.93$ ($P < 0.01$). Data are presented to show typical diurnal and grain-enforced changes in pH recorded in a rumen over a 70-day period. The development of the Well Cow pH bolus device potentially enables researchers, dairy farmers and feedlot managers to monitor rumen function of any ruminant over prolonged periods without the need for invasive sampling. Enemark *et al.* (2003) considered that a 14–21-day observational period is required to properly monitor for conditions such as subacute ruminal acidosis. Whilst significant correlation ($P < 0.01$; $r = 0.982$) existed between the two readings for the first 40 days of continuous recording, the Well Cow pH bolus reading started to deviate significantly from the directly measured value thereafter. Regardless, a continuously measuring functional life of up to 40 days indicates that the current prototype has the capacity to accurately detect subacute ruminal acidosis.

Additional keywords: acidosis, bovine, datalogger, rumen.

Introduction

Current feeding systems that aim to satisfy the energy requirements of peak lactation dairy cows and the growth rates of feedlot cattle have implications for both ruminal pH and microbial health. Both factors ultimately impact on ruminant health. The normal rumen pH is in the range 5.5–6.8, depending on the type of diet (Cunningham 2002). More specifically, Smith (2009) considered a pH value of 5.5–7.0 as normal for cattle grazing high-quality forage that includes up to 50% concentrate supplement. Subacute ruminal acidosis (SARA) is defined as an extended period where rumen pH falls below pH 5.5 and is increasingly seen as a major nutritional problem affecting dairy cows (Nordlund 2001) and feedlot cattle. Economic consequences are considerable with the cost of SARA to the North American dairy industry calculated at US\$1.12 per cow per day (Stone 1999), or more recently US\$500 million to US\$1 billion per year (Krause and Oetzel 2006).

Various methods have been used to measure the pH of ruminal fluid. Smith (1941) reported the first *in vivo* measurement of bovine rumen pH. Later, spot sampling for rumen fluid samples via rumenocentesis (Duffield 1999), oro-ruminal probes (Geishauer 1993) and rumen cannulae were used but proved unsatisfactory for accurately characterising the dynamic pattern in rumen pH. The first attempts to continuously measure pH in sheep (Matscher *et al.* 1957) and in cattle (Lampila 1955) used

in-dwelling glass electrodes in cannulated animals connected by a wire to a receiver located outside the rumen. In 1993, Dado and Allen developed a system enabling constant measurement of rumen pH in animals maintained in stanchions but reported difficulties in maintaining calibration of the electrode due to static build up, faulty solid-core electrode leads, and rumen fluid leakage. This electrode–transmitter combination required cleaning and recalibration every 2 days to avoid electrode drift, although subsequent changes to tubing, connectors, and electrode leads have improved electrode performance (Dado and Allen 1993).

Later researchers (Keunen *et al.* 2002; Maekawa *et al.* 2002; Cottee *et al.* 2004; Beauchemin and Yang 2005; Rustomo and Al Zahal 2006; Al Zahal *et al.* 2007) have gradually improved the accuracy of remote measurements of pH over time and reduced the degree of confinement required of the animal. Stand-alone systems that continuously measure rumen pH without using external cables have recently been developed by Enemark *et al.* (2003), Graf *et al.* (2005) and Penner *et al.* (2006) enabling use in grazing and unrestrained animals. The Lethbridge Research Centre pH measurement system (Penner *et al.* 2006) is a much neater and more compact in-dwelling electrode and datalogger system than the predecessor (Beauchemin and Yang 2005) but still requires daily removal for data transfer and recalibration. Enemark *et al.* (2003) trialled

the continuous 8-day measurement of pH by an electrode located in the reticulum and reported only minor drift of the electrode readings over this period.

Wireless communication and telemetric systems appear feasible for a range of other online data-collection tasks and animal monitoring (Lowe *et al.* 2007). Peters (1997) described digestive processes in penguins using a novel pH sensor with a free-flowing junction to compensate for pressure changes. The operating principle of this device is similar to the autonomous acid reflux monitoring probe developed for humans (Bravo™ pH monitoring system, Medtronic Minneapolis, http://www.medtronic.com/downloadablefiles/Gastro-GastroFranchise_Background.pdf, verified 17 November 2009). Probes of this type employ 'gastric telemetry' to transmit data detected by a sensor in the probe via radio signals. Recently, Mottram *et al.* (2008) reported the development of a prototype intraruminal bolus capable of measuring pH continuously, storing the data and transmitting it telemetrically via an in-built radio transmitter to a remote receiver station.

The purpose of the current study was to establish the accuracy over time of this prototype bolus system (Well Cow Rumen pH Datalogger and Transponder; WCpH). This paper compares the data obtained using the WCpH bolus system to that obtained from measurements of samples derived directly from fistulated animals in terms of reliability of data download, variation between individual boluses and the duration of accurate measurement by the bolus.

Materials and methods

Location

The study was conducted during the Australian autumn at the University of Queensland's Mt Cotton Research Farm, Brisbane, Australia (latitude 27.3°S; longitude 153.1°E; 42 m average elevation).

Animals

This study was conducted with the approval of the University of Queensland Animal Ethics and Welfare Committee (approval #SVS/692/05). The WCpH boluses were calibrated before use, scheduled to measure rumen pH at 15-min intervals and inserted directly into the ventral sac of the rumen of each of four fistulated mature *Bos indicus*-cross steers. Intervals of 15 min were selected to reduce the memory required for data storage over the long periods of measurement; battery power usage by the datalogger increases with memory use. Four animals were used to assess interbolus accuracy. The steers were maintained on improved tropical pasture (predominantly *Digitaria eriantha*) for the entire trial period apart from the individual test days that were conducted 1 week apart. In preparation for each test day, the steers were weighed, a sample of rumen fluid was collected for testing, and the steers were fasted overnight in individual pens. The following morning, each animal was moved into a standard cattle crush, the archived pH data were downloaded via the transceiver and a rumen sample was collected and measured as described below.

Cracked barley grain at 2% bodyweight was mixed directly into the rumen material through the fistula to ensure accurate dosage and a more immediate rumen response. The period of

fasting followed by the rapid addition of grain was designed to result in a marked increase and then a marked decrease in rumen pH to enable assessment of the reading range and accuracy of the bolus. Manual collection and assessment of rumen fluid was repeated hourly for 5–6 h after grain treatment. Finally, the recorded WCpH bolus data for the day were downloaded allowing direct comparison against those reference readings taken during the day using the standard bench-top pH meter (described below). Bicarbonate (500 g) was then mixed into the rumen contents to prevent development of ruminal acidosis and the animals were observed for normal feeding behaviour after return to their paddock.

Rumen pH measurement

For manual pH (MANpH) measurements, each steer was held in a cattle crush, the rumen fistula plug was removed and a stiff gauze-covered aspiration tube was directed to a position immediately adjacent to the WCpH bolus; this accounted for potential differences in pH at different rumen sites (Garrett *et al.* 1999). Approximately 10 mL of rumen fluid was aspirated and discarded to flush the tubing before a 50-mL sample was collected and immediately measured using a standard bench-top pH meter (pH Cube, TPS, Brisbane, Qld, Australia). This reading was considered the reference value. The bench-top pH meter was calibrated daily using the same pH 4.0 and 7.0 buffers (TPS) used to calibrate the WCpH boluses.

WCpH system

The WCpH bolus (Mottram *et al.* 2008) comprises a sensor, an electronics component to transduce and condition the signal and store the data, a radio transceiver, aerial and battery. The pH electrode is a single-junction glass bulb electrode (Mottram *et al.* 2008). The glass formulation and reference junction of these prototype boluses was designed to remain stable for at least 60 days, slowing the effects of electrode degradation that occurs. This is all sealed within an enclosed container small enough to pass down the throat of an animal. The bolus is heavy duty, designed for submersible applications and contains in-built weighting so that it remains in the reticulum. Once sealed, the bolus is complete and the electronics and battery begin to operate from this date.

The identity of each bolus is individual and it is programmed to respond only when its unique identity number is interrogated. The sensor was calibrated using standard pH buffers 4.0 and 7.0, the calibration constants were entered into the bolus microprocessor by linked computer software (Well Cow Ltd, <http://www.wellcow.co.uk/overview.html>, verified 17 November 2009) and the bolus was directly transferred into the ventral sac of the rumen of the animal. For WCpH measurements, the bolus identity number for each steer was entered into the computer software, the receiving device was held near to but behind the animal's left shoulder and the pH data were downloaded within seconds. Within the bolus, the analogue-to-digital converter was programmed to sample every minute and the pH was averaged every 15 min. This reading interval was selected to reduce the amount of memory required for data storage and to reduce the usage of battery reserves over the 70-day monitoring period. The data was transmitted wirelessly to a transceiver

connected to a laptop computer using a half-duplex radio link. At each download the data was cleared and the bolus automatically reset to continue monitoring pH levels.

Statistical analyses

Absolute deviances were analysed, allowing the estimation of bias as well as variability. Deviances proved to be normally distributed, so no transformations were required. Linear, bent-stick and non-linear regressions were fitted to test the accuracy over time of the WCpH bolus results against the directly measured rumen fluid pH using the GENSTAT Statistical program (GENSTAT for Windows, release 9.1, VSN International, Oxford, UK).

Results and discussion

Download reliability

The boluses reliably downloaded the archived data upon interrogation. Any failure to initially identify the bolus and download data was readily corrected through adjustment of the transceiver position, allowing for minor bolus relocation due to normal rumen movement. However, based on the position at which the transceiver established bolus contact, no bolus appeared to have migrated more than 20 cm from its original placement in the ventral rumen.

Variation between boluses and steers

Fig. 1 describes between-steer WCpH bolus and MANpH measurements from four identically treated steers over a 70-day test period. The similar profiles of the MANpH readings in Fig. 1a (mean \pm s.d. pH difference of 0.40 ± 0.22 ; range 0.17–1.1) suggest consistency between rumen environments (animals) and treatments. Apart from the aberrant initial reading for steer #147 and a noticeably higher tracking pattern by the bolus in steer #149, the average difference in pH values between individual WCpH boluses across all readings was 0.57 (\pm s.d. 0.23; range 0.25–1.16). This wider mean pH deviation between WCpH readings is likely due to differences between individual boluses and should be addressed by the manufacturer.

The values in Table 1 show that whilst variation exists between the boluses in terms of difference from the respective reference values (MANpH), apart from bolus #142 they are quite consistent. Table 1 also shows the appreciable difference from the reference value that occurs after 40 days of bolus insertion.

A major problem with in-dwelling electrodes is the sensor drift that occurs due to a deterioration of the bolus electrode over time. Along with results in Table 1, an example of this drift can be seen in Fig. 2 after sampling occasion 21 (40 days post-insertion)

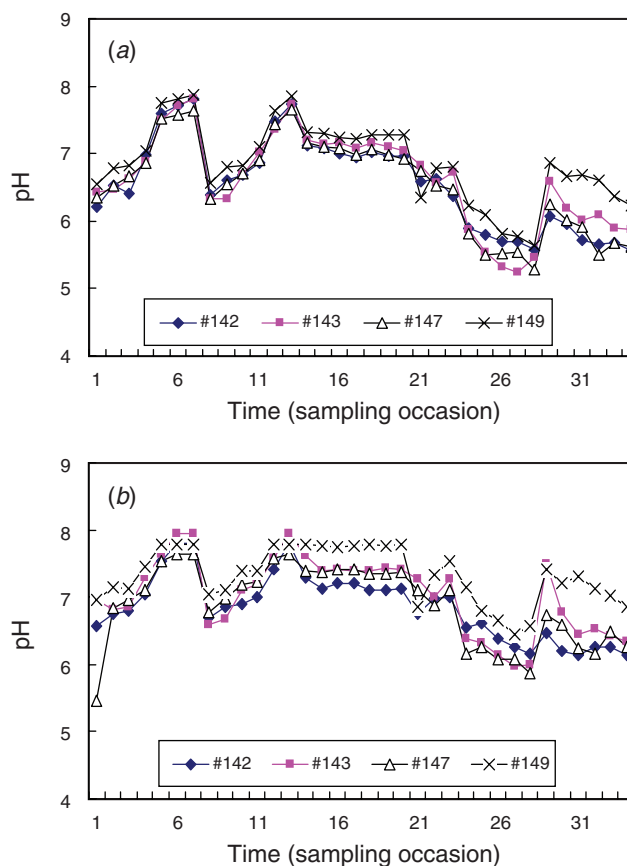


Fig. 1. (a) Comparison of the manually collected and analysed ruminal pH readings for each of four identically treated steers at various times over a 70-day period. (b) Comparison of the pH readings downloaded from a Well Cow pH bolus within each steer on the same occasions.

where the MANpH and WCpH readings are compared for just one of the boluses. A slight over-reading by the bolus was evident throughout with a mean difference in bolus and pH readings of 0.31 (\pm s.d. 0.23). This contrasts with findings from other laboratories (Smith 1941; McArthur and Miltimore 1968; Dado and Allen 1993; Al Zahal *et al.* 2007) that reported mean pH values recorded by continuous pH systems to be slightly lower (0.28, 0.1, 0.11 and 0.07, respectively) than the reference values. However, these groups did not maintain boluses untouched in the rumen for longer than 8 days.

Significantly, across the four boluses used, there was close agreement ($P < 0.01$; $r = 0.982$) between the WCpH bolus and related MANpH readings for up to ~40 days (mean \pm s.d.

Table 1. Average difference (\pm s.d.) between manual and bolus pH readings for the whole 70-day test period, for the period up to 40 days, and for the period between 40 and 70 days

Within rows, means followed by the same letter are not significantly different at $P = 0.05$

Bolus insertion period	Steer #142	Steer #143	Steer #147	Steer #149	Across-bolus pH average
70-day period	0.31a \pm 0.23	0.43ab \pm 0.2	0.41ab \pm 0.3	0.47b \pm 0.24	0.39 \pm 0.25
Up to 40 days	0.15a \pm 0.12	0.29b \pm 0.10	0.22ab \pm 0.3	0.35b \pm 0.21	0.25 \pm 0.20
After 40 days	0.55a \pm 0.14	0.62a \pm 0.16	0.59a \pm 0.15	0.69a \pm 0.13	0.60 \pm 0.15

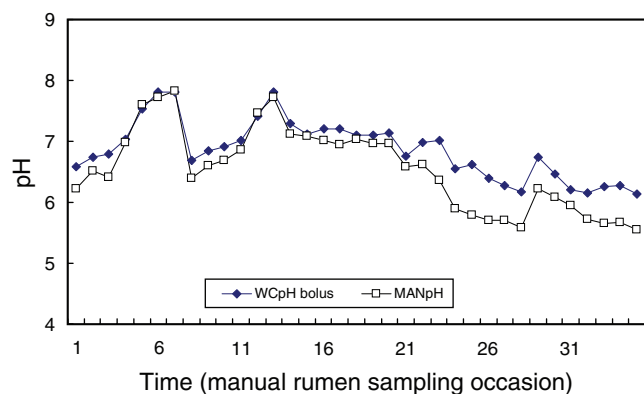


Fig. 2. Same-time comparison between the manually collected pH meter (MANpH) reference results (squares) and the corresponding Well Cow pH (WCpH) bolus readings (diamonds) for one steer over a 70-day period. The bolus appears to lose its accuracy from approximately sampling occasion 21, which relates to the time 40 days after activation.

difference of 0.25 ± 0.20) after which the margin increased appreciably to $0.60 (\pm \text{s.d. } 0.15)$. This initial margin is consistent with the values reported by the aforementioned researchers. The appreciable drift observed thereafter was seen in all four boluses examined (Table 1), suggesting that the period around 40 days post-insertion is the 'break point', or the point of deviation, from reliable pH measurement. This is a major advance on current bolus capacity but also presents a major challenge for future work.

Duration of reliable measurement

Fig. 3 displays a complete download of WCpH readings for one steer taken at 15-min intervals over a 10-week period and includes MANpH readings taken on various corresponding occasions.

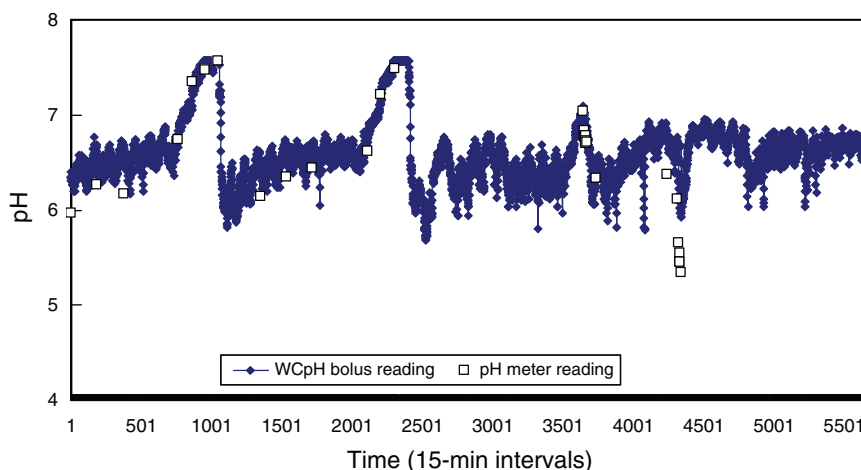


Fig. 3. An example of a complete analogue output of pH readings taken every 15 min (diamonds) for 70 days by a Well Cow pH (WCpH) bolus. The corresponding occasions when manual pH readings were taken from ruminal fluid samples (squares) are included. Stand-out features are the normal daily fluctuation in pH in pasture-fed beef cattle (e.g. points 1000–2000 equate to 1 week) and the periods of overnight fasting followed by rapid grain intake (e.g. points 800–1000 and 2200–2400). The electrode appeared to lose its accuracy from approximately point 4000 (6 weeks after activation).

Overall, the mean ($\pm \text{s.d.}$) pH recorded on the manually collected samples (pH 6.64 ± 0.67) was generally less than that of the continuously measured telemetric system (pH 7.03 ± 0.54) with a correlation of $r = 0.93$ ($P < 0.01$). The normal diurnal variation is clearly evident, as are the fasting (marked increase in pH) and grain treatment occasions (rapid fall in pH) at around 800, 2200 and 3550 min. As already discussed, there appears to be a loss of accuracy (sensor drift) from ~ 40 days after bolus insertion (i.e. 4000 min in Fig. 3). This was particularly noticeable during a period when a significant fall in rumen pH (below 6.0) occurred as detected by the MANpH system at around 4400 min.

In contrast, up to 40 days after insertion, very low pH levels were able to be detected as occurred in a lead-up experiment (Fig. 4). This animal demonstrated the significant fall in ruminal pH that occurs with SARA; the readings enabled immediate treatment with sodium bicarbonate to occur even before behavioural signs became evident.

Up to ~ 40 days after insertion, the WCpH bolus readings closely approximated the pH meter readings and showed the advantage of continuous measurements that allow detection of normal diurnal and other fluctuations in rumen pH that spot sampling may not detect. Probes for pH measurement are inherently unstable and normally require frequent cleaning and calibrating. Normally, holding the pH electrode in materials that differ from neutral for long periods appears to desensitise the sensor and this is overcome by recalibration. These prototype boluses were designed to remain stable for at least 60 days. Data presented has demonstrated that the WCpH bolus accuracy tended to drift after ~ 40 days, which is still appreciably longer than any other versions.

The errors over time are measured as absolute deviations (i.e. the bolus minus the metered pH values). Whilst these exhibit some degree of variability, Fig. 5 shows that there is no evidence of a break point in these patterns, but rather a steady increase. Statistically, there was a significant ($P < 0.05$) main

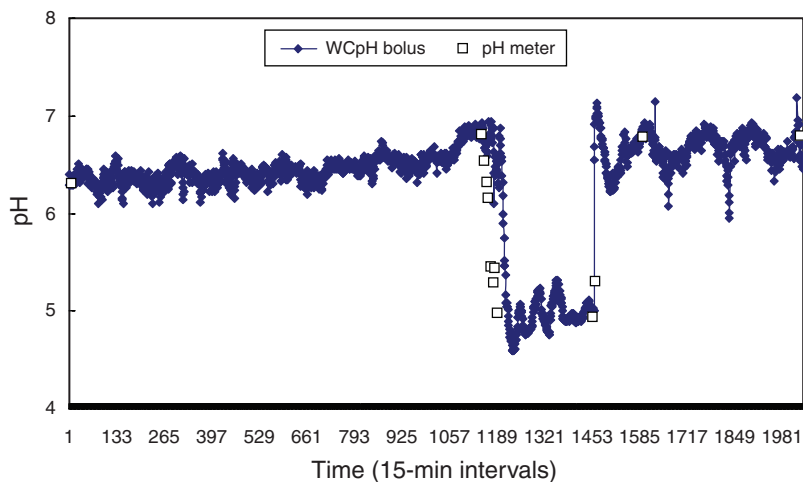


Fig. 4. Well Cow pH (WCpH) bolus (diamonds) and manual pH readings (squares) reflecting the incidence of subacute ruminal acidosis in a steer. Immediate treatment with 1000 g sodium bicarbonate saw an equally rapid ruminal resolution.

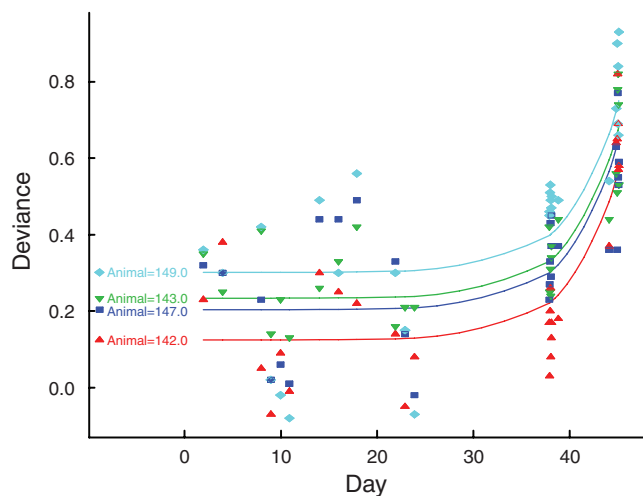


Fig. 5. Absolute deviations (Well Cow pH bolus minus manual pH values) over time for ruminal pH readings taken from four steers, plus fitted exponential regressions.

effect of ‘bolus’ indicating different initial precisions for the boluses. The interaction of the exponential slopes with ‘bolus’ was not significant ($P > 0.05$); hence, the relative position of the errors of these boluses was maintained over time.

Conclusion

The principal difference between the Well Cow in-dwelling continuous-reading data logger and traditional pH meters deployed by fistula is the ease and simplicity with which large amounts of archived data can be collected from a difficult and easily perturbed location. Minimal variation between WCpH and MANpH values was identified during the first 40 days of insertion. Application-wise, this period of time would provide ample opportunity to remotely monitor a dairy or feedlot animal’s performance or introduction to a new or higher concentrate ration.

The capacity to accurately detect and monitor pH levels below 5.5 was demonstrated (Fig. 4). It is at these levels of subclinical acidosis that most interest lies for feedlot and dairy cattle scientists. At this stage, the authors have demonstrated a novel technology platform that may have major implications for research and production systems in the future.

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