Evaluation of Kahne rumen sensors in fistulated sheep and cattle under contrasting feeding conditions

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Abstract

The Kahne rumen sensor (bolus) is a device developed to measure temperature, pressure and pH in non-fistulated animals. This bolus allows real-time monitoring of the rumen environment, which could help preventing health problems such as rumen acidosis in cows. It is less invasive to use boluses compared to other technologies that measure the ruminal pH (e.g. rumenocentesis). Kahne boluses and transceivers are commercially available in the market.

Several studies on the relationships between data recorded by the bolus and actual data recorded by independent devices were conducted. The bolus temperature and pressure were compared with actual temperature and pressure under controlled conditions. The pH drift was studied by comparing the difference between bolus and direct measurement over time. The capture of the data was calculated for each bolus in various experiments to examine the factors affecting the data capture rate of the boluses. Animal to animal variation was studied using boluses in a group of cows fed and managed under uniform conditions. An animal experiment involving fistulated cows eating two different diets was performed using boluses to monitor the changes of ruminal pH.

There was no apparent interruption to normal animal behaviour as a result of using boluses. Regression relationships between bolus measurements and actual data for both temperature and pressure were developed and used for calibration of bolus data. The pH drift was a problem, as the regression relation between the pH difference and the time for one bolus from one experiment could not represent this bolus on other experiment. The data capture rate on the hourly basis ranged from 0 to 100%, but was usually between 30 to 70%. The data capture rate was affected by many factors and further studies to identify these factors are needed. A study of animal to animal variation suggests that in a comparison of 2 treatments, a minimum 3 cows per group would be required to detect the standard deviation of 0.11 for a pH difference of

5% of the mean (approximately 0.35 pH units). Seventeen cows per group would be required to detect the standard deviation of 0.33 for the same difference. The boluses effectively monitored the ruminal pH change in cows easting two different diets and the profile of change of pH was successfully analysed. Feeding 7.6 kg baleage twice a day cause pH to decrease at 0.009 pH units per minute during feeding, while offering a similar quantity of grass and hay once a day resulted in a decrease of 0.0009 pH units per minute during feeding. The beginning of pH increase was about 1 hour following feeding and continuous during resting and rumination. The level of pH increase did not differ significantly for two diets..

The Kahne devices appear to have advantages compared to other technologies for the measurement of parameters of the rumen environment on a real-time basis. Boluses are especially good at intensively monitoring the temperature, pressure, and pH in the rumen. The major limitations of this technology to be used are the data capture rate and the pH drift. By improving the limitations found in the experiment, the Kahne rumen sensor could become very useful for both scientific research and under commercial conditions for monitoring animal health.

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Chapter 1: Introduction and Literature Review

1.1 Introduction

Rumen sensor (bolus) developed by Kahne Ltd is designed to monitor the rumen environment with minimum influence on animal behaviour. It is a technology alternative to the methods used to measure rumen environment such as oral stomach tube, rumenocentesis, and rumen fistula. It is a less invasive method compared to using oral stomach tube and rumenocentesis. It does not require a surgical process, which is required by rumen fistula. The bolus is also commercially available in the market for monitoring the whole herd if required.

Careful and thorough tests are needed to evaluate this device before it can be used, as once the bolus is in the rumen, the only way to get it out is to slaughter the animal. Since the bolus works in the rumen to collect data such as ruminal pH, temperature, and pressure, the understanding of the rumen environment is essential for testing the device. The knowledge of ruminal pH, especially acidosis is also necessary because pH is used as a parameter for determining the healthiness of the rumen and acidosis causes significant financial loss to the farm. The pH data collected by the bolus is important for monitoring the health of both individuals and the herd.

The following reviews the rumen, subacute rumen acidosis (SARA), the methods used to monitor the rumen environment, and the bolus from Kahne Ltd.

1.2 Ruminal pH and subacute rumen acidosis (SARA)

1.2.1 Rumen

The rumen, together with the reticulum, omasum and abomasum form the stomach of the ruminant animal. It serves as the primary site for microbial fermentation of ingested feed.

The rumen is the largest of the four compartments of the stomach of ruminant animals. It is connected to the esophagus and reticulum. The rumen is composed of 4 muscular sacs: dorsal sac, ventral sacs, caudo-dorsal blind sac and caudo-ventral blind sac (Church, 1979). The lining of the rumen wall is covered in small finger like projections (approximately 5 mm in length and 3 mm wide in cattle) called papillae, which increase the surface area of the rumen wall and assist in absorbing volatile fatty acids (VFAs).

The rumen is heterogeneous environment in terms of temperature, pH, pressure, etc. The rumen environment (temperature, pH, and pressure) is affected by many factors such as diet, water intake and rumination. The digestion of food causes pH to decrease and the heat from fermentation causes a temperature increase. The absorption of VFAs from the rumen and the inflow of saliva to the rumen lead to an increase in the ruminal pH. The food and water intake causes a temperature drop. During rumination the rumen undergoes cycles of contraction up to 1-3 times per minutes leading to changes to the pressure.

A large range of ruminant and non-ruminant animals rely on rumen microbes to digest forage and convert feed components into useable sources of energy and protein. The rumen provides an environment that is very favourable for the growth of microbes as the ruminal pH ranges from 5.5 to 7 and temperature ranges from 39 to 41ºC (Church, 1979). Conditions in the rumen are anaerobic. As a result, the microbial forms found in the rumen are nearly all anaerobes or facultative anaerobes (Hungate, 1966). Rumen microbes are mainly bacteria and protozoa; others include fungi, archaea and viruses. Bacteria have an important function of digesting structural carbohydrates (hemicelluloses and cellulose), non-structural carbohydrates (starch, sugar, and pectin), and protein to form nutrients for host animals. Bacteria degradation results in the moderation of VFAs that are absorbed and used by the animal. The bacteria themselves are degraded in the small intestine and are absorbed. Rumen protozoa can digest other microbes and ferment constituents of plant materials for energy (Hungate, 1966). Protozoa are not essential for rumen functioning. Rumen fungi help break down digesta particles and hydrolyze ester linkages between lignin and hemicelluloses or cellulose. Rumen Archaea help in lowering ruminal pressure by reducing carbon dioxide to methane. Rumen viruses help microbial recycling by lysing microbes (Hobson and Stewart, 1997).

The rumen provides an anaerobic environment, constant temperature and pH for microbes to ferment digesta and an absorption site for nutrients.

1.2.2 Ruminal pH

Ruminal pH is an important parameter of digestion and rumen health. It has relationships with rumen fermentation and animal production. An ideal ruminal pH provides an optimal rumen environment for digestion (Wales et al, 2004). De Veth and Kolver (1999) established in their study that dry matter digestibility of pasture was optimized at pH 6.35. Wales et al (2004) suggested large gains in productivity could be achieved by increasing average daily ruminal pH from 5.6 to 6.1. When ruminal pH decreased to a certain level, the digestion of food ingested would be affected. De Veth and Kolver (1999) suggested that a large reduction in digestion occurred when ruminal pH was less than 5.8. Beauchemin (2007) also suggested pH 5.8 as it caused a decrease in fibre digestion and feed efficiency. The decrease of ruminal pH could cause digestion disorders that lead to health problems such as acidosis.

1.2.3 Ruminal acidosis

Acidosis is a pathological condition resulting from accumulation of acid or depletion of the alkaline reserve in the blood and body tissues and characterized by an increase in hydrogen ion concentration (Bramley et al, 2003). Rumen acidosis is a syndrome related to a metabolic disorder of the rumen causing a decrease in pH in the rumen. There are two types of acidosis: acute and subacute acidosis. Acute acidosis is severe and less common in the field. The affected cattle are depressed, dehydrated, show anorexia, diarrhoea, abdominal pain, lethargy, staggering, recumbency and may die (Bramley et al, 2003; Krause and Oetzel, 2006). Acute acidosis is caused by unusual sudden dietary changes leading to uncompensated drop in ruminal pH (Krause and Oetzel, 2006). Subacute ruminal acidosis (SARA) is less severe and more common compared to acute acidosis. It has a greater economic impact to the herd as it is harder to detect and causes long term health issues such as whole herd lameness problems (Bramley et al, 2003).

Subacute rumen acidosis (SARA) is defined as periods of moderately depressed ruminal pH. The threshold ruminal pH value varied in different studies depending on the measuring technology. For example, ruminal pH 5.5 was suggested by Garrett et al. (1999) using rumenocentesis to collect ruminal fluid samples, while ruminal pH 6.0 was suggested by Plaizier (2004) using stomach tube at approximately 4 hours after feeding. Beauchemin (2007) used a threshold value of 5.8 because cellulolytic ruminal bacteria do not grow below pH 6.0, which causes a decrease in fibre digestion and feed conversion efficiency.

Subacute rumen acidosis occurs when VFA production from fermented diets exceeds the ability of the rumen environment to neutralize or absorb. Absorption of VFA occurs passively across the ruminal wall and is enhanced by papillae, which provides a large surface area for VFA absorption. Dirksen and Smith (1987) found that when cattle were fed highly fermentable diets (diets that are high in water soluble carbohydrate), ruminal papillae increased in length to enlarge the surface area for VFA absorption. It was suggested to allow a three-week period on a high energy diet for transition cows to grow ruminal papillae to a length that allow sufficient absorption of VFAs during calving. Passive VFA absorption through rumen wall and papillae was also increased when ruminal pH drop to thresholds value (pH 5.5) because ruminal VFAs are rapidly shifted toward the undissociated form and VFAs are passively absorbed only in the undissociated form. Besides increasing VFA absorption, cattle could maintain ruminal pH by regulating their food intake, increasing buffer production i.e. saliva production, and through microbial adaptation. All these responses are short-term responses. If the amount of VFAs produced is still excessive, ruminal pH will decrease to a dangerous level (pH 5.5) and SARA occurs (Krause and Oetzel, 2006). When ruminal pH is below 5.5, ruminal glucose from the structural and non-structural carbohydrates (cellulose and starch) is fermented to lactate instead of VFAs by *Streptococcus boives*. Lactate has a lower absorption rate than VFAs in low pH because lactate is less dissociated than VFAs. For example, at pH 5.0, lactate is 5.2 times less dissociated than VFA. Lactate then remains in the rumen causing more reduction in ruminal pH. When ruminal pH drops under 5.0, growth of lactate-utilizing bacteria like *Megasphaera elsdenii* is inhibited causing further decrease in pH.

When cattle are affected by SARA, the microbial balance is disrupted and the rumen environment is ruined. During SARA, the numbers and range of species of microbes are largely reduced, and protozoal populations are decreased close to zero as protozoa do not survive extended exposure to pH below 5.5. Even after cattle are recovered from SARA, they have less capacity to adapt to sudden dietary changes in the future because the ruminal microbes are less stable.

1.2.4 Economic damage of SARA

The economic damage of SARA comes from reduced milk production, decreased milk solid (fat and protein), the cost of treatment, the cost of extra feed due to low feed efficiency, the cost of lameness and other health problems, and the cost of culling. In New Zealand, it was estimated that $54 per cow per year was lost from reduced milk production caused by SARA. Donovan (1997) estimated that SARA cost US$ 500 million to US$ 1 billion a year to the U.S. dairy industry alone. Stone (1999) in a study calculated US$ 400 to 475 lost income per cow per year from SARA.

1.2.5 Signs of SARA

Cattle experiencing SARA do not show very clear symptoms. Signs associated with

SARA include: low feed intake, lameness, faeces foamy and contains gas bubbles, and faeces contains large particles of fibre (>12.7 mm) that appear to be undigested (Hall, 2002; Beauchemin, 2007; Plaizier et al, 2008).

Low feed intake is the result of a low ruminal pH causing the cattle to eat less in order to reduce the production of VFA.

Lameness is caused by laminitis. SARA is one of the facts that could result in laminitis. When bacteria and toxins produced by bacteria enter blood stream through damaged rumen wall, laminitis occurs (Stone, 2004; Cook et al, 2004; Krause and Oetzel, 2006; Beauchemin, 2007).

Bubbly faeces are a sign of abnormal microbial products in the rumen caused by SARA.

Large fibre particles in faeces are caused by the feed passing through the rumen too quickly to be properly digested.

Above are the signs that may indicate SARA in the herd, however, the most effective way to detect SARA is by measuring ruminal pH.

1.2.6 Prediction of SARA

Prediction and prevention of SARA is considered to be more important than treating SARA because the economic damage of SARA has already occurred when the signs are observed.

The factors that influence the occurrence of SARA include time, animal, and feed. Time is when SARA is most likely to strike. Animal refers to the individuals that have the highest risk. Feed means the diets that most likely to induce SARA.

Time of changing diets is the most important factor because all cattle are at risk of SARA when there is a sudden change of diet. The high risk time is when cattle eat a large amount of a high energy rapidly fermentable diet. In the situation of dairy cows, SARA normally occurs in the postpartum period. During the postpartum period cows often have a high intake of easily fermentable feed after a period of lower feed intake during pregnancy (Nordlund, 2003). For beef cattle, the first few weeks following relocation to a feedlot and transition to a high energy finishing diet are also important because the rumen and rumen microbes need to adapt to the high energy diet. The prediction of SARA could be made during these times, and would be beneficial to animal health and welfare.

Diet is another important factor and it is the easiest factor to be manipulated. The high energy diets that are high in easily fermentable material and low in neutral detergent fibre (NDF) can cause rapid fermentation and produce excessive amount of VFA, which causes a decrease in ruminal pH (Beauchemin, 2007). Therefore, it is easy to predict SARA during a change of diet.

The animal factors should be considered especially for the cows that just recovered from SARA. In a recovering animal, the rumen wall and rumen environment could not sustain a high energy diet. So, if no buffer or additives are added to the diet, the animal is highly likely to get SARA again (Garrett et al, 1999).

1.2.7 Methods of preventing SARA

Several methods of prevention could be used. The methods include adding dietary buffers and neutralizing agents in the diet, adding fibre in the diet, adding direct fed microbials in the feed, using rumen modifiers and antibiotics, and feeding management (Bramley et al, 2003; Nordlund, 2003; Krause and Oetzel, 2006; Beauchemin, 2007; Enemark, 2008).

Adding dietary buffers and neutralizing agents in the feed is a popular choice as it provides a positive effect on preventing SARA. Dietary buffers such as sodium bicarbonate are widely used in North American feedlots and dairy farms, as it has been available in the market for over 40 years and many studies have confirmed it efficacy

(Bramley et al, 2003; Enemark, 2008). Neutralizing agents include magnesium oxide (causmag), sodium bentonite, and calcium carbonate (limestone). The use of dietary buffers together with neutralizing agents proves to have positive effects on controlling ruminal pH and increasing production (Bramley et al, 2003). However, it is not recommended to add buffer in every meal.

Adding direct-fed microbials (DFM) is a method and an example of this is the use of yeast culture as additives in the feed rations. The additives could reduce diurnal rumen acidity and improve digestion of corn silage (Nocek and Kautz, 2006). Bach et al (2007) suggested that live yeasts had a beneficial effect on ruminal pH of cows in a study.

Using rumen modifiers and antibiotics such as sodium monensin (Rumensin™), lasalocid, virginiamycin, and tylosin could reduce the risk of SARA (Bramley et al, 2003). In the Australian dairy industry the rumen modifier sodium monensin has been proven to improve the digestive efficiency and reduce the risk of acidosis (Lean et al, 2000). Some antibiotics control the production of lactate and hence reduce the risk of SARA occurring (Enemark, 2008).

Management of feeding includes control eating rate and salivation rate by increasing the proportion of physically effective fibre (peNDF) in the diet (Beauchemin, 2007). Feed management is important, especially at high-risk times for SARA such as the postpartum period. A good management would allow the rumen to adapt to a high energy diet gradually and prevent the ruminal pH falling to a dangerously low level (Enemark, 2008).

1.2.8 Treating SARA

When SARA occurs, the aim of using treatments is to reduce the acidity and increase the microbes to restore the normal rumen environment. Since the economic losses have already happened when the symptoms of SARA was observed, treatment is less favourable than prevention. Therapeutic treatment is needed in severe cases of SARA to reduce the amount of acid-producing carbohydrates in the rumen (Kleen et al, 2003; Enemark, 2008). Therapy for SARA uses NaCl, multivitamin (mostly vitamin B12), and medicine (e.g. Eucarvet). The dosage of the medicine used for treating SARA is different depending on the symptom.

1.3 Technologies for measuring ruminal pH

Ruminal pH is important in the pathogenesis of SARA as the decrease of ruminal pH to critical levels results in disorder of ruminal environment and the onset of SARA.

Even in well-managed farms, SARA can still be a problem. The high energy diet required for high production could put animals at risk of SARA. Therefore, the monitoring of rumen pH is very important in preventing and managing the disorder (Enemark et al, 2003).

Different technologies can be for measuring ruminal pH in different situations. Veterinarians use a rumen stomach tube or rumenocentesis to measure ruminal pH in herds. Fistulated cattle are used in scientific research for the direct measurement of ruminal pH.

1.3.1 Using rumen stomach tube for measuring ruminal pH

The rumen stomach tube is also known as an oral stomach tube. The rumen stomach tube was developed to collect ruminal fluid and to administer water soluble drugs through mouth in cattle (Geishauser, 1993). A rumen stomach tube is a solid, tube-like probe containing rows of small holes on the end to work as the suction head (Duffield et al, 2004). The multiple holes at the suction head help to reduce blockage during collecting ruminal fluid. The earliest device was described by Pounden (1954). The probe must be at least 2.3 meters long and should have internal diameter larger than 8 mm to avoid being plugged. Modifications were done during the time to make the end of probe flexible and the suction head heavy enough for entering the ventral sac of the rumen (Dirksen and Smith, 1987). Bramley et al (2003) suggested the device could be constructed using a 3.5-meter long, 19 mm diameter plastic reinforced garden hose riveted to a 10 to 15 cm length of copper pipe with multiple holes drilled along all sides. The rumen stomach tube could be connected to a suction pump for the collection of ruminal fluid and to a funnel for the administration of liquids (Geishauser, 1993).

In order to collect ruminal fluid using the rumen stomach tube, the length (usually 1.8 to 2.25 meters) of inserting rumen stomach tube should be calculated using the distance from the incisivi (incisor teeth: the teeth for cutting or gnawing, located in the front of the mouth in both jaws) to the caudodistal end of the ventral sac of the rumen and the distance between the incisors and the most caudal point of the left 9^{th} rib (Geishauser, 1993). The animal should be restrained and the head should be pulled by a nose leader during insertion. A suction pump is used for obtaining ruminal fluid. If a blockage appeared, the tube should be withdrawn by approximately 30 cm to 1 m and then reintroduced to the original depth. The measurement of pH is done right after the collection using a pH probe.

Geishauser (1993) suggested that the best time for collecting ruminal fluid is before morning feeding and 3-4 hours after food intake.

The benefits of this technology are:

1. The device is simple to use. The procedure for acquiring ruminal fluid is straight forward. The clean up for the device is also very easy: just connect to a water tap.
2. It takes few minutes to collect enough ruminal fluid not only for pH measurement but also for other use such as analysis of rumen microbes. Geishauser (1993) required an average of 40 seconds for introducing the probe and an average of 2 minutes for collection 1.2 litres of ruminal fluid. The tube could collect over 2 litres of fluid with no problem. Therefore, it is suitable for collection of representative samples of ruminal fluid for diagnostic purposes and also for collection of larger

volumes of ruminal fluid for transfer (Geishauser, 1993).

3. Water soluble drugs could be administered into rumen with this technology. It makes easy for some treatments.

The limitations of this technology are:

1. The procedure could make animal uncomfortable as first, the introduction of device is via the mouth. Second, animal's head should be restrained during the procedure. Third, when a blockage happens, the withdrawal and reintroduction of the probe is very unpleasant to animal.
2. The position of the suction head in the rumen could not be determined or controlled completely as the rumen stomach tube only allows limited control of position (Nordlund and Garrett, 1995). There is a slight chance that the probe would not enter the rumen and if that happened, the measurement of pH would not represent the ruminal pH.
3. The contamination from saliva could not be eliminated. Some studies showed the affection from saliva was not significant while others suggested ruminal pH measured from this technology is higher than the pH measured by rumenocentesis or taken via rumen fistula (Geishauser and Gitzel, 1996; Duffield et al, 2004).

1.3.2 Using rumenocentesis to measure Ruminal pH

Rumenocentesis is another method to collect ruminal fluid using percutaneous needle aspiration (Bramley et al, 2003; Nordlund, 2003). Rumenocentesis is useful in determining whether SARA exists in a herd (Nordlund and Garrett, 1995).

Rumenocentesis was described in 1984 in German literature (Nordlund and Garrett, 1995).

In order to obtain a sample using rumenocentesis, the animal should be restrained and its rear legs should be tied together. To avoid the major muscle masses and for the needle to enter the ventral sac of the rumen easily, the needle should be inserted through the skin approximately 15 to 20 cm caudoventral to the costochondral junction of the last rib on the left side of the animal (Nordlund, 1994; Bramley et al, 2003). When the needle is thrust into the rumen, ruminal fluid is collected with very slight aspiration. Bramley et al (2003) suggested a minimum of 4 ml could be obtained using rumenocentesis. Nordlund (2003) stated that 3-5 ml of rumen fluid can be collected with minimal difficulty. When collection is done, pH should be measured immediately using a field-ready pH meter that requires only a small volume of fluid.

The timing of sample collection using this method should be when the pH reaches a nadir in order to evaluate potential acidosis. Nordlund and Garrett (1995) gave a guideline that in herds where rations are fed as separate components, samples should be collected 2-5 hours following the primary concentrate meal. While in herds fed a total mixed ration twice a day, samples should be collected 5-8 hours post morning feeding.

The benefits of using rumenocentesis are:

1. Rumenocentesis collects samples from the cranial-ventral rumen. Therefore, each sample can be collected from almost the same location. Multiple samples can be taken without difficulty.
2. The contamination of ruminal fluid collected by rumenocentesis is minimized as the only possible affection is from CO_2 leaving the ruminal fluid caused by the negative pressure within the syringe.
3. Animal feel less pain with sedation during rumenocentesis. Even without sedation, the animal calms down after the needle is inserted through the skin.
4. The effect of rumenocentesis on animal's health is small. A survey, involving 800 cows in Australia, reported only one incident involving a mild abscess being formed around the surgical site (Bramley et al, 2003).

The limitations of using rumenocentesis are:

1. An experienced person is required to perform rumenocentesis as the procedures

include needle insertion.

2. The obstruction by ingesta will occasionally happen. Even though it could be cleared by forcing a small volume of air or fluid back through the needle, the creation of a negative pressure within the syringe would lead to an increase of the pH as CO_2 will leave the fluid (Nordlund and Garrett, 1995; Nordlund, 2003).
3. The amount of ruminal fluid collected is comparatively small. The pH meter suitable for measurement is limited.
4. Even though the effect of rumenocentesis on a cow is comparatively minor, this method may not be completely benign in healthy cattle (Bramley et al, 2003).

1.3.3 Using a rumen fistula for measurement of ruminal pH

A rumen fistula is an artificial opening that allows scientists to look inside the rumen of an animal. Insertion of the rumen fistula is usually performed using a modified surgical technique involving a rumen clamp (Duffield, 1999). A rumen fistula allows rumen fluid to be taken out for measurement of pH. Ruminal fluid that is sampled by rumen fistula was used in experiments to evaluate and compare the performance of rumenocentesis and rumen stomach tube (Geishauser and Gitzel, 1996; Duffield et al, 2004). However, while a fistula is very good for scientific investigation of the rumen environment, it is not practical to be used on commercial dairies (Garrett et al, 1999).

There are many technologies that have been developed for continuously measuring ruminal pH in fistulated animals (Johnson and Sutton, 1968; McArther and Miltimore, 1968; Dado and Allen, 1993; Enemark et al, 2003; Duffield et al, 2004; Al Zahal et al, 2007). The early continuous recording systems had two problems: animal's movement during the recording was restricted as the data logging device could not be moved with the animal and the pH electrode had to be protected by a rigid casing to prevent breakage in the rumen (Johnson and Sutton, 1968; McArther and Miltimore, 1968).

Al Zahal et al (2007) described a recently developed continuous recording system used in fistulated cows. The system was composed of 2 main components : an indwelling pH electrode and a portable data logger. The pH electrode was heavy-duty and designed for submersible applications. The pH electrode cable was connected to a data logger using a rigid plastic protective tubing that went through the rubber stopper of the cannula. A stainless steel weight was connected to the pH electrode to keep it within the ventral sac of the rumen. The data logger was put in a waterproof box to be tied on the back of the animal with a belt to allow free movement for animal.

The procedures of pH monitoring using this system were:

1. The pH electrode was calibrated using standard buffer solution of pH 4.00 and 7.00 before put into rumen. The data logger was configured.
2. The pH electrode was put into rumen and the data logger was mounted on animal's back. The animal was then set free.
3. The pH data was logged out using a personal digital assistant.
4. The pH electrode was calibrated weekly to ensure the accuracy of the data.

The benefits of using fistulated animal for continuous recording of ruminal pH are:

1. Animals are allowed normal activity during recording as the system would not limit any movement.
2. The continuous data provides an excellent tool for monitoring and analyzing ruminal pH.
3. The device also allows recording of temperature for the study of pH-temperature interaction associated with ruminal disorders such as SARA (Al Zahal et al, 2008).

The limitation of the continuous recording system is that it can only be used on fistulated animals, which makes it commercially impracticable for farms.

There are ethical costs associated with using invasive such as rumen stomach tube, rumenocentesis, and rumen fistula to enable measurements on the rumen environment. These methods may also have an effect on animal's welfare as well as on animal behaviour. In order to minimize the ethical cost and the influence on animal behaviour, Kahne Ltd developed devices that aim to cause the least disruption to cows while measuring rumen environments (ruminal pH, pressure and temperature). These devices potentially allow for measurement on a large number of animals in a commercial production system, whereas the current alternatives tend to be confined to a research setting. Furthermore, they potentially enable measurements over larger period of time, and minimise the costs associated with frequent handling of animals.

1.4 Kahne device for continuously recording ruminal pH

Kahne Ltd has developed a wireless sensor network for continuous telemetry of data recorded in the rumen. The aim of these devices is to enable measurement of ruminal pH, pressure and temperature in intact non-fistulated animals. The commercial products were launched in August 2007. The concept of wireless sensor network is to measure, and even record the data. A receiver was used to capture the data measured by the rumen sensor through low power radio. The captured data was stored or transmitted to the computer for analysis using cable or internet (GSM modem).

The rumen sensor (bolus) is the key component of the system as it measures pH, temperature and pressure and transmits the data. The bolus is designed for animal weighing over 150 kg. The bolus has a shape of “T” (Figure 1.3.1). The sensor body is similar to that used for other purposes, e.g. the Rumensin bolus from ELANCO Animal Health.

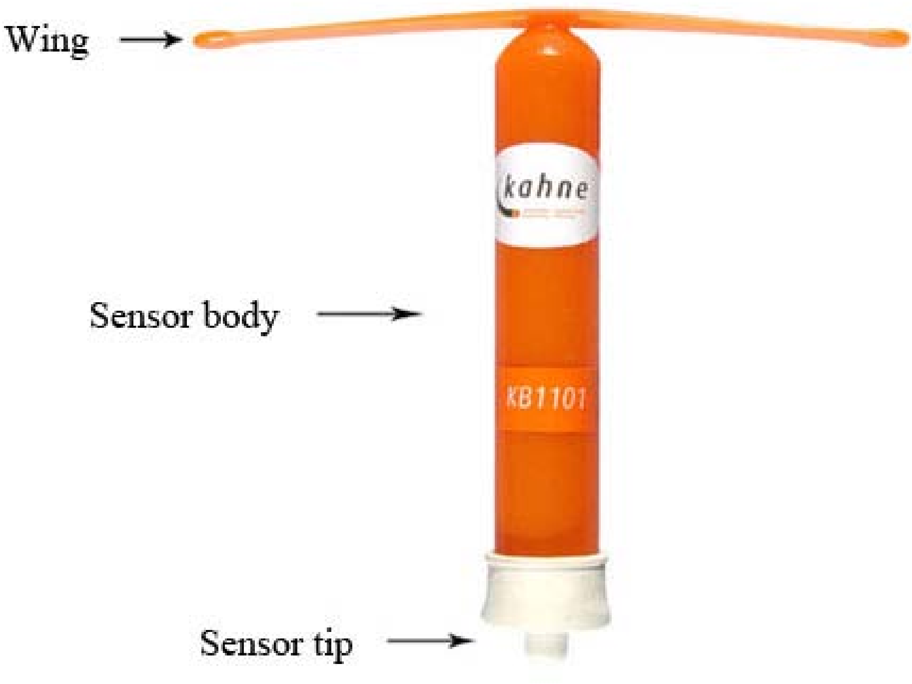


Figure 1.4.1 Kahne rumen sensor (bolus): type KB1101 (measures pH, pressure and temperature); wings are to prevent bolus to be regurgitated; sensor tip is covered by a plastic cap for protection before use.

The sensor body is designed to be inserted via mouth using standard balling gun. The wings are held against the sensor body during dosing, and return to the spread position once in the rumen. The wings of the sensor are to prevent regurgitation. There is a low power FSK radio transceiver, an inbuilt data logging system and a lithium ion battery in the body. The multiple sensors in the sensor tip allow the measurement of temperature, pressure and pH. Table 1.3.1 showed the property of the sensor on measurement of pressure, temperature and pH (unpublished data by Kahne Ltd).

Table 1.4.1 The measurement range, resolution, accuracy and drift of pressure, temperature and pH of rumen sensor:

	Measurement range	Resolution	Accuracy	Drift
Pressure	10-1100 mbar	0.1 mbar	+/- 1.5 mbar	<1 mbar / year
Temperature	-40 to 125℃	0.01℃	< 0.8℃	< 0.8℃ / volt
pH	2-12	0.01	+/- 0.02	Span: < +/- 0.02 / month

The Kahne devices had been used in several animal trials with intact beef cattle or cows (unpublished data by Kahne Ltd). The sensor was able to show change of pH, pressure and temperature dynamics due to feed changes. The relationships of ruminal pH to ruminal temperature and ruminal pressure could be drawn using data collected by rumen sensor. The animal showed no distress attributable to the sensors during trial. There was no rumen damage noted in the animal at the time of slaughter in a three month trial. The boluses were found in the raft of the rumen at slaughter. The boluses were physically intact and complete. The field receiver was 20 to 30 meters away from animals during trials. On one occasion, the receiver was able to capture the data from animals that was up to 200 meters away. The transmission intervals were different in different trials: 5 minutes being the shortest and 2 hours being the longest. The pH drift was higher than expected as a drift of 3 pH value in a month was found in an experiment (unpublished data by Kahne Ltd).

1.5 Aim and objectives

1.5.1 Aim

To characterise the Kahne devices (transceivers and boluses) and to use them to study the diet effects on pH change in rumen.

1.5.2 Objectives

To validate the pH, temperature, and pressure data that was collected by the boluses.

To test the data capture rate of Kahne devices (transceivers and boluses) and the factors affecting the data capture rate.

To use Kahne devices in a 7-day animal trial studying the effect on two diets to the pH change in cows.

Chapter 2: Materials and Methods

2.1 Introduction

The experiments were conducted at AgResearch Grasslands from autumn to spring 2008 using fistulated sheep and cows that were involved in other experiments. The fistulated sheep were from Massey University. Two fistulated cows (No.722 and 723) were retained at AgResearch as rumen fluid donors. Other fistulated cows (No. 713 to 721) were from AgResearch Ballantrae Hill Country Research station.

2.2 Kahne devices and the operation process

The data recording devices used in the experiments were from Kahne Ltd in Auckland, New Zealand. Kahne Ltd is a company that develops scientific instrumentation for rumen nutrition, animal behaviour and animal health research. Their technologies are also involved in animal welfare and animal emissions analyses.

The devices were consisted of software (Kahne Data Processing System V1.1, for enabling the configuration and communication of boluses and transceivers via a computer), transceivers (KR2001, KR2002, and KR2105 for capturing the data signals send by the boluses and transmitting the data to the storage device) and rumen boluses (KB1001 and KB1101, for measuring rumen environment and sending signals to the transceivers) that were made for *in vivo* measurement from the rumen of unrestrained cattle under commercial conditions.

Kahne Data Processing System V1.1 was installed in a laptop computer before the experiments were started. This software enabled the configuration of transceivers and boluses. It assists data collection, pH calibration and the connection of GSM modem.

There were a total of four kinds of Kahne transceiver used in experiments. The transceivers were KR2001, KR2002, KR2003 and KR2105. Transceiver KR2001, KR2002 and KR2003 needed to be connected to computer during data recording. Transceiver KR2105 only needed to be connected to computer during the property setup phase, because it has its own battery (to support data collection) and its own memory card (to store the data). The features of each transceiver are summarized in Table 2.2.1.

Table 2.2.1 The usage and the features of different transceivers:

Transceiver	Usage	Features
KR2001	Property configuration, pH calibration, data collection	USB cable, receiver, short whip antenna
KR2002	Data collection	USB cable, receiver, 5 element *Yagi antenna
KR2003	Data collection	RS232/USB cable, USB cable, receiver, 7 element Yagi antenna, pole mount, microprocessor, SD card, power supply
KR2105	Data collection	RS232/USB cable, USB cable, receiver, 7 element Yagi antenna, GSM modem, **field trailer, ***solar panel, ***6-volt rechargeable battery, microprocessor, SD card,

* Yagi antenna: a directional antenna system consisting of an array of a dipole and additional closely coupled parasitic elements. The reception distance is determined by the number of elements in the Yagi antenna. The area within a 60 degree range towards where the Yagi antenna is pointing is the best reception area. ** Field trailer: the trailer that enables KR2105 to be pulled by ATV into the field. *** Solar panel and rechargeable battery: it allows continuous usage in the field without the supply of electricity from a power cord.

There were two kinds of boluses used. KB1001 was for measuring temperature and pressure, while KB1101 was for measuring pH, temperature and pressure.

The configuration of Kahne devices included configuration of transceiver property and configuration of bolus property. The steps in the configuration process were:

1. The transceiver was connected to the computer through USB cable (KR2001 and KR2002) or through RS232/USB cable and USB cable (KR2003 and KR2105).
2. The Kahne operation system in the computer was turned on by double clicking the icon.
3. In the opening window, “Setting” was clicked and then “Configuring Kahne Device” was chosen. The “Configuration Setup” box would show in the computer. In the box, “Main Transceiver ID” (a) was set to 1 as a default. “Communicate with Bolus Directly” (b) was ticked and “Set Property” (c) was clicked to confirm the setting and to check if the transceiver was connected to computer successfully. This completed the configuration of the transceiver (Figure 2.2.1).

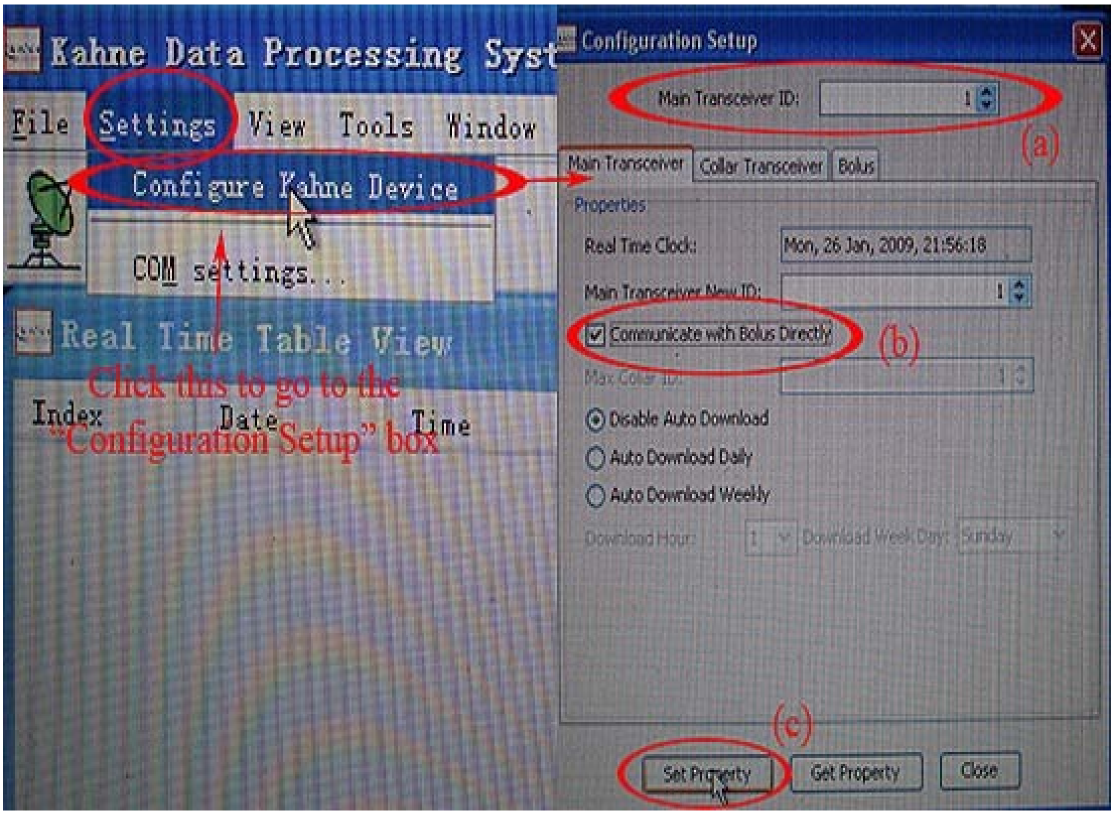


Figure 2.2.1 The process of using Kahne Data Processing System to get to “Configuration setup” box and configure the transceiver property (a→b→c).

4. "Bolus" tab (d) was selected (Figure 2.2.2). In the "Bolus" box, "Configure Bolus via Main transceiver" (e) was ticked. Bolus ID was entered into "Bolus Current ID" (f).
5. Bolus was turned on by activating the internal magnetic reed switch by removing the magnet that was taped onto the bolus away from the bolus.
6. "Get Property" (g) was clicked to see if the bolus and the transceiver had registered contact.
7. Values were entered to "Bolus New ID" (f) and "Sampling Frequency" (h). The default of the "Bolus New ID" was the same as "Bolus Current ID". The value of "Bolus New ID" was recorded in a log book if it was changed. The "Sampling Frequency" could be selected in increments of 1 second over the range 5 seconds to 59 seconds and 1 minute over the range 1 minute to 24 hours or as required.
8. "Set Property" (i) was clicked to confirm the setup. Bolus was then able to be used for data collection (Figure 2.2.2).

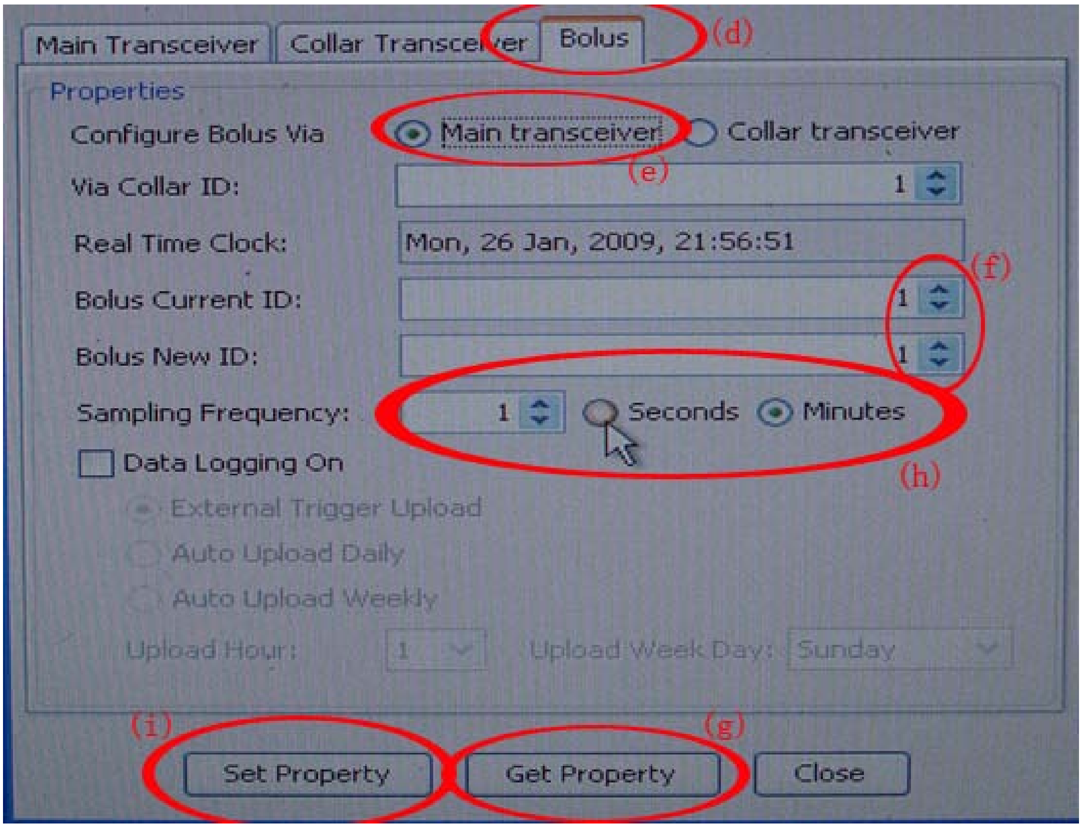


Figure 2.2.2 The process of setting the bolus property in the "Configuration setup" box

(d→e→f→g→h→i).

The data downloading processes for KR2001 and KR2002 required a direct connection to the computer and the Kahne software. For the KR2003 and KR2105, the data was stored in the SD card and could be downloaded directly into computer. The data downloading processes was:

1. “View” (j) (Figure 2.2.3) was clicked and then “Logged Data Table” (k) was selected. “Logged Data Table View” box was shown
2. The time that data recording was started was entered into “From Data Time” (l) and the time that data recording was finished was entered into “To Date Time” (m). The ID of Bolus that was used was entered into “All Boluses” (n). “Apply” (o) was clicked to show the data.
3. “Export” (p) was clicked to download the data that were shown in the box into an Excel file (Figure 2.2.3).

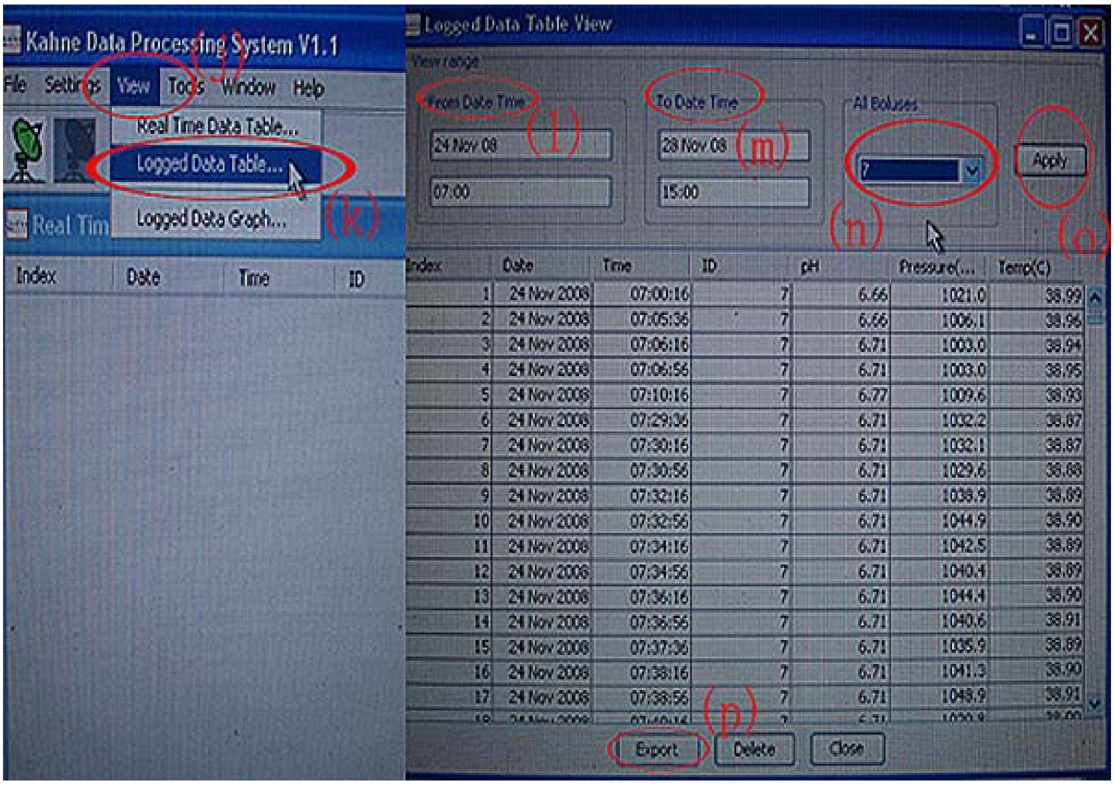


Figure 2.2.3 The process of downloading recorded data using Kahne Data Processing System (j→k→l→m→n→o→p).

2.3 Temperature calibration

The calibration of Kahne rumen boluses is important as the data captured using the boluses need to be validated before they could be used for analysis.

To calibrate the temperature of each bolus, a water bath (Grant water bath), a reference thermometer (Hanna thermometer that was calibrated by a manufacturing calibrated thermometer), a computer and a transceiver KR2001 were used (Figure 2.3.1).

The measurement range of temperature for boluses is -40 to 125℃ and the sensor measures up to 0.1℃ difference. There is no problem for boluses to measure the temperature in the rumen (37.8℃ to 40℃) (MacDonald, 1984).

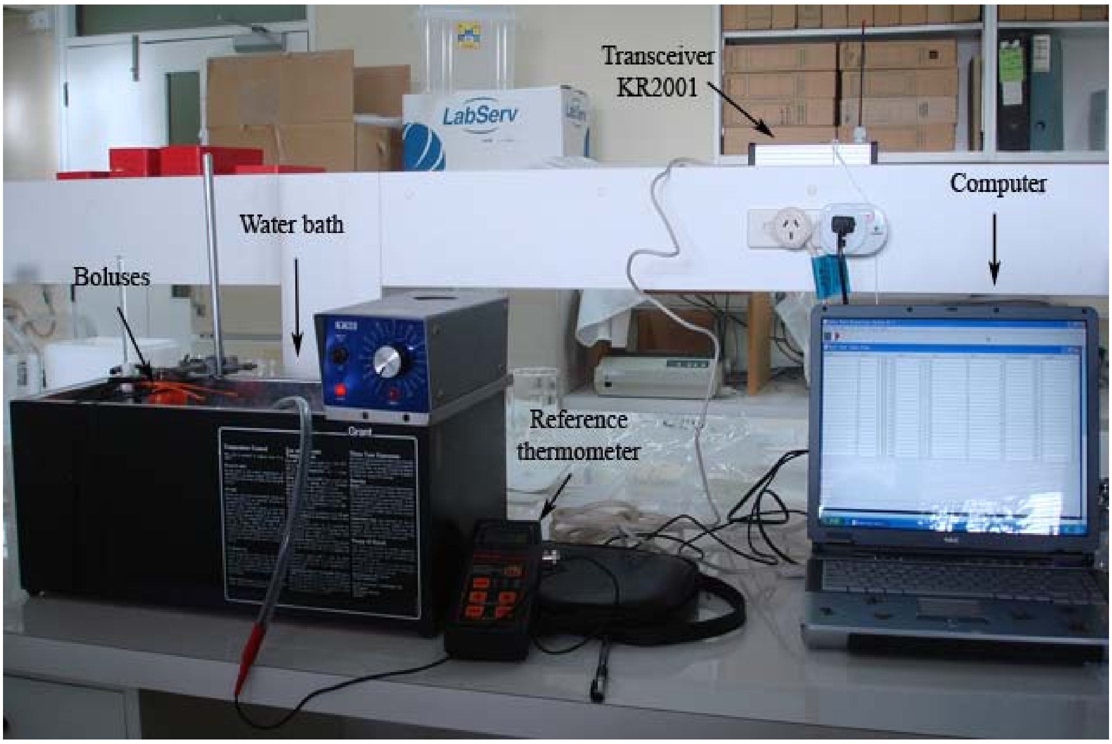


Figure 2.3.1 Equipments used for Temperature calibration of boluses: water bath, reference thermometer, computer, transceiver KR2001 and boluses.

Boluses were set at 1 minute transmission interval during calibration. Boluses were put into water bath with tip side down. The temperature probe of reference thermometer was placed at approximately same location as the boluses (Figure 2.3.2).

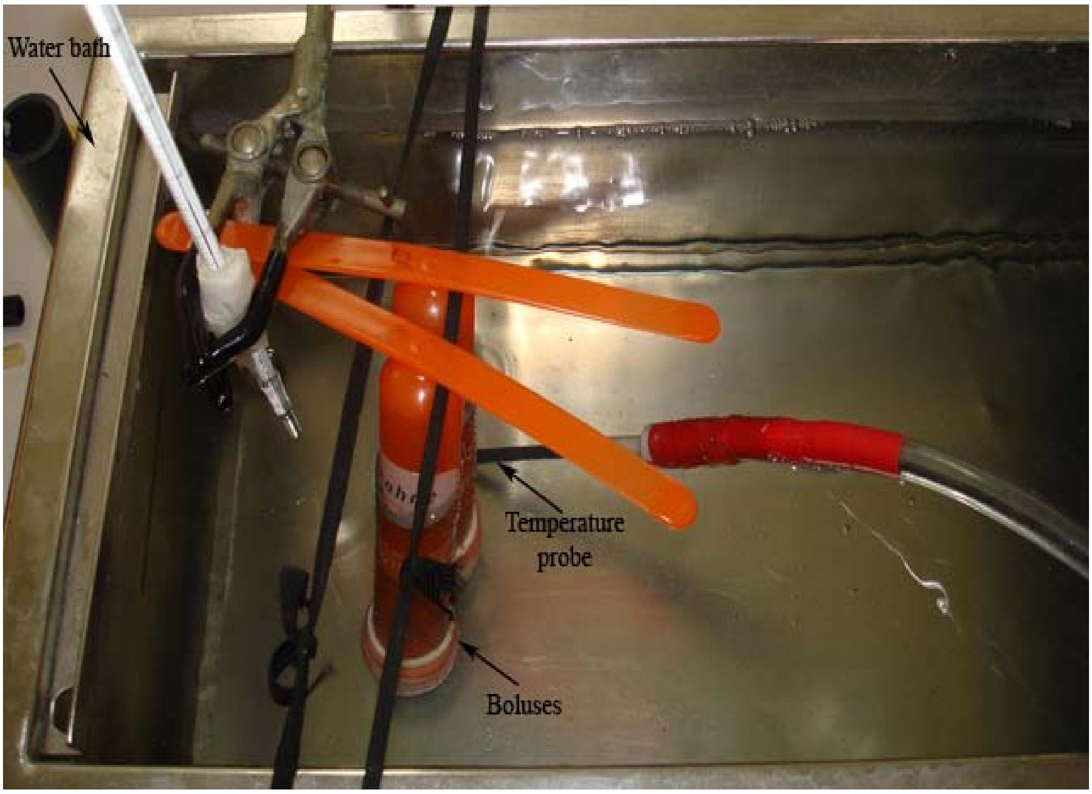


Figure 2.3.2 Boluses and temperature probe were put very close to each other in the water bath to ensure the bolus temperature and actual temperature was measured at very similar conditions.

Temperature in the water bath was stabilized initially at 30℃ (to allow a sufficient range to calibrated over) when calibration started. The boluses were kept in the water bath until the bolus temperature that was shown in computer, was stable. The elapsed time spent was recorded to determine how long each bolus needed to reach a stable temperature (Ts) and this temperature was recorded. The difference between the bolus temperature and the real temperature was calculated. The temperature in the water bath was then turned up to 35℃. After waiting for the same amount of time (Ts), the temperature shown in computer was recorded. This procedure was repeated at 36℃, 37℃, 38℃, 39℃, 40℃, 41℃ and 42℃. Calibration was done in steps of 1 degree between 35℃ to 42℃ as this temperature range includes the normal range of temperature in the rumen (37.8℃ to 40℃) (MacDonald, 1984). The bolus temperature was then plotted against actual temperature using Excel to get a trend line and slope from regression equation for each bolus (Figure 2.3.3, page 28). By using these formulas, the temperature recorded by boluses could be calibrated to actual temperature.

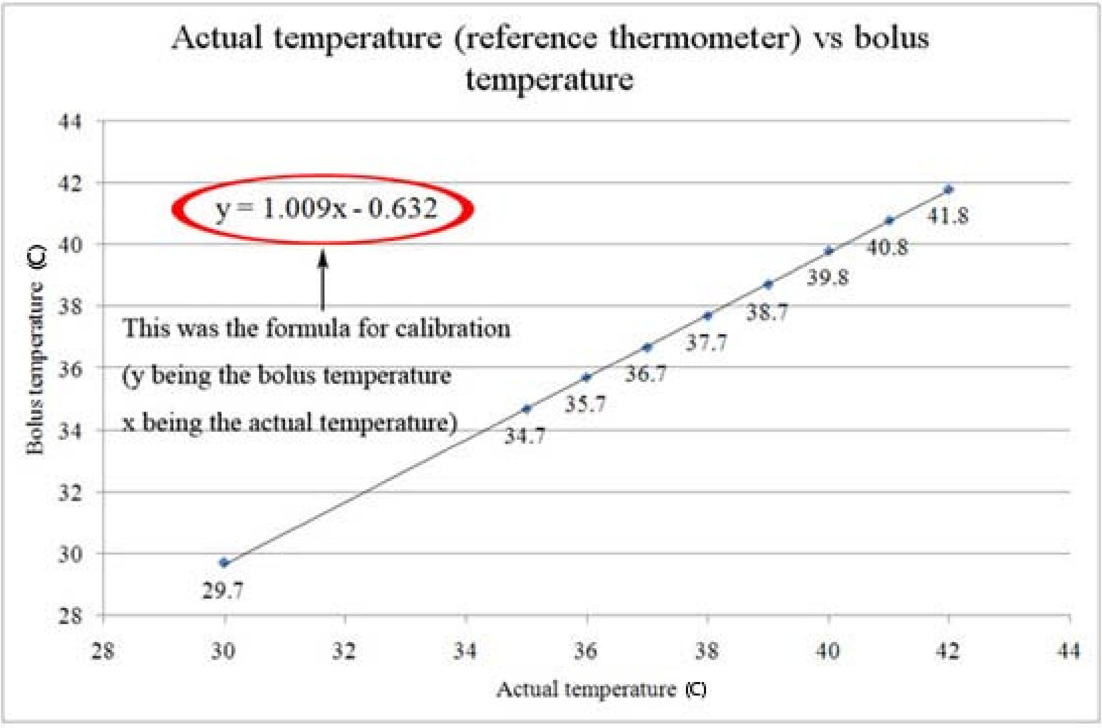


Figure 2.3.3 Graph of plotting bolus temperature against actual temperature for one bolus using Excel. A trend line and formula (red cycle) from regression analysis was formed for calibration of bolus temperature.

2.4 Pressure calibration

The equipment used for pressure calibration included a reference barometer (VAISALA digital barometer) that was calibrated by the manufacturer (Figure 2.4.1), a computer, boluses and transceiver KR2001.

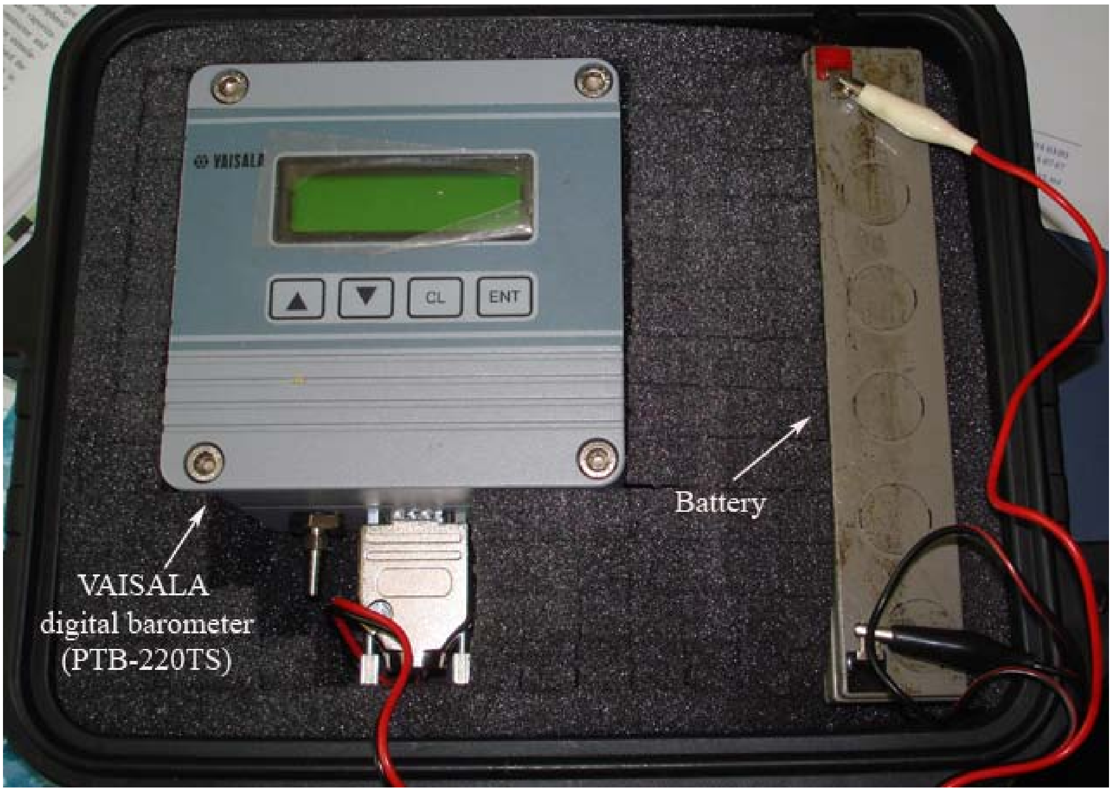


Figure 2.4.1 The reference barometer (VAISALA digital barometer [PTB-220TS]) and battery. The barometer and battery is fitted in a portable box.

Calibration was done in different weather conditions (pressure from high to low: sunny > cloudy > rainy) and different location (high pressure: AgResearch Palmerston North animal building block A; lower pressure 5/20 Ranfurly Street, Palmerston North) as significant pressure occurred (1024.75 mbar to 982.9 mbar). Boluses and reference barometer were put close to each other during data recording in order to make sure they were under same atmospheric pressure. This was to examine the relationship between pressure measured by boluses and the actual pressure measured by reference barometer. Both barometer reading and boluses reading were recorded and bolus pressure was plotted against barometer pressure using Excel. A trend line and slope were determined for each bolus (Figure 2.4.2). The formula was then used for to calibrate the pressure data collected.

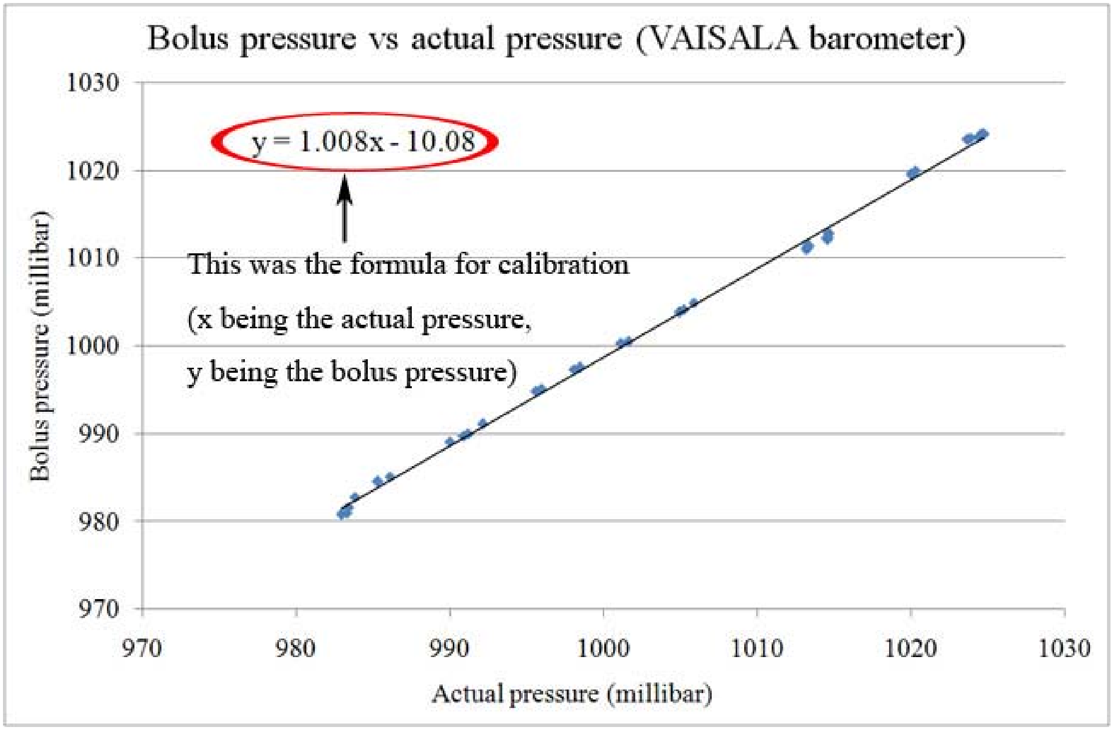


Figure 2.4.2 Graph of plotting bolus pressure against actual pressure for one bolus using Excel and a trend line and formula (red cycle) were formed suing regression analysis for calibration of bolus pressure.

The limitation of this pressure calibration process is that unlike reference thermometer which could be placed in the rumen through rumen fistula, the reference barometer could not be placed in the rumen to measure the pressure. However, it could still provide a relationship between bolus pressure and actual pressure under atmospheric pressure.

2.5 pH calibration

2.5.1 pH calibration for short-term (up to 24 hours) measurement

According the manufacturer, the pH drift would only occur in long-term (over 24 hours) base. Therefore, using the factory calibration process before each short-term experiment is sufficient for ensuring the accuracy of the pH data.

The factory calibration process was done immediately before each experiment. The factory pH calibration followed calibration process on "User Guide: KB1101 Bolus pH Calibration" (Kahne, 2008). The materials used were a water bath (Grant water bath), a computer, a transceiver KR2001, a thermometer, beakers, Kimwipe (laboratory wipes), magnetic stirrer (VELP heating magnetic stirrer ARE) and stir bars, Milli-Q water (deionised water), pH 7 buffer solution (Eutech Instruments Pte Ltd; batch Number M487/2; April, 2008) and pH 4.01 buffer solution (Eutech Instruments Pte Ltd; batch number M048/2; April, 2008) (Figure 2.5.1).

Figure 2.5.1 Equipments used for pH calibration: pH 4.01 buffer solution, pH 7.00 pH buffer solution, beaker, magnetic stirrer and stir bar.

The factory calibration process was as follows:

1. All boluses (protection caps removed) were put into Milli-Q water for a minimum of 20 minutes.
2. Two beakers were cleaned using Milli-Q water and dried with Kimwipes.
3. 150 ml pH 7 buffer solutions was poured into one beaker and 150 ml pH 4 buffer solutions were poured into the other beaker.
4. One magnetic stirrer was put into each of those beakers.
5. These two beakers were put into a water bath. The temperature of water bath was adjusted to 40+/-0.5℃.
6. Computer and Kahne software were turned on.
7. One of the boluses was switched on by removing the magnet.
8. The bolus was blot dried using Kimwipe and was put into the beaker that filled with pH 7 buffer solution.
9. The bolus was manually circulated in the solution for 20 seconds to ensure sure no air bubbles stayed at the sensor tip.
10. The magnetic stirrer was turned on for 5 minutes to stabilize.
11. The Kahne software was used to check the connection between the bolus and transceiver KR2001 and to set up transceiver property as described in Figure 2.2.1 (page 21).
12. Bolus property was set up through “Bolus” tab on “configuration Setup”: (a) “Main transceiver” was chosen, and then (b) “Get Property” was clicked. After “Get Property successful” was shown, the bolus property was reviewed; (c) Bolus ID number was entered into “Bolus New ID” and (d) 10 seconds was chosen in the “Sampling Frequency”, (e) “Set Property” was clicked and after “Set Property Successful” was shown, the bolus property was successfully set to transmit data every 10 seconds (Figure 2.5.2).

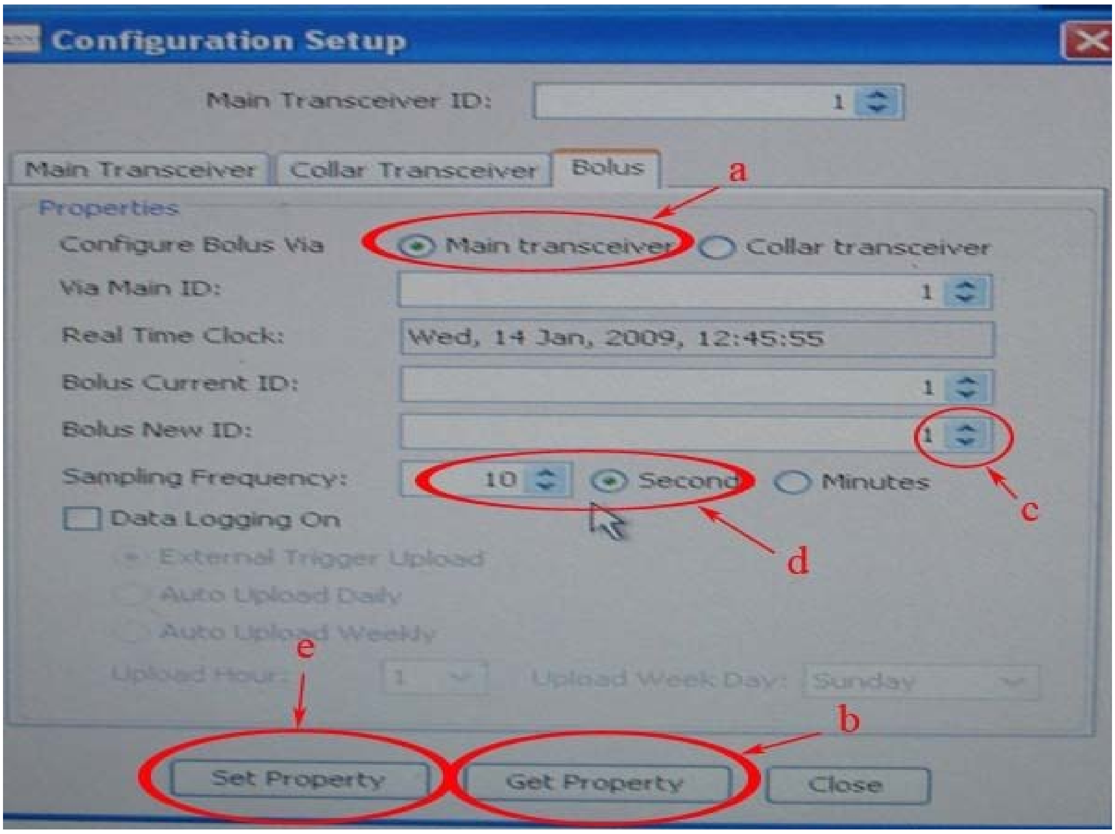


Figure 2.5.2 Steps of setting up bolus property through Kahne software before factory calibration process (a→b→c→d→e). Note, the transmission interval was set at 10 seconds (d).

13. "Tools" was selected and "Calibrate pH7" was clicked. Bolus ID was entered to calibrate the selected bolus in pH 7 buffer solution. This step was repeated until successful (Figure 2.5.3).

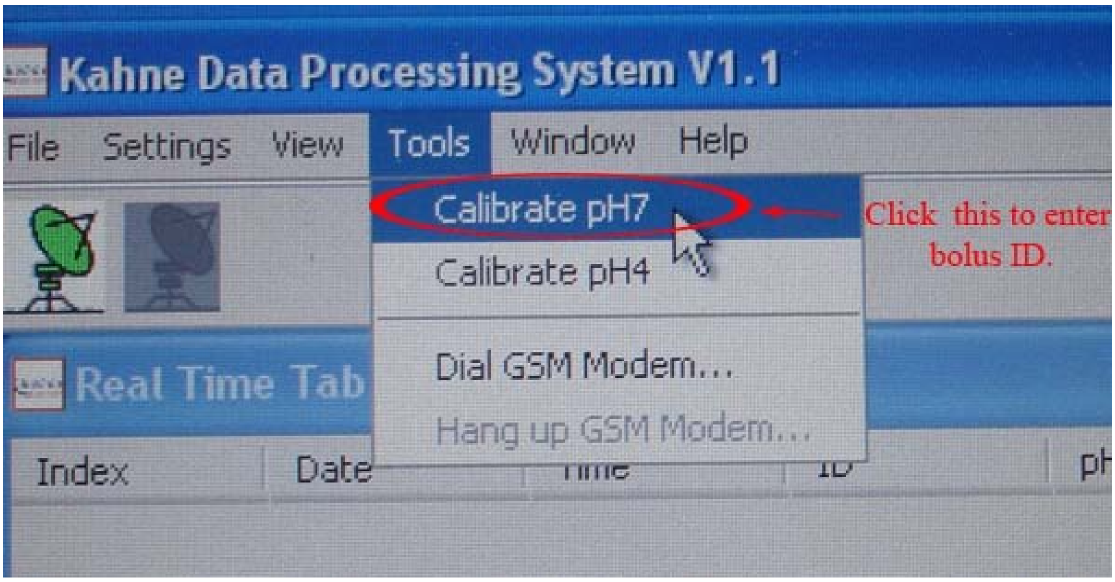


Figure 2.5.3 "Calibrate pH7" was clicked in order to enter the bolus ID for calibration.

14. The bolus was removed from the pH7 buffer solution, rinsed with Milli-Q water and blot dried with Kimwipe.
15. The bolus was put into the beaker that filled with pH 4 buffer solution.
16. Steps 9 to 12 were repeated.
17. In Kahne software, "Tools" was selected and "Calibrate pH4" was clicked. Bolus ID was entered to calibrate selected bolus in pH 4 buffer solution. This step was repeated until successful.

This process was done to each bolus before each experiment to ensure that at the beginning of experiment the pH of each bolus was calibrated.

2.5.2 Validation for pH drift

The pH drift that occurs during the long-term experiment affects the accuracy of the data collected. Therefore, it is essential to validate the pH drift. The pH validation process performed during each experiment required a calibrated pH meter (Hanna pH meter). The process was as followed:

1. The rumen-fistulated cow was restrained in a cattle crate (Figure 2.5.4).

Figure 2.5.4 A fistulated cow was confined in a cattle crate for pH validation process.

2. The cap (rubber stopper) of the cannula was removed.
3. The bolus in the rumen was located manually (which could take up to 20 minutes).
4. The Hanna pH probe was turned on before direct measurement (Figure 2.5.5).

Figure 2.5.5 The pH probe was ready to be inserted into rumen through cannula (probe showed pH 7.24 before inserted into rumen).

5. The pH probe was inserted into rumen and was placed close to the bolus tip. The pH probe was kept in the rumen until the pH reading was stabilized (Figure 2.5.6). The pH reading and the time that reading was recorded was written down on the lab book.

Figure 2.5.6 The pH reading (6.59) was recorded when it reached a stabilized phase in order to get the actual pH in the rumen.

2.5.3 pH calibration for long-term experiments

When the experiment was finished, the difference between bolus pH and the probe pH at the time of direct measure was calculated (there should be two measurements a day during the experiment). These values were used in the graph as y axis. The time of each measurement was converted to minutes elapsed from the start. Elapsed time was used on the x axis in the regression analysis. A trend line and slope and intercept were determined (Figure 2.5.7). This formula was used to correct for the drift in pH that occurred in the experiment. The calibrated pH data was then used in the data analysis.

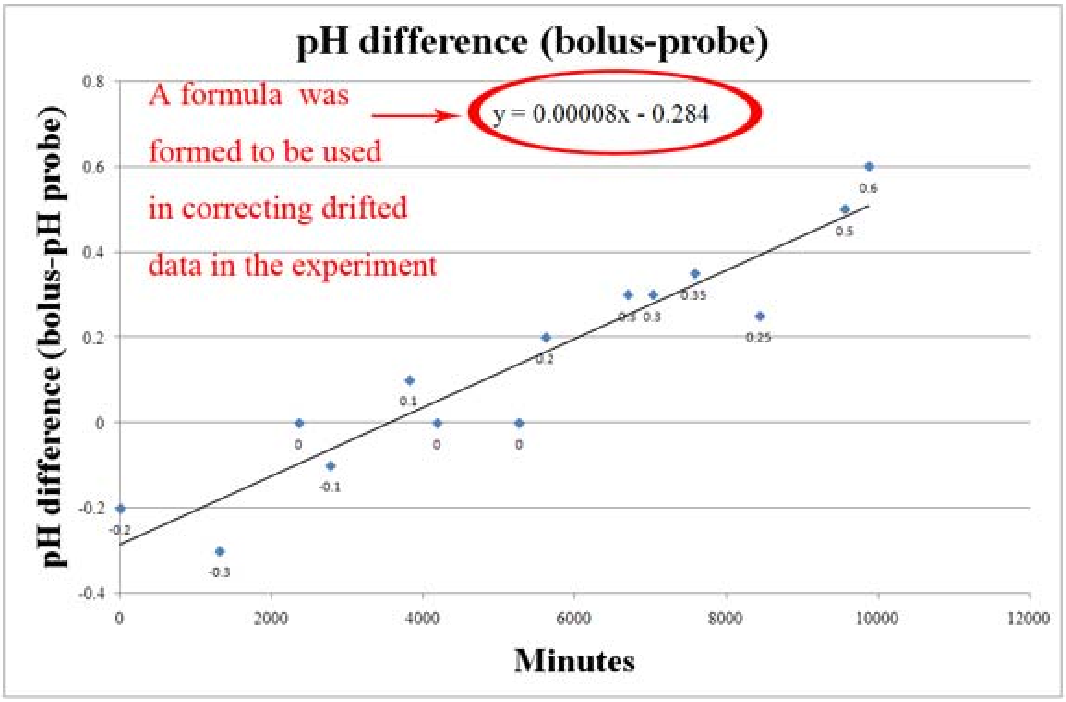


Figure 2.5.7 Graph of plotting pH difference against time for one bolus using Excel and a trend line and a formula (red cycle) was formed from regression analysis to correct for the drift in pH occurred in the experiments.

2.6 Data recording correlated indoor with boluses external to the animal

The experiment to determine data capture rate when the boluses were outside of the animal body was done in the same yard where cows were confined during the indoor experiments (section 2.8). The experiment used the KR2105 transceiver and 7 boluses. The steps in this procedure were as follows:

1. The KR2105 was setup and placed 3 meters away from one side of the yard (Location A, Figure 2.8.2). All seven boluses were set up to transmit at 1-minute interval.
2. Boluses were placed in the yard (approximately 5 meters away from the KR2105 transceiver) for 20 minutes and the number of data captured for each bolus was recorded. The capture rate for each bolus was calculated by dividing the actual numbers of transmission captured by the expected number of 20 (1-minute interval at 20 minutes)
3. Boluses were placed randomly in the yard (the distance to KR2105 ranged from 5 to 15.8 meters) for 20 minutes and data capture rate was recorded. The capture rate was calculated in the same way as described is procedure 2.
4. Step 2 was repeated 4 times with the distance from the boluses to KR2105 ranging from 6.7 to 20.2 meters.
5. Boluses were placed at the edge of other side of the yard (22 meters from KR2105) for 20 minutes and the data capture rate was calculated.
6. The mean data capture rate for each bolus was calculated to compare among boluses. The mean capture rate at various distances was calculated using the capture rate calculated for each bolus from different distance. This was used to compare the effect of distance on the data capture rate when the boluses were placed external to the animal.

2.7 Indoor data recording from sheep

Indoor data recording from sheep was done using fistulated sheep, boluses (KB1001 and KB1101), transceiver KR2001, and a laptop computer. The experiments were done to test the data transmission of the boluses in small animals.

There were four fistulated Romney sheep in the experiment (labeled as No. 12, 269, 315, and Y10). All sheep were over 5 years old, and weighed 45.43 ± 2.44 kg (mean ± standard deviation).

The sheep were kept in a 5-meter x 5-meter pen in animal building block A in AgResearch Palmerston North. The computer and transceiver KR2001 were set in the middle of one side of the holding area (Figure 2.7.1). The maximum distance between the transceiver and the bolus that was inserted in the rumen of sheep was 6.7 meters during the experiments.

Figure 2.7.1 The pen (5m x 5m), the location of the sheep, computer and the transceiver KR2001.

Each sheep was offered 500g of food twice daily at 08:45 and 15:45. The information of experiments using sheep was summarized in Table 2.7.1.

Table 2.7.1 Summary of the measurements with boluses inserted in sheep:

Date	Sheep ID	Equipment used	Transmission interval	Diet
17th to 19th April	No. 12, Y10	KR2001 and 2xKB1001	2 minutes	hay
21st to 23rd April	No. 269, 315	KR2001 and 2xKB1001	2 minutes	hay
8th to 10th May	No. 12, 269, 315, Y10	KR2001, 2xKB1001 and 2xKB1101	2 minutes	*synthetic diet
13th to 14th June	No. 12, 269, 315	KR2105 and 3xKB1101	5 minutes	hay

* The main components of the synthetic diet were hay, casein, fructan and barley straw. The synthetic diet also contains trace amounts of sunflower oil, mineral mix and NaCl. The ratio of protein and carbohydrate was altered in the synthetic diet by changing the amounts of casein and fructan.

The insertion of boluses and the direct measurement of rumen pH and temperature were done before feeding. Sheep was restrained by one person, while another person opened the fistula cap and inserted the bolus and the temperature probe.

Data were monitored during the experiment by observing the “real time Table view” in the Kahne software. The collected data were logged out through Kahne software at the end of each experiment.

2.8 Indoor data recording from stall-fed cows

The indoor data recording from cows was done in the yard that was behind the animal building block A in AgResearch Palmerston North (Figure 2.8.1). The experiments were done to test the data transmission in large animals in the indoor conditions

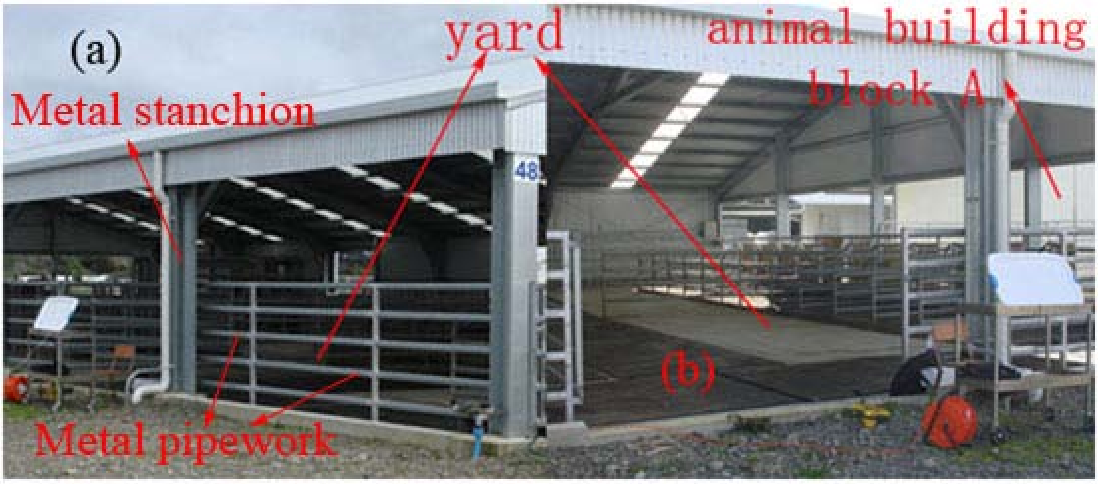


Figure 2.8.1 View of yard that is behind animal building block A from two different angles (a and b, which were also showed in Figure 2.8.2). There are metal pipework surrounds the holding area.

The yard was 19 meters long and 8.5 meters wide. There were 12 feeding stalls and 1 water trough in the yard. The cows were free to roam the remainder of the holding area when they were not eating (Figure 2.8.2).

In Figure 2.8.2, the distance ranges between boluses that were inserted in cows in the yard and transceivers that located in positions A, B, and C were 3 to 22 meters for position A, 2 to 13 meters for position B, and 0 to 20 meters for position C. Positions A, B, and C also differed with respected to the metal pipework of the sides of the yard. Positions A and B were external to the yard, and as a result, the line-of-sight for transmission from the cows to the transceiver was through the metal pipework and metal framework. There was a metal stanchion and a metal pipework (5 pipes as shown in Figure 2.8.1) between position A and the holding area, while for position B, there was a metal pipework (3 pipes) as obstacle (it is suspected that the metal pipework might affect the data transmission). Position C was internal and there was no line-of-sight obstruction to transmission. To compare location factors for transceiver KR2105, positions A and C were used. Position B was used only when 2 transceivers were recording simultaneously, with another transceiver in position C.

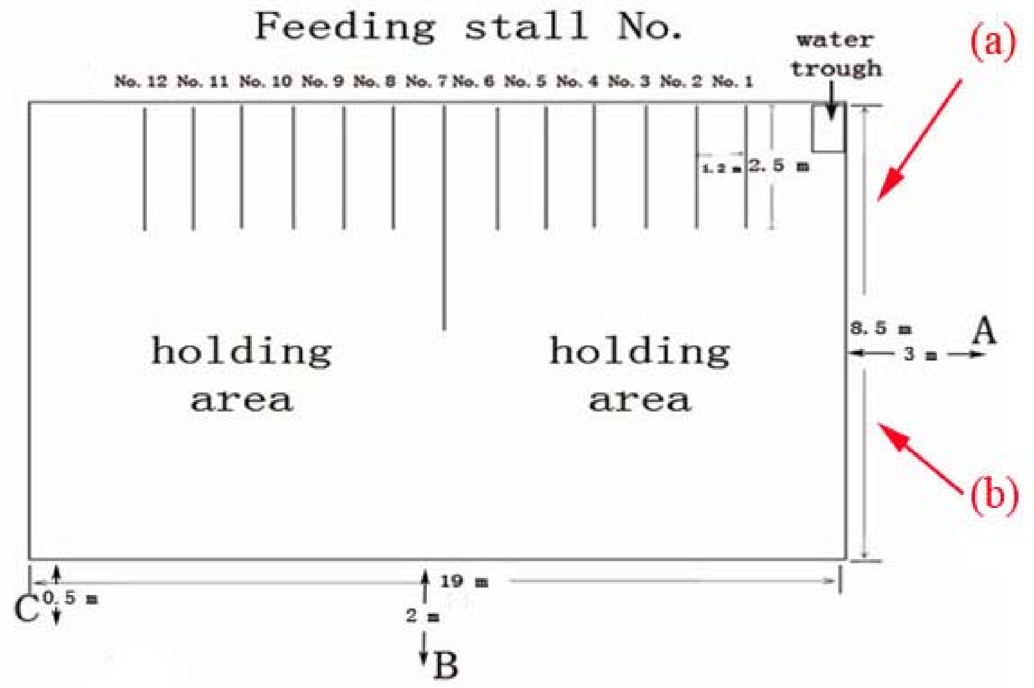


Figure 2.8.2 Overhead view of yard. Positions A, B, and C represented the different locations of transceivers during different experiment. (a) and (b) represented the two different angles of view to the yard showed in Figure 2.8.1.

There were total 11 fistulated cows (labeled from No. 713 to 723) used. All cows were non-lactating Holstein-Friesian x Jersey crossbred, over 9 years old. The mean body weight of cows was 516.55 ± 23.18 kg.

During the measurements, cows were fed twice a day (around 09:00 and 16:00). Cows were secured in feeding stalls for 40 minutes. Each cow was offered a similar quantity of baleage (7.6 kg for each cow). When finished eating, the cows were released from the feeding stall into the holding area. The experiments were summarized in the Table 2.8.1.

Table 2.8.1 Summary of indoor measurements with boluses inserted in cows:

Date	Equipments	Cows ID	Diet	Transmission interval	Transceiver position
21st to 22nd May	KR2001 and 2xKB1101	No.722 and 723	grass	5 minutes	B
14th to 17th June	KR2105 and 5xKB1101	No.713, 714, 715, 716, 717, 718, 719, 720 and 721	baleage	5 minutes	A
28th to 30th June	KR2105 and 6xKB1101	No. 713, 715, 716, 717, 718 and 721	baleage	5 minutes	A
27th to 30th September	*KR2105, KR2002, 1xKB1001 and 4xKB1101	No.713, 714, 715, 716, 717, 718, 719 and 720	baleage	**interval was varied (from 55 seconds to 35 seconds)	B (KR2002) and C (KR2105)
25th to 28th November	*KR2105, KR2002 and 4xKB1101	No.716, 717, 718, 719, 720 and 721	baleage	**interval was varied (from 55 seconds to 40 seconds)	B (KR2002) and C (KR2105)

* Two transceivers (KR2105 and KR2002) were used because of the suggestion made by Kahne Ltd that two transceivers could help increase the data capture rate and hence increase the accuracy of the experiment results. The two transceivers were placed in different locations to cover the most area with good reception as one transceiver could have poor reception in some places in the area.

** The transmission interval was varied because Kahne Ltd suggested that when more than 4 boluses with same transmission interval were used at same time, some signals could overlap to each other, causing loss of data. In extreme cases, some boluses could lose signal during the entire experiment. The varied transmission interval for each bolus could reduce the chance of that happening.

2.9 Outdoor data recording from grazing cows

Experiments of outdoor data recording were done in various paddocks (depending on which one was available) in AgResearch Palmerston North. A small area of the required shape and dimensions were formed using temporary electric fences. The experiments were done to test the data transmission in large animals in the outdoor conditions

8 different cows were used in outdoor experiments with some experiments using two cows while for other measurements, the remaining 6 cows were used. All cows were non-lactating Holstein-Friesian x Jersey crossbred, over 9 years old. The mean body weight of cows was 519.36±22.22 kg Table 2.9.1 summarizes the experiments. During the experiments cows stayed in the holding area but were free to move around and graze at will. Cows were offered a new break of grass and hay once a day at approximately 10:00 in the morning.

Table 2.9.1 Summary of outdoor measurements with boluses inserted in cows:

Date	Equipments	Cows ID	Diet	Transmission interval	Transceiver position
15th to 16th May	KR2001, 1xKB1001 and 1xKB1101	No. 722 and 723	grass	5 minutes	A
12th to 13th June	KR2105 and 2xKB1101	No. 722 and 723	grass	5 minutes	B
21st to 25th November	KR2105, KR2002 and 4xKB1001	No. 716, 717, 718, 719, 720 and 721	grass and hay	interval varied (55 to 40 seconds)	C (KR2105) and D (KR2002)

The holding area in the outdoor experiments was constructed to be as similar as possible in area and shape to the yard in indoor experiments. The maximum distance between the transceiver and the boluses that were inserted in the rumen of cows was 17 meters during the experiments.

The overhead view of holding area and the location of transceiver in each outdoor experiment was shown in Figure 2.9.1 and Figure 2.9.2.

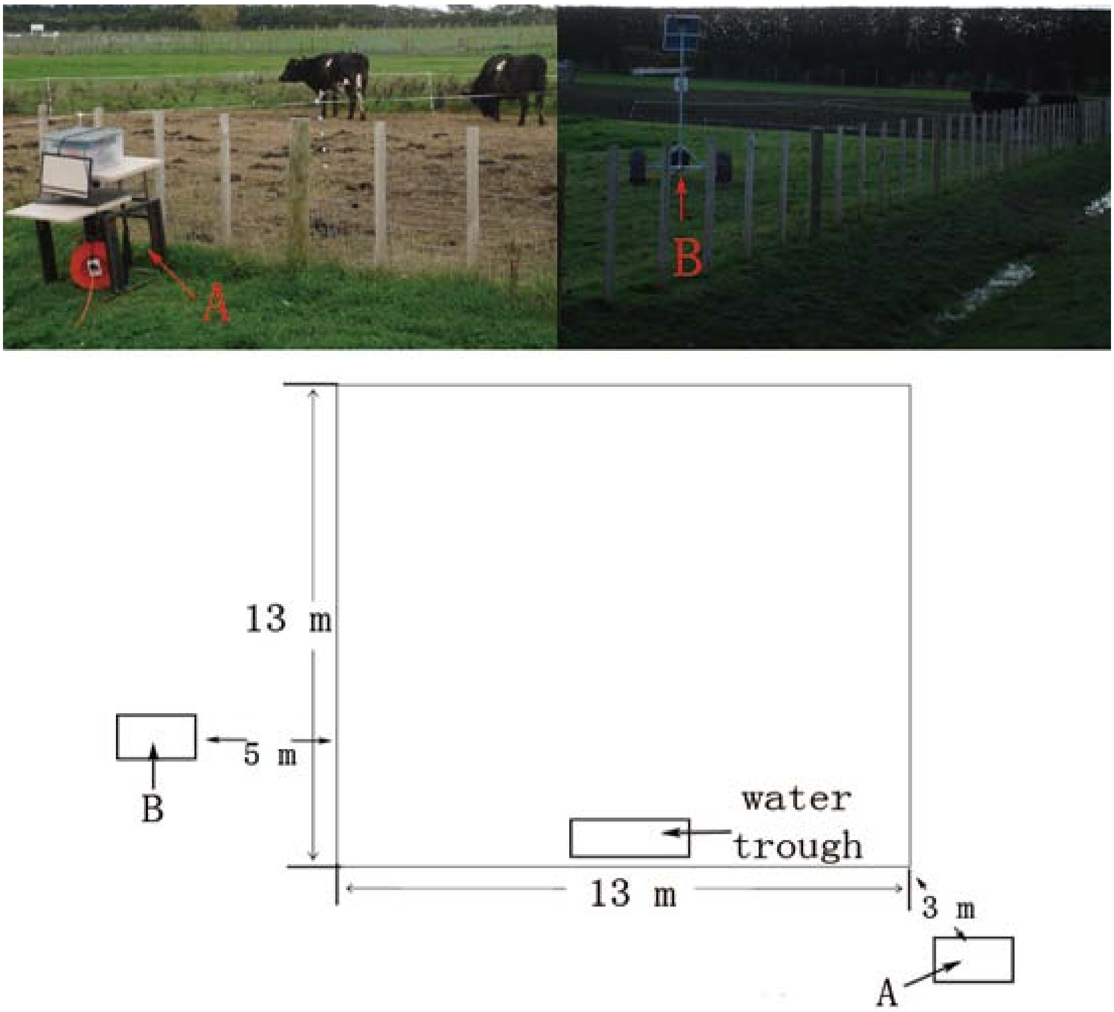
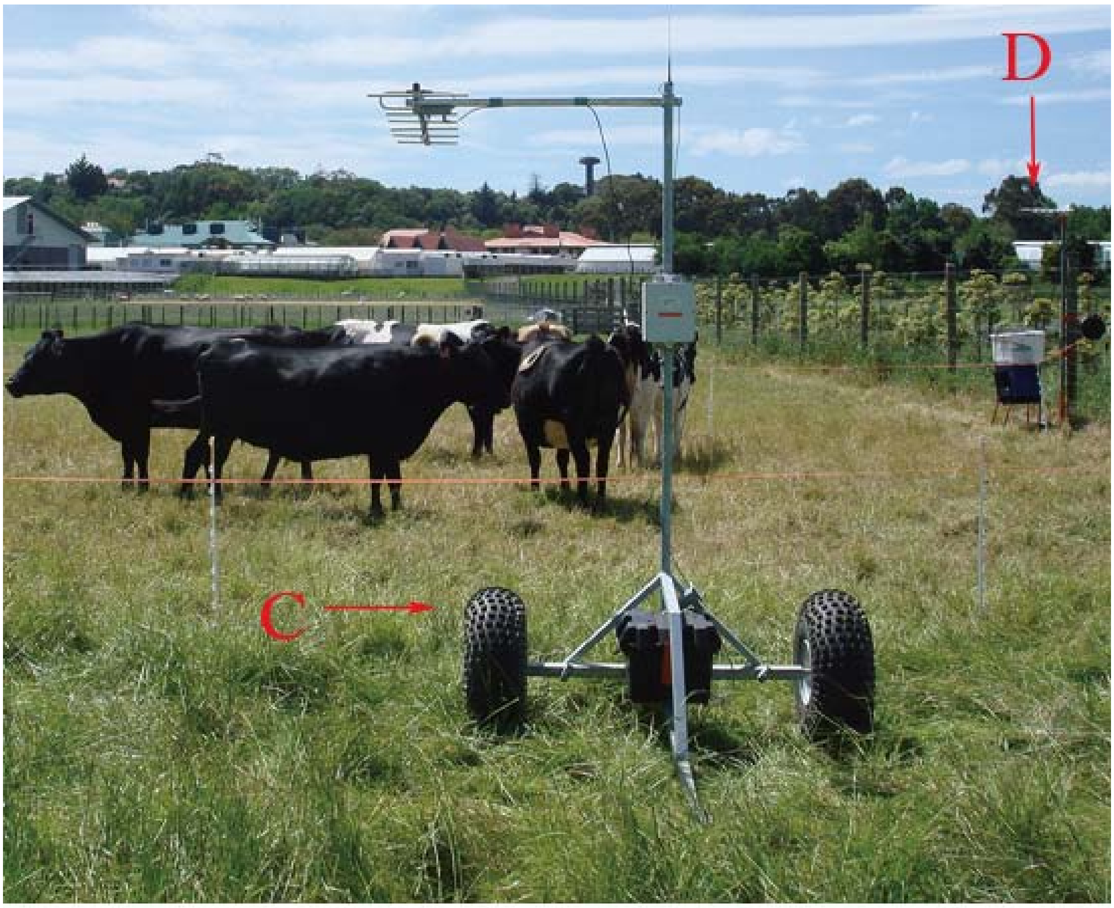


Figure 2.9.1 Pictures of holding area (13m x 13m) of measurements in May and June (Table 2.9.1, page 42) and the overhead view of holding area. Positions A and B indicated the location of the two transceivers.



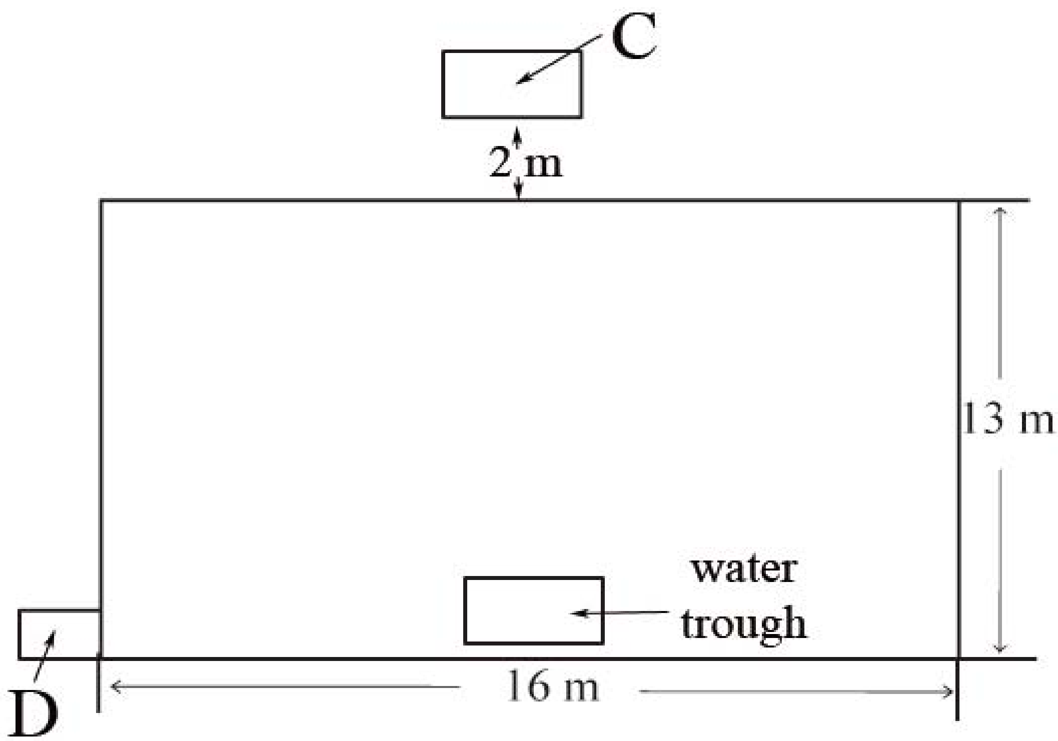


Figure 2.9.2 Picture of holding area (13m x 16m) measurements on November (Table 2.9.1, page 42) and the overhead view. Positions C and D indicated the location of the two transceivers.

2.10 Calculation of data capture rate

For each experiment, the data capture rate was calculated. This was based on the observation that the data captured were seldom complete, possibly due to the animal moving out of transmission range and other undetermined factors such as the effect of rumen contents. The data capture rate was used as a factor for evaluating the characteristic of the equipment under different experiment conditions.

Listed are the descriptors used for evaluating the capture rate under different measurement conditions:

1. The data capture rate outside the animal. This was calculated by dividing the actual number of transmission data captured by the transceiver by the number of data transmissions that were expected to be captured (i.e. the number determined from the product of the transmission interval and duration). It was used to separate the effects of the animal and rumen contents from distance *per.se.* on capture rate, and to determine if the building structure (in the indoor experiment) itself was causing local interference to data transmission
2. The mean data capture rate when transmitting from the rumen. This was calculated by dividing the actual number of data transmissions captured by the transceiver by the number of data transmissions that were expected to be captured. Under conditions where more than one transceiver was used (in order to improve the capture rate), the data for the same bolus from each transceiver were combined using Excel and the overlaps were deleted. The mean data capture rate reflects the transceiver's ability to capture data signals sent by the boluses. It can be used to compare between different transceivers or the same transceiver under different experiment conditions.
3. Hourly data capture rate. This was calculated by dividing the actual number of data transmission captured per hour by the number of data that were expected to be captured in that hour. This was to determine the transceiver performance in hourly intervals. It was used as a measure of the variability of the data capture rate.

From this data a frequency distribution of data capture rate was prepared. The data capture rate on an hourly basis was divided into ten groups with each group covering a 10% capture rate range (i.e. 0-10%, 10.01-20%, 90.01-100%). This was to examine the hour to hour consistency of the data captured during the experiments.

2.11 Use Kahne device for detecting animal to animal variation

The experiment to study the animal to animal variation was done on 14th to 17th June (Table 2.8.1, page 41). The experiment using 9 cows (ID: 713 to 721), 5 boluses (KB1101) and transceiver KR2105. The cows were held in the yard and fed equal amounts of baleage, twice daily, at same time of the day. The transceiver KR2105 was located in position A in Figure 2.8.2 (page 40) to receive the signals sent by boluses. The transmission interval was set at 5 minutes, which means there were 12 data signals sent per hour. The boluses were switched between different cows for each 24-hour period during the experiment to enable 48-hour recording for 6 cows and 24-hour recording for 3 cows. The recording started from 9:00 14th and finished at 8:00 17th, which gave a total period of 71 hours. The mean pH of the whole period and the mean pH for each hour were calculated for each cow. The data capture rate was also calculated to determine the reliability of the data.

2.12 The effect of diet on pH change in cows

To determine the effect of diet on rumen pH, 6 fistulated cows (non-lactating Holstein-Friesian x Jersey crossbred, ID: No.716 to No.721), 4 boluses (KB1101) and 2 transceivers (KR2105 and KR2002) were used. With 4 boluses and 6 cows to measure, the boluses were switched to different cows for each 24-hour period during the experiment. This was to ensure that over a 7-day period there were equal numbers of 24-hr recordings for each cow. The 24-hour period was started from 09:00 on the first day and finished at 09:00 the next day. This was determined by the feeding time of the cows and the time that the boluses were switched among. The transmission interval of each bolus was set differently from 40 to 55 seconds as advised by Kahne Ltd in order to increase the data capture rate (Table 2.8.1, page 41).

The experiment involved two parts: indoor (Table 2.8.1 date: 25th to 28th November, page 41) and outdoor (Table 2.9.1 date: 21st to 25th November, page 42). This data set was not only for comparing pH change from different diets (grass and hay fed outdoors vs. baleage fed in doors) but also for comparing the difference of data capture rates (indoor vs. outdoor).

For the indoor part of the experiment, cows were kept in the yard behind the animal building Block A at AgResearch Palmerston North (Figure 2.8.2, page 40). Cows were fed twice a day at approximately 09:45 (varied between 09:35 and 10:25) and 15:45 (varied between 15:45 and 15:55). Each cow was offered 7.6 kg baleage at each meal (total of approximately 6.1 kg dry matter per day). Cows were allowed to eat for 40 minutes in the feeding stall and were then returned to the holding area. This was sufficient time for the cows to consume their ration. For the cows with boluses inserted in the rumen, direct measurements of rumen pH were taken twice a day before each meal in order to monitor the pH drift for each bolus. Cows were moved to and restrained in a cattle crate for access to the rumen via the fistula. The time of taking the direct measurement was before the meal in the morning (between 09:20 and 10:00), and in the afternoon (between 15:25 and 15:35)

For the outdoor part of the experiment, cows were kept in a restricted area (13x16 m^2) in a paddock at AgResearch Palmerston North (Figure 2.9.2, page 44). Cows were fed once a day at approximately 10:00 in the morning (varied between 10:00 and 10:20). Each cow was offered 2 m^2 of fresh grass and 6 kg of hay per day (total of approximately 6 kg of dry matter). Cows were free to choose the time and the order in which they ate the grass or hay. For those cows with a bolus inserted, direct measurements of ruminal pH were taken twice a day in order to monitor the pH drift that occurred in each bolus. Cows were walked to the nearby yard, and restrained in a cattle crate for access to the rumen via the fistula. The time of taking the direct measurement was before the grass and hay feed in the morning (between 09:20 and 09:50), and in the afternoon (between 15:25 and 15:40), which was approximately the same time as the afternoon measurement with cows fed baleage

For each diet, the mean rumen pH of different cows at each day was calculated using the data recorded by the boluses and transceivers. The results were then used to calculate the mean pH for each day, the mean pH for each cow, and the overall mean pH for each diet.

To simply presentation of the daily pattern of pH recording from short transmission interval (ranged from 55 to 40 seconds) giving a large number of data points (ranged from 1570 to 2160) in a 24-hour period, to show the contrast between diets, the pH for each 15 minutes was aggregated into one data point by taking the mean pH over successive 15 minute intervals. This reduced the number of data points to 96 (4 per hour times 24 hours). It is considered that the accuracy of the pH pattern could be affected. However, the observation showed that using 15 minutes as the interval for aggregation is appropriate as it had an insignificant effect on the change of pH. It was also noticed that by aggregating the data, the sudden change of pH caused by by the moving of the bolus in the rumen from rumen contractions, rumination, and the heterogeneous nature of the rumen environment can be minimized.

The 24-hour pH pattern for each cow at two diets was plotted using the aggregated data. Because there was a difference in the time of feeding for each day, the data was processed (if the variation of time was large) to present the pH pattern more precisely. If the difference in time at which feed was offered each day was small, the effect of this variation on the aggregated mean for each 15-minute interval was considered insignificant, and in deriving the rumen pH across days, the data were meaned according to actual clock-time. If time variation in time of feeding was large, this was considered a significant difference in relation to the time-scale of pH changes in the rumen and for this reason, pH across days were aggregated on the basis of elapsed time from feeding (i.e. data points for each cow were transformed from actual time to elapsed time from feeding and averaged on this basis).

For grass and hay diet, the time of which feed was offered varied from day to day by less than 20 minutes, this was considered small.

For baleage diet, the time of which feed was offered in the morning varied from day to day for different cows. Cow No.716, 719 and 720 had small variations (less than 10 minutes) from day to day, while cow No. 717, 718, and 721 had larger variations (40 minutes) from day to day. For the time of feed in the afternoon, the variation was small (less than 10 minutes) for all cows.

The 24-hour pH pattern for each diet was then plotted using the mean pH calculated from all cows.

SAS was used as the statistical analysis tool for determine the effect of different factors on the pH change. The analysis of diet effect was focused on the patterns of pH change when cows ate their meal and when they were resting (i.e. not eating). For baleage diet, the food was available from 09:45 to 10:25 in the morning and from 15:45 to 16:25 in the afternoon (40 minutes per meal). Cows did not eat again until the next meal. For the grass and hay diet, the food availability was predominantly from 10:00 to11:00 in the morning (60 minutes per meal). However, there would have been residual grass and hay available during the remainder of the 24-hour period for the cows to eat. It was assumed that for the grass and hay diet a large proportion of the daily allocation was consumed during the first hour, with comparatively small amounts consumed during the remainder of the day. This was considered in the regression analysis and hence has an effect on the pattern of pH change.

The estimates for each cow within each diet were analysed by regression, fitting terms for animal and feed availability, and weighted by the number of observations. The slope for pH change with or without food was analysed and predictions from the regression model showed the pH decrease when food was available and the pH increase when animals were resting. The slope of pH change for each cow was calculated to test the variation between different cows (i.e. the effect of animal)

Chapter 3: Results

3.1 Temperature calibration

The temperature calibration of each bolus was conducted using linear regression analysis to form a relationship (Figure 3.1.1) and enables the temperature recorded from each bolus to be corrected to actual temperature by using the slope and intercept (Table 3.1.1).

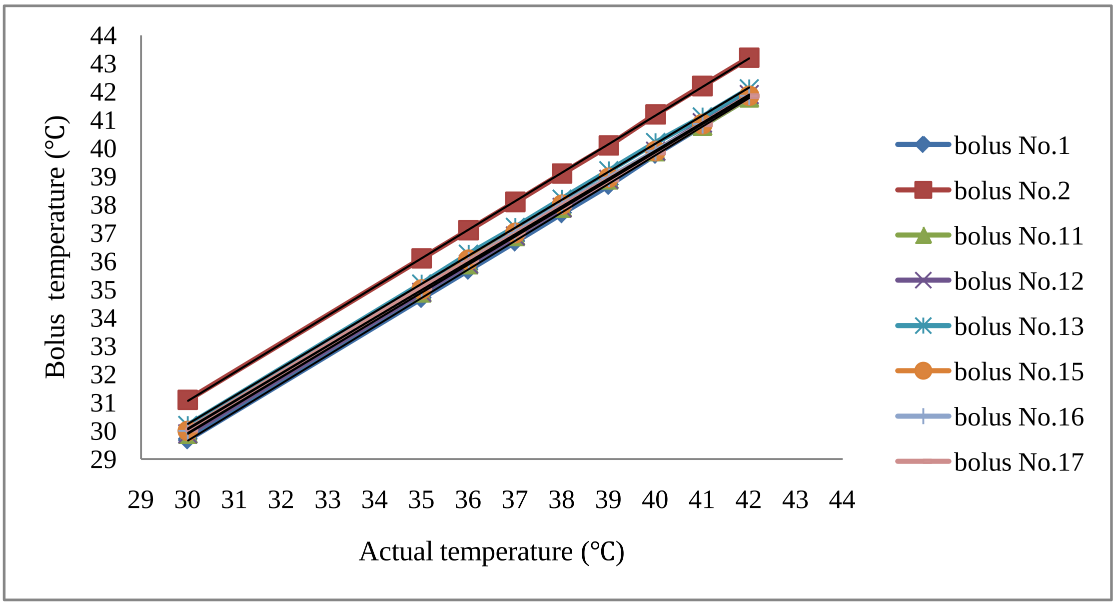


Figure 3.1.1 The regression relationship between bolus temperature and actual temperature, as determined using a reference thermometer, over a range of 30 to 42°C for 8 boluses.

Table 3.1.1 The slopes, intercepts, and their standard errors (SE) of 8 boluses tested in the experiment:

Bolus ID	slope	SE	intercept	SE
1	1.01	<0.01	-0.63	0.14
2	1.01	<0.01	0.77	0.14
11	0.99	<0.01	0.16	0.14
12	1.00	<0.01	-0.10	<0.01
13	0.99	<0.01	0.49	0.16
15	0.99	0.01	0.51	0.19
16	0.99	<0.01	0.48	0.16
17	0.98	<0.01	0.71	0.13

Table 3.1.2 The linear regression analysis using the slope and intercept of bolus No.1 (b1) as parameter, the difference of slopes and interceptors of other boluses was compared:

Parameter	slope	SE	intercept	SE
(b1)	1.01	<0.01	-0.63	0.14
Difference to the parameter				
**b2	<0.01	<0.01	*1.39	0.2
b11	*0.02	<0.01	*0.78	0.2
b12	<0.01	<0.01	*0.53	0.2
b13	*0.02	<0.01	*1.12	0.2
b15	*0.02	<0.01	*1.14	0.2
b16	*0.02	<0.01	*1.1	0.2
b17	*0.03	<0.01	*1.36	0.2

* There was a significant difference ($p < 0.05$). ** b2 to b17 represents bolus No.2 to No.17

Linear regression (using SAS) that included different intercepts and different slopes for each line using bolus No.1 (b1) as parameter intercept and slope (Table 3.1.2) showed:

1. B1 had a slope of 1.01 and an intercept of 0.63. Over the range of temperature used for calibration (12℃), the slope had no practical difference to 1.
2. The slope of 5 of the 7 other boluses were significantly different to b1. The largest difference was 0.03 from B17. However, within the range of 30 to 42℃, the slope difference was of no practical significance.
3. The intercept for all of the 7 other boluses was significantly different to b1.

However, the linear regression may not describe the relationship the best. Figure 3.1.2 shows the difference between the bolus temperature and the actual temperature plotted against the actual temperature.

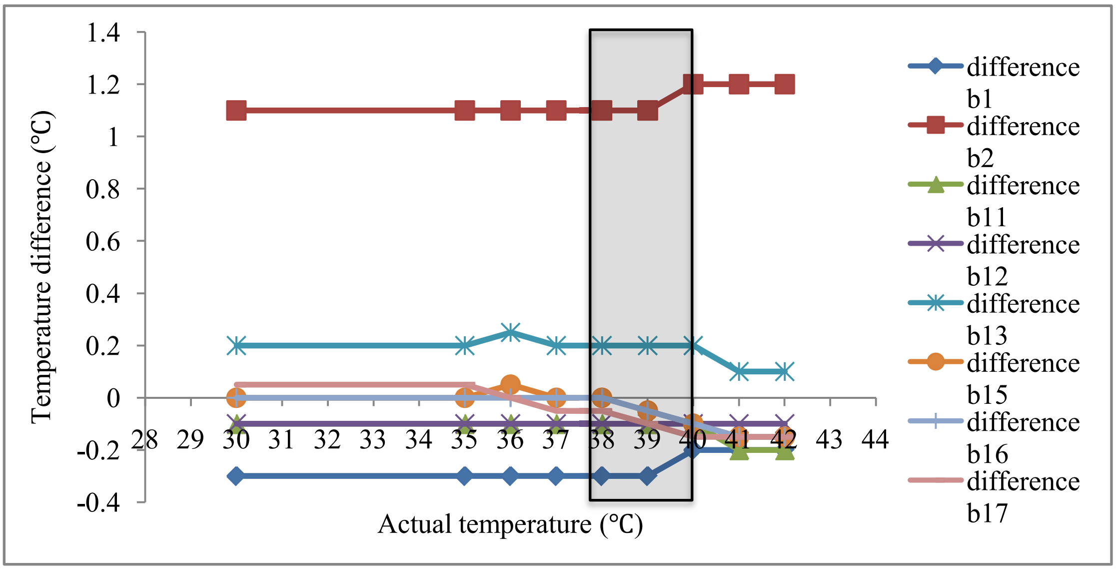


Figure 3.1.2 The temperature difference (the bolus temperature minus the actual temperature) plotted against actual temperature for 8 boluses over the temperature range from 30 to 42°C, record according to previous example in Figure 3.1.1 (page 51), the shadowed area is temperature 37.8 to 40°C. "Difference b1" to "difference b17" represents the temperature difference against actual temperature for different boluses (b1 to b17).

The expectation was for a straight line at y = 0 with departures from y = 0 indicating bias and a slope≠ 0 indicating that th e bias was not consistent over the range of temperature. It was observed from Figure 3.1.2 that the most changes occurred at temperature 40°C (5 out of 8 boluses had a change in temperature difference at or near this temperature). Between 30 and 40°C, 4 out of 8 boluses had no change in temperature difference. The other 4 boluses had small variations in temperature difference (from 0.05 to 0.15°C). The temperature of a cow is between 37.8 and 40°C (MacDonald, 1984), and outside that temperature range, the variation in temperature difference was minor (0.05°C). The small variation of the temperature difference between 37.8 and 40°C means that for these 8 boluses, one measurement of difference between bolus temperature and actual temperature in the range of 37.8 to 40°C is sufficient for adjusting bolus temperature to actual temperature. Therefore, the formula for calibration between 37.8 to 40°C is: actual temperature = bolus temperature + the temperature difference measured (the bolus temperature minus the actual temperature).

3.2 Pressure calibration

Bolus pressure was calibrated by regressing bolus pressure on actual pressure, determined using a barometer (Figure 3.2.1). The data range of actual pressure was from 982.9 to 1024.75 millibars. Note, the range for the x and y axes cover the data range only, and they are not continuous from zero.

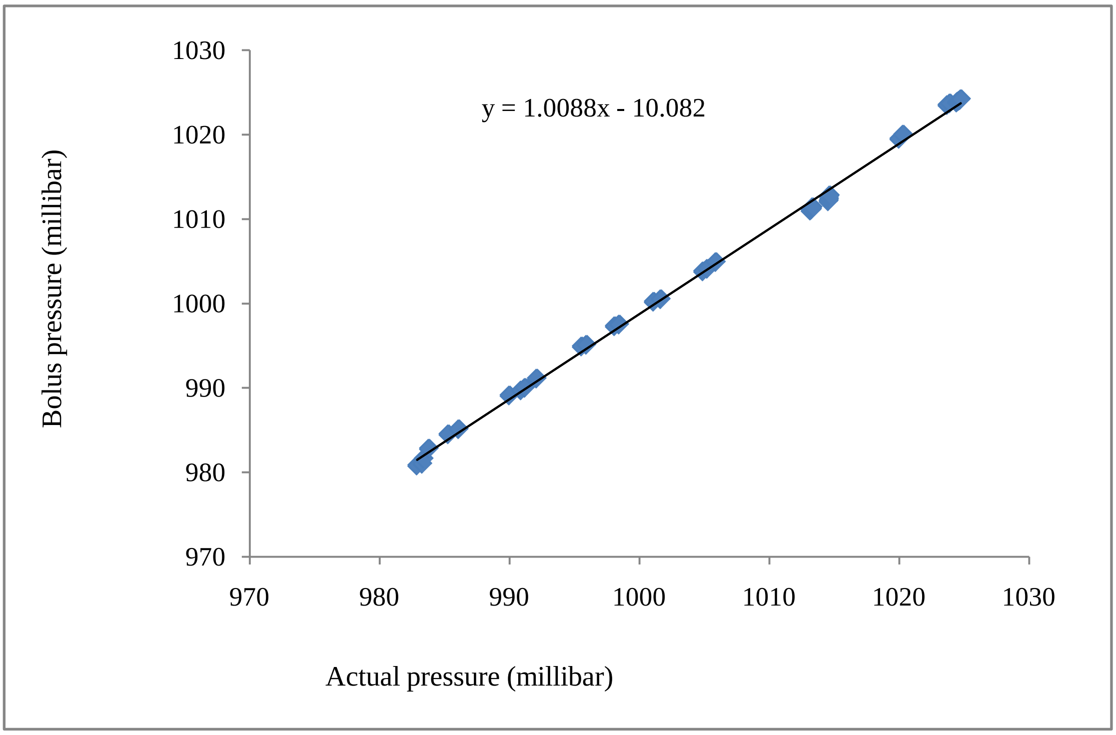


Figure 3.2.1 The regression relationship between bolus pressure and actual pressure, as determined using a reference barometer, over a range of atmospheric pressure from 982.9 to 1024.75 used for calibration of one bolus.

A regression line was drawn from the data. The slope of the regression relationship was 1.0088 and the intercept was -10.08. The slope means that with the increase of 1 millibar of actual pressure, the bolus pressure increases at 1.0088 millibars. By using these values, the actual pressure within the pressure range (982.9 to 1024.75 millibars) could be calculated from the bolus pressure.

3.3 pH calibration

For each bolus, the pH indicated immediately after the calibration process was less than ±0.1 pH unit different at both pH 4 and pH 7 buffer solution. Drift problems occurred in all the boluses examined in the longer period (4 to 7 days), and to some boluses in the shorter period (1 to 3 days).

The bolus pH was calibrated by regressing the difference between the bolus pH and the actual pH (measured by reference pH probe) on time. The time unit for transmission of pH during calibration was 1 minute. For each time point of direct measurement of pH, transmitted bolus pH data was interrogated and the pH corresponding to that time point was extracted and used for calibration. The x axis of elapsed time was converted from minutes to days from start to better align with biologically meaningful units of pH drift (Figure 3.3.1).

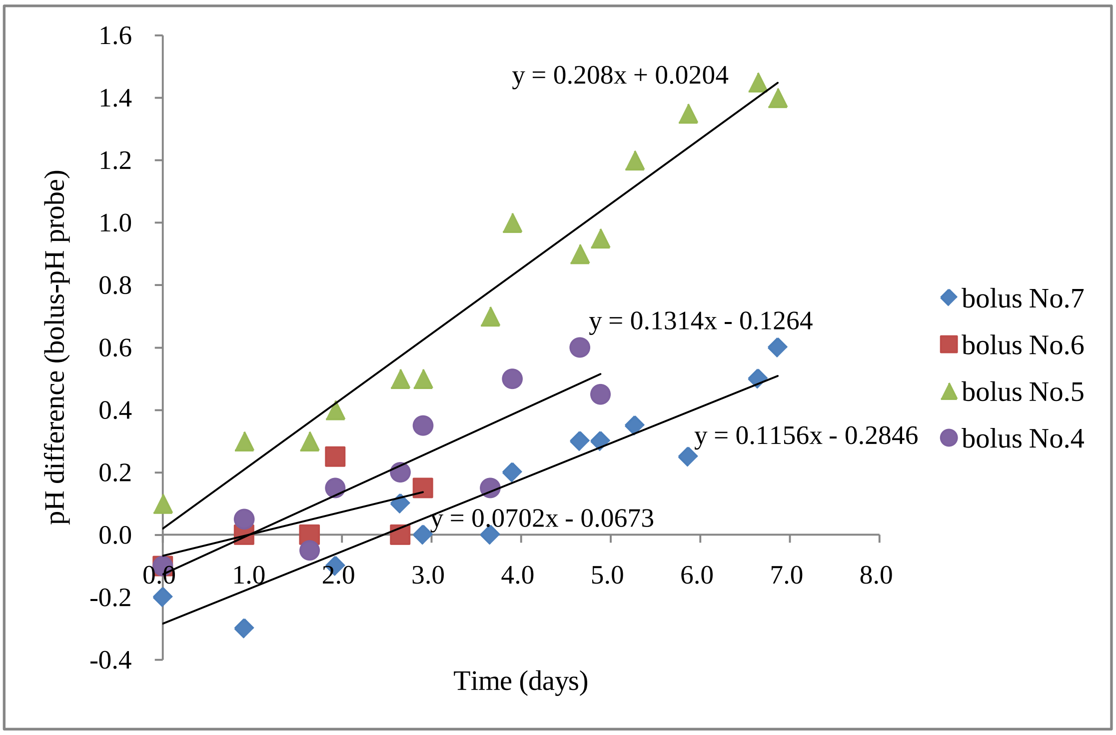


Figure 3.3.1 The regression relationship between pH difference, as calculated using bolus pH minus the pH measured by the reference pH probe, and time, as determined by the days elapsed from the first recording for 4 boluses.

The slope and the intercept of the regression relationship were different for each bolus. It could be observed from Figure 3.3.1 that the biggest slope value was 0.208, which means that the bolus pH drifted at 0.208 pH units per day in a 6.9 day period. The smallest slope value in Figure 2 was 0.0702, which means the pH drift was less than 0.1 pH unit but for this bolus the duration was on 2.9 days. For the shorter period (1 to 3 days), the slope of the regression relationship will be less reliable than for the longer period (4 to 7 days) as the longer the calibration period, the more direct measurement could be performed giving more data points to be used in the regression relationship. The intercept ranged from -0.2846 to 0.02. This represents the pH difference at time zero, i.e. for bolus No.7, -0.2846 means the bolus pH was 0.2846 lower than actual pH, while for bolus No.5, 0.02 means the bolus pH was 0.02 higher than actual pH.

3.4 Data capture rate

3.4.1 The characters of Kahne bolus and transceivers on capturing data signals

During the use of Kahne bolus and transceivers, the characters of these device on capturing data signals was observed. Some characters have important influence on capture rate:

1. When the transmission interval was set at seconds, the real time table view showed an interval that was 3 seconds longer (e.g. when set at 10 seconds, the real time view showed 13 seconds). When interval was set at minutes, the real time table view also showed minutes.
2. When the transceiver was linked to the computer and used the software to process the received data and show the data in the computer real time table view, the time unit was showed in second base (e.g. when set at 20 seconds and start transmission at 09:00:00, the time of the first data is 09:00:00. The time of the second data is 09:00:23). And the time shown had some delays (e.g. 09:00:23 in real time view showed at 09:00:28 in the computer clock).

3. When the data was exported to an excel file, the precision in time recording was reduced. For example, in the real time view the time showed as 09:00:00, 09:00:23, and 09:00:46, but in the exported data it showed as 09:00:00, 09:00:00, and 09:00:00.
4. When using the KR2105 transceiver (with the SD card as the storage system to store data in an excel file format), the data in Excel file showed the exact time of transmission.

It is possible the above character had effects on data capture rate, especially when the delays happened in processing the data, some data that transmits within seconds could overlap and get lost.

3.4.2 Data capture rate in the experiments

The data capture rate determined with boluses outside the animal, in the yard behind the animal building block A in AgResearch Palmerston North where the indoor experiment was conducted. Table 3.4.1 shows the mean data capture rate for experiment with boluses either inside or outside the cows. This was to determine the effect of the animal itself (rumen contents, body mass) on data transmission and capture.

Table 3.4.1 The mean data capture rate of transceiver KR2105 from boluses there were either external to the animal or in the rumen:

Location of boluses	Mean capture rate	Range of capture rate	Duration of recording
External	80.5%	70% to 84%	2hours 20 minutes
In the rumen	*26.5%	15.6% to 32%	**71 hours and 48 hours

* This capture rate does not include three boluses for which the capture rate was zero. ** Include one 71-hour period and two 48-hour period.

The mean data capture rate was 80.5% for measurements with the boluses outside of the animal. One bolus had a lower data capture rate (70%) because it had a 0% recording for 20 minutes in the experiment (the reason for this break in data capture is not known). However, when the 0% was moved, the data capture rate for this bolus was 82% and the overall mean data capture rate was 83%. There was very small difference (≤ 4%) among boluses in the data capture rate if the period of 0% capture rate was removed for that bolus. In the experiment with boluses outside the animals, the relationship between mean data capture rate and the distance is shown in Figure 3.4.1.

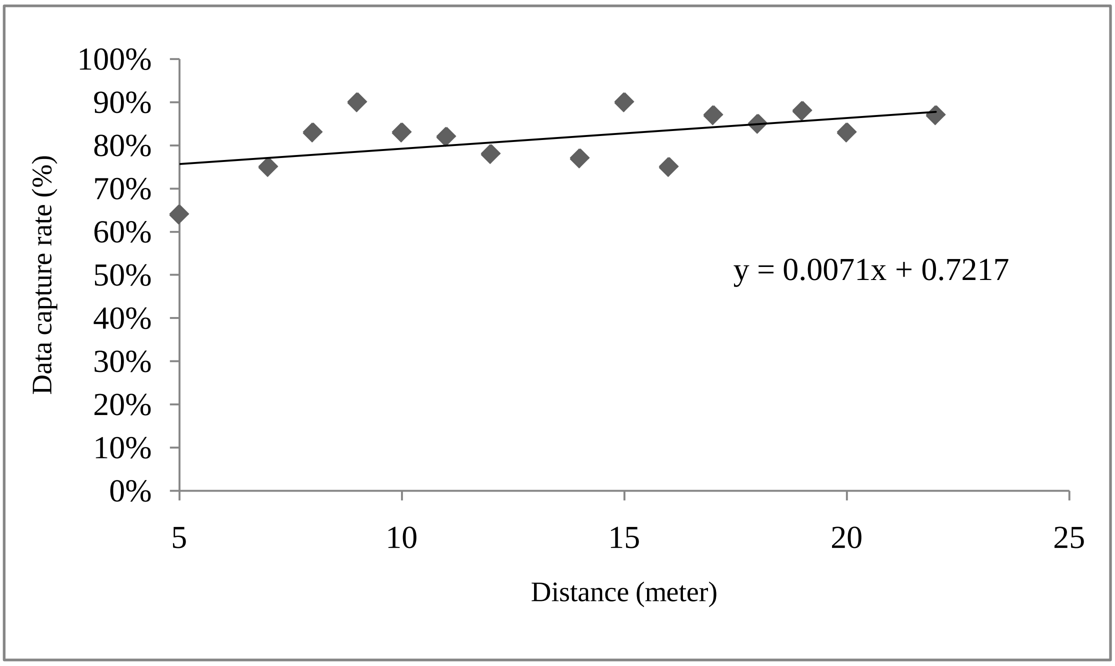


Figure 3.4.1 The linear regression relationship between distance, as determined by the distance between boluses and transceiver KR2105, and data capture rate as calculated using the method in section 2.6.

The regression analysis showed that there were significant effect ($p < 0.05$) on the distance between boluses and transceiver KR2105, when measured indoors with boluses external to the animal. However, if the 0% capture rate was removed as an abnormal recording (because it was not consistent with other capture rate), there were no significant effect ($p > 0.1$) of the distance between boluses and transceiver KR2105.

The mean capture rate indoors with boluses external to the animal also suggests that there were other factors preventing the data capture rate from reaching 100%.

The effects of animals on data capture rate could be observed from Table 3.4.1 (page 58). The mean data capture rate from boluses outside the cows was 80.5%, while the mean data capture rate from experiments in the same location using boluses inserted in fistulated cows showed a mean data capture rate of 26.5%. Three boluses showed 0% data capture rate for a very long period (1 for 68 hours and 2 for 47 hours) (others showed a range in data capture rate of 15.6% to 32%). This suggests that when inserted in cows, the data capture rate was decreased by a huge amount even though other factors remained constant.

The effects of transceivers on data capture rate were studied by comparing the data capture rate for different transceivers involving the same location and the same animals. The transceivers compared were KR2105 combined with KR2002 (Table 2.8.1, page 41), KR2105 alone and KR2001 alone. As showed in Table 2.2.1 (page 20), KR2001 was used mainly for configuration and calibration. The short whip antenna limited the transmission range to an estimated 5 meters. KR2105 is the transceiver that has the most features. A 7 element Yagi antenna enabled it to have transmission range estimated at 25 meters, with 15 to 25 meters being the most efficient zone for data capture. KR2002 has a 5 element Yagi antenna. The estimated transmission range is around 15 meters, with 5 to 15 meters being the most efficient zone for data capturing. Table 3.4.2 shows the mean capture rate from different transceivers in the same location and using the same animals.

Table 3.4.2 The mean data capture rate of transceiver KR2001 alone, KR2105 alone, and KR2105 and KR2002 operating together in various situations:

Location (animal)	Mean capture rate (capture rate range)		
	KR2001	KR2105	KR2105 and KR2002
† pen (sheep)	72.6% (65% to 76%)	84.1% (83% to 86%)	N/A
††yard (cows)	8.3% (6.9% to 9.8%)	26.5% (15.6% to 32%)	60.8% (55% to 69.9%)
†††paddock (cows)	1.8% (0.9% to 2.6%)	46.4% (43% to 49%)	*53.6% (50.5% to 55.5%)

†, ††, ††† The experiments were summarized in Table 2.7.1 (page 38), 2.8.1 (page 41), and 2.9.1 (page 42). * The area was slightly larger 13x16 for this experiment.

There was a big difference between the transceivers KR2001 and KR2105. There was also a big difference between the KR2105 and the KR2105 combined with KR2002 in the indoor experiment. It was expected that the KR2105 alone would be sufficient for capturing data. However, the low capture rate in the yard indicated this was not the case. Results showed that a higher capture rate was achieved by combining KR2105 and KR2002. It was also observed in the experiments that the duration of 0% capture rate was significantly lower when the KR2105 was combined with KR2002.

Table 3.4.2 also shows that the range of transmission with placement of the bolus within the rumen and the location (building structure) could influence the data capture rate for a single transceivers. For the short distance ranges using transceiver KR2001, when the area was increased from pen (5x5m^2) to yard (19x8.5 m^2) or paddock (13x13m^2), the capture rate decreased significantly ($p < 0.05$). For KR2105 alone, the decrease of capture rate from a small area to a larger area was large. This could not be explained by a single factor as both size of the area and the species of animal were different.

The effects of location for KR2105 and KR2105 with KR2002 are also showed in Table 3.4.2. There was significant difference ($p < 0.05$) of capture rate from KR2105 between two locations (26.5% for the yard vs. 46.4% for the paddock). When both KR2105 and KR2002 were used together, the difference was smaller (60.8% vs. 53.6%). The difference could also be caused by the location of the transceivers. When KR2105 was used alone, it was positioned in location A in Figure 2.8.2 (page 40). When both KR2105 and KR2002 were used together, KR2105 was positioned in location C in Figure 2.8.2. The data capture rates from KR2105 in these two locations were significantly different, as the mean capture rate in location A was 26.5%, while in location C the mean capture rate was 41.3%.

It was observed that there were a large number of hours in the experiments where the data capture rate was 0%. When this occurred, the mean data capture was low (less than 30%). The apparent cause was setting the boluses with the same transmission interval. When the transmission interval was set at intervals that differ using a scale of seconds rather than minutes, the number of hours with 0% transmission was substantially reduced.

The hourly capture rate and the frequency distribution of capture rate indicate the pattern of data transmission. Figure 3.4.2 shows an example of hourly data capture rate for 2 boluses (bolus No.12 and No.13) over a single 24-hour period using two cows outdoor eating grass. Figure 3.4.3 shows the frequency distribution of data capture rate in the same measurement. The hourly capture rate and the frequency distribution of capture rate assist in the analysis of the data capture rate by showing the amount of low data capture rate, and when these occurred. These measures help determine the consistency of the capture rate.

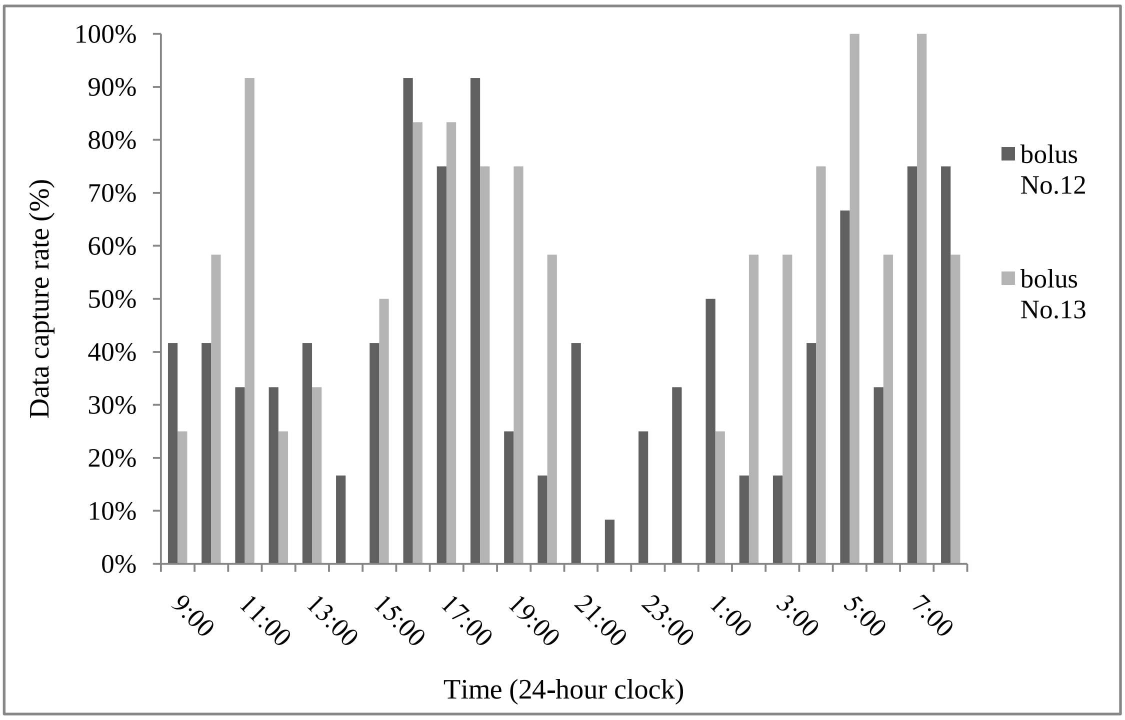


Figure 3.4.2 Hourly data capture rate for two boluses (Bolus No.12 and No.13) over a 24-hour period of measurement from 09:00 12th to 09:00 13th June (Appendix 1 Table A.1.11.1).

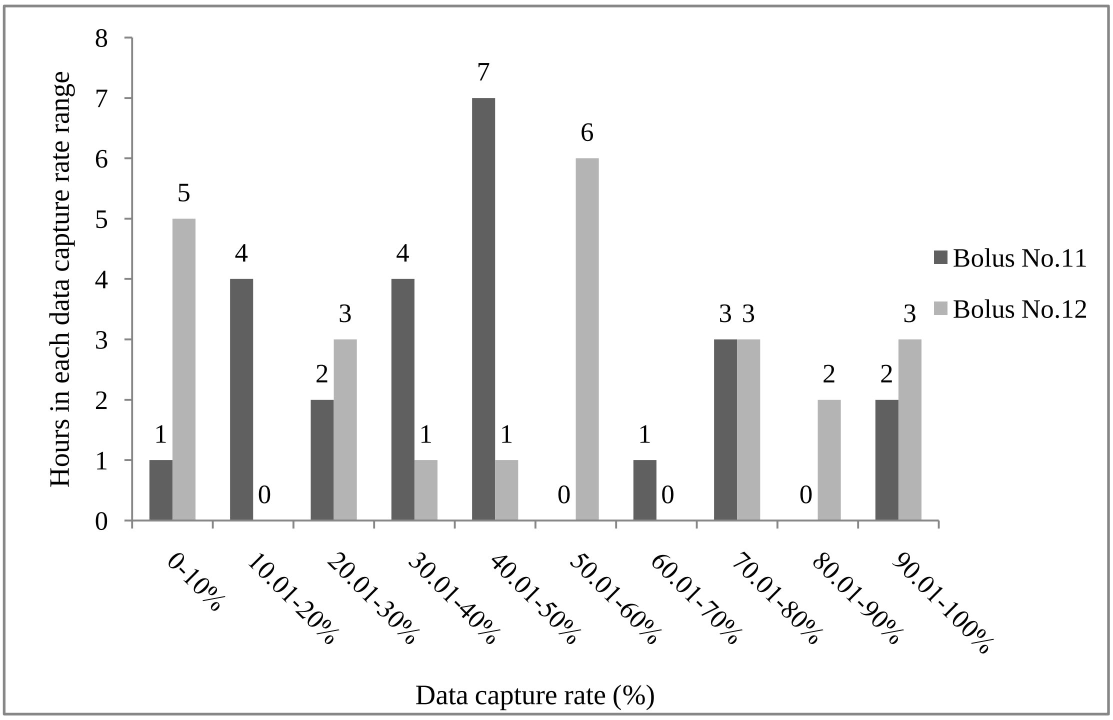


Figure 3.4.3 Frequency distribution of data capture rate for two boluses (Bolus No.12 and No.13) over a 24-hour period measurement same as shown in Figure 3.4.2 (Appendix 1 Table A.1.11.2).

This 24-hour period experiment using bolus No.11 (b11) and 12 (b12) had a mean data capture rate of 43% for b11 and 49.6% for b12. However, it was observed from Figure 3.4.2 (page 62) and 3.4.3, b11 had only one hour at less than 10% capture rate, while b12 had 5 hours at less than 10% capture and the capture rate for these 5 hours was 0%. Therefore, even though bB12 had a higher mean capture rate, b11 had a more consistent data capture rate and hence produced more reliable data.

3.5 Animal to animal variation

This experiment used transceiver KR2105 and 5 boluses to collected pH data for determination of animal to animal variation of ruminal pH. It was expected to have pH data from all cows with 6 x 48-hour recording and 3 x 24-hour recording.

Bolus No.15 only transmitted 4 data signals in the entire experiment. It resulted in no pH data collected from cow No.721 (expected one 24-hour period) and only one 24-hour period of pH data from cow No.714 (expected 2 x 24-hour periods). The data recording for cow No.715 was also affected as only four pH data was recorded in a 24-hour period (Appendix 3). The mean data capture rate over the 71-hour period for five boluses was 20.82%±11.67%. Table 3.5.1 showed the frequency distribution of data capture rate for five boluses (ID: 12, 13, 15, 16, and 17) in a 71-hour period measurement.

Table 3.5.1 The frequency distribution of data capture rate for five boluses (ID: 12, 13, 15, 16, and 17) in a 71-hour period (09:00 14th to 08:00 17th June) measurement:

	Bolus ID				
*DCR	12	13	15	16	17
0-10%	24	18	**69	18	24
10.01-20%	12	12	2	10	13
20.01-30%	9	18	0	9	10
30.01-40%	6	13	0	9	6
40.01-50%	11	6	0	13	10
50.01-60%	3	3	0	4	3
60.01-70%	3	1	0	5	3
70.01-80%	3	0	0	2	1
80.01-90%	0	0	0	1	1
90.01-100%	0	0	0	0	0

* DCR: Data Capture Rate

** Bolus No.15 had no signals recorded for 69 hours in the experiment, which resulted in no pH data from two cows in a 24-hour period and only 4 data points from one cow in a 24-hour period.

The average capture rate was low. For 2 boluses there were 24 hours with 0-10% capture rate and for 2 others there were 18 hours with 0-10% capture rate. A high hourly capture rate (e.g. above 50%) only accounted for an average of less than 12% of the time in the 71-hour period.

Table 3.5.2 The mean, standard deviation (SD), and coefficient variation (CV) for each hour in the 24-hour period and for the whole 24-hour period:

Time	mean	SD	CV
9:00	6.65	0.2	3.07%
10:00	6.49	0.19	2.89%
11:00	6.59	0.11	1.65%
12:00	6.75	0.19	2.78%
13:00	6.8	0.16	2.34%
14:00	6.85	0.15	2.24%
15:00	6.9	0.18	2.61%
16:00	6.76	0.28	4.14%
17:00	6.64	0.26	3.89%
18:00	6.47	0.19	2.99%
19:00	6.52	0.21	3.17%
20:00	6.54	0.24	3.70%
21:00	6.65	0.23	3.48%
22:00	6.71	0.21	3.19%
23:00	6.76	0.33	4.88%
0:00	6.75	0.24	3.57%
1:00	6.79	0.18	2.64%
2:00	6.86	0.17	2.52%
3:00	6.87	0.15	2.23%
4:00	6.87	0.14	2.01%
5:00	6.89	0.18	2.68%
6:00	6.95	0.18	2.65%
7:00	7.09	0.18	2.54%
8:00	7.11	0.21	2.97%
mean	6.76		
SD	0.17		
CV	2.54%		

Table 3.5.2 showed the mean pH of all cows in an hour. For the situation when two 24-hour periods existed, the mean pH for that hour was the mean of the estimates for the same hour from two successive 24-hour periods. Table 3.5.2 listed the mean, standard deviation, and coefficient variation for pH in cows in each hour. The mean

hourly pH differed from 7.11 at 08:00 to 6.49 at 18:00 (mean ± SD = 6.76 ± 0.17). The mean pH for cows differed from 7.04 for cow No.714 to 6.57 for cow No 720 (Appendix 3 Table A.3.2). The coefficient variations for each hour and for each cow were not big as for most hours the CV was around 3%, with highest being 4.88% (at 23:00 hours) and lowest being 1.65% (at 11:00 hours). The overall CV was comparatively low (2.52%) (Appendix 3 Table A.3.2).

3.6 The effect of diet on pH change in cows

Over 7 days there were 4 x 24-hour (09:00 to 09:00) recording for 3 cows, 3 x 24-hour recording for 2 cows, and 2 x 24-hour recording for 1 cow. There were 7 occasions when the 24-hour data recording was incomplete caused by two reasons. Firstly, the beginning of data recording was from 15:30 at the first day. Secondly, one of the bolus stopped transmitting data due to low battery. Table 3.6.1 summarized the mean pH from the experiment.

Table 3.6.1 Mean pH for each cow in each 24-hour period (09:00 to 09:00, named Day 1, Day 2, etc), mean pH for all cows recorded in each 24-hour period, mean pH for each cow in each diet, and mean pH for all cows in each diet:

Mean pH		Cow ID						Mean pH
		716	717	718	719	720	721	
Diet 1 (grass and hay)	Day 1	*6.50	*6.71	*6.51	**N/A	N/A	*6.76	6.62
	Day 2	6.47	6.85	6.51	N/A	N/A	6.70	6.63
	Day 3	N/A	6.85	6.69	6.63	6.68	N/A	6.71
	Day 4	†6.61	N/A	N/A	6.61	6.83	6.88	6.73
	Mean for grass/hay diet	6.53	6.80	6.57	6.62	6.76	6.78	6.67
Diet 2 (baleage)	Day 5	†6.72	6.65	6.67	N/A	N/A	6.71	6.69
	Day 6	N/A	6.76	N/A	6.60	†6.72	6.66	6.69
	Day 7	6.66	N/A	6.68	N/A	6.75	N/A	6.70
	Mean for baleage diet	6.69	6.71	6.67	6.60	6.74	6.68	6.69

* The mean of the pH was from an incomplete 24-hour data set because the beginning of data recording was from the afternoon. ** The N/A means that there was no bolus available to be inserted in the rumen for that 24-hour period. † The mean of the pH was from an incomplete 24-hour data set as there was data missing due to the loss of power from one bolus.

For the grass and hay diet, the mean pH for day 1 was significantly lower ($p < 0.05$) than the mean pH of 4 days combined, while the 3 other days showed no significant difference from it. The mean pH for cow No.716 was significantly lower than the combined mean pH, while the 5 other cows showed no significant difference to it. The mean pH for cows No.716, 718, and 719 was significantly lower than the other three cows.

For the baleage diet, there was no significant difference in mean pH between different days. The mean pH for cow No.719 was significantly lower than the combined mean pH, while the 5 other cows showed no significant difference to it. There was a significant pH difference between two cows (719 and 720) for the baleage diet.

The difference in mean pH between two diets is very small (6.67 vs. 6.69). The mean pH for day 2, 3, and 4 showed no significant difference to the mean pH in day 5, 6, and 7. However, mean pH for day 1 was significantly lower than the mean pH for 3 days on baleage diet. The difference in mean pH of the same cow between two diets ranged from 0 to 0.15 pH unit.

The pattern of 24-hour pH change was different for two different diets for each cow and for all cows combined (Appendix 2). Figure 3.6.1 shows the mean 24-hour pH pattern for cows offered grass and hay diet.

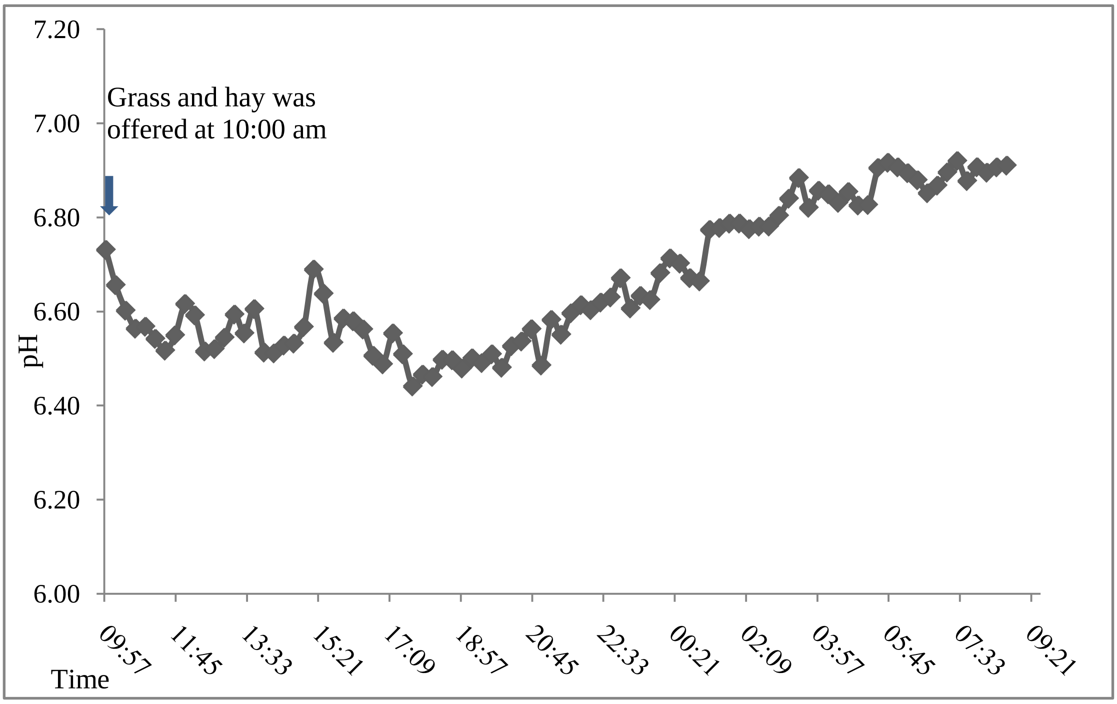


Figure 3.6.1 Profile of mean 24-hour pH (10:00 to 9:30) taken from all cows. The grass and hay diet was offered at 10:00. The mean pH decrease was 0.15 pH unit from 10:00 to 11:00.

After the initial drop for one hour following feeding at 10:00 in Figure 3.6.1, during which the pH decreased from 6.73 at 10:00 to 6.58 at 11:00, the pH dropped continuously to 6.5 at 11:30. The pH fluctuated between 11:30 and 17:30 and then increased steadily until just before feeding time on the next day.

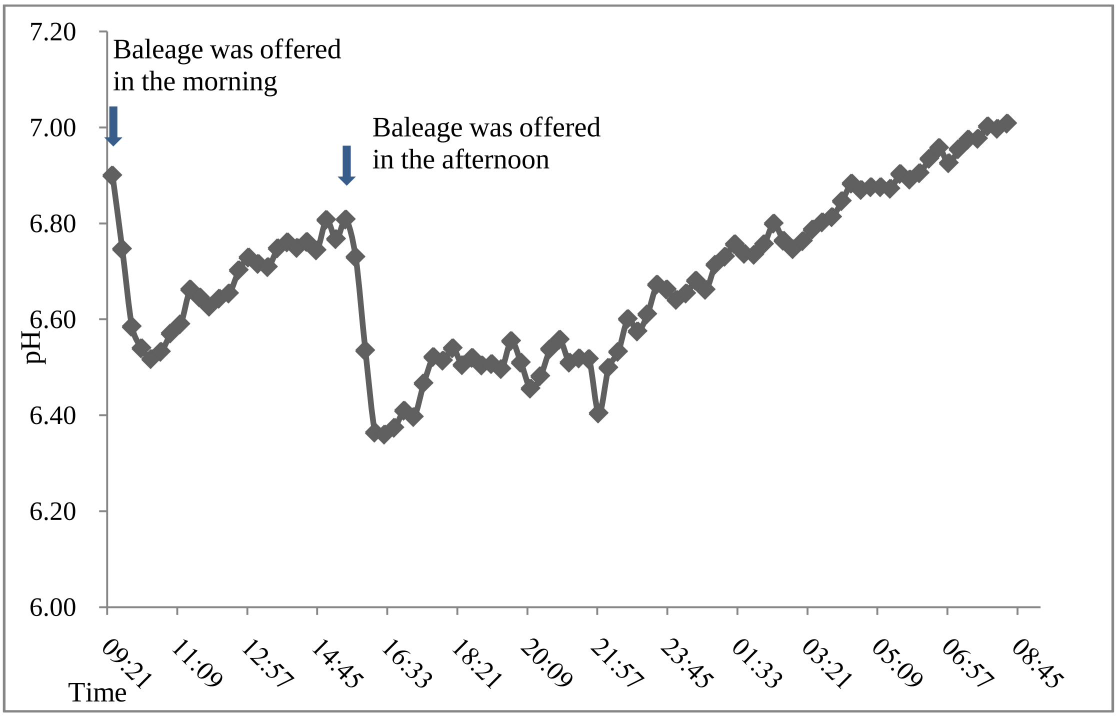


Figure 3.6.2 Profile of mean 24-hour pH (09:30 to 08:45) taken from all cows. The baleage diet was offered at 09:45 and 15:45. The mean pH dropped 0.35 pH unit from 09:45 to 10:30, and 0.45 pH unit from 15:45 to 16:30.

In Figure 3.6.2, when the meal was offered at 09:45, the pH dropped 0.35 pH unit from 6.9 to 6.55 in 45 minutes. When the meal was offered at 15:45, the pH dropped 0.45 pH unit from 6.81 to 6.36 in 45 minutes. The pH started to increase steadily from one hour after the baleage meal was offered until the next meal.

The regression analysis using SAS showed that when feed was available, the pH decreased at 0.00090 (± 0.00022) per minute or 0.054 per hour. When the feed was not available, the pH increased at 0.00052 (± 0.00012) per minute or 0.031 per hour.

When feed was available for baleage diet, the analysis showed pH decreased at 0.00909 (± 0.00095) per minute or 0.545 per hour. After this initial decrease, and when the feed was no longer available, the pH increased at 0.00070 (± 0.00029) per minute or 0.042 per hour.

The regression analysis of the slope of the pH change from each cow showed that there was very little variation between animals (i.e. the effect of animal was minor).

Chapter 4: Discussions

4.1 Introduction

The aim of this project was to validate the pH, temperature, and pressure data that were measured using the Kahne rumen boluses by comparing the data collected and the actual temperature, pressure, and pH recorded from reference thermometer, barometer, and pH probe. The data capture rate was tested to determine the factors that affect the Kahne transceivers' ability to capture data signals transmitted from the boluses. The boluses and transceivers were then used in a trial on studying the effect of two diets on the pH change in cows.

It was found that the temperature and pressure measured in the experiment by the boluses could be calibrated using the formula established in the calibration process. The pH drift found in the experiments pose a threat on the accuracy of the data as to calibrate the pH drift, direct measurement of the ruminal pH is required. The data capture rate varied in different situations. To ensure a sufficient capture rate in the animal experiment, two transceivers should be used and placed in assured locations. The effect of two diets to the pH change in cows was significant when the data was regression analysed using SAS.

This discussion will cover the calibration of temperature, pressure, pH, the factors affecting the data capture rate, how to improve the transmission, and the effect of two diets with aspects of experimental conditions.

4.2 Temperature calibration

Among all eight boluses tested in the calibration experiments, linear regression analysis using SAS showed significant difference among boluses in slope or intercept.

However, the linear regression analysis is only the simplest way to describe the relationship between bolus temperature and actual temperature. There was an assumption that it was the most appropriate model.

When combining the linear regression results for the boluses with the temperature range over which the boluses were tested, the narrow range (12℃ difference) could lead to no practical difference even though the linear regression showed significant difference in slopes and intercepts. In the experiment of measuring rumen temperature, the narrower temperature range (37.8 to 40℃) may make the calibration process very simple: bolus temperature would only need to be measured once against the actual temperature in the range between 30 and 42℃ to calculate the intercept, while the slope could be treated as 1.

In order to look for more detailed relationship of bolus and actual temperature, the temperature difference was plotted against actual temperature. By plotting the temperature difference versus the actual temperature, only minor changes in temperature difference in the physiological range were observed, especially within the range between 30 to 39℃. Therefore, an arbitrary way to describe the relationship is to divide the temperature range into groups. For example, in this experiment, groups should be from 30 to 40℃ and from 40 to 42℃, with 37.8 to 40℃ being emphasized. This leads to an easy way to calibrate the temperature for each experiment (e.g. in cow's rumen). For instance, only one measurement is required in the normal physiological range of rumen temperature (37.8 to 40℃) and this difference can be used as the correcting factor.

It could be suggested that for these 8 boluses, the actual temperature can be calculated using the intercepts gathered using the calibration process and the bolus temperature.

However, for any new boluses in the future, it is recommended that the calibration process be repeated to investigate the relationship between the bolus temperature and the actual temperature, over a range of temperature, to confirm if a simple calibration is sufficient.

4.3 Pressure calibration

The calibration of bolus pressure was done over the pressure range 982.9 and 1024.75 millibars (bolus pressure range 980.8 to 1024.2 millibars). The regression relationship was linear, which enabled the actual pressure to be determined using the slope and intercept of the regression line. However, as the pressure range for calibration was limited, any pressure that is out of the range could only be adjusted to actual pressure by extrapolating outside the calibration range. There were on average 70% (45% to 88%) of the rumen pressure recordings that were beyond the calibration range, with the highest bolus pressure recorded being 1100 millibars (the limit of the bolus pressure range). This means that an average 70% of the pressure data were adjusted based on the extrapolation.

The pressure calibration was done under conditions that a pressure chamber was not available. The experiment used factors such as altitude (location) and weather to influence the pressure under atmospheric conditions. This was the reason that the actual pressure data points for calibration were not uniformly spaced within the range and this is also a limitation associated with using atmospheric variation for calibration. However, after the pressure data that were transmitted from the rumen under experimental conditions were examined, it was clear that the pressure range under atmospheric conditions was not wide enough for calibration of rumen pressure. In order to enable the correction of rumen pressure, the experiment for pressure calibration could be done using a pressure chamber to ensure the wider pressure range (up to 1100 millibars). If a pressure chamber was not available, another method might be to use a 1-meter-deep water tank (for every meter under water, the pressure increase 101 millibars) to put the boluses under water for increasing the pressure range to 1100 millibars. However, the way to keep the bolus under water (because boluses float) at a precisely determined depth, and to move boluses from one depth to another (to change the pressure) requires a lot of planning. Kahne Ltd should also extend the pressure range of its boluses if possible to over 1100 millibars as there could be pressure peaks in the rumen that are higher than the bolus limitation.

To use these boluses for pressure measurement in the rumen in the future, a wider range of pressure should be used to establish the linear regression relationship between bolus pressure and actual pressure for calibration.

4.4 pH calibration

After factory calibration process, the difference of pH between bolus and reference pH probe was small ($<\pm0.1$). However, when put into the rumen, some boluses immediately showed a larger difference such as -0.2. This partially led to the intercept of the regression relationship being larger than 0.1 (-0.126 and -0.284 in two boluses) This initial increase in the difference between the bolus pH and actual pH is possible because even though the pH probe was pushed as close to the bolus sensor tip, these two could still be measuring different ruminal fluid with different pH.

The pH drift from the bolus has a huge impact on the accuracy of the data captured, hence affecting the analysis of the data. If calibration processes were not carried on, the drift could cause abnormality of the data (e.g. pH 8.2 during feeding).

In order to examine whether the regression relationship for one bolus from the pH calibration process could be used to calibrate pH drift of the same bolus in other experiments, extra test was done. The test included comparing the actual drift over 3 days with the drift that would be predicted for 3 days, using the calibration relationship, for the same boluses in a different experiment. The results indicated that 2 of the 4 boluses showed $\leq$0.1 pH difference between actual drift and predicted drift, while other two boluses showed a high pH difference (0.5 and 0.7). This means that for some boluses, it is necessary to calibrate pH drift in each experiment using the calibration relationship obtained from that experiment. It was also possible that other factors such as animal to animal and dietary difference (could lead to different patterns of pH drift.

If boluses were used in experiments involving pH data collection, a pH drift calibration should be carried out for each bolus. It is recommended that the calibration relationship should not be used in other experiments unless more tests were done to establish the relationship between bolus pH drift and the factors associated with it. Any new technologies to reduce the pH drift in the boluses should be used to improve the accuracy of the pH data.

4.5 Data capture rate

There are several possible reasons for the loss of data transmissions under the conditions that the boluses were external to the animals. The boluses maybe out of transmission range, extraneous electronic “noise” may interfere with the signal transmission and affect the range, the reduction of the signal from bouncing around the metal framework structure indoors, and the overlapping of the signals during the processing by the software (e.g. one signal comes at 9:00:23 and the other comes at 9:00:26, but during the processing by the software [as there are delays] these two signals may be recognized as one, resulting the other being lost)

In the experiment that boluses were tested under external conditions, the average data capture rate was 80.5%. The transmission distance can be ruled out as a reason for the loss of approximately 20% transmission because all boluses were in the effective range (within 30 meters as stated by the user guide). The similar capture rate for different transmission range showed that with 22-meter range, the distance between boluses and transceivers was not a factor that affected the capture rate. The reason for not reaching 100% could be a combination of extraneous electronic “noise”, location (metal framework of the building structure), and successive data signals overlapping. Kahne Ltd used a device to test for electronic interference in the indoor experiment and suggested that there could be some “noise” that affected the transmission. The bounce of the signals around metal framework could decrease the strength of the signals and hence reduce the data received by transceiver. The weak signals could be overlapped by stronger signals and not be received by transceivers.

When the boluses were placed in the rumen, it was found that there was a significant decrease in the data capture rate compared with the boluses external to the rumen. In each case the same location was used with the same transceiver (KR2105) located in same place (position A in Figure 2.8.2, page 40) but the boluses in fistulated cows rather than free-standing resulted in an average capture rate of 26.5%, compared to the capture rate outside the cows of 80.5%. It is possible that the animal body mass combined with the rumen contents contributed to the decrease.

It was not clear whether the distance between boluses and transceivers was affecting capture rate when the boluses were put in cows’ rumen. No tests were done to determine this because the cows were not restrained to a fixed position and were free to roam around their pen or paddock. It was also worth noting that when left outside the cows, there was only a short period of 0% capture rate (20 minutes) for 1 bolus. In contrast, when in the cows, there were three long periods of 0% capture rate recorded in 3 boluses (one lasted for 69 hours and other two lasted for 47 hours) (appendix 1). A battery problem could be ruled out as the boluses were checked after the experiment and all boluses showed no sign of low power or any other abnormality. It was suggested by Kahne Ltd that by using different transmission intervals for each boluses, the chances of overlapping and the elimination of one transmission by another could be largely reduced. This practice increased the capture rate for transceiver KR2105 (41.3% compared to 26.5%) and the mean number of hours of 0% capture rate was reduced largely (2 ± 1.5 hours in 0% capture rate in a 24-hour period [8% ± 6% of the time] when used different transmission interval compared to 18 ± 6 hours in 0% capture rate in a 48-hour period [37% ± 12% of the time] when used same transmission interval).

The animal factor combined with the size of area (transmission range) had a significant effect on data capture rate. However, it was not clear whether animal impedance or transmission range affects the capture rate more, as the experiments did not separate the two factors. This was despite the experiment showing that the distance between boluses and transceivers did not affect data capture rate as long as the distance does not exceed the limit (e.g. for KR2105, 30 meters in the user guide, 25 meters from the Kahne technician, and 22 meters when tested in the experiment). When the bolus was in the cows, the separated effects of the animal impedance and transmission could not be tested. However, in this situation when the transmission is affected by the animal, the transmission range may be reduced and become a limiting factor for obtaining a high data capture rate.

The difference of data capture rate between indoor and outdoor experiments (26.5% for indoor vs. 46.4% for outdoor) could be the result of location, distance combined with animal factors. It is obvious that when cows stayed in the paddock there was no metal framework which could cause interferes of the signal transmission, but the electric fence could interfere with the signal. It was not clear whether the transmission range of boluses inserted in cows was reduced causing the loss of data in the experiment.

For a given set of boluses, animal, and transceiver, the effect of transceiver location was tested at 2 different positions (position A and C in Figure 2.8.2, page 40).The position of the transceiver KR2105 around the test area caused the difference of capture rate (26.5% for position A vs. 41.3% for position C). The effect of the distance range, as in different positions, the distance ranges between the boluses and transceiver changed (22 meters for position A vs. 20 meters for position C). It is also worth noticing that when the transceiver KR2105 was located in position A in Figure 2.8.2, there was a metal pipework and a metal stanchion in the line of sight between the transceiver and the boluses, In contrast, when the transceiver KR2105 was located in position C in Figure 2.8.2 (page 40), there was no metal pipework between the transceiver and the boluses. It is likely that the interference of the transmission signal from the metal pipework could affect the data capture rate.

By comparing the data capture rate in different experiments, it can be concluded that some factors or a combination of factors were affecting the capture rate. Factors such as location, animal, transceiver was observed to have a large effect on capture rate. Animal combined with distance, transceiver combined with transmission interval and all factors combined also had obvious effects on data capture rate. More experiments on confirming and proving the above factors are required in order to determine ways to improve the data capture rate. The other factors which need to be studied are the diet factor and animal to animal variation.

The hourly data capture rate and the frequency distribution of data capture rate is useful for determining the consistency of the data. It would be helpful to find out the number of hours in low data capture rate and in which hour the low capture rate occurred. This information is important especially for deciding whether the data captured is representative of the period of measurement. Even with a high average data capture rate (over 70%), there still could be hours of low capture rate. If periods of low or zero capture rates coincide with periods of rapid change in pH, for example, during and immediately following a meal, the data could be biased. Therefore, the consistency level of the data capture rate was very important. A high consistency level (data capture rate for most hours were close to average capture rate) means the captured data could represent what had happened in the experiment in an unbiased manner and all data could be used for analysis purpose even if the average capture rate was not high. A low consistency level (data capture rate for each hour varied, leaving hours of low and high capture rate) could mean the data collected could not represent the whole situation. It may not be appropriate to use the data from hours in low capture rate for data analysis, while data from hours in high capture rate could still be used but only to represent these hours, but not the whole period.

4.6 Animal to animal variation

The possibility that bolus No.15 had a flat battery was ruled out, as a test after the experiment showed normal readings and transmissions of bolus No.15.

The low data capture rate and the uneven distributed hourly capture rate could cause biases if the pH data was used to describe the variation of each cow especially if pH pattern was required because the mean pH calculated from limited data for each hour may not represent the actual pH. There are always chances that the “important” pH points were missing.

The daily pH and the hourly pH for most cows could be calculated despite the problem of low data capture rate with the concern that the data may not present the real situation. However, we were not confident to use these data for analysis purpose because of the biases of pH data from low capture rate.

It was good to see that the coefficient variation was small because the larger the CV the more animals are required to determine the difference of pH via animals.

Based on these data, for a simple 2 treatment comparison, aiming to detect a difference between treatment of 5% of the mean (0.34 pH units), 3 animals would be required per treatment group for a standard deviation of 0.11 (the lowest recorded for any hour). Seventeen animals per group would be required to detect the same difference when the standard deviation was 0.33 (the highest recorded for any hour). 6 animals per group would be required to detect the same difference for standard deviation of 0.17, which was the standard deviation of the daily mean. The assumptions used were a power of 80% and a significance level of 5%. More animals per group would be required with more stringent criteria.

4.7 The effect of diet on pH change in cows

It was a surprise to lose transmission from one of the boluses due to low battery, but not unexpected as it was not known at the start how long the battery could last. The battery life is defined by the numbers of transmissions sent from a bolus. The bolus that lost its power had sent approximate 14,000 signals. However, during the course of this study, the maximum number of data transmitted from any bolus was over 22,000. When the battery went flat, the bolus had to be withdrawn from use. The battery has to be changed by the manufacturer in Auckland and it could take several days for the bolus to return.

The reason for a similar pH for the two different diets could be explained by the similar intake of dry matter (6 kg DM for grass and hay and 6.1 kg DM for baleage). The nutritional requirements for these cows were low as they were mature, non-pregnant, and non-lactating. Because the cows were fed at a low level just to maintain their body condition, it is unlikely that the intake of either diet would cause a substantial decrease in pH in the rumen.

It was expected that within in the mean pH over different days would remain constant. This was the case for baleage diet. When baleage was fed, each cow was offered the same amount of dry matter (7.6 kg baleage) for a similar duration (40 minutes). Observation showed that these cows finished their meal within 35 minutes, and all cows ate the same amount dry matter.

For the grass and hay diet the mean pH for the first day was lower than for the other 3 days. This could be the result of incomplete data as the time covered was from17:30 at the first day to 09:00 the next day. The mean pH for day 2 was also lower than day 3 and 4 by approximate 0.1 pH unit. This could be caused by the combination of eating behaviour of the cows on the different diet, and the different cows that contributed to the mean pH for each day as only the pH of 4 out of 6 cows was recorded on any single day. When grass and hay was fed, the total amount of feed offered was 12 m^2 grass and 36 kg of hay per day (2 m^2 grass and 6 kg of hay per cow per day). However, the cows were allowed to choose when to eat, and in which order they ate, and some cows may have eaten more dry matter in total than others or some cows may have differed in the proportion of grass and hay that they ate. This could lead to the difference of mean pH for each cow within a day. Because only 4 boluses were used in 6 cows, the mean pH for each day was actually the mean pH from 4 cows instead of 6 cows. This could lead to lower daily pH if the pH recording was from the 4 cows that had the lower mean pH or higher daily pH if it was from the 4 cows that had the higher pH. This is suggested by comparing cow No.716, 718 and 719 with other cows on different diets. Although No.716, 718 and 719 showed a significantly lower pH on the grass and hay diet, they showed similar mean pH to other cows on the baleage diet.

The significant difference in daily mean pH between two diets could be explained by several factors including the nature of their eating behaviour and the diet. When cows were offered grass and hay, they did not eat as fast as when they were offered baleage, as they learned that they did not need to finish the meal in a certain amount of time. This contributed to the slower pH decrease in the first hour of the meal was offered. The grass and hay diet was probably fermented more slowly than baleage diet because there was more neutral detergent fibre (NDF) in the grass and hay diet (Varga et al, 1984). Hay also causes less of a pH drop and a slower rate of decrease than silage (Schingoethe, 1975). The nature of the eating behaviour on the grass and hay diet could also contribute to the small decrease in ruminal pH after feed was offered and the fluctuation of pH after the initial drop in Figure 3.6.1 (page 68).

When cows were offered baleage, they ate quickly because they learned that they had limited time to eat and also because it takes less time to ingest baleage and baleage take less space than pasture in the rumen (Krause and Combs, 2002). It was observed that it takes an average of 35 minutes for cows to finish eating a 7.6 kg baleage meal. On the occasion when the boluses were switched among cows after feeding, it was observed that there was still plenty of space in the rumen to be filled. Baleage was fermented much faster causing a significant decrease in rumen pH (Bargo et al, 2002). The fast eating and rapid fermentation of baleage could be expected to cause the large and rapid decrease of ruminal pH which started after the meal was consumed. After the initially large drop of pH caused by the fermentation of the feed, there was a trend of increasing ruminal pH that commenced from about one hour after the meal was offered (Figure 3.6.2, page 69). The trend of pH increase lasted until the next meal because there was no feed available between two meals. The initial decrease of pH and the steadily increase in pH after the meal was finished was consistent with the pH pattern of all cows (Appendix 2). The nature of the rumen environment and its ability to restore the pH to optimum level also contributed to the pH pattern shown in Figure 3.6.2 (Krause and Combs, 2002).

The SAS regression analysis showed a significant difference of ruminal pH change for the two different diets. The comparison was focused on the availability of the food. It showed that the rate of pH decrease from eating baleage is 10 times greater than the rate of decline from eating grass and hay (0.054/hour for grass and hay diet vs. 0.54/hour for baleage diet). It also showed that the rate of pH recovery from the initial decrease was slower for grass and hay diet but not significantly different between diets (0.03/hour for grass and hay vs. 0.04/hour for baleage). The decrease and recovery of the ruminal pH represent the general trend for the daily profile of the pH in rumen for all cows used in experiment. Each cow would have its own unique eating behaviour and its own profile of pH throughout the day.

The regression results on the effect of baleage on pH change in the rumen were consistent with Figure 3.6.2 (pH decrease 0.54 per hour from regression analysis vs. pH decrease 0.4 in 40 minutes from Figure 3.6.2, page 69). However, the regression results on the effect of grass and hay diet on pH change was different from Figure 3.6.1 (pH decrease 0.054 per hour from regression analysis vs. pH decrease 0.15 per hour from Figure 3.6.1, page 68). This is because in the regression analysis there was an assumption that some food remained available beyond one hour following allocation of the grass and hay. Therefore, the time of food availability of grass and hay was elongated in comparison with the baleage. The observation that there were food still available in the field matches the fluctuation of pH after the initial drop during the first hour that food was offered as shown in Figure 3.6.1 (page 68).

4.8 The comparison of Kahne devices with other technologies

The Kahne boluses and transceivers showed the ability for measuring the temperature, pressure, and pH in the rumen of cows and sheep on a real time bases. This technology is useful on not only in scientific research but also for commercial use. The ruminal pH, temperature, and pressure are important parameters for health in ruminants. Ruminal pH is used as a parameter of health in cattle (Bramley et al, 2003; Kleen et al, 2003). Monitoring the pH in the rumen could assist in preventing ruminal acidosis (Oetzel, 2003; Krause and Oetzel, 2006; Al Zahal et al, 2007; Nagaraja and Titgemeyer, 2007). Temperature in the rumen also indicates the healthiness of the animal. Disease events such as Bovine Viral Diarrhea Virus (BVD) and Bovine Respiratory Disease (BRD) commonly result in an increase in core body temperature (Dye et al, 2007). It was suggested that ruminal temperature may aid in the detection of SARA (Al Zahal et al, 2008). Monitoring ruminal pressure could help in detecting bloat in cattle, especially for cattle in the feedlot that are offered large amount of highly fermentable grains (Theurer, 1986; Cheng et al, 1998). Using Kahne boluses and transceivers that enable the real time observation of ruminal pH, temperature, and pressure could allow first hand treatment of problems when they were detected.

There are other technologies developed to measure rumen environment. These technologies enable the measurement of signals or multiple aspects in the rumen (i.e. pH, temperature, and pressure).

4.8.1 Technologies of measuring rumen temperature

The technologies developed for measurement of rumen temperature including using fistulated animal (Bhattacharya and Warner,1968; Brod et al, 1982; Al Zahal et al, 2007; Al Zahal et al, 2008), using Radiosonde equipment (Dracy et al, 1963) and using FM-AM temperature-sensitive radio transmitting units and telemetering system (Kurtenbach and Dracy, 1965; Darcy and Kurtenbach, 1968), and using the temperature boluses that are available in the United States for measurement of the rumen temperature of feedlot cattle (Dye and Richards, 2007; Dye et al, 2007). Among these technologies, only temperature boluses were designed for both scientific and commercial use. The rest were for scientific research only. In 1963, Darcy et al reported an experiment using radiosonde equipment and transmitter to measure the intra-reticular temperature of intact cattle. The radiosonde equipment has a range of 5 feet (1.67 meters) and had to be placed close to animal. The use of a U.S. Army signal receiver and the close range meant that it could only be used for scientific research. Darcy and Kurtenbach (1968) described the FM-AM temperature telemetering system for monitoring ruminal temperature in intact, unrestrained cattle. The telemetry system covers a $30m^2$ area for animal to move freely and the transmission units have battery lifetimes of 60 days. The limitations for this technology are the expensive equipment (at that time) and the temperature drift, which happens when the power is low in the transmission unit. The use of rumen fistula is very popular as a research practice to monitor the rumen environment, for example the measurements of ruminal temperature through the fistula. Bord et al (1970) used thermistor probes through the fistula of cattle and a scanning tele-thermometer to monitor the ruminal temperature in one study. Al Zahal et al (2007) described a system for continuous recording of pH through rumen fistula. The electrode was able to measure pH and temperature simultaneously. The receiver for that device was mounted on cows back to allow them to move freely and the battery allows one year of data recording. Calibration was performed to eliminate drift if necessary. Dye and Richards (2007) tested temperature boluses and used them to monitor rumen temperature of feedlot cattle (Dye et al, 2007). The studies gave positive feedback on using temperature boluses in commercial feedlots. They identified an advantage of detecting core body temperature, an early warning of health events. It was also concerned with bolus problems including the data from boluses that were not received, and no reading or abnormal readings for the boluses.

The Kahne boluses have the advantages of being able to be used in intact animals and being able to measure pH, temperature, and pressure. The technology described by Al Zahal et al (2007) with fistulated animal may have the advantage of more accurate data recording, but the use of a fistula is not appropriate for a large number of animals or commercially. Temperature boluses (Dye and Richards, 2007) have many similarities to the rumen boluses but they lack the ability to study the relationship between pH and temperature in the rumen.

4.8.2 Technologies of measuring ruminal pressure

Rumen pressure or rumen motility was measured by technologies such as using radiosonde telemetry transmitters (Darcy and Kurtenbach, 1965) and a radio telemetering capsule and demodulator (Cook and Riley, 1970; Riley and Cook, 1974; Riley, 1986). The radiosonde telemetry transmitters described by Darcy and Kurtenbach (1965) can send a signal approximately 20 feet (6.67 meters), allowing the animal to move freely within that range. Riley (1986) used a pressure sensitive capsule for monitoring rumen activity in an intact animal. The transmission units were inexpensive, commercially available and could last for several weeks. Kahne boluses are similar to the pressure sensitive capsule (Riley, 1986) in the sense of measuring ruminal pressure data and transmitting the signals. But with the ability of measuring pressure with pH and temperature simultaneously, the boluses are much more useful in both scientific research and commercial use.

4.8.3 Technologies of measuring ruminal pH

Ways of measuring ruminal pH were described in section 1.3 in the literature review. The boluses are less invasive than rumen stomach tube, rumenocentesis and rumen fistula. The ethical cost is minimized if boluses were used. In scientific research, both rumen stomach tube, rumenocentesis and rumen fistula have proved to measure ruminal pH effectively (Nordlund and Garrett, 1995; Nordlund, 2003; Al Zahal et al, 2007). The Kahne boluses are still in the testing phase but the results are positive. The Kahne boluses are also the only commercial available products suitable to be used on a large number of animals for pH monitoring (e.g. feedlot).

4.8.4 Overall comparison

In scientific research, only the continuous recording system of pH through rumen fistula (Al Zahal et al, 2007) and the Kahne boluses have the ability of allowing animal to move freely and measuring more than one parameter in the rumen (i.e. ruminal pH and temperature). Temperature boluses (Dye and Richards, 2007) also allow free movement but only measures rumen temperature. Both technologies are good at measuring and collection data in an intensive period.

In commercial business such as a dairy farm or a feedlot, only the Kahne boluses and the temperature boluses enable measurements over longer periods of time for a large number of animals. By using these two technologies, the costs associated with frequent handling of animals could be minimised.

The Kahne bolus appears a better technology compared to other existed technologies. Firstly, it is available for both scientific research and commercial use. Secondly, it can simultaneously measure three parameters in the rumen. Thirdly, it can measure in short intervals (minimums 5 seconds) to closely monitor changes over short time frames or with less frequent transmission over longer intervals, allowing a long period of recording (up to 3 months). Fourthly, the reliability of transmission should be improved further and the pH drift problem should be rectified to enable measurement over longer periods.

Chapter 5: Conclusions and Future work

5.1 Conclusions

Each bolus tested showed a difference in temperature, pressure, or pH. It is clear that no two boluses were the same. Therefore, each boluses should be tested and calibrated before use.

For the boluses tested in these experiments, they are reliable for measuring temperature in animal experiments. It is not certain if these boluses could be used for measuring pressure in animal experiments. This is because there was a linear regression relationship between bolus pressure and actual pressure but the limited range of pressure that boluses were tested for did not give enough information on whether the relationship is consistent over a greater range of pressures. However, if the range for testing was increased and the linear regression relationship was consistent, these boluses are definitely able to be used in future experiment. Data on rumen pH is satisfactory over short durations (e.g. less than 3 days). However, they are not able to produce useable data for pH measurement in experiments unless direct measurement from reference pH probe is used to correct the pH drift occurred during long-term experiments (over 3 days). The regression relationships for each bolus under different experimental conditions were observed to differ, which suggests that the boluses were not ready for use in intact animals even if there were a formula available to correct pH drift from the previous experiments. It would need to be derived separately for each experiment.

There was no significant difference between data capture rate of each boluses tested in a control environment, which means the ability of transmitting data from each bolus were similar. In practice, the data capture rate was affected substantially by the animal body. However, other factors did affect the capture rate as well. The improvement of capture rate by using two transceivers was significant.

The experiment on testing the effect of diet on pH showed that the pH data from boluses were reliable if the process of correcting pH drift was carried along with the experiment. It was found in this experiment that even though the daily mean pH for each diet was similar, the pattern of pH change was very different between different diets. Feeding baleage caused a significantly larger drop in ruminal pH (0.54 pH unit per hour) compared with feeding grass and hay. It was also observed that the pH decrease began within 15 minutes after feed was given in both diet and pH began to increase about 1 hour following feed and continuous during resting and rumination.

The boluses could be very useful for scientific research. To be used in commercial farms the data capture rate and the pH drift should be improved. It could be a useful technology if the problems identified during this study are fixed.

5.2 Future work

A testing system could be developed to check the bolus property for measurement of temperature, pressure and pH. This includes finding out if there is a linear regression relationship between bolus pressure and actual pressure in a wide pressure range (up to 1100 millibars). It also includes testing same bolus in various experiments to find the model describe the pH drift in assorted situations. This needs to be done to allow boluses to be used in experiments with intact animals, where direct measurement of pH for calibration purposes is not possible.

Further experiments are needed to determine how much each factor affects the data capture rate and the relationship between those different factors. It is also important to work out the number of transceivers needed and the location of where they should be placed to get a reasonable amount of data from animals in a confined area. This is because this information is essential for commercial buyers.

A formula could be developed to relate the pH measured by the bolus to the health of the animal for easily detecting any adverse health events. Instead of shown actual pH data to the farm through computer, a warning program could be developed to advise the farmer to take action when critical predetermined pH points were reached.

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Appendix 1 The tables of hourly data capture rate (DCR) and frequency distribution of data capture rate for each indoor and outdoor experiment

A.1.1 Indoor experiment with boluses inserted in sheep from 17th to 19th April

There were a total of 49 hours continuous recording for this study. The parameter recorded was temperature. The transmission interval was 2 minutes, which means that there should be 30 data signals captured per hour. The mean data capture rate was 75.03% for bolus No.1 (b1) and 73.88% for bolus No.2 (b2).

Table A.1.1.1 Hourly data capture rate for 2 boluses in the indoor experiment using sheep from 17th to 19th April:

Date and time	b1	b2
2008-04-17 8:00 to 8:59	57%	43%
2008-04-17 9:00 to 9:59	63%	70%
2008-04-17 10:00 to 10:59	67%	70%
2008-04-17 11:00 to 11:59	97%	87%
2008-04-17 12:00 to 12:59	93%	80%
2008-04-17 13:00 to 13:59	80%	77%
2008-04-17 14:00 to 14:59	90%	60%
2008-04-17 15:00 to 15:59	90%	77%
2008-04-17 16:00 to 16:59	97%	77%
2008-04-17 17:00 to 17:59	90%	77%
2008-04-17 18:00 to 18:59	80%	83%
2008-04-17 19:00 to 19:59	77%	80%
2008-04-17 20:00 to 20:59	63%	7%
2008-04-17 21:00 to 21:59	70%	13%
2008-04-17 22:00 to 22:59	60%	73%
2008-04-17 23:00 to 23:59	77%	67%
2008-04-18 0:00 to 0:59	83%	90%
2008-04-18 1:00 to 1:59	87%	80%
2008-04-18 2:00 to 2:59	63%	77%
2008-04-18 3:00 to 3:59	77%	90%
2008-04-18 4:00 to 4:59	97%	83%
2008-04-18 5:00 to 5:59	87%	80%
2008-04-18 6:00 to 6:59	90%	77%
2008-04-18 7:00 to 7:59	77%	83%
2008-04-18 8:00 to 8:59	27%	23%
2008-04-18 9:00 to 9:59	87%	73%
2008-04-18 10:00 to 10:59	77%	83%
2008-04-18 11:00 to 11:59	73%	87%
2008-04-18 12:00 to 12:59	87%	83%

Table A.1.1.1 (cont.)

Date and time	b1	b2
2008-04-18 13:00 to 13:59	87%	83%
2008-04-18 14:00 to 14:59	93%	73%
2008-04-18 15:00 to 15:59	80%	77%
2008-04-18 16:00 to 16:59	43%	80%
2008-04-18 17:00 to 17:59	63%	77%
2008-04-18 18:00 to 18:59	0%	60%
2008-04-18 19:00 to 19:59	93%	77%
2008-04-18 20:00 to 20:59	70%	70%
2008-04-18 21:00 to 21:59	80%	87%
2008-04-18 22:00 to 22:59	50%	97%
2008-04-18 23:00 to 23:59	37%	80%
2008-04-19 0:00 to 0:59	97%	83%
2008-04-19 1:00 to 1:59	83%	87%
2008-04-19 2:00 to 2:59	97%	87%
2008-04-19 3:00 to 3:59	83%	100%
2008-04-19 4:00 to 4:59	57%	80%
2008-04-19 5:00 to 5:59	83%	83%
2008-04-19 6:00 to 6:59	70%	80%
2008-04-19 7:00 to 7:59	80%	70%
2008-04-19 8:00 to 8:59	70%	40%

Table A.1.1.2 Frequency distribution of data capture rate (DCR) for 2 boluses in the indoor experiment using sheep from 17th to 19th April:

DCR	b1	b2
0-10%	1	1
10.01-20%	0	1
20.01-30%	1	1
30.01-40%	1	1
40.01-50%	2	1
50.01-60%	3	2
60.01-70%	9	5
70.01-80%	11	20
80.01-90%	13	15
90.01-100%	8	2

A.1.2 Indoor experiment with boluses inserted in sheep from 21st to 23rd April

There were a total of 49 hours continuous recording for this study. The parameter recorded was temperature. The transmission interval was 2 minutes, which means that there should be 30 data signals captured per hour. The mean data capture rate was 76.19% for bolus No.1 (b1) and 75.92% for bolus No.2 (b2).

Table A.1.2.1 Hourly data capture rate for 2 boluses in the indoor experiment using sheep from 21st to 23rd April:

date and time	b1	b2
2008-04-21 8:00 to 8:59	30%	33.33%
2008-04-21 9:00 to 9:59	83.33%	66.67%
2008-04-21 10:00 to 10:59	86.67%	80%
2008-04-21 11:00 to 11:59	50%	36.67%
2008-04-21 12:00 to 12:59	23.33%	40%
2008-04-21 13:00 to 13:59	80%	83.33%
2008-04-21 14:00 to 14:59	80%	76.67%
2008-04-21 15:00 to 15:59	70%	76.67%
2008-04-21 16:00 to 16:59	80%	63.33%
2008-04-21 17:00 to 17:59	76.67%	90%
2008-04-21 18:00 to 18:59	93.33%	76.67%
2008-04-21 19:00 to 19:59	83.33%	83.33%
2008-04-21 20:00 to 20:59	86.67%	76.67%
2008-04-21 21:00 to 21:59	86.67%	83.33%
2008-04-21 22:00 to 22:59	86.67%	63.33%
2008-04-21 23:00 to 23:59	100%	73.33%
2008-04-22 0:00 to 0:59	90%	86.67%
2008-04-22 1:00 to 1:59	96.67%	66.67%
2008-04-22 2:00 to 2:59	73.33%	83.33%
2008-04-22 3:00 to 3:59	86.67%	80%
2008-04-22 4:00 to 4:59	90%	70%
2008-04-22 5:00 to 5:59	83.33%	80%
2008-04-22 6:00 to 6:59	86.67%	70%
2008-04-22 7:00 to 7:59	86.67%	80%
2008-04-22 8:00 to 8:59	80%	83.33%
2008-04-22 9:00 to 9:59	80%	76.67%
2008-04-22 10:00 to 10:59	90%	86.67%
2008-04-22 11:00 to 11:59	70%	86.67%
2008-04-22 12:00 to 12:59	76.67%	83.33%
2008-04-22 13:00 to 13:59	93.33%	73.33%
2008-04-22 14:00 to 14:59	83.33%	76.67%
2008-04-22 15:00 to 15:59	56.67%	73.33%
2008-04-22 16:00 to 16:59	90%	100%
2008-04-22 17:00 to 17:59	66.67%	86.67%
2008-04-22 18:00 to 18:59	83.33%	80%
2008-04-22 19:00 to 19:59	83.33%	83.33%
2008-04-22 20:00 to 20:59	50%	80%
2008-04-22 21:00 to 21:59	10%	86.67%
2008-04-22 22:00 to 22:59	76.67%	83.33%

Table A.1.2.1 (cont.)

date and time	b1	b2
2008-04-22 23:00 to 23:59	73.33%	83.33%
2008-04-23 0:00 to 0:59	93.33%	66.67%
2008-04-23 1:00 to 1:59	83.33%	80%
2008-04-23 2:00 to 2:59	63.33%	80%
2008-04-23 3:00 to 3:59	76.67%	76.67%
2008-04-23 4:00 to 4:59	73.33%	83.33%
2008-04-23 5:00 to 5:59	66.67%	83.33%
2008-04-23 6:00 to 6:59	83.33%	83.33%
2008-04-23 7:00 to 7:59	96.67%	80%
2008-04-23 8:00 to 8:59	43.33%	43.33%

Table A.1.2.2 Frequency distribution of data capture rate for 2 boluses in the indoor experiment using sheep from 21st to 23rd April:

DCR	b1	b2
0-10%	1	0
10.01-20%	0	0
20.01-30%	2	0
30.01-40%	0	3
40.01-50%	3	1
50.01-60%	1	0
60.01-70%	5	7
70.01-80%	12	19
80.01-90%	19	18
90.01-100%	6	1

A.1.3 Indoor experiment with boluses inserted in sheep from 8th to 10th May

There were a total of 49 hours continuous recording for this study. The parameter recorded was temperature and pH. The transmission interval was 2 minutes, which means that there should be 30 data signals captured per hour. The mean data capture rate was 70.54% for bolus No.1 (b1) and 65.03% for bolus No.11 (b11); b2 and b12 were recording abnormal data and was stopped.

Table A.1.3.1 Hourly data capture rate for 2 boluses in the indoor experiment using sheep from 8th to 10th May:

date and time	b1	b11
2008-05-08 15:00 to 15:59	80%	70%
2008-05-08 16:00 to 16:59	93.33%	80%
2008-05-08 17:00 to 17:59	66.67%	80%
2008-05-08 18:00 to 18:59	86.67%	73.33%
2008-05-08 19:00 to 19:59	56.67%	73.33%
2008-05-08 20:00 to 20:59	76.67%	73.33%
2008-05-08 21:00 to 21:59	73.33%	76.67%
2008-05-08 22:00 to 22:59	86.67%	76.67%
2008-05-08 23:00 to 23:59	76.67%	76.67%
2008-05-09 0:00 to 0:59	86.67%	66.67%
2008-05-09 1:00 to 1:59	76.67%	63.33%
2008-05-09 2:00 to 2:59	80%	70%
2008-05-09 3:00 to 3:59	86.67%	83.33%
2008-05-09 4:00 to 4:59	46.67%	56.67%
2008-05-09 5:00 to 5:59	23.33%	63.33%
2008-05-09 6:00 to 6:59	13.33%	16.67%
2008-05-09 7:00 to 7:59	33.33%	50%
2008-05-09 8:00 to 8:59	13.33%	13.33%
2008-05-09 9:00 to 9:59	40%	76.67%
2008-05-09 10:00 to 10:59	83.33%	73.33%
2008-05-09 11:00 to 11:59	83.33%	83.33%
2008-05-09 12:00 to 12:59	56.67%	46.67%
2008-05-09 13:00 to 13:59	93.33%	76.67%
2008-05-09 14:00 to 14:59	70%	63.33%
2008-05-09 15:00 to 15:59	73.33%	60%
2008-05-09 16:00 to 16:59	76.67%	60%
2008-05-09 17:00 to 17:59	33.33%	76.67%
2008-05-09 18:00 to 18:59	36.67%	50%
2008-05-09 19:00 to 19:59	73.33%	70%
2008-05-09 20:00 to 20:59	60%	50%
2008-05-09 21:00 to 21:59	33.33%	43.33%
2008-05-09 22:00 to 22:59	76.67%	53.33%
2008-05-09 23:00 to 23:59	96.67%	80%
2008-05-10 0:00 to 0:59	86.67%	83.33%
2008-05-10 1:00 to 1:59	53.33%	46.67%
2008-05-10 2:00 to 2:59	80%	66.67%
2008-05-10 3:00 to 3:59	86.67%	20%
2008-05-10 4:00 to 4:59	76.67%	13.33%

2008-05-10	5:00 to 5:59	90%	93.33%
2008-05-10	6:00 to 6:59	83.33%	86.67%
2008-05-10	7:00 to 7:59	83.33%	83.33%

Table A.1.3.1 (cont.)

date and time		b1	b11
2008-05-10	9:00 to 9:59	90%	83.33%
2008-05-10	10:00 to 10:59	86.67%	83.33%
2008-05-10	11:00 to 11:59	90%	63.33%
2008-05-10	12:00 to 12:59	80%	20%
2008-05-10	13:00 to 13:59	76.67%	73.33%
2008-05-10	14:00 to 14:59	86.67%	83.33%
2008-05-10	15:00 to 15:59	83.33%	76.67%

Table A.1.3.2 Frequency distribution of data capture rate for 2 boluses in the indoor experiment using sheep from 8^{th} to 10^{th} May:

DCR	b1	b11
0-10%	0	0
10.01-20%	2	5
20.01-30%	1	0
30.01-40%	5	0
40.01-50%	1	6
50.01-60%	4	4
60.01-70%	2	9
70.01-80%	15	15
80.01-90%	16	9
90.01-100%	3	1

A.1.4 Indoor experiment with boluses inserted in sheep from 13^{th} to 14^{th} June

There were a total of 22 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was 5 minutes, which means that there should be 12 data signals captured per hour. The mean data capture rate was 86.36% for bolus No.15 (b15), 82.95% for bolus No.16 (b16), and 82.95% for bolus No.17 (b17).

Table A.1.4.1 Hourly data capture rate for 3 boluses in the indoor experiment using sheep from 13^{th} to 14^{th} June:

date and time		b15	b16	b17
2008-06-13	10:00 to 10:59	75.00%	91.67%	58.33%
2008-06-13	11:00 to 11:59	91.67%	83.33%	83.33%
2008-06-13	12:00 to 12:59	83.33%	83.33%	100%
2008-06-13	13:00 to 13:59	100%	66.67%	83.33%
2008-06-13	14:00 to 14:59	75.00%	66.67%	83.33%

Table A.1.4.1 (cont.)

date and time	b15	b16	b17
2008-06-13 15:00 to 15:59	83.33%	75.00%	100%
2008-06-13 16:00 to 16:59	91.67%	100%	75.00%
2008-06-13 17:00 to 17:59	83.33%	66.67%	83.33%
2008-06-13 18:00 to 18:59	91.67%	75.00%	100%
2008-06-13 19:00 to 19:59	91.67%	91.67%	75.00%
2008-06-13 20:00 to 20:59	83.33%	91.67%	75.00%
2008-06-13 21:00 to 21:59	83.33%	66.67%	66.67%
2008-06-13 22:00 to 22:59	91.67%	83.33%	83.33%
2008-06-13 23:00 to 23:59	83.33%	100%	91.67%
2008-06-14 0:00 to 0:59	83.33%	91.67%	75.00%
2008-06-14 1:00 to 1:59	83.33%	91.67%	83.33%
2008-06-14 2:00 to 2:59	83.33%	83.33%	83.33%
2008-06-14 3:00 to 3:59	91.67%	83.33%	100%
2008-06-14 4:00 to 4:59	91.67%	100%	91.67%
2008-06-14 5:00 to 5:59	66.67%	66.67%	75.00%
2008-06-14 6:00 to 6:59	91.67%	91.67%	66.67%
2008-06-14 7:00 to 7:59	100%	75.00%	91.67%

Table A.1.4.2 Frequency distribution of data capture rate for 3 boluses in the indoor experiment using sheep from 13th to 14th June:

DCR	b15	b16	b17
0-10%	0	0	0
10.01-20%	0	0	0
20.01-30%	0	0	0
30.01-40%	0	0	0
40.01-50%	0	0	0
50.01-60%	0	0	1
60.01-70%	1	5	2
70.01-80%	2	3	5
80.01-90%	9	5	7
90.01-100%	10	9	7

A.1.5 Indoor experiments with boluses inserted in cows from 21st to 22nd May

There were a total of 25 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was 5 minutes, which means that there should be 12 data signals captured per hour. The mean data capture rate was 0.87% for bolus No.11 (b11) and 2.60% for bolus No.12 (b12).

Table A.1.5.1 Hourly data capture rate for 2 boluses in the indoor experiment using cows from 21st to 22nd May:

time	b11	b12
2008-05-21 9:00 to 9:59	0.33%	0%
2008-05-21 10:00 to 10:59	0.56%	0%
2008-05-21 11:00 to 11:59	0.11%	1.44%
2008-05-21 12:00 to 12:59	0%	2.56%
2008-05-21 13:00 to 13:59	1.11%	2.33%
2008-05-21 14:00 to 14:59	0.44%	2.67%
2008-05-21 15:00 to 15:59	1.22%	1.89%
2008-05-21 16:00 to 16:59	0%	5.56%
2008-05-21 17:00 to 17:59	0%	6.94%
2008-05-21 18:00 to 18:59	0%	3.47%
2008-05-21 19:00 to 19:59	0%	4.17%
2008-05-21 20:00 to 20:59	1.39%	0.69%
2008-05-21 21:00 to 21:59	5.56%	2.08%
2008-05-21 22:00 to 22:59	6.25%	5.56%
2008-05-21 23:00 to 23:59	4.86%	6.94%
2008-05-22 0:00 to 0:59	0%	1.39%
2008-05-22 1:00 to 1:59	0%	2.78%
2008-05-22 2:00 to 2:59	0%	1.39%
2008-05-22 3:00 to 3:59	0%	0%
2008-05-22 4:00 to 4:59	0%	0%
2008-05-22 5:00 to 5:59	0%	0%
2008-05-22 6:00 to 6:59	0%	4.17%
2008-05-22 7:00 to 7:59	0%	4.17%
2008-05-22 8:00 to 8:59	0%	1.39%
2008-05-22 9:00 to 9:59	0%	3.47%

Table A.1.5.2 Frequency distribution of data capture rate for 2 boluses in the indoor experiment using cows from 21st to 22nd May:

DCR	b11	b12
0-10%	25	25
10.01-20%	0	0
20.01-30%	0	0
30.01-40%	0	0
40.01-50%	0	0
50.01-60%	0	0
60.01-70%	0	0
70.01-80%	0	0
80.01-90%	0	0

90.01-100%	0	0

A.1.6 Indoor experiments with boluses inserted in cows from 14th to 17th June

There were a total of 71 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was 5 minutes, which means that there should be 12 data signals captured per hour. The mean data capture rate was 24.41% for bolus No.12 (b12), 23.94% for bolus No.13 (b13), 0.47% for bolus No.15 (b15), 30.40% for bolus No.16 (b16), and 24.88% for bolus No.17 (b17).

Table A.1.6.1 Hourly data capture rate for 5 boluses in the indoor experiment using cows from 14th to 17th June:

time		b12	b13	b15	b16	b17
2008-06-14	9:00 to 9:59	16.67%	25.00%	16.67%	66.67%	0%
2008-06-14	10:00 to 10:59	16.67%	25.00%	16.67%	16.67%	25.00%
2008-06-14	11:00 to 11:59	0%	25.00%	0%	8.33%	41.67%
2008-06-14	12:00 to 12:59	0%	8.33%	0%	50%	83.33%
2008-06-14	13:00 to 13:59	16.67%	0%	0%	66.67%	0%
2008-06-14	14:00 to 14:59	66.67%	25.00%	0%	66.67%	25.00%
2008-06-14	15:00 to 15:59	16.67%	50%	0%	8.33%	33.33%
2008-06-14	16:00 to 16:59	16.67%	33.33%	0%	33.33%	66.67%
2008-06-14	17:00 to 17:59	75.00%	25.00%	0%	25.00%	50%
2008-06-14	18:00 to 18:59	41.67%	58.33%	0%	16.67%	50%
2008-06-14	19:00 to 19:59	25.00%	25.00%	0%	0%	16.67%
2008-06-14	20:00 to 20:59	8.33%	33.33%	0%	25.00%	66.67%
2008-06-14	21:00 to 21:59	66.67%	66.67%	0%	33.33%	0%
2008-06-14	22:00 to 22:59	33.33%	33.33%	0%	33.33%	25.00%
2008-06-14	23:00 to 23:59	41.67%	25.00%	0%	0%	16.67%
2008-06-15	0:00 to 0:59	25.00%	0%	0%	16.67%	0%
2008-06-15	1:00 to 1:59	33.33%	33.33%	0%	50%	50%
2008-06-15	2:00 to 2:59	25.00%	25.00%	0%	25.00%	8.33%
2008-06-15	3:00 to 3:59	25.00%	25.00%	0%	58.33%	41.67%
2008-06-15	4:00 to 4:59	41.67%	25.00%	0%	0%	41.67%
2008-06-15	5:00 to 5:59	41.67%	33.33%	0%	8.33%	50%
2008-06-15	6:00 to 6:59	0%	16.67%	0%	33.33%	58.33%
2008-06-15	7:00 to 7:59	16.67%	8.33%	0%	25.00%	8.33%
2008-06-15	8:00 to 8:59	8.33%	8.33%	0%	0%	0%
2008-06-15	9:00 to 9:59	50%	25.00%	0%	8.33%	25.00%
2008-06-15	10:00 to 10:59	16.67%	25.00%	0%	8.33%	66.67%
2008-06-15	11:00 to 11:59	0%	16.67%	0%	58.33%	16.67%
2008-06-15	12:00 to 12:59	33.33%	50%	0%	25.00%	8.33%
2008-06-15	13:00 to 13:59	16.67%	25.00%	0%	66.67%	8.33%
2008-06-15	14:00 to 14:59	16.67%	16.67%	0%	8.33%	58.33%
2008-06-15	15:00 to 15:59	8.33%	16.67%	0%	41.67%	16.67%

2008-06-15	16:00 to 16:59	58.33%	33.33%	0%	50%	33.33%
2008-06-15	17:00 to 17:59	50%	8.33%	0%	83.33%	16.67%
2008-06-15	18:00 to 18:59	8.33%	16.67%	0%	16.67%	33.33%
2008-06-15	19:00 to 19:59	33.33%	16.67%	0%	16.67%	75.00%

Table A.1.6.1 (cont.)

time		b12	b13	b15	b16	b17
2008-06-15	20:00 to 20:59	66.67%	0%	0%	50%	16.67%
2008-06-15	21:00 to 21:59	41.67%	8.33%	0%	41.67%	16.67%
2008-06-15	22:00 to 22:59	16.67%	0%	0%	41.67%	0%
2008-06-15	23:00 to 23:59	0%	16.67%	0%	50%	8.33%
2008-06-16	0:00 to 0:59	8.33%	16.67%	0%	0%	33.33%
2008-06-16	1:00 to 1:59	16.67%	25.00%	0%	0%	0%
2008-06-16	2:00 to 2:59	58.33%	33.33%	0%	0%	25.00%
2008-06-16	3:00 to 3:59	41.67%	33.33%	0%	0%	16.67%
2008-06-16	4:00 to 4:59	75.00%	8.33%	0%	33.33%	25.00%
2008-06-16	5:00 to 5:59	58.33%	58.33%	0%	33.33%	8.33%
2008-06-16	6:00 to 6:59	41.67%	8.33%	0%	8.33%	16.67%
2008-06-16	7:00 to 7:59	33.33%	41.67%	0%	25.00%	41.67%
2008-06-16	8:00 to 8:59	8.33%	16.67%	0%	50%	25.00%
2008-06-16	9:00 to 9:59	41.67%	33.33%	0%	25.00%	33.33%
2008-06-16	10:00 to 10:59	8.33%	50%	0%	58.33%	0%
2008-06-16	11:00 to 11:59	0%	33.33%	0%	25.00%	0%
2008-06-16	12:00 to 12:59	0%	25.00%	0%	41.67%	25.00%
2008-06-16	13:00 to 13:59	0%	41.67%	0%	16.67%	25.00%
2008-06-16	14:00 to 14:59	16.67%	58.33%	0%	16.67%	8.33%
2008-06-16	15:00 to 15:59	8.33%	33.33%	0%	41.67%	16.67%
2008-06-16	16:00 to 16:59	8.33%	8.33%	0%	66.67%	33.33%
2008-06-16	17:00 to 17:59	0%	25.00%	0%	41.67%	50%
2008-06-16	18:00 to 18:59	0%	33.33%	0%	16.67%	0%
2008-06-16	19:00 to 19:59	0%	0%	0%	8.33%	58.33%
2008-06-16	20:00 to 20:59	0%	0%	0%	75.00%	50%
2008-06-16	21:00 to 21:59	33.33%	33.33%	0%	0%	8.33%
2008-06-16	22:00 to 22:59	25.00%	16.67%	0%	16.67%	8.33%
2008-06-16	23:00 to 23:59	25.00%	50%	0%	16.67%	0%
2008-06-17	0:00 to 0:59	25.00%	8.33%	0%	25.00%	0%
2008-06-17	1:00 to 1:59	0%	8.33%	0%	75.00%	8.33%
2008-06-17	2:00 to 2:59	25.00%	16.67%	0%	33.33%	8.33%
2008-06-17	3:00 to 3:59	41.67%	25.00%	0%	33.33%	16.67%
2008-06-17	4:00 to 4:59	8.33%	25.00%	0%	8.33%	16.67%
2008-06-17	5:00 to 5:59	25.00%	0%	0%	33.33%	25.00%
2008-06-17	6:00 to 6:59	8.33%	16.67%	0%	41.67%	8.33%
2008-06-17	7:00 to 7:59	16.67%	8.33%	0%	58.33%	16.67%

Table A.1.6.2 Frequency distribution of data capture rate for 5 boluses in the indoor experiment using cows from 14th to 17th June:

DCR	b12	b13	b15	b16	b17
0-10%	24	18	69	18	24
10.01-20%	12	12	2	10	13
20.01-30%	9	18	0	9	10
30.01-40%	6	13	0	9	6
40.01-50%	11	6	0	13	10
50.01-60%	3	3	0	4	3
60.01-70%	3	1	0	5	3
70.01-80%	3	0	0	2	1
80.01-90%	0	0	0	1	1
90.01-100%	0	0	0	0	0

A.1.7 Indoor experiments with boluses inserted in cows from 28th to 30th June

There were a total of 48 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was 5 minutes, which means that there should be 12 data signals captured per hour. The mean data capture rate was 15.63% for bolus No.12 (b12), 27.95% for bolus No.13 (b13), 31.42% for bolus No.14 (b14), 0% for bolus No.15 (b15), 31.94% for bolus No.16 (b16), and 0.87% for bolus No.17 (b17).

Table A.1.7.1 Hourly data capture rate for 6 boluses in the indoor experiment using cows from 28th to 30th June:

time		b12	b13	b14	b15	b16	b17
2008-06-28	9:00 to 9:59	33.33%	33.33%	33.33%	0%	50%	41.67%
2008-06-28	10:00 to 10:59	41.67%	33.33%	16.67%	0%	16.67%	0%
2008-06-28	11:00 to 11:59	0%	0%	8.33%	0%	0%	0%
2008-06-28	12:00 to 12:59	0%	0%	16.67%	0%	0%	0%
2008-06-28	13:00 to 13:59	0%	16.67%	58.33%	0%	25.00%	0%
2008-06-28	14:00 to 14:59	16.67%	41.67%	50%	0%	66.67%	0%
2008-06-28	15:00 to 15:59	8.33%	41.67%	41.67%	0%	8.33%	0%
2008-06-28	16:00 to 16:59	16.67%	33.33%	41.67%	0%	41.67%	0%
2008-06-28	17:00 to 17:59	8.33%	16.67%	50%	0%	50%	0%
2008-06-28	18:00 to 18:59	0%	0%	8.33%	0%	16.67%	0%
2008-06-28	19:00 to 19:59	0%	66.67%	25.00%	0%	66.67%	0%
2008-06-28	20:00 to 20:59	8.33%	25.00%	16.67%	0%	41.67%	0%
2008-06-28	21:00 to 21:59	0%	33.33%	33.33%	0%	25.00%	0%

2008-06-28	22:00 to 22:59	0%	41.67%	16.67%	0%	16.67%	0%
2008-06-28	23:00 to 23:59	0%	16.67%	41.67%	0%	25.00%	0%
2008-06-29	0:00 to 0:59	0%	16.67%	41.67%	0%	25.00%	0%
2008-06-29	1:00 to 1:59	0%	33.33%	25.00%	0%	25.00%	0%

Table A.1.7.1 9 (cont.)

time		b12	b13	b14	b15	b16	b17
2008-06-29	2:00 to 2:59	16.67%	16.67%	25.00%	0%	16.67%	0%
2008-06-29	3:00 to 3:59	0%	66.67%	50%	0%	16.67%	0%
2008-06-29	4:00 to 4:59	16.67%	16.67%	58.33%	0%	16.67%	0%
2008-06-29	5:00 to 5:59	8.33%	25.00%	58.33%	0%	66.67%	0%
2008-06-29	6:00 to 6:59	58.33%	8.33%	50%	0%	83.33%	0%
2008-06-29	7:00 to 7:59	25.00%	50%	50%	0%	16.67%	0%
2008-06-29	8:00 to 8:59	25.00%	25.00%	41.67%	0%	50%	0%
2008-06-29	9:00 to 9:59	0%	66.67%	0%	0%	0%	0%
2008-06-29	10:00 to 10:59	8.33%	8.33%	33.33%	0%	50%	0%
2008-06-29	11:00 to 11:59	16.67%	0%	0%	0%	66.67%	0%
2008-06-29	12:00 to 12:59	8.33%	41.67%	8.33%	0%	16.67%	0%
2008-06-29	13:00 to 13:59	0%	58.33%	25.00%	0%	50%	0%
2008-06-29	14:00 to 14:59	33.33%	8.33%	16.67%	0%	25.00%	0%
2008-06-29	15:00 to 15:59	33.33%	8.33%	16.67%	0%	0%	0%
2008-06-29	16:00 to 16:59	66.67%	58.33%	0%	0%	50%	0%
2008-06-29	17:00 to 17:59	41.67%	25.00%	25.00%	0%	50%	0%
2008-06-29	18:00 to 18:59	0%	41.67%	58.33%	0%	8.33%	0%
2008-06-29	19:00 to 19:59	0%	66.67%	25.00%	0%	66.67%	0%
2008-06-29	20:00 to 20:59	8.33%	33.33%	8.33%	0%	16.67%	0%
2008-06-29	21:00 to 21:59	8.33%	75.00%	33.33%	0%	8.33%	0%
2008-06-29	22:00 to 22:59	16.67%	75.00%	91.67%	0%	8.33%	0%
2008-06-29	23:00 to 23:59	16.67%	16.67%	83.33%	0%	16.67%	0%
2008-06-30	0:00 to 0:59	0%	25.00%	25.00%	0%	16.67%	0%
2008-06-30	1:00 to 1:59	83.33%	0%	16.67%	0%	25.00%	0%
2008-06-30	2:00 to 2:59	33.33%	25.00%	8.33%	0%	50%	0%
2008-06-30	3:00 to 3:59	25.00%	8.33%	8.33%	0%	41.67%	0%
2008-06-30	4:00 to 4:59	41.67%	0%	33.33%	0%	41.67%	0%
2008-06-30	5:00 to 5:59	0%	33.33%	58.33%	0%	91.67%	0%
2008-06-30	6:00 to 6:59	0%	8.33%	58.33%	0%	50%	0%

Table A.1.7.2 Frequency distribution of data capture rate for 6 boluses in the indoor experiment using cows from 28th to 30th June:

DCR	b12	b13	b14	b15	b16	b17
0-10%	27	14	11	48	10	47
10.01-20%	8	7	7	0	11	0
20.01-30%	3	6	7	0	7	0
30.01-40%	4	7	5	0	0	0
40.01-50%	3	6	10	0	13	1

50.01-60%	1	2	6	0	0	0
60.01-70%	1	4	0	0	5	0
70.01-80%	0	2	0	0	0	0
80.01-90%	1	0	1	0	1	0
90.01-100%	0	0	1	0	1	0

A.1.8 Indoor experiments with boluses inserted in cows from 27th to 30th September

There were a total of 72 hours continuous recording for this study. The parameter recorded was temperature and pH. The transmission interval was varied from 35 to 55 seconds for different boluses, which means that there should be 65 to 102 data signals captured per hour for different boluses. The mean data capture rate was 69.87% for bolus No. 3 (b3), 62.67% for bolus No.4 (b4), 65.50% for bolus No.5 (b5), 58.72% for bolus No.6 (b6), and 64.83% for bolus No.7 (b7)

Table A.1.8.1 Hourly data capture rate for 5 boluses in the indoor experiment using cows from 27th to 30th September:

time		b3	b4	b5	b6	b7
2008-09-27	15:00 to 15:59	47.69%	48.61%	35.00%	48.89%	48.54%
2008-09-27	16:00 to 16:59	64.62%	65.28%	86.25%	74.44%	31.07%
2008-09-27	17:00 to 17:59	89.23%	63.89%	41.25%	51.11%	45.63%
2008-09-27	18:00 to 18:59	90.77%	68.06%	46.25%	53.33%	92.23%
2008-09-27	19:00 to 19:59	87.69%	65.28%	77.50%	57.78%	90.29%
2008-09-27	20:00 to 20:59	69.23%	73.61%	93.75%	15.56%	96.12%
2008-09-27	21:00 to 21:59	60%	77.78%	81.25%	38.89%	67.96%
2008-09-27	22:00 to 22:59	81.54%	81.94%	8.75%	0%	84.47%
2008-09-27	23:00 to 23:59	78.46%	83.33%	51.25%	70%	78.64%
2008-09-28	0:00 to 0:59	92.31%	77.78%	77.50%	64.44%	93.20%
2008-09-28	1:00 to 1:59	72.31%	77.78%	92.50%	34.44%	89.32%
2008-09-28	2:00 to 2:59	86.15%	58.33%	83.75%	51.11%	90.29%
2008-09-28	3:00 to 3:59	61.54%	52.78%	77.50%	18.89%	78.64%
2008-09-28	4:00 to 4:59	80%	69.44%	62.50%	34.44%	94.17%
2008-09-28	5:00 to 5:59	95.38%	80.56%	50%	64.44%	81.55%
2008-09-28	6:00 to 6:59	73.85%	80.56%	61.25%	60%	87.38%
2008-09-28	7:00 to 7:59	67.69%	63.89%	70%	55.56%	86.41%
2008-09-28	8:00 to 8:59	72.31%	59.72%	91.25%	77.78%	84.47%
2008-09-28	9:00 to 9:59	61.54%	30.56%	70%	77.78%	70.87%
2008-09-28	10:00 to 10:59	78.46%	62.50%	37.50%	32.22%	45.63%
2008-09-28	11:00 to 11:59	81.54%	56.94%	62.50%	14.44%	92.23%
2008-09-28	12:00 to 12:59	69.23%	27.78%	52.50%	73.33%	43.69%
2008-09-28	13:00 to 13:59	40%	68.06%	82.50%	41.11%	79.61%
2008-09-28	14:00 to 14:59	76.92%	68.06%	45.00%	71.11%	67.96%
2008-09-28	15:00 to 15:59	63.08%	47.22%	47.50%	56.67%	68.93%
2008-09-28	16:00 to 16:59	70.77%	62.50%	71.25%	58.89%	87.38%

2008-09-28	17:00 to 17:59	64.62%	69.44%	92.50%	65.56%	17.48%
2008-09-28	18:00 to 18:59	89.23%	65.28%	98.75%	46.67%	37.86%
2008-09-28	19:00 to 19:59	72.31%	79.17%	17.50%	68.89%	78.64%
2008-09-28	20:00 to 20:59	67.69%	26.39%	0%	63.33%	53.40%
2008-09-28	21:00 to 21:59	81.54%	72.22%	47.50%	74.44%	64.08%
2008-09-28	22:00 to 22:59	75.38%	68.06%	71.25%	52.22%	54.37%

Table A.1.8.1 (cont.)

time		b3	b4	b5	b6	b7
2008-09-28	23:00 to 23:59	86.15%	76.39%	62.50%	60%	84.47%
2008-09-29	0:00 to 0:59	46.15%	51.39%	28.75%	78.89%	83.50%
2008-09-29	1:00 to 1:59	58.46%	38.89%	46.25%	73.33%	26.21%
2008-09-29	2:00 to 2:59	72.31%	76.39%	71.25%	87.78%	89.32%
2008-09-29	3:00 to 3:59	81.54%	77.78%	80%	64.44%	86.41%
2008-09-29	4:00 to 4:59	84.62%	80.56%	77.50%	48.89%	89.32%
2008-09-29	5:00 to 5:59	73.85%	80.56%	48.75%	57.78%	56.31%
2008-09-29	6:00 to 6:59	86.15%	61.11%	82.50%	58.89%	80.58%
2008-09-29	7:00 to 7:59	83.08%	90.28%	46.25%	68.89%	87.38%
2008-09-29	8:00 to 8:59	80%	61.11%	36.25%	62.22%	91.26%
2008-09-29	9:00 to 9:59	80%	34.72%	65.00%	73.33%	79.61%
2008-09-29	10:00 to 10:59	66.15%	51.39%	58.75%	65.56%	51.46%
2008-09-29	11:00 to 11:59	61.54%	73.61%	66.25%	56.67%	54.37%
2008-09-29	12:00 to 12:59	69.23%	58.33%	56.25%	83.33%	32.04%
2008-09-29	13:00 to 13:59	78.46%	66.67%	71.25%	67.78%	79.61%
2008-09-29	14:00 to 14:59	21.54%	68.06%	7.50%	17.78%	24.27%
2008-09-29	15:00 to 15:59	83.08%	83.33%	71.25%	53.33%	72.82%
2008-09-29	16:00 to 16:59	78.46%	81.94%	58.75%	94.44%	57.28%
2008-09-29	17:00 to 17:59	36.92%	51.39%	81.25%	71.11%	69.90%
2008-09-29	18:00 to 18:59	18.46%	44.44%	63.75%	55.56%	70.87%
2008-09-29	19:00 to 19:59	86.15%	68.06%	78.75%	67.78%	72.82%
2008-09-29	20:00 to 20:59	93.85%	62.50%	66.25%	74.44%	51.46%
2008-09-29	21:00 to 21:59	89.23%	25.00%	86.25%	76.67%	52.43%
2008-09-29	22:00 to 22:59	66.15%	56.94%	87.50%	72.22%	0.97%
2008-09-29	23:00 to 23:59	76.92%	66.67%	78.75%	41.11%	68.93%
2008-09-30	0:00 to 0:59	89.23%	69.44%	87.50%	71.11%	38.83%
2008-09-30	1:00 to 1:59	53.85%	56.94%	93.75%	71.11%	34.95%
2008-09-30	2:00 to 2:59	23.08%	2.78%	93.75%	75.56%	54.37%
2008-09-30	3:00 to 3:59	53.85%	22.22%	81.25%	58.89%	31.07%
2008-09-30	4:00 to 4:59	70.77%	73.61%	86.25%	53.33%	38.83%
2008-09-30	5:00 to 5:59	66.15%	72.22%	71.25%	60%	50.49%
2008-09-30	6:00 to 6:59	76.92%	73.61%	60%	77.78%	58.25%
2008-09-30	7:00 to 7:59	75.38%	62.50%	76.25%	71.11%	66.02%
2008-09-30	8:00 to 8:59	70.77%	63.89%	92.50%	74.44%	83.50%
2008-09-30	9:00 to 9:59	53.85%	62.50%	50%	90%	64.08%
2008-09-30	10:00 to 10:59	93.85%	76.39%	77.50%	50%	64.08%

2008-09-30	11:00 to 11:59	44.62%	47.22%	88.75%	57.78%	20.39%
2008-09-30	12:00 to 12:59	60%	52.78%	80%	37.78%	63.11%
2008-09-30	13:00 to 13:59	53.85%	62.50%	87.50%	62.22%	76.70%
2008-09-30	14:00 to 14:59	23.08%	73.61%	28.75%	16.67%	27.18%

Table A.1.8.2 Frequency distribution of data capture rate for 5 boluses in the indoor experiment using cows from 27th to 30th September:

DCR	b3	b4	b5	b6	b7
0-10%	0	1	3	1	1
10.01-20%	1	0	1	5	1
20.01-30%	3	4	2	0	4
30.01-40%	2	3	3	5	7
40.01-50%	3	4	10	6	4
50.01-60%	7	11	6	19	11
60.01-70%	14	25	10	13	10
70.01-80%	21	15	16	19	11
80.01-90%	16	8	13	2	15
90.01-100%	5	1	8	2	8

A.1.9 Indoor experiments with boluses inserted in cows from 25th to 28th November

There were a total of 78 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was varied from 40 to 55 seconds for different boluses, which means that there should be 90 to 102 data signals captured per hour for different boluses. The mean data capture rate was 58.76% for bolus No.4 (b4), 54.99% for bolus No.5 (b5), 60% for bolus No.6 (b6), and 56.05% for bolus No.7 (b7); bolus No.6 lost power for 71 out of 78 hours.

Table A.1.9.1 Hourly data capture rate for 4 boluses in the indoor experiment using cows from 25th to 28th November:

time		b4	b5	b6	b7
2008-11-25	9:00 to 9:59	18.46%	27.78%	0%	5.56%
2008-11-25	10:00 to 10:59	35.38%	26.39%	0%	16.67%
2008-11-25	11:00 to 11:59	3.08%	22.22%	0%	5.56%
2008-11-25	12:00 to 12:59	69.23%	52.78%	0%	55.56%
2008-11-25	13:00 to 13:59	73.85%	48.61%	0%	57.78%
2008-11-25	14:00 to 14:59	29.23%	59.72%	0%	28.89%
2008-11-25	15:00 to 15:59	49.23%	38.89%	43.75%	84.44%
2008-11-25	16:00 to 16:59	84.62%	45.83%	71.25%	23.33%
2008-11-25	17:00 to 17:59	60%	84.72%	67.50%	36.67%
2008-11-25	18:00 to 18:59	50.77%	87.50%	81.25%	76.67%
2008-11-25	19:00 to 19:59	93.85%	58.33%	62.50%	35.56%

2008-11-25	20:00 to 20:59	47.69%	50%	36.25%	81.11%
2008-11-25	21:00 to 21:59	78.46%	15.28%	57.50%	20%
2008-11-25	22:00 to 22:59	46.15%	8.33%	0%	73.33%
2008-11-25	23:00 to 23:59	56.92%	22.22%	0%	60%
2008-11-26	0:00 to 0:59	46.15%	2.78%	0%	36.67%
2008-11-26	1:00 to 1:59	63.08%	27.78%	0%	70%
2008-11-26	2:00 to 2:59	66.15%	68.06%	0%	47.78%

Table A.1.9.1 (cont.)

time		b4	b5	b6	b7
2008-11-26	3:00 to 3:59	67.69%	69.44%	0%	83.33%
2008-11-26	4:00 to 4:59	67.69%	8.33%	0%	32.22%
2008-11-26	5:00 to 5:59	64.62%	33.33%	0%	60%
2008-11-26	6:00 to 6:59	47.69%	9.72%	0%	72.22%
2008-11-26	7:00 to 7:59	83.08%	58.33%	0%	36.67%
2008-11-26	8:00 to 8:59	69.23%	63.89%	0%	56.67%
2008-11-26	9:00 to 9:59	29.23%	16.67%	0%	35.56%
2008-11-26	10:00 to 10:59	53.85%	52.78%	0%	91.11%
2008-11-26	11:00 to 11:59	60%	54.17%	0%	92.22%
2008-11-26	12:00 to 12:59	47.69%	70.83%	0%	55.56%
2008-11-26	13:00 to 13:59	35.38%	69.44%	0%	27.78%
2008-11-26	14:00 to 14:59	53.85%	55.56%	0%	45.56%
2008-11-26	15:00 to 15:59	9.23%	30.56%	0%	50%
2008-11-26	16:00 to 16:59	69.23%	72.22%	0%	52.22%
2008-11-26	17:00 to 17:59	84.62%	52.78%	0%	17.78%
2008-11-26	18:00 to 18:59	75.38%	77.78%	0%	93.33%
2008-11-26	19:00 to 19:59	86.15%	52.78%	0%	70%
2008-11-26	20:00 to 20:59	92.31%	94.44%	0%	37.78%
2008-11-26	21:00 to 21:59	80%	40.28%	0%	13.33%
2008-11-26	22:00 to 22:59	76.92%	55.56%	0%	25.56%
2008-11-26	23:00 to 23:59	32.31%	59.72%	0%	57.78%
2008-11-27	0:00 to 0:59	58.46%	73.61%	0%	76.67%
2008-11-27	1:00 to 1:59	67.69%	26.39%	0%	18.89%
2008-11-27	2:00 to 2:59	75.38%	4.17%	0%	58.89%
2008-11-27	3:00 to 3:59	70.77%	73.61%	0%	60%
2008-11-27	4:00 to 4:59	80%	86.11%	0%	77.78%
2008-11-27	5:00 to 5:59	70.77%	75.00%	0%	1.11%
2008-11-27	6:00 to 6:59	76.92%	52.78%	0%	71.11%
2008-11-27	7:00 to 7:59	56.92%	59.72%	0%	46.67%
2008-11-27	8:00 to 8:59	80%	66.67%	0%	50%
2008-11-27	9:00 to 9:59	75.38%	63.89%	0%	72.22%
2008-11-27	10:00 to 10:59	58.46%	81.94%	0%	90%
2008-11-27	11:00 to 11:59	63.08%	84.72%	0%	31.11%
2008-11-27	12:00 to 12:59	43.08%	43.06%	0%	56.67%
2008-11-27	13:00 to 13:59	67.69%	80.56%	0%	82.22%

2008-11-27	14:00 to 14:59	61.54%	61.11%	0%	73.33%
2008-11-27	15:00 to 15:59	55.38%	65.28%	0%	93.33%
2008-11-27	16:00 to 16:59	64.62%	73.61%	0%	84.44%
2008-11-27	17:00 to 17:59	87.69%	70.83%	0%	47.78%
2008-11-27	18:00 to 18:59	52.31%	66.67%	0%	41.11%
2008-11-27	19:00 to 19:59	86.15%	68.06%	0%	27.78%
2008-11-27	20:00 to 20:59	12.31%	27.78%	0%	72.22%

Table A.1.9.1 (cont.)

time		b4	b5	b6	b7
2008-11-27	22:00 to 22:59	53.85%	76.39%	0%	76.67%
2008-11-27	23:00 to 23:59	67.69%	59.72%	0%	84.44%
2008-11-28	0:00 to 0:59	41.54%	79.17%	0%	51.11%
2008-11-28	1:00 to 1:59	43.08%	58.33%	0%	62.22%
2008-11-28	2:00 to 2:59	46.15%	79.17%	0%	82.22%
2008-11-28	3:00 to 3:59	69.23%	41.67%	0%	58.89%
2008-11-28	4:00 to 4:59	55.38%	70.83%	0%	63.33%
2008-11-28	5:00 to 5:59	69.23%	87.50%	0%	36.67%
2008-11-28	6:00 to 6:59	40%	66.67%	0%	51.11%
2008-11-28	7:00 to 7:59	70.77%	75.00%	0%	88.89%
2008-11-28	8:00 to 8:59	66.15%	62.50%	0%	80%
2008-11-28	9:00 to 9:59	30.77%	62.50%	0%	68.89%
2008-11-28	10:00 to 10:59	26.15%	44.44%	0%	67.78%
2008-11-28	11:00 to 11:59	41.54%	58.33%	0%	68.89%
2008-11-28	12:00 to 12:59	46.15%	51.39%	0%	75.56%
2008-11-28	13:00 to 13:59	72.31%	62.50%	0%	72.22%
2008-11-28	14:00 to 14:59	63.08%	59.72%	0%	54.44%

Table A.1.9.2 Frequency distribution of data capture rate for 4 boluses in the outdoor experiment using cows from 25th to 28th November:

DCR	b4	b5	b6	b7
0-10%	2	5	71	3
10.01-20%	2	2	0	5
20.01-30%	3	7	0	5
30.01-40%	5	3	1	9
40.01-50%	12	8	1	7
50.01-60%	14	18	1	15
60.01-70%	18	14	2	7
70.01-80%	14	13	1	14
80.01-90%	6	7	1	9
90.01-100%	2	1	0	4

A.1.10 outdoor experiments with boluses inserted in cows from 15th to 16th May

There were a total of 23 hours continuous recording for this study. The parameter recorded was temperature and pH. The transmission interval was 5 minutes, which means that there should be 12 data signals captured per hour. The mean data capture rate was 9.78% for bolus No.1 (b1) and 6.85% for bolus No.11 (b11).

Table A.1.10.1 Hourly data capture rate for 2 boluses in the outdoor experiment using cows from 15th to 16th May:

date and time	b1	b11
2008-05-15 16:00 to 16:59	34.17%	0%
2008-05-15 17:00 to 17:59	10.83%	6.67%
2008-05-15 18:00 to 18:59	0%	10.83%
2008-05-15 19:00 to 19:59	0%	0%
2008-05-15 20:00 to 20:59	39.17%	0%
2008-05-15 21:00 to 21:59	35.00%	0%
2008-05-15 22:00 to 22:59	0%	2.50%
2008-05-15 23:00 to 23:59	3.33%	9.17%
2008-05-16 0:00 to 0:59	0%	15.00%
2008-05-16 1:00 to 1:59	3.33%	0.83%
2008-05-16 2:00 to 2:59	0%	0%
2008-05-16 3:00 to 3:59	0.83%	2.50%
2008-05-16 4:00 to 4:59	0%	3.33%
2008-05-16 5:00 to 5:59	0%	0%
2008-05-16 6:00 to 6:59	0%	0%
2008-05-16 7:00 to 7:59	13.33%	0%
2008-05-16 8:00 to 8:59	0.83%	0.83%
2008-05-16 9:00 to 9:59	1.67%	17.50%
2008-05-16 10:00 to 10:59	1.67%	6.67%
2008-05-16 11:00 to 11:59	35.00%	30.83%
2008-05-16 12:00 to 12:59	6.67%	5.83%
2008-05-16 13:00 to 13:59	10.83%	6.67%
2008-05-16 14:00 to 14:59	28.33%	38.33%

Table A.1.10.2 Frequency distribution of data capture rate for 2 boluses in the outdoor experiment using cows from 15th to 16th May:

DCR	b1	b11
0-10%	15	18
10.01-20%	3	3
20.01-30%	1	0
30.01-40%	4	2
40.01-50%	0	0
50.01-60%	0	0

60.01-70%	0	0
70.01-80%	0	0
80.01-90%	0	0
90.01-100%	0	0

A.1.11 outdoor experiments with boluses inserted in cows from 12th to 13th June

There were a total of 24 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was 5 minutes, which means that there should be 12 data signals captured per hour. The mean data capture rate was 43.06% for bolus No.12 (b12) and 49.65% for bolus No.13 (b13)

Table A.1.11.1 Hourly data capture rate for 2 boluses in the outdoor experiment using cows from 12th to 13th June:

date and time	b12	b13
06-12 9:00 to 9:59	42%	25%
06-12 10:00 to 10:59	42%	58%
06-12 11:00 to 11:59	33%	92%
06-12 12:00 to 12:59	33%	25%
06-12 13:00 to 13:59	42%	33%
06-12 14:00 to 14:59	17%	0%
06-12 15:00 to 15:59	42%	50%
06-12 16:00 to 16:59	92%	83%
06-12 17:00 to 17:59	75%	83%
06-12 18:00 to 18:59	92%	75%
06-12 19:00 to 19:59	25%	75%
06-12 20:00 to 20:59	17%	58%
06-12 21:00 to 21:59	42%	0%
06-12 22:00 to 22:59	8%	0%
06-12 23:00 to 23:59	25%	0%
06-13 0:00 to 0:59	33%	0%
06-13 1:00 to 1:59	50%	25%
06-13 2:00 to 2:59	17%	58%
06-13 3:00 to 3:59	17%	58%
06-13 4:00 to 4:59	42%	75%
06-13 5:00 to 5:59	67%	100%
06-13 6:00 to 6:59	33%	58%
06-13 7:00 to 7:59	75%	100%
06-13 8:00 to 8:59	75%	58%

Table A.1.11.2 Frequency distribution of data capture rate for 2 boluses in the outdoor experiment using cows from 12th to 13th June:

DCR	b11	b12
0-10%	1	5
10.01-20%	4	0
20.01-30%	2	3
30.01-40%	4	1
40.01-50%	7	1
50.01-60%	0	6
60.01-70%	1	0
70.01-80%	3	3
80.01-90%	0	2
90.01-100%	2	3

A.1.12 outdoor experiments with boluses inserted in cows from 21st to 25th November

There were a total of 88 hours continuous recording for this study. The parameter recorded was pH. The transmission interval was varied from 40 to 55 seconds for different boluses, which means that there should be 90 to 102 data signals captured per hour for different boluses. The mean data capture rate was 55.49% for bolus No.4 (b4), 54.15% for bolus No.5 (b5), 50.56% for bolus No.6 (b6), and 54.18% for bolus No.7 (b7); bolus No.6 lost power for 19 out 88 hours.

Table A.1.12.1 Hourly data capture rate for 4 boluses in the outdoor experiment using cows from 21st to 25th November:

date and time		b4	b5	b6	b7
2008-11-21	17:37 to 17:59	36.92%	37.50%	38.75%	36.67%
2008-11-21	18:00 to 18:59	61.54%	95.83%	88.75%	94.44%
2008-11-21	19:00 to 19:59	41.54%	55.56%	91.25%	92.22%
2008-11-21	20:00 to 20:59	61.54%	56.94%	91.25%	95.56%
2008-11-21	21:00 to 21:59	61.54%	84.72%	98.75%	97.78%
2008-11-21	22:00 to 22:59	95.38%	0%	100%	25.56%
2008-11-21	23:00 to 23:59	60%	27.78%	100%	57.78%
2008-11-22	0:00 to 0:59	0%	72.22%	95.00%	83.33%
2008-11-22	1:00 to 1:59	0%	68.06%	28.75%	35.56%

2008-11-22	2:00 to 2:59	15.38%	5.56%	60%	87.78%
2008-11-22	3:00 to 3:59	73.85%	50%	83.75%	58.89%
2008-11-22	4:00 to 4:59	66.15%	86.11%	83.75%	52.22%
2008-11-22	5:00 to 5:59	20%	22.22%	53.75%	70%
2008-11-22	6:00 to 6:59	63.08%	31.94%	27.50%	65.56%
2008-11-22	7:00 to 7:59	80%	51.39%	21.25%	30%
2008-11-22	8:00 to 8:59	49.23%	75.00%	55.00%	58.89%
2008-11-22	9:00 to 9:59	60%	86.11%	62.50%	82.22%
2008-11-22	10:00 to 10:59	87.69%	88.89%	55.00%	57.78%

Table A.1.12.1 (cont.)

date and time		b4	b5	b6	b7
2008-11-22	12:00 to 12:59	73.85%	93.06%	33.75%	60%
2008-11-22	13:00 to 13:59	63.08%	95.83%	92.50%	53.33%
2008-11-22	14:00 to 14:59	67.69%	84.72%	67.50%	53.33%
2008-11-22	15:00 to 15:59	0%	2.78%	7.50%	6.67%
2008-11-22	16:00 to 16:59	63.08%	54.17%	56.25%	82.22%
2008-11-22	17:00 to 17:59	78.46%	95.83%	63.75%	48.89%
2008-11-22	18:00 to 18:59	73.85%	66.67%	18.75%	64.44%
2008-11-22	19:00 to 19:59	53.85%	91.67%	47.50%	91.11%
2008-11-22	20:00 to 20:59	50.77%	69.44%	35.00%	88.89%
2008-11-22	21:00 to 21:59	78.46%	47.22%	6.25%	73.33%
2008-11-22	22:00 to 22:59	92.31%	38.89%	5.00%	94.44%
2008-11-22	23:00 to 23:59	63.08%	48.61%	77.50%	61.11%
2008-11-23	0:00 to 0:59	52.31%	34.72%	8.75%	50%
2008-11-23	1:00 to 1:59	90.77%	27.78%	7.50%	57.78%
2008-11-23	2:00 to 2:59	61.54%	54.17%	26.25%	96.67%
2008-11-23	3:00 to 3:59	26.15%	22.22%	35.00%	76.67%
2008-11-23	4:00 to 4:59	89.23%	40.28%	21.25%	52.22%
2008-11-23	5:00 to 5:59	58.46%	41.67%	3.75%	18.89%
2008-11-23	6:00 to 6:59	60%	86.11%	48.75%	83.33%
2008-11-23	7:00 to 7:59	61.54%	55.56%	50%	73.33%
2008-11-23	8:00 to 8:59	83.08%	43.06%	43.75%	61.11%
2008-11-23	9:00 to 9:59	18.46%	27.78%	20%	20%
2008-11-23	10:00 to 10:59	76.92%	75.00%	41.25%	68.89%
2008-11-23	11:00 to 11:59	73.85%	83.33%	51.25%	57.78%
2008-11-23	12:00 to 12:59	64.62%	38.89%	98.75%	43.33%
2008-11-23	13:00 to 13:59	67.69%	75.00%	91.25%	94.44%
2008-11-23	14:00 to 14:59	87.69%	97.22%	48.75%	44.44%
2008-11-23	15:00 to 15:59	50.77%	38.89%	60%	35.56%
2008-11-23	16:00 to 16:59	69.23%	75.00%	47.50%	43.33%
2008-11-23	17:00 to 17:59	63.08%	61.11%	48.75%	64.44%
2008-11-23	18:00 to 18:59	76.92%	66.67%	80%	83.33%
2008-11-23	19:00 to 19:59	70.77%	52.78%	88.75%	87.78%

2008-11-23	20:00 to 20:59	26.15%	27.78%	67.50%	62.22%
2008-11-23	21:00 to 21:59	52.31%	70.83%	13.75%	43.33%
2008-11-23	22:00 to 22:59	1.54%	62.50%	2.50%	97.78%
2008-11-23	23:00 to 23:59	0%	66.67%	35.00%	30%
2008-11-24	0:00 to 0:59	58.46%	48.61%	75.00%	94.44%
2008-11-24	1:00 to 1:59	83.08%	56.94%	77.50%	23.33%
2008-11-24	2:00 to 2:59	84.62%	23.61%	42.50%	45.56%
2008-11-24	3:00 to 3:59	63.08%	59.72%	11.25%	18.89%
2008-11-24	4:00 to 4:59	53.85%	62.50%	13.75%	32.22%
2008-11-24	5:00 to 5:59	70.77%	12.50%	71.25%	37.78%

Table A.1.12.1 (cont.)

date and time		b4	b5	b6	b7
2008-11-24	6:00 to 6:59	70.77%	72.22%	52.50%	73.33%
2008-11-24	7:00 to 7:59	80%	34.72%	65.00%	57.78%
2008-11-24	8:00 to 8:59	84.62%	69.44%	37.50%	28.89%
2008-11-24	9:00 to 9:59	13.85%	31.94%	3.75%	7.78%
2008-11-24	10:00 to 10:59	63.08%	20.83%	67.50%	43.33%
2008-11-24	11:00 to 11:59	44.62%	75.00%	60%	84.44%
2008-11-24	12:00 to 12:59	72.31%	56.94%	53.75%	17.78%
2008-11-24	13:00 to 13:59	15.38%	66.67%	27.50%	45.56%
2008-11-24	14:00 to 14:59	61.54%	55.56%	0%	27.78%
2008-11-24	15:00 to 15:59	12.31%	15.28%	0%	7.78%
2008-11-24	16:00 to 16:59	12.31%	13.89%	0%	53.33%
2008-11-24	17:00 to 17:59	35.38%	37.50%	0%	52.22%
2008-11-24	18:00 to 18:59	15.38%	58.33%	0%	65.56%
2008-11-24	19:00 to 19:59	49.23%	36.11%	0%	42.22%
2008-11-24	20:00 to 20:59	40%	26.39%	0%	7.78%
2008-11-24	21:00 to 21:59	72.31%	77.78%	0%	28.89%
2008-11-24	22:00 to 22:59	87.69%	90.28%	0%	2.22%
2008-11-24	23:00 to 23:59	1.54%	63.89%	0%	70%
2008-11-25	0:00 to 0:59	23.08%	33.33%	0%	34.44%
2008-11-25	1:00 to 1:59	41.54%	62.50%	0%	43.33%
2008-11-25	2:00 to 2:59	80%	63.89%	0%	50%
2008-11-25	3:00 to 3:59	81.54%	48.61%	0%	26.67%
2008-11-25	4:00 to 4:59	76.92%	45.83%	0%	3.33%
2008-11-25	5:00 to 5:59	55.38%	26.39%	0%	44.44%
2008-11-25	6:00 to 6:59	76.92%	40.28%	0%	27.78%
2008-11-25	7:00 to 7:59	10.77%	18.06%	0%	42.22%
2008-11-25	8:00 to 8:59	52.31%	52.78%	0%	24.44%

Table A.1.12.2 Frequency distribution of data capture rate for 4 boluses in the outdoor experiment using cows from 21st to 25th November:

DCR	b4	b5	b6	b7
0-10%	6	3	27	6

10.01-20%	9	4	5	4
20.01-30%	3	10	6	10
30.01-40%	3	11	6	6
40.01-50%	5	10	9	14
50.01-60%	13	13	10	14
60.01-70%	19	13	6	10
70.01-80%	18	9	5	4
80.01-90%	9	7	5	9
90.01-100%	3	8	9	11

Appendix 2 24-hour pH patterns for all cows and for each cow eating two different diets and the tables of the aggregated pH data

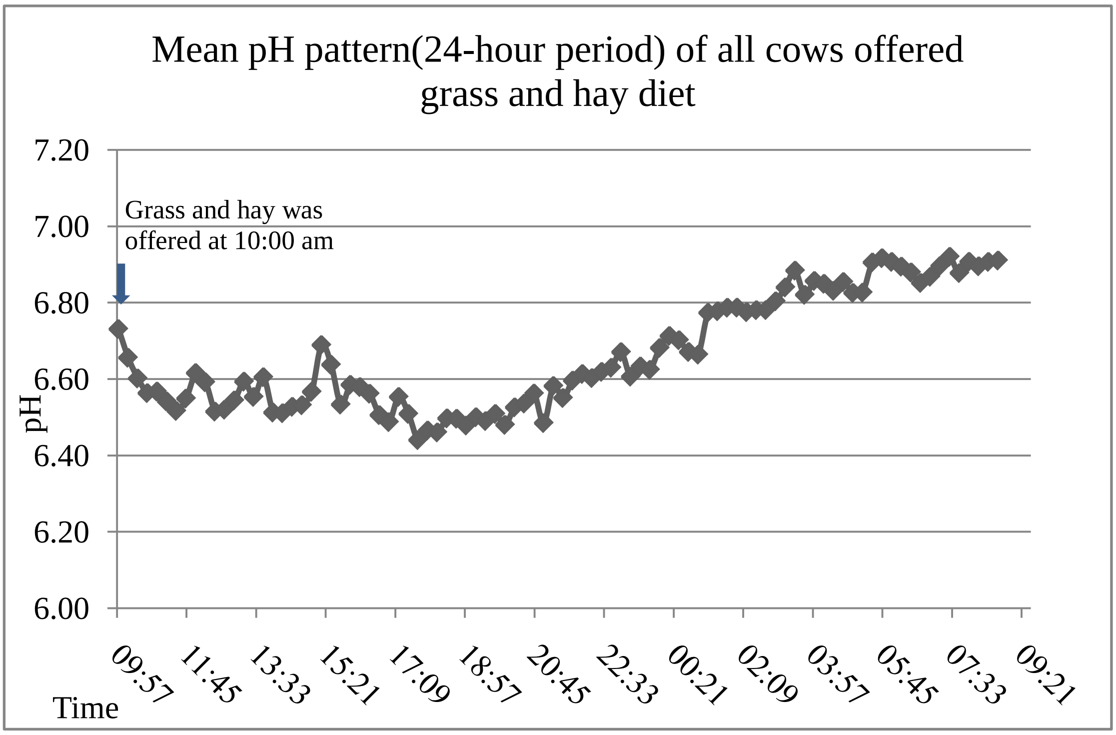

Figure A.2.1 Profile of mean 24-hour pH taken from all cows offered the grass and hay diet.

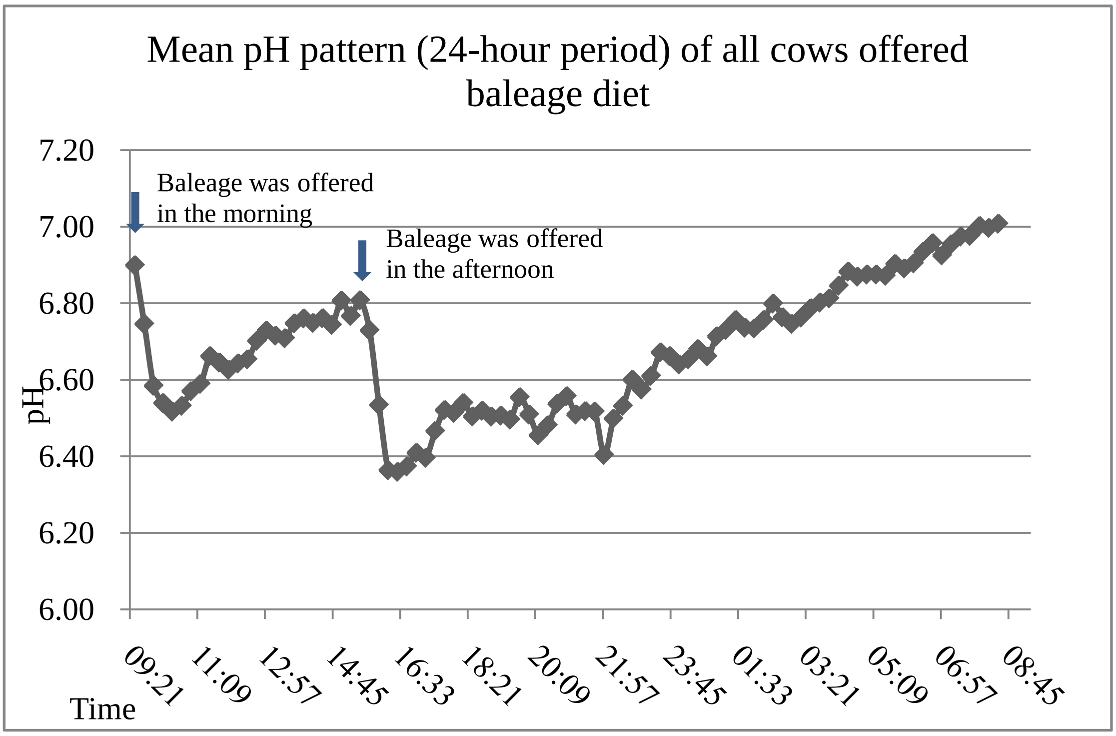

Figure A.2.2 Profile of mean 24-hour pH taken from all cows offered the baleage diet.

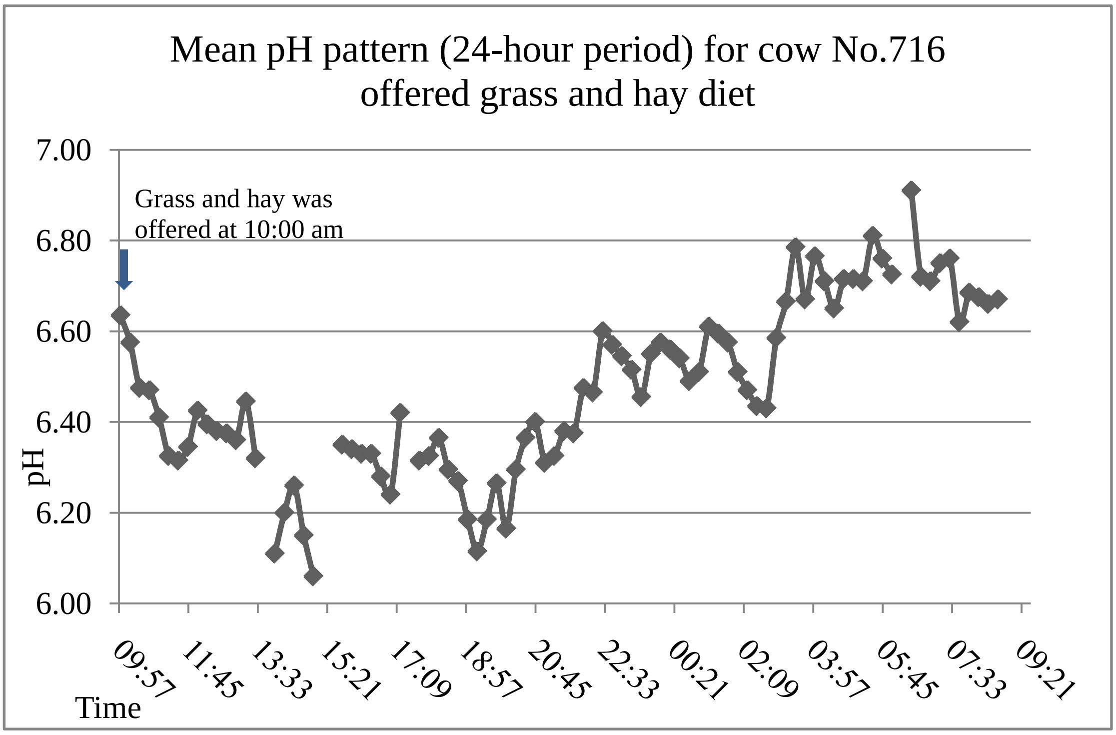

Figure A.2.3 Profile of mean 24-hour pH taken from cow No.716 offered the grass and hay diet.

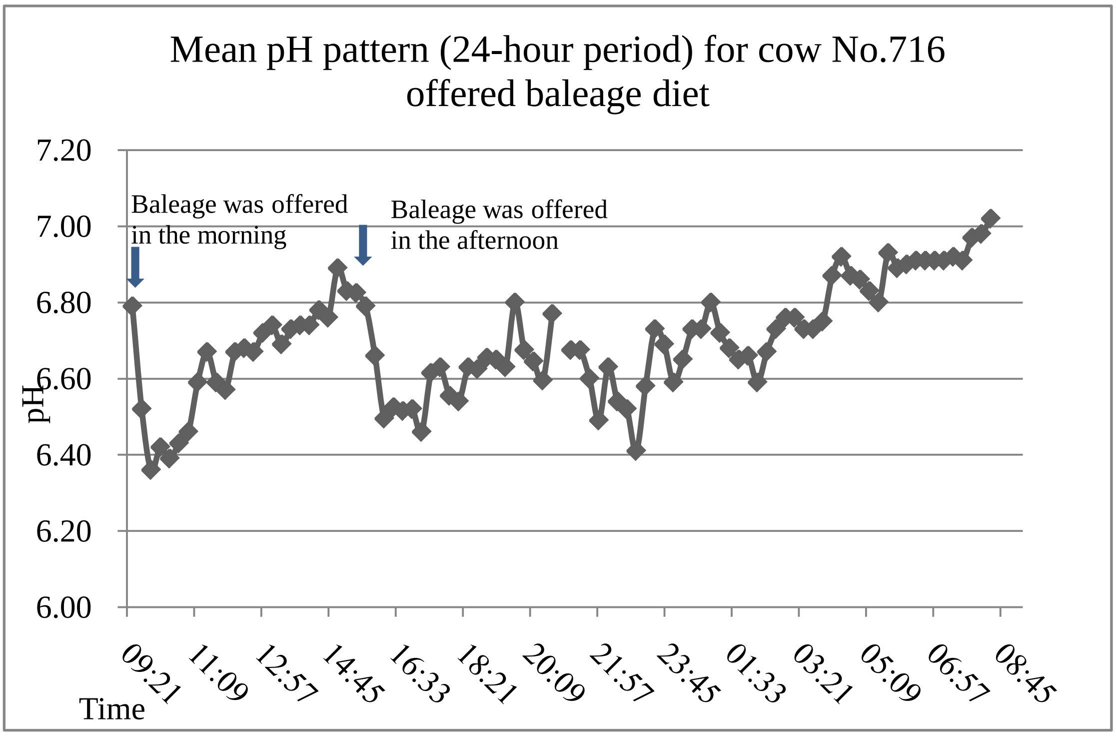

Figure A.2.4 Profile of mean 24-hour pH taken from cow No.716 offered the baleage diet.

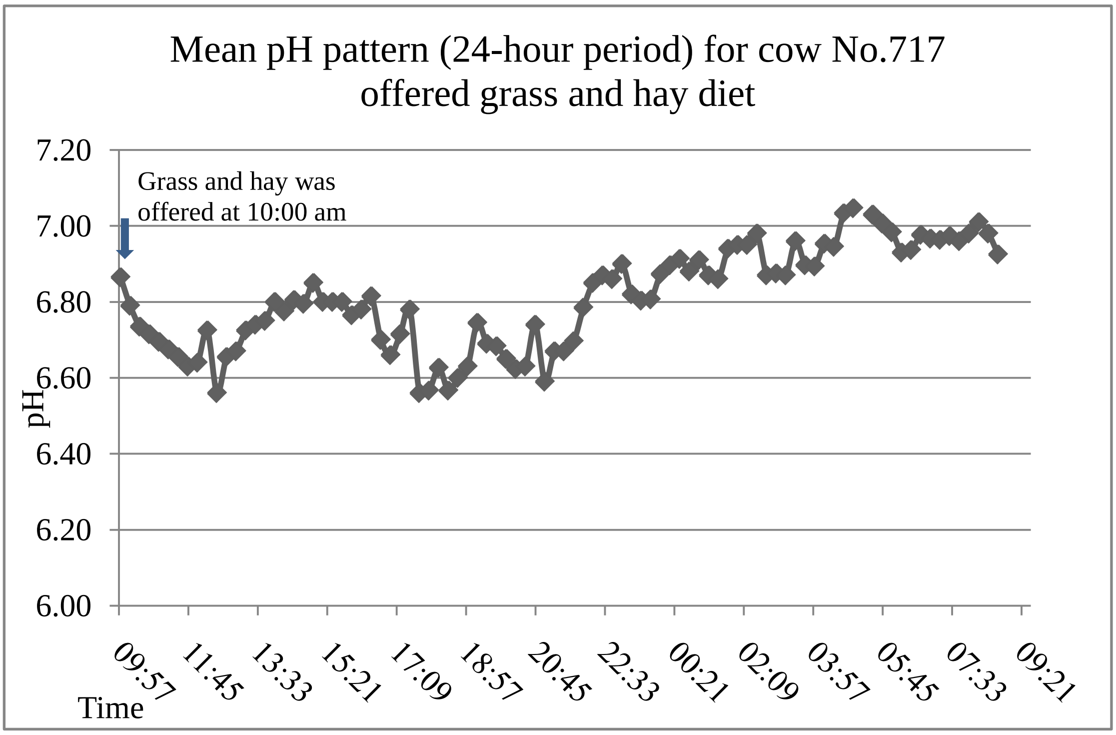

Figure A.2.5 Profile of mean 24-hour pH taken from cow No.717 offered the grass and hay diet.

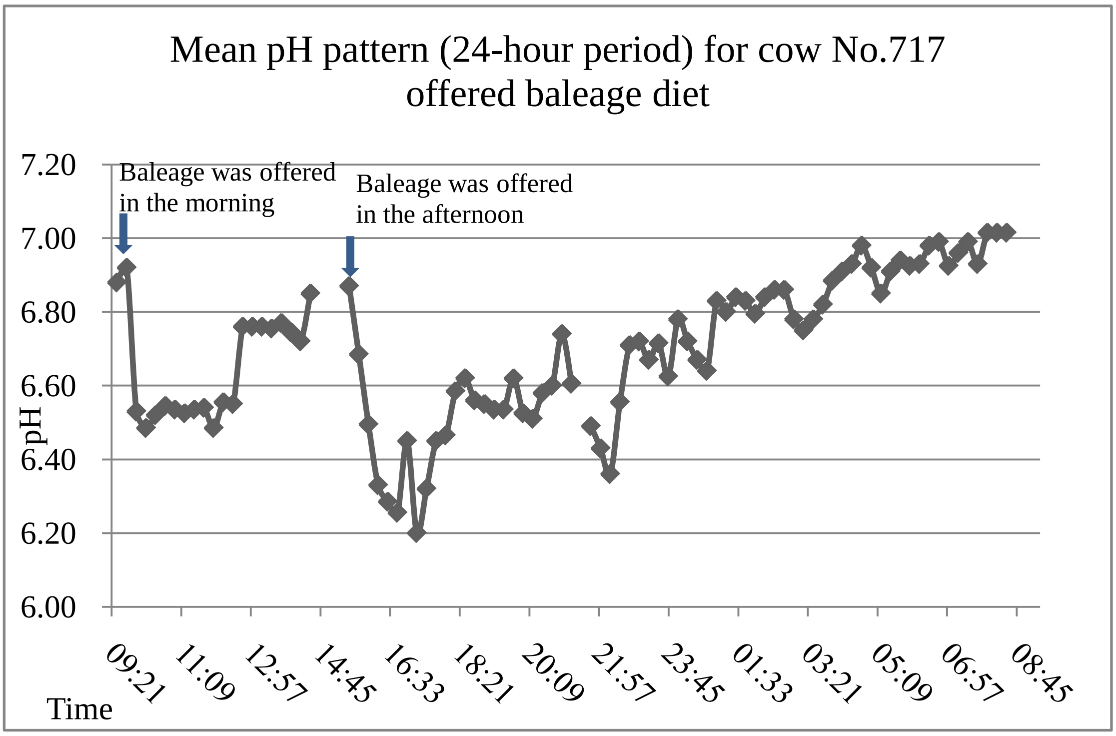

Figure A.2.6 Profile of mean 24-hour pH taken from cow No.717 offered the baleage diet.

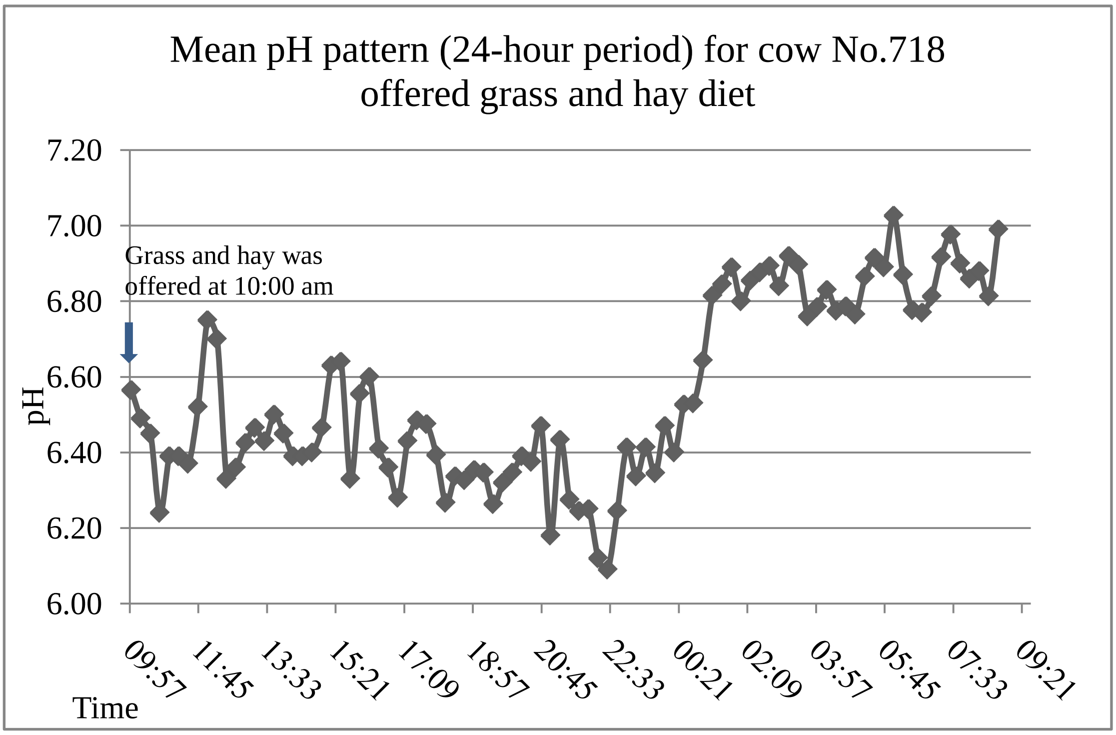

Figure A.2.7 Profile of mean 24-hour pH taken from cow No.718 offered the grass and hay diet.

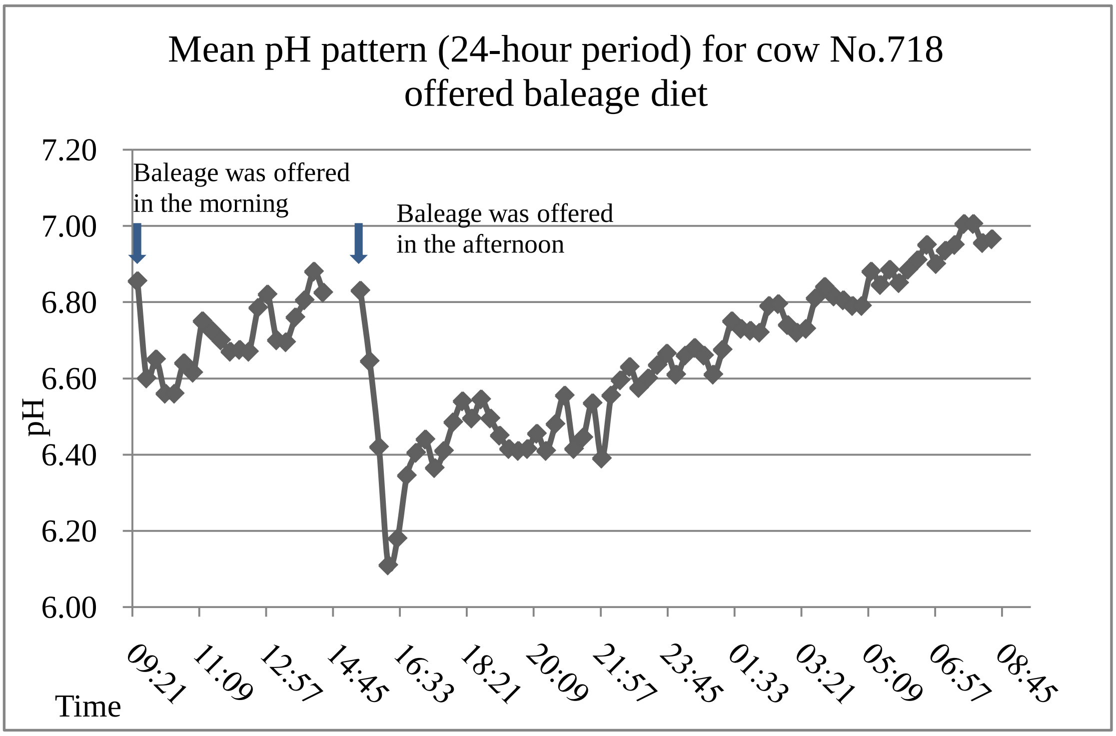

Figure A.2.8 Profile of mean 24-hour pH taken from cow No.718 offered the baleage diet.

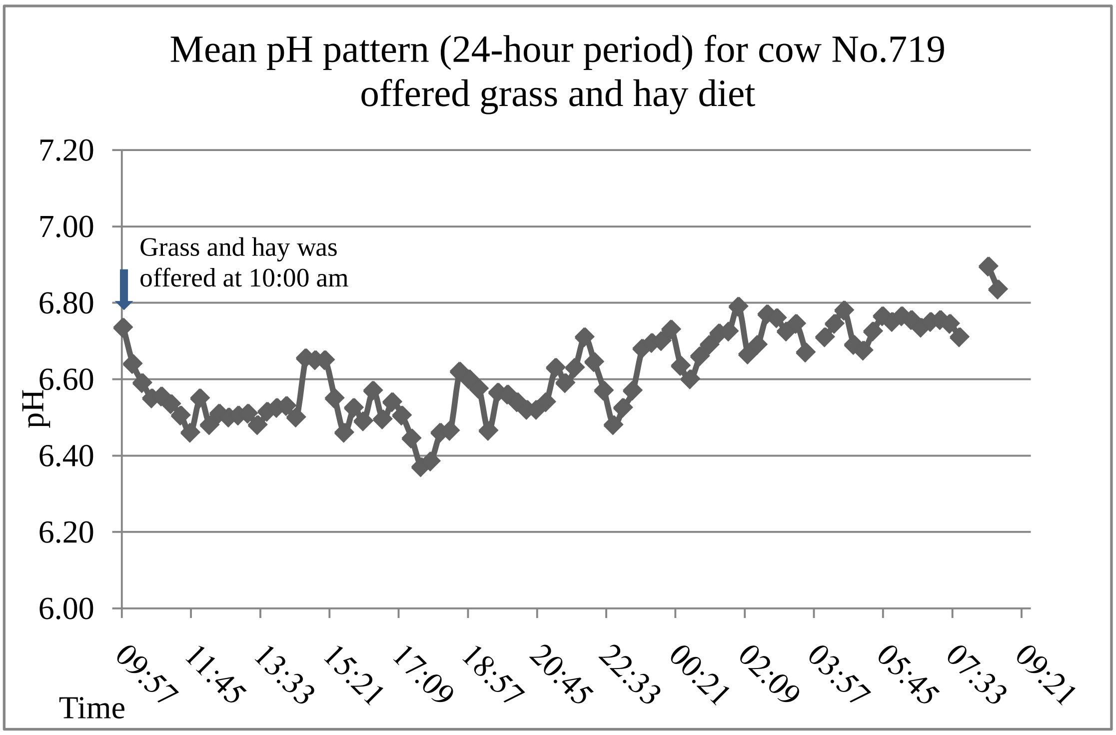

Figure A.2.9 Profile of mean 24-hour pH taken from cow No.719 offered the grass and hay diet.

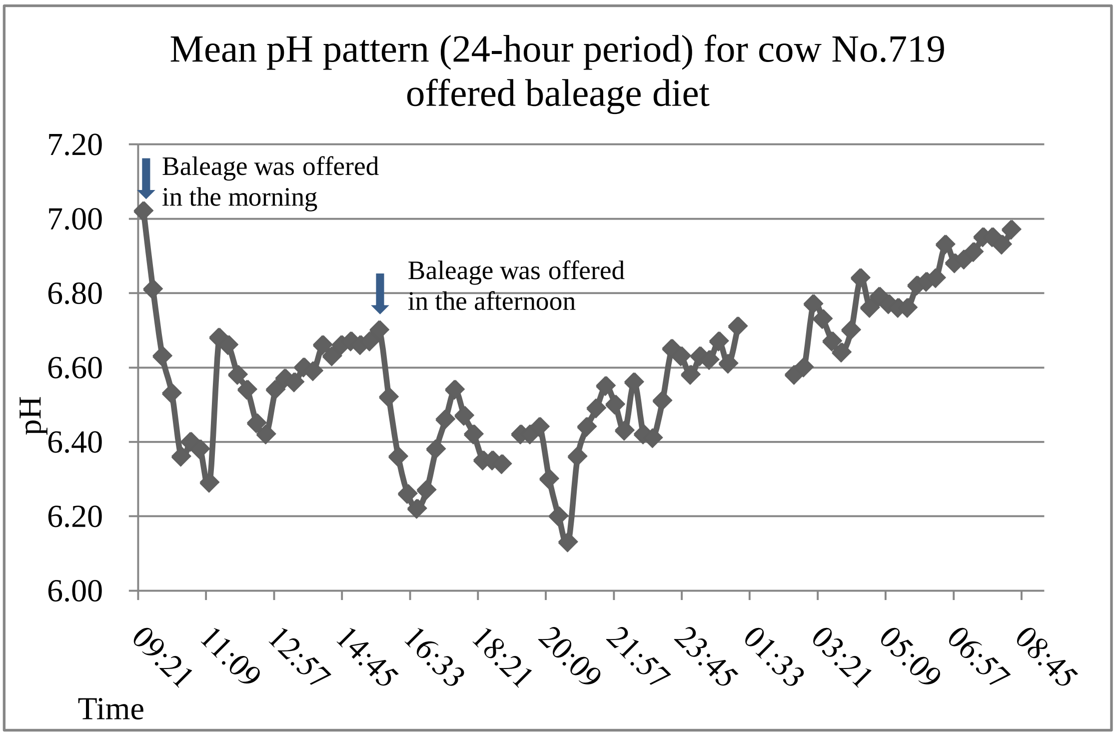

Figure A.2.10 Profile of mean 24-hour pH taken from cow No.719 offered the baleage diet.

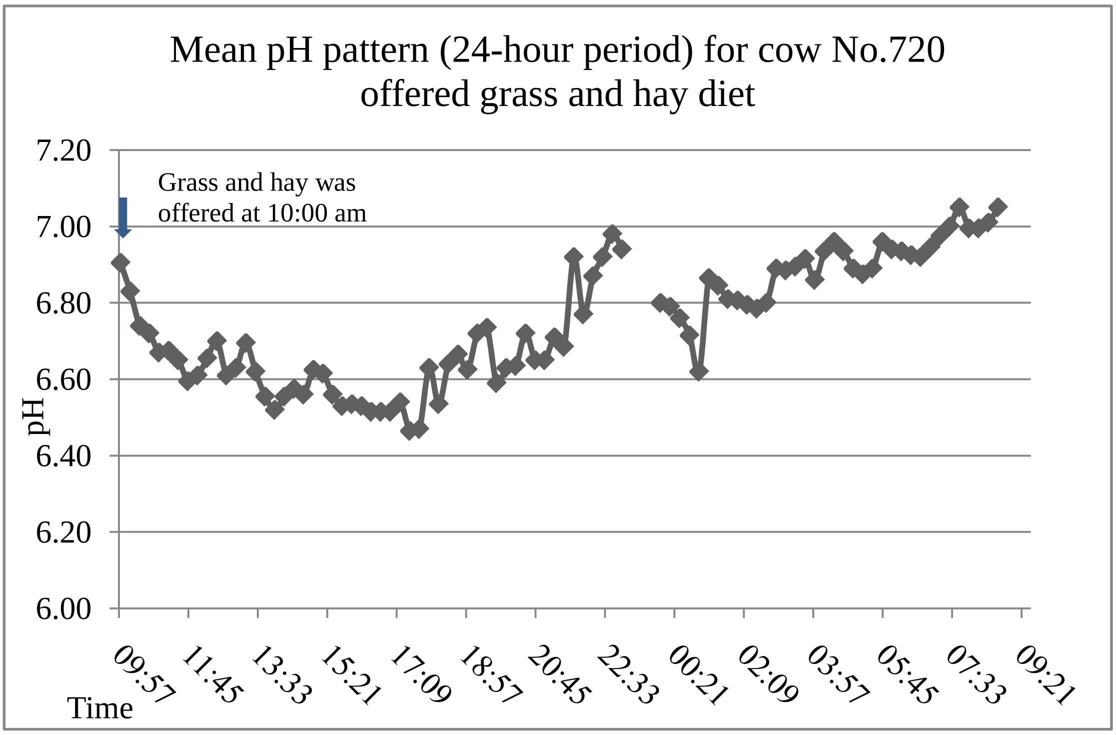

Figure A.2.11 Profile of mean 24-hour pH taken from cow No.720 offered the grass and hay diet.

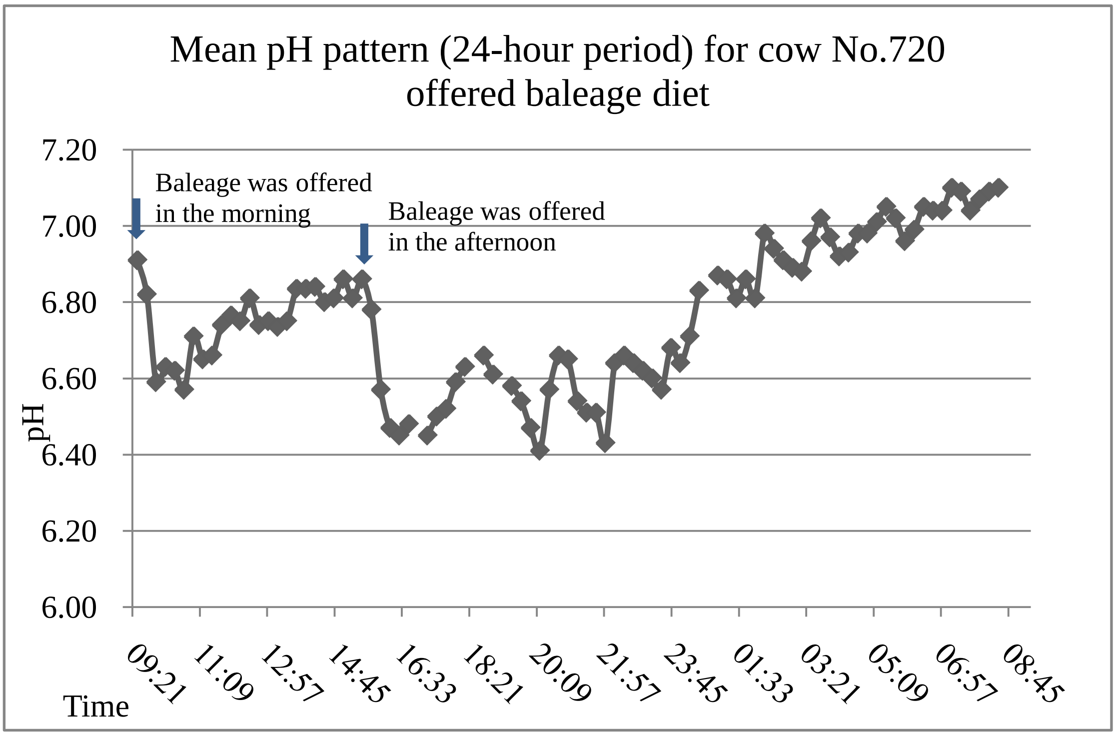

Figure A.2.12 Profile of mean 24-hour pH taken from cow No.720 offered the baleage diet.

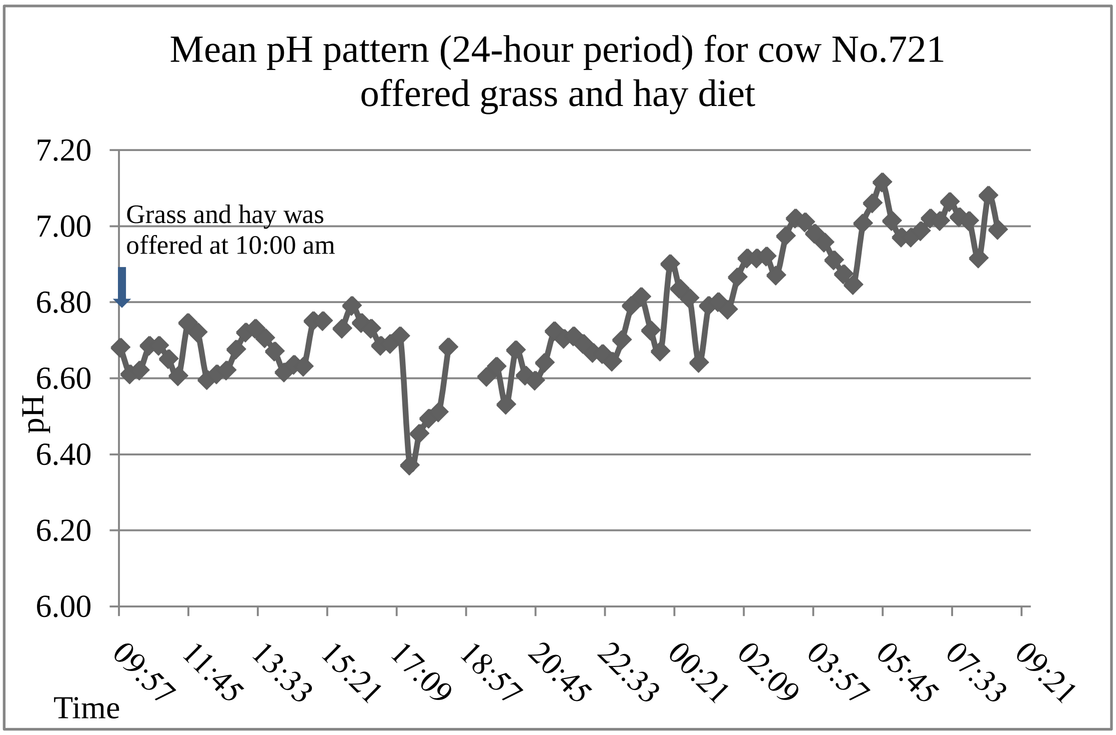

Figure A.2.13 Profile of mean 24-hour pH taken from cow No.721 offered the grass and hay diet.

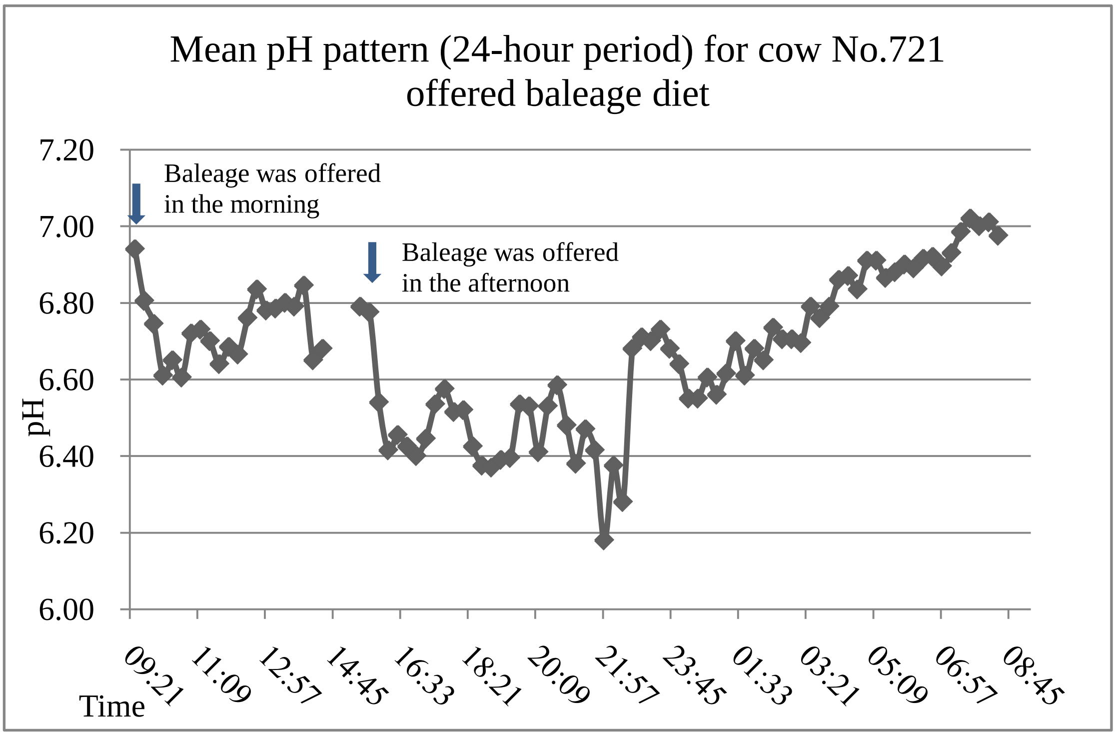

Figure A.2.14 Profile of mean 24-hour pH taken from cow No.721 offered the baleage diet.

Table A.2.1 The mean pH for cows offered grass and hay diet using aggregated data (15-minute was used as the interval for aggregation) and the mean pH calculated from all cows in each 15-minute interval:

Time	Cow ID						mean pH
	716	717	718	719	720	721	
10:00	6.64	6.87	6.57	6.74	6.91	6.68	6.73
10:15	6.58	6.79	6.49	6.64	6.83	6.61	6.66
10:30	6.48	6.74	6.45	6.59	6.74	6.62	6.60
10:45	6.47	6.72	6.24	6.55	6.72	6.69	6.56
11:00	6.41	6.70	6.39	6.56	6.67	6.69	6.57
11:15	6.33	6.68	6.39	6.54	6.68	6.65	6.54
11:30	6.32	6.66	6.37	6.51	6.65	6.61	6.52
11:45	6.35	6.63	6.52	6.46	6.60	6.75	6.55
12:00	6.43	6.64	6.75	6.55	6.61	6.72	6.62
12:15	6.40	6.73	6.70	6.48	6.66	6.60	6.59
12:30	6.38	6.56	6.33	6.51	6.70	6.61	6.52
12:45	6.38	6.66	6.36	6.50	6.61	6.62	6.52
13:00	6.36	6.67	6.43	6.51	6.63	6.68	6.54
13:15	6.45	6.73	6.47	6.51	6.70	6.72	6.59
13:30	6.32	6.74	6.43	6.48	6.62	6.73	6.55
13:45	*n/r	6.75	6.50	6.52	6.56	6.71	6.61
14:00	6.11	6.80	6.45	6.53	6.52	6.67	6.51

14:15	6.20	6.78	6.39	6.53	6.56	6.62	6.51
14:30	6.26	6.81	6.39	6.50	6.58	6.64	6.53
14:45	6.15	6.80	6.40	6.66	6.56	6.63	6.53
15:00	6.06	6.85	6.47	6.65	6.63	6.75	6.57
15:15	n/r	6.80	6.63	6.65	6.62	6.75	6.69
15:30	n/r	6.80	6.64	6.55	6.56	n/r	6.64
15:45	6.35	6.80	6.33	6.46	6.53	6.73	6.53
16:00	6.34	6.77	6.56	6.53	6.54	6.79	6.59
16:15	6.33	6.78	6.60	6.49	6.53	6.75	6.58
16:30	6.33	6.82	6.41	6.57	6.52	6.73	6.56
16:45	6.28	6.70	6.36	6.50	6.52	6.69	6.51
17:00	6.24	6.66	6.28	6.54	6.52	6.69	6.49
17:15	6.42	6.72	6.43	6.51	6.54	6.71	6.55
17:30	n/r	6.78	6.49	6.45	6.47	6.37	6.51
17:45	6.32	6.56	6.48	6.37	6.47	6.45	6.44
18:00	6.33	6.57	6.39	6.39	6.63	6.49	6.47
18:15	6.37	6.63	6.27	6.46	6.54	6.51	6.46
18:30	6.30	6.57	6.34	6.47	6.64	6.68	6.50
18:45	6.27	6.60	6.33	6.62	6.67	n/r	6.50
19:00	6.19	6.63	6.35	6.60	6.63	n/r	6.48
19:15	6.12	6.75	6.35	6.58	6.72	n/r	6.50

Table A.2.1 (cont.)

Time	Cow ID						mean
	716	717	718	719	720	721	pH
19:30	6.19	6.69	6.26	6.47	6.74	6.60	6.49
19:45	6.27	6.68	6.32	6.57	6.59	6.63	6.51
20:00	6.17	6.65	6.35	6.56	6.63	6.53	6.48
20:15	6.30	6.62	6.39	6.54	6.64	6.67	6.53
20:30	6.37	6.63	6.38	6.52	6.72	6.61	6.54
20:45	6.40	6.74	6.47	6.52	6.65	6.59	6.56
21:00	6.31	6.59	6.18	6.54	6.65	6.64	6.49
21:15	6.33	6.67	6.43	6.63	6.71	6.72	6.58
21:30	6.38	6.67	6.28	6.59	6.69	6.70	6.55
21:45	6.38	6.70	6.25	6.63	6.92	6.71	6.60
22:00	6.48	6.79	6.25	6.71	6.77	6.69	6.61
22:15	6.47	6.85	6.12	6.65	6.87	6.67	6.60
22:30	6.60	6.87	6.09	6.57	6.92	6.66	6.62
22:45	6.57	6.86	6.25	6.48	6.98	6.64	6.63
23:00	6.55	6.90	6.41	6.53	6.94	6.70	6.67
23:15	6.52	6.82	6.34	6.57	n/r	6.79	6.61
23:30	6.46	6.80	6.41	6.68	n/r	6.81	6.63
23:45	6.55	6.81	6.35	6.70	n/r	6.73	6.62

00:00	6.58	6.87	6.47	6.70	6.80	6.67	6.68
00:15	6.56	6.90	6.40	6.73	6.79	6.90	6.71
00:30	6.54	6.91	6.53	6.64	6.76	6.84	6.70
00:45	6.49	6.88	6.53	6.60	6.72	6.81	6.67
01:00	6.51	6.91	6.64	6.66	6.62	6.64	6.66
01:15	6.61	6.87	6.82	6.69	6.87	6.79	6.77
01:30	6.60	6.86	6.85	6.72	6.85	6.80	6.78
01:45	6.58	6.94	6.89	6.73	6.81	6.78	6.79
02:00	6.51	6.95	6.80	6.79	6.81	6.87	6.79
02:15	6.47	6.95	6.86	6.67	6.80	6.92	6.78
02:30	6.44	6.98	6.88	6.69	6.79	6.92	6.78
02:45	6.43	6.87	6.89	6.77	6.80	6.92	6.78
03:00	6.59	6.88	6.84	6.76	6.89	6.87	6.80
03:15	6.67	6.87	6.92	6.73	6.89	6.97	6.84
03:30	6.79	6.96	6.90	6.75	6.90	7.02	6.88
03:45	6.67	6.90	6.76	6.67	6.92	7.01	6.82
04:00	6.77	6.89	6.79	n/r	6.86	6.98	6.86
04:15	6.71	6.95	6.83	6.71	6.94	6.96	6.85
04:30	6.65	6.95	6.78	6.75	6.96	6.91	6.83
04:45	6.72	7.03	6.79	6.78	6.94	6.87	6.85
05:00	6.72	7.05	6.77	6.69	6.89	6.85	6.83
05:15	6.71	n/r	6.87	6.68	6.88	7.01	6.83
05:30	6.81	7.03	6.92	6.73	6.89	7.06	6.91

Table A.2.1 (cont.)

Time	Cow ID						mean pH
	716	717	718	719	720	721	
05:45	6.76	7.01	6.89	6.77	6.96	7.12	6.92
06:00	6.73	6.98	7.03	6.75	6.94	7.01	6.91
06:15	n/r	6.93	6.87	6.77	6.94	6.97	6.89
06:30	6.91	6.94	6.78	6.76	6.93	6.97	6.88
06:45	6.72	6.98	6.77	6.74	6.92	6.99	6.85
07:00	6.71	6.97	6.81	6.75	6.95	7.02	6.87
07:15	6.75	6.96	6.92	6.76	6.98	7.01	6.90
07:30	6.76	6.97	6.98	6.75	7.00	7.06	6.92
07:45	6.62	6.96	6.90	6.71	7.05	7.02	6.88
08:00	6.69	6.98	6.86	n/r	7.00	7.01	6.91
08:15	6.68	7.01	6.88	n/r	7.00	6.92	6.90
08:30	6.66	6.98	6.81	6.90	7.01	7.08	6.91
08:45	6.67	6.93	6.99	6.84	7.05	6.99	6.91
09:00	6.55	6.92	6.66	6.80	6.94	6.89	6.79
09:15	6.40	6.82	6.48	6.81	n/r	6.89	6.68
09:30	6.42	6.88	6.54	n/r	n/r	6.64	6.62
09:45	6.49	6.92	6.67	6.71	6.91	6.68	6.73

* n/r means that there was no data recorded in the 15-minute interval.

Table A.2.2 The mean pH for cows offered baleage diet using aggregated data (15-minute was used as the interval for aggregation) and the mean pH calculated from all cows in each 15-minute interval:

Time	Cow ID						mean
	716	717	718	719	720	721	pH
09:30	6.79	6.88	6.86	7.02	6.91	6.94	6.90
09:45	6.52	6.92	6.60	6.81	6.82	6.81	6.75
10:00	6.36	6.53	6.65	6.63	6.59	6.75	6.58
10:15	6.42	6.49	6.56	6.53	6.63	6.61	6.54
10:30	6.39	6.52	6.56	6.36	6.62	6.65	6.52
10:45	6.43	6.55	6.64	6.40	6.57	6.61	6.53
11:00	6.46	6.54	6.62	6.38	6.71	6.72	6.57
11:15	6.59	6.53	6.75	6.29	6.65	6.73	6.59
11:30	6.67	6.54	6.73	6.68	6.66	6.70	6.66
11:45	6.59	6.54	6.70	6.66	6.74	6.64	6.65
12:00	6.57	6.49	6.67	6.58	6.77	6.69	6.63
12:15	6.67	6.56	6.68	6.54	6.75	6.67	6.64
12:30	6.68	6.55	6.67	6.45	6.81	6.76	6.65
12:45	6.67	6.76	6.79	6.42	6.74	6.84	6.70

Table A.2.2 (cont.)

Time	Cow ID						mean
	716	717	718	719	720	721	pH
13:00	6.72	6.76	6.82	6.54	6.75	6.78	6.73
13:15	6.74	6.76	6.70	6.57	6.74	6.79	6.72
13:30	6.69	6.76	6.70	6.56	6.75	6.80	6.71
13:45	6.73	6.77	6.76	6.60	6.84	6.79	6.75
14:00	6.74	6.75	6.81	6.59	6.84	6.85	6.76
14:15	6.74	6.72	6.88	6.66	6.84	6.65	6.75
14:30	6.78	6.85	6.83	6.63	6.80	6.68	6.76
14:45	6.76	n/r	*n/r	6.66	6.81	n/r	6.74
15:00	6.89	n/r	n/r	6.67	6.86	n/r	6.81
15:15	6.83	n/r	n/r	6.66	6.81	n/r	6.77
15:30	6.83	6.87	6.83	6.67	6.86	6.79	6.81
15:45	6.79	6.69	6.65	6.70	6.78	6.78	6.73
16:00	6.66	6.50	6.42	6.52	6.57	6.54	6.53
16:15	6.50	6.33	6.11	6.36	6.47	6.42	6.36
16:30	6.53	6.29	6.18	6.26	6.45	6.46	6.36
16:45	6.52	6.26	6.35	6.22	6.48	6.43	6.37
17:00	6.52	6.45	6.41	6.27	n/r	6.40	6.41

17:15	6.46	6.20	6.44	6.38	6.45	6.45	6.40
17:30	6.62	6.32	6.37	6.46	6.50	6.54	6.47
17:45	6.63	6.45	6.41	6.54	6.52	6.58	6.52
18:00	6.56	6.47	6.49	6.47	6.59	6.52	6.51
18:15	6.54	6.59	6.54	6.42	6.63	6.52	6.54
18:30	6.63	6.62	6.50	6.35	n/r	6.43	6.50
18:45	6.63	6.56	6.55	6.35	6.66	6.38	6.52
19:00	6.66	6.55	6.50	6.34	6.61	6.37	6.50
19:15	6.65	6.54	6.45	n/r	n/r	6.39	6.51
19:30	6.63	6.54	6.42	6.42	6.58	6.40	6.50
19:45	6.80	6.62	6.41	6.42	6.54	6.54	6.55
20:00	6.68	6.53	6.42	6.44	6.47	6.53	6.51
20:15	6.65	6.51	6.46	6.30	6.41	6.41	6.46
20:30	6.60	6.58	6.41	6.20	6.57	6.53	6.48
20:45	6.77	6.60	6.48	6.13	6.66	6.59	6.54
21:00	n/r	6.74	6.56	6.36	6.65	6.48	6.56
21:15	6.68	6.61	6.42	6.44	6.54	6.38	6.51
21:30	6.68	n/r	6.45	6.49	6.51	6.47	6.52
21:45	6.60	6.49	6.54	6.55	6.51	6.42	6.52
22:00	6.49	6.43	6.39	6.50	6.43	6.18	6.40
22:15	6.63	6.36	6.56	6.43	6.64	6.38	6.50
22:30	6.54	6.56	6.60	6.56	6.66	6.28	6.53
22:45	6.52	6.71	6.63	6.42	6.64	6.68	6.60

Table A.2.1 (cont.)

Time	Cow ID						mean
	716	717	718	719	720	721	pH
23:00	6.41	6.72	6.58	6.41	6.62	6.71	6.57
23:15	6.58	6.67	6.60	6.51	6.60	6.70	6.61
23:30	6.73	6.72	6.64	6.65	6.57	6.73	6.67
23:45	6.69	6.63	6.67	6.63	6.68	6.68	6.66
00:00	6.59	6.78	6.61	6.58	6.64	6.64	6.64
00:15	6.65	6.72	6.66	6.63	6.71	6.55	6.65
00:30	6.73	6.67	6.68	6.62	6.83	6.55	6.68
00:45	6.73	6.64	6.66	6.67	n/r	6.61	6.66
01:00	6.80	6.83	6.61	6.61	6.87	6.56	6.71
01:15	6.72	6.80	6.68	6.71	6.86	6.62	6.73
01:30	6.68	6.84	6.75	n/r	6.81	6.70	6.76
01:45	6.65	6.83	6.73	n/r	6.86	6.61	6.74
02:00	6.66	6.80	6.73	n/r	6.81	6.68	6.73
02:15	6.59	6.84	6.72	n/r	6.98	6.65	6.76
02:30	6.67	6.86	6.79	n/r	6.94	6.74	6.80
02:45	6.73	6.86	6.80	6.58	6.91	6.71	6.76
03:00	6.76	6.78	6.74	6.60	6.89	6.71	6.75

03:15	6.76	6.75	6.72	6.77	6.88	6.70	6.76
03:30	6.73	6.78	6.73	6.73	6.96	6.79	6.79
03:45	6.73	6.82	6.81	6.67	7.02	6.76	6.80
04:00	6.75	6.89	6.84	6.64	6.97	6.79	6.81
04:15	6.87	6.91	6.82	6.70	6.92	6.86	6.85
04:30	6.92	6.93	6.81	6.84	6.93	6.87	6.88
04:45	6.87	6.98	6.79	6.76	6.98	6.84	6.87
05:00	6.86	6.92	6.79	6.79	6.98	6.91	6.88
05:15	6.83	6.85	6.88	6.77	7.01	6.91	6.88
05:30	6.80	6.91	6.85	6.76	7.05	6.87	6.87
05:45	6.93	6.94	6.89	6.76	7.02	6.88	6.90
06:00	6.89	6.93	6.85	6.82	6.96	6.90	6.89
06:15	6.90	6.93	6.89	6.83	6.99	6.89	6.90
06:30	6.91	6.98	6.91	6.84	7.05	6.92	6.93
06:45	6.91	6.99	6.95	6.93	7.04	6.92	6.96
07:00	6.91	6.93	6.90	6.88	7.04	6.90	6.93
07:15	6.91	6.96	6.94	6.89	7.10	6.93	6.95
07:30	6.92	6.99	6.95	6.91	7.09	6.99	6.97
07:45	6.91	6.93	7.01	6.95	7.04	7.02	6.98
08:00	6.97	7.02	7.01	6.95	7.07	7.00	7.00
08:15	6.98	7.02	6.96	6.93	7.09	7.01	7.00
08:30	7.02	7.02	6.97	6.97	7.10	6.98	7.01
08:45	6.79	7.03	6.84	6.91	6.91	6.97	6.91

Table A.2.1 (cont.)

Time	Cow ID						mean
	716	717	718	719	720	721	pH
09:00	6.52	7.03	6.76	6.98	6.82	6.98	6.85
09:15	6.36	7.07	7.11	6.94	6.59	6.98	6.84

* n/r means that there was no data recorded in the 15-minute interval.

Appendix 3 Tables of the mean and standard deviation of pH data for the experiment to determine animal to animal variation

Table A.3.1 Mean and standard deviation of each cow in three 24-hour period from 14th to 17th June and the overall mean and standard deviation (SD) (calculated using all the data collected from one cow):

Duration		cow ID								
		713	714	715	716	717	718	719	720	721
14th to 15th June	Mean	*N/A	7.02	***6.29	N/A	N/A	6.57	6.69	6.60	N/A
	SD	N/A	0.27	0.22	N/A	N/A	0.24	0.18	0.21	N/A
15th to 16th June	Mean	6.98	N/A	N/A	6.82	6.59	N/A	N/A	6.55	n/r
	SD	0.24	N/A	N/A	0.21	0.21	N/A	N/A	0.20	n/r
16th to 17th June	Mean	7.02	**n/r	6.73	N/A	N/A	6.60	6.73	N/A	N/A
	SD	0.18	n/r	0.28	N/A	N/A	0.21	0.30	N/A	N/A
Overall	Mean	6.96	7.04	6.73	6.81	6.62	6.61	6.75	6.57	
	SD	0.22	0.24	0.22	0.18	0.19	0.19	0.20	0.21	

* N/A means the bolus was not available, i.e. bolus was not put into this cow. ** n/r means the bolus signals were not recorded, i.e. bolus was in the rumen but no signal was caught. *** Only four pH data was recorded for cow No.715 from 09:00 14th to 08:00 15th June.

Table A.3.2 The mean pH for each cow at each hour (if two 24-hour period existed, the mean of two periods was used). The mean, standard deviation (SD), and coefficient variation (CV) calculated for each cow:

	Cow ID								
Time	713	714	715	716	717	718	719	720	721
9:00	6.98	6.83	6.62	6.58	6.3	6.52	6.74	6.65	n/r
10:00	6.72	6.49	6.36	6.8	6.51	6.43	6.42	6.22	n/r
11:00	*n/r	n/r	6.73	6.65	6.61	6.51	6.6	6.42	n/r
12:00	6.94	n/r	7.04	6.76	6.61	6.64	6.72	6.51	n/r
13:00	6.92	7.08	6.69	6.9	6.67	6.78	6.77	6.59	n/r
14:00	7.06	7.08	6.8	6.89	6.67	6.76	6.84	6.69	n/r
15:00	7.21	7.1	6.97	6.89	6.72	6.85	6.78	6.71	n/r
16:00	7.05	7.13	6.35	6.97	6.72	6.44	6.66	6.79	n/r
17:00	6.96	6.72	6.46	7.05	6.62	6.31	6.5	6.48	n/r
18:00	6.3	6.79	6.74	6.38	6.37	6.32	6.5	6.35	n/r
19:00	6.78	6.73	6.6	6.62	6.36	6.43	6.52	6.15	n/r
20:00	6.73	6.94	6.35	6.53	6.21	6.4	6.72	6.41	n/r
21:00	6.79	7.02	n/r	6.78	6.45	6.5	6.65	6.36	n/r
22:00	6.94	6.91	6.74	n/r	n/r	6.56	6.76	6.38	n/r
23:00	6.91	7.48	6.77	6.73	6.61	6.53	6.67	6.39	n/r
0:00	6.89	7.23	6.56	6.67	6.64	n/r	6.73	6.54	n/r
1:00	6.92	7.08	6.77	6.85	n/r	6.58	6.76	6.59	n/r
2:00	7	7.21	6.71	6.8	6.8	6.7	6.77	6.9	n/r
3:00	7.03	7.15	6.85	6.92	6.72	6.75	6.78	6.75	n/r
4:00	7	7.08	6.88	6.94	6.8	6.69	6.89	6.7	n/r
5:00	7.14	7.17	6.64	6.92	6.78	6.88	6.82	6.76	n/r
6:00	7.26	n/r	6.85	6.94	6.83	6.84	7.16	6.79	n/r
7:00	7.2	7.29	7.12	7.08	6.93	6.93	7.32	6.83	n/r
8:00	7.34	7.37	7.08	7.05	n/r	n/r	6.97	6.83	n/r
mean	6.96	7.04	6.73	6.81	6.62	6.61	6.75	6.57	n/r
SD	0.22	0.24	0.22	0.18	0.19	0.19	0.2	0.21	n/r
CV	3.12%	3.35%	3.25%	2.62%	2.84%	2.81%	2.96%	3.15%	n/r

* n/r means no pH data were recorded in that hour for any 24-hour period for this cow.